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SERODIAGNOSIS OF TOXOCARA ANTIBODIES AMONG INFANTS AND PREGNANT WOMEN SUSPECTED OF OCULAR OR VISCERAL TOXOCARIASIS USING TWO TYPES ELISA ANTIGENS [TEE & TEX]

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ABSTRACT

The diagnosis of toxocariasis depends heavily on immunological tests because parasites may be few in the tissue of those infected and, unless situated in an organ such as the eye, may be difficult or impossible to locate. In general, patients with ocular toxocariasis have serum anti-*T canis* antibody titres that are significantly lower than those with visceral toxocariasis. An enzyme linked immunosorbent assay (ELISA) using *T canis* embryonated egg antigen (TEE) and (TEX) were used for diagnosis of toxocariasis. This assay showed a high degree of sensitivity and specificity. Aim of work, diagnosis of asymptomatic toxocariasis in infants before two years old and suspected infection in pregnant women by ELISA with comparison between two antigens TEE & capture TEX. This work was done between 8/2005 and 4/2006. Specimens of serum collected from 79 infants (apparent healthy) aged between 4 weeks to 30 moths (51 females and 28 males) Also, 28 specimens of serum collected from asymptomatic pregnant women aged between 18-32 years old and their infants (28)(17 females and 11 males) at the same age of infants above . The baseline laboratory studies that were done included WBCs, differential count and circulating eosinophil count. Examination of faeces for ova and any parasites. Serodignosis by ELISA using two of antigens , *Toxocara canis* embryonated egg antigen (TEE) and *Toxocara canis* antigen capture ELISA. Results, *Toxocara* antibodies found in 7 &12 pregnant women serum when tested by TEE & capture TEX ELISA respectively. The serum samples of infants (28) which taken from infant's pregnant mothers given positive for *Toxocara* antibodies 3/28 and 7/28 when tested by TEE ELISA and capture TEX ELISA respectively. Active ocular toxocariasis diagnosed in one mother only in left eye. All in active ocular toxocariasis diagnosed by capture TEX ELISA except one baby's serum only diagnosed by TEE ELISA . In conclusion, the capture TEX ELISA was better able to discriminate between positive and negative samples than TEE ELISA. In addition, testing samples by both capture TEX ELISA and TEE ELISA. Toxocariasis should be given more attention and that the ophthalmologists should be more aware of this disease-especially in children and young adults-and should more often include toxocariasis in the differential diagnosis of the ocular diseases.

INTRODUCTION

Human visceral and ocular toxocariasis, which is caused by nematode larvae of the genus *Toxocara*, can occasionally be a serious or life-threatening condition (Schantz, 1989). *Toxocara canis*, an intestinal parasite dogs, foxes and other canids, has a worldwide distribution and is regarded as the main cause of human toxocariasis (Barriga, 1988). Infection in human is caused by ingestion of embryonated *Toxocara* eggs present in soil, water, food, dirty hands, on vegetables or by ingestion of larvae from undercooked giblets. The larvae hatch in the small intestine and migrate through somatic organs, preferably the liver and eyes. Visceral larva migrans is characterized by chronic weakness, abdominal pain, diverse signs of allergy or hypereosinophilia (Taylor et al. 1988). Eosinophilia typically is not detected in children with ocular manifestation (Berrocal, 1980). Ocular larva migrans occurs when larvae become trapped in the eyes, leading to uveitis and optic papillitis (Shields, 1984), when a single larva became trapped in the retina the resulting inflammatory response may lead to impaired vision (Gillespie et al. 1993).Duguid, 1961 and Ashton, 1967 have reported the three more frequently encountered ocular manifestations: chronic endophthalmitis (most prevalent), solitary granuloma and peripheral retinitis (rarely). Ashton believes that three ocular manifestations vary only in severity and location but are the result of the same underlying

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cause. Wilkinson and Weldh reported that a peripheral retinal mass was the most frequent manifestation of ocular toxocariasis. They noticed that elevated retinal folds commonly extended from the peripheral mass to the disc. They concluded that *Toxocara* infection should be suspected in any patient with unilateral pars planitis. In 1972, O'Connor found additional patients with peripheral lesions and a subretinal tube-like structure running from the area of the disc to the peripheral mass. He was convinced that this retinal fold in the from of a tube-like structure was specific for *Toxocara* infection, and was able to confirm the diagnosis in the only eye obtained for microscopic examination. Toxocariasis has also been proposed as a potential aetiology in neurologic disorders when the larvae migrate in the central nervous system (Nicoletti et al. 2002). *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. Sprent, in 1958, suggested that *Toxocara* may carry other diseases, especially into the central nervous system, thereby transporting viruses and other microorganisms across the blood-brain barrier. In 1965, Hutchinson demonstrated that toxoplasma gondii may be faecally transmitted inside the ova of *Toxocara*. This was confirmed in 1967 by Jacobs and his co-works who have shown also that *Toxocara* nematoes obtained from cats are naturally infected with T. gondii. The confirmation of the diagnosis of toxocariasis depends heavily on immunological tests because parasites may be few in the tissue of those infected and, unless situated in an organ such as the eye, may be difficult or impossible to locate (Schantz & Glickman, 1978). Arising from the early work was the development of the toxocariasis skin test (Woodruff et al. 1964) followed by the adaptation of the fluorescent antibody test to toxocariasis (Bisseru and Woodruff, 1968) and the subsequent modification which improved its specificity (Woodruff, 1970). The adaptation by Voller et al. (1976a) of the highly sensitive enzyme-immunoassay procedures for the serological diagnosis of a variety of infectious diseases stimulated this enquiry as to whether the enzyme-linked immunosorbent assay technique could be adapted with advantage for toxocariasis. An enzyme linked immunosorbent assay (ELISA) using *T canis* embryonated egg antigen (TEE) was found to be more sensitive for diagnosis of visceral toxocariasis than either bentonite flocculation or indirect haemagglutination using antigen prepared from adult *T canis* worms (Glickman et al. 1978). Experiences during the few past years have shown several shortcomings in the use 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 (Glickman et al. 1981). As a result, at least one patient with a suspected retinoblastoma and a negative serum ELISA titer for *T canis* has had an eye enucleated, with a pathologist subsequently finding a *T canis* larva on microscopical examination (Searl et al, 1981). A possible solution to this problem was suggested when higher anti-*T canis* antibody titres were found in the vitreous than in the serum of patients with clinically diagnosed toxocaral ophthalmitis (Biglan et al. 1979). It was noted, however, that vitreous aspiration poses some risks and, in the clinically setting, may limit testing to serum only. In 1975 de Savigny described a technique for in vitro maintenance of *T canis* larvae with concomitant production of excretory/secretory or exoantigen (TEX). TEX was used with ELISA for testing patients with visceral toxocariasis. This assay showed a high degree of sensitivity and specificity (Glickman et al. 1985). Gillespie et al, (1993) described an antigen capture ELISA which detects a repeating carbohydrate epitope found in the ES antigens of *T canis.* The authors reported 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.

MATERIAL AND METHOD

Aim of work

Diagnosis of asymptomatic toxocariasis in infants before two years old and suspected infection in pregnant women by ELISA with comparison between two antigens TEE & capture TEX.

Study area

This work was done between 8/2005 and 4/2006. Specimens of serum collected from 79 infants (apparent healthy) aged between 4 weeks to 30 moths (51 females and 28 males) came to The Japan (Abu-El-Reash) Teaching Hospital for examination or vaccination. Also, 28 specimens of serum collected from

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asymptomatic pregnant women aged between 18-32 years old and their infants (28)(17 females and 11 males) at the same age of infants above which attending to the Planning Family centre in El-Basatin area.

Patients and sera

Information about the patient's socioeconomic background, the presence of pets in the household, age, sex, pica history, blood count were prospectively collected in a vacutainer tube, centrifuged twice, and separated immediately. The serum were placed in a special fiberglass refrigerator and covered with dry ice to prevent thawing while being delivered to the laboratory. All infants examined for general health, external evaluation of the conjunctiva, cornea and pupils, motility examination and a complete paediatric evaluation by podiatrist and ophthalmologist. The baseline laboratory studies that were done included WBCs, differential count and circulating eosinophil count. Examination of faeces for ova and any parasites, using both direct and concentration methods.

ELISA antigens and procedure

Toxocara canis embryonated egg antigen (TEE)

Tubes are coated with a known antigen. The adult *T canis* organism were obtained from puppies. The gravid uteri of the live worms were removed by dissection after having washed the worm repeatedly with 0.15 M Nacl. Eggs were removed from the uteri and placed in 1% formalin at room temperature for 21 days to induce embryonization . The embryonated eggs were homogenizwd separately in 0.05 M borate buffer (PH 8.6), using glass Ten-Broeck homogenizers. This mixture was then centrifuged at 2,000 g for 30 minutes. The supernatant fractions were used as antigens. The ELISA used in this study for the detection of antibodies in the serum of patients is a modification (Glickman et al, 1979) of the method developed by Engvall and Perlman, 1971. Microtitre plates were used to perform the assay. The plates were made of polystyrene and the wells coated with the appropriate antigen.

The serum to be tested is added, and the reactions are incubated. If the test serum contains the specific antibody to the antigen, it will attach to the fixed antigen.

An enzyme-linked anti-immunoglobulin (either whole or species-specific IgG) is added and will become fixed to any antibody that had combined with the antigen.

The enzyme substrate (alkaline phosphatase) is then added, yielding, through hydrolysis, the chromogenic component that is measured colorimetrically. The intensity of the colour reaction generated by the enzyme-anti-immunoglobulin (or antispecific globulin, IgG) will be proportional to the amount of antibody present in the original test serum (Bottone,1979).

Toxocara canis antigen capture ELISA

For the preparation of excretory/secretory antigen (TEX), hatched *T canis* larvae were obtained from second stage larvae were hatched from aliquots of the embryonated eggs and maintained in culture following the method of de Savigny, 1975. Hatched larvae were added to serial 50 ml flasks in 10 ml of RPML1640 (KC Biological, Lenexa, KA), PH 7.2, supplemented with 1% glutamine at a concentration of (1X10)3 larvae per millilitre. Cultures were incubated in 5% CO2 at 37C and 95% relative humidity. At weekly intervals cultures were examined microscopically for contamination and larval viability. Cultures with contamination or those with greater than5% larval mortality were discarded. At weekly intervals larvae were allowed to settle for 15 min and the culture medium aspirated aseptically and transferred to sterile centrifuge tubes. Fresh culture medium was added to the flasks and cultures were incubated as before. Conditioned culture medium was centrifuged at 1000 rpm for 5 min and the supernatant fluid was collected aseptically. Weekly samples were collected for up to 16 weeks, pooled and stored at -70C. Medium was exhaustively dialyzed against 0.05 M Tris buffer, PH8.0 containing 0.02% sodium azide, and concentrated using an ultrafiltration system with a 10 000 molecular weight exclusion limit (Amicon Corp, Lexington, MA). An ELISA method was used to detection the presence of IgG antibody directed against the ES antigens of *Toxocara* sp. 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 labeled mouse anti-human IgG monoclonal antibody (de Savigny et al 1979).

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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 optimized by checkerboard titration. Polystyrene microtiter plates (Dynatech) were coated with a mouse monoclonal antibody (1 µg/ml) T cn-2 by incubation over-night 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 labeled T cn-2 with hydrogen peroxide and ortho phenylene diamine (Sigma Chemica Co) as substrate (Gillespie et al. 1993).

Monoclonal antibody : T cn-2 was selected from a panel of anti-*Toxocara* ES monoclonal antibodies because it shows no reaction with other ascarid species (Maizels et al. 1987 and Page et al. 1991). This antibody was purified from ascetic fluid by sepharose 6 FPLC gel filtration. T cn-2 antibody was labeled with horse-radish peroxidase using the two step glutaraldehyde conjugation method (Avrameas et al. 1971).

All serum samples were preabsorbed with AEE (*Ascaris* emberyonated egg antigen). Each 50 µl of specimen be preabsorbed with 20 µl of AEE to remove non specific reactivity to *Ascaris* and to prevent cross reaction (Cypess et al. 1977).

Relative protein concentrations of TEE and TEX antigen were determined by spectrophotometric absorbence at 280 nm with comparison to a bovine albumin standard curve.

Level of positive titers as follows:

1-ELISA , any titre higher than 1:2 as consistent with past or present *T canis* infection.

2-A titer greater than 1:4 as diagnostic in a child with clinical ocular toxocariasis.

3-A titer greater than 1:16 as diagnostic of visceral toxocariasis (Berrocal, 1980).

Statistical analysis

Statistical differences in ELISA values between TEE ELISA & capture TEX ELISA were determined by application of the 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 (Snedecor and Cochran, 1967). The values derived from this analysis are not measures of statistical significance but rather are probabilities of misclassification using TEE ELISA or capture TEX ELISA and were used for comparative purposes.

RESULTS

Toxocara antibodies found only in 7/28 pregnant women when tested by TEE ELISA. One only had ocular toxocariasis when examined by ophthalmologist. By capture TEX ELISA the serum given positive for toxocariasis for 12 pregnant women and only one patient had ocular toxocariasis when examined. The serum samples of infants (28) which taken from infant's pregnant mothers given positive for *Toxocara* antibodies 3/28 and 7/28 when tested by TEE ELISA and capture TEX ELISA respectively. One baby only had ocular toxocariasis by testing capture TEX ELISA. Positive resulting for *Toxocara* antibodies in serum samples of infants had no pregnant mothers which tested by TEE ELISA given 13/79 and 20/79 given positive when tested by capture TEX ELISA. Ocular toxocariasis found only in one baby which his serum tested by TEE ELISA but 2 babies had ocular toxocariasis when their serum tested by capture TEX ELISA and examined by ophthalmologist. All ocular toxocariasis were inactive and had no ocular manifestation except one pregnant mother had active toxocariasis in one eye only (left eye) when examined by ophthalmologesit

DISCUSSION

In this study, the serum taken from pregnant mothers and their infants (28 & 28) and also, taken from 79 infants had no pregnant mother . The children were aged between one and 30 months. The infants before six months had no any titer for ELISA TEE OR capture TEX. Also, we neglected any low titre for antibodies because acquired antibodies IgG through placental transfer from the mother. IgG is catabolized with a half-life of 30 days. A fall in IgG titre over the first three months is registered, acerbated by the increase in the blood volume of the growing infant. Thereafter, the rate of synthesis over takes the rate of breakdown of

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maternal IgG and the over-all concentration increases steadily (Tiselius et al. 1974). Since the other immunoglobulins do not cross the placenta, only traces are present in the circulation of the newborn the exception is IgM low but significant levels of IgM in cord blood are present in the newborn. These are synthesized by the body, and reach adult levels after nine months (Char, 1978). The children of six months or more, given titre for antibodies with two ELISA tests (tables 3&4) and (tables 5&6). At age of six months the infant started crawling and eating pica or any thing on the floor and their handsare not frequently washed. Also, they pretend to play with animals like dogs and cats which increase the risk of *Toxocara* infection in this population group. They were not grouped by sex as no important differences in *Toxocara* seropositivity were found between males and females and, also, there were no significant differences between patients from different regions either and no significant difference in number of positive sera between the younger and older age (logar et al. 2004). Pregnant mothers may crave for eating the pica and also, presence of dogs and cats in their houses or in the neighborhood increasing the risk of *Toxocara* infection, that showed in our data with two ELISA tests tables (1& 2). The ELISA technique takes advantage of chromogenic substrates, which are colorless prior to enzymatic stimulation but develop a colour product after enzymatic degradation the intensity of the liberated chromogen, as determined colorimetrically, is directly proportional to the concentration of antigen or antibody present in the test sample (Berrocal, 1980). 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. It binds to a repeating carbohydrate epitope found on all the major components of the ES antigens (Gillespie et al. 1993). Specificity is important when other parasites are capable of producing similar clinical manifestations but where medical management may differ significantly. Such is the case with ocular toxocariasis. Whereas children with visceral toxocariasis typically have pronounced eosinophilia and anti-*T canis* specific antibody titres in their serum. Patients with ocular toxocariasis are usually asymptomatic and often have lower concentrations of eosinophils and circulating anti –*T canis* specific antibody (Glickman et al. 1985). These differences have been attributed to the level of infection; children with visceral toxocariasis are often geophagic or coprophagic and consume many eggs, while children with ocular toxocariasis lack this habit and have probably been inadvertently infected with few eggs (Glickman et al. 1981). Results of our studies have shown that TEE and capture TEX ELISA are comparably sensitive. Capture TEX ELISA, however, resulted in better discrimination between the positive and negative sera than TEE ELISA. That agreement reported study by Glickman and his team, 1985, when using TEE and TEX ELISA for immunological diagnosis of ocular toxocariasis sera were available from 11 patient with clinically diagnosed unilateral ocular toxocariasis and negative *Toxocara* specimens were obtained fron 12 patients with clinically diagnosed unilateral retinoblastoma . Sakai et al (1998), found out in the macula lesion of the patient's eye, who lost visual acuity caused by a *Toxocara* spp., that the serum of this patient did not show strong reaction to excretory/secretory (ES) antigen from *T canis* larvae, but did show a strong reaction in ELISA with antigens of *T canis* larvae (Fisher, 2003). So it is suggested that some of our *Toxocara* positive patients could be infected by the eggs of *T cati*. Logar et al. 1993, found out that the incidence rate of *Toxocara* infection in ocular patients of Slovenia had increased from 21 to 28% when compared period 1990/1991. Another explanation for high percent of *Toxocara* antibodies in ocular patients found in Logar, 2004, study might also, be higher quality of diagnostic tests which are more specific and sensitive than 12 years ago when they used the ELISA test with *Toxocara* excretory/secretory (ES) antigen. *Toxocara* ELISA values greater than 1.50 indicate significant levels of specific anti-body and are associated with active or recent clinical toxocariasis (de Savigny et al. 1979) . In Brazil, Lima Coe'lho et al.2005 reported that prevalence rates reached approximately 40% in different study areas. However, when compared with results obtained from other studies this seems to be extremely high. The high prevalence rates could be due to the cross-reactivity antigens used. However, no cross-reaction was observed in an ELISA using serum samples from mice experimentally infected with *A suum* (Yamasaki et al. 2000)

CONCLUSION

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The capture TEX ELISA was better able to discriminate between 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. Such an assay might facilitate study of the natural history of infection. However, the small amounts of antigens required and the comparatively large numbers of tests. Thus, the use of the recombinant *T canis* antigen, even in different diagnostic methods, would be recommended not only for routine diagnosis, but also for seroepidemiologic surveys of toxocariasis in humans. *Toxocara* infection in humans can be ascribed to generally inadequate knowledge about the round worms of dogs and cats, the route of infection and about preventive measures which include better personal hygiene and elimination of intestinal parasites from pets. It is suggested that in high prevalence of *Toxocara* seropositive cases in ocular patients, toxocariasis should be given more attention and that the ophthalmologists should be more aware of this disease-especially in children and young adults-and should more often include toxocariasis in the differential diagnosis of the ocular diseases.

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Table 1 : *Toxocara canis* antibodies titer measured by TEE ELISA in pregnant women

No. of cases	ELISA titer	Pit presence in their house		Pica	WBCs	E%	Ova and other parasites
		Dogs	Cats				
1	1: 8	-	-	+	7000	2	-
2	1: 16	-	+	-	9200	11	*E histolytica*
3	1: 32	+	-	-	11000	6	*G lamblia*

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4	1: 128	+	+	-	12700	18	*S. mansoni*
5	1: 8	+	-	-	9100	1	-
6	1: 32*	-	+	+	8500	5	-
7	1: 8	-	-	-	6500	3	-

*Active ocular toxocariasis

Table 2 : *Toxocara canis* antibodies titer measured by capture TEX ELISA in pregnant women

No. of cases	ELISA titer	Pit presence in their house		Pica	WBCs	E%	Ova and other parasites
		Dogs	Cats				
1	1: 32	+	-	-	10900	8	*E histolytica*
2	1: 16	-	-	+	9500	6	-
3	1: 8	-	-	-	7000	1	-
4	1: 8	-	-	+	8500	2	-
5	1: 32	-	+	-	9800	9	*S. mansoni*
6	1: 8	-	-	-	8000	6	-
7	1: 16	+	-	-	11000	3	*G lamblia*
8	1: 128	+	-	-	10500	12	*S. mansoni*
9	1: 64**	-	-	-	9500	9	-
10	1: 8	-	-	-	7500	0	-
11	1: 8	-	+	-	9300	5	-
12	1: 16	-	-	+	6800	2	-

** Inactive ocular toxocariasis

Table 3 : *Toxocara canis* antibodies titer measured by TEE ELISA in pregnant mothers's infants

No. of cases	Age by months	ELISA titer	Pit presence in their house		Pica	WBCs	E%	Ova and other parasites	Sex
			Dogs	Cats					
1	9	1: 32	+	-	-	9500	11	*H nana*	Female
2	15	1: 4	-	-	+	8900	2	-	Female
3	26	1: 16	-	-	-	8500	5	-	Female

Table 4 : *Toxocara canis* antibodies titer measured by capture TEX ELISA in pregnant mothers's infants

No. of cases	Age by months	ELISA titer	Pit presence in their house		Pica	WBCs	E%	Ova and other parasites	Sex
			Dogs	Cats					
1	10	1: 16	+	-	-	9500	1	-	Female
2	7	1: 32**	-	-	+	8300	3	-	Male
3	22	1: 128	+	-	+	12000	11	*E vermicularis*	Male
4	24	1: 8	-	-	-	7400	0	-	Female

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5	9	1: 8	+	-	-	7800	5	*G lamblia*	Female
6	9	1: 32	-	+	+	6200	6	*E histolytica*	Female
7	30	1: 16	-	-	+	8500	0	-	Male

** Inactive ocular toxocariasis

Table 5: *Toxocara canis* antibodies titer measured by TEE ELISA in infants

No. of cases	Age by months	ELISA titer	Pit presence in their house		Pica	WBCs	E%	Ova and other parasites	Sex
			Dogs	Cats					
1	10	1: 8	-	-	-	7000	6	-	Female
2	6	1: 32	-	+	-	6500	3	-	Female
3	12	1: 8	-	-	+	7500	0	-	Male
4	9	1: 16	+	-	-	9000	7	*H nana*	Female
5	28	1: 8	-	-	+	8800	5	-	Female
6	30	1: 128	+	-	+	11300	13	*H nana*	Male
7	22	1: 8	-	-	-	8500	1	-	Female
8	18	1: 16	-	-	-	9300	6	-	Female
9	24	1: 32	+	-	-	8500	8	*E histolytica*	Male
10	30	1: 8	-	-	-	7500	1	-	Female
11	7	1: 8**	-	-	+	8700	2	-	Female
12	9	1: 8	+	-	-	9000	5	-	Male
13	18	1: 32	-	+	+	10500	9	*G lamblia*	Female

** Inactive ocular toxocariasis

Table 6 : *Toxocara canis* antibodies titer measured by capture TEX ELISA in infants

No. of cases	Age by months	ELISA titer	Pit presence in their house		Pica	WBCs	E%	Ova and other parasites	Sex
			Dogs	Cats					
1	18	1: 16**	-	+	-	8500	2	-	Female
2	22	1: 2	-	-	-	7300	0	-	Female
3	7	1: 32	+	-	-	9300	1	-	Male
4	9	1: 8	-	-	-	8000	5	*E histolytica*	Female
5	21	1: 8	-	-	-	6200	6	-	Female
6	24	1: 8	-	-	-	9100	3	-	Female
7	30	1: 32	+	-	+	9500	8	*E vermicular*	Male
8	8	1: 16	+	-	-	8700	1	*G lamblia*	Male
9	7	1: 8	-	-	+	7500	0	-	Female
10	25	1: 128	+	-	+	11500	14	*H nana*	Male
11	13	1: 8	-	+	-	5900	9	-	Female
12	18	1: 64	+	-	-	16700	11	-	Female

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13	24	1: 8	-	+	-	8500	0	-	Female
14	22	1: 32	+	-	-	9200	3	-	Female
15	10	1: 8	-	-	+	8500	5	-	Male
16	12	1: 16	+	+	-	8500	6	*G lamblia*	Female
17	24	1: 32	+	-	-	9700	2	*E histolytica*	Female
18	18	1: 8	-	-	-	5600	8	-	Male
19	9	1: 8	+	-	-	7800	1	-	Female
20	12	1: 32**	+	+	+	9900	5	-	Female

** Inactive ocular toxocariasis