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RECOGNITION OF EXCRETED-SECRETED AND SOMATIC ANTIGENS FROM *TOXOCARA CANIS* LARVAE BY SERA OF PATIENTS WITH DIFFERENT CLINICAL MANIFESTATIONS OF HUMAN TOXOCARIASIS

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Abstract: Human toxocariasis is one of the most reported zoonotic helminth infections in the world, whose etiological agent is *Toxocara canis*. Depending on the affected organ, several clinical manifestations can be distinguished: visceral toxocariasis, pulmonary toxocariasis, cutaneous toxocariasis, ocular toxocariasis or asymptomatic toxocariasis, among others. This makes the diagnosis very difficult. The purpose of this study is to contribute to the knowledge of human toxocariasis, especially in the serodiagnosis, analysing the recognition of specific antigens by sera from patients with different clinical manifestations of the disease. Western blot technique (WB) was used to analyse larval somatic antigens (LS) and excreted-secreted (ES) antigens of larvae of *T. canis*, recognized by IgA and IgG class of immunoglobulins. Thus, four detection systems were used: LS/IgG, LS/IgA, ES/IgG, and ES/IgA. The antigen detection was greater with LS antigens than ES antigens using Western blot. The highest sensitivity (81%) was found with the LS / IgG system, and the lowest (19%) with the ES/IgA one; in relation to sera detected by ELISA. Very interesting, with almost all antigen/antibody systems were detected different sets of antigens; depending of kind of toxocariasis were the sera came from. Even more, in several cases a unique antigen, was found only with one kind of toxocariasis sera. However, it is necessary further studies in order to confirm these results with a more high number of patients presenting these four form of toxocariasis. The greater percentage of detection (78%) with a specific antigen of 40 kDa, was obtained using ES / IgA, in asyntomatic toxocariasis. This result is relevant since this antigen could guide diagnosis in the acute phase of the disease. However, the finding of specific antigens to some of the clinical manifestations opens the possibility of optimizing techniques using purified antigens to maximize their detection, in order to improve the diagnosis of the different toxocariasis forms.

Keywords: Human toxocariasis, antigens, western blot, clinical manifestations.

INTRODUCTION

Toxocariasis is one of the most commonly reported zoonotic helminth infections in the world with a higher prevalence in tropical settings and in rural populations (MACPHERSON 2013; MA et al. 2018; FAKHRI et al., 2018). According to worldwide epidemiological studies, toxocariasis would infect tens of millions of people annually and more than 100 million dogs (MA et al. 2018; FAKHRI et al., 2018; ROSTAMI et al., 2020; SCHWARTZ et al., 2022). In most cases infection by *Toxocara* spp. is asymptomatic. However, when they present as a disease, the clinical manifestations can be presented as a different syndromes such as: visceral larva migrans (VLM), ocular larva migrans (OLM), neurotoxocariasis, and covert or common toxocariasis (MAGNAVAL et al., 1997; FINSTERER et al., 2007; PIVETTI-PEZZI et al., 2009; MA et al., 2018; BARRIOS et al., 2020; CHAMORRO 2022).

A definitive diagnosis of human toxocariasis is often a challenge for the clinician since the clinical signs and symptoms of the disease are non-specific. Within the paratenic host, *Toxocara* spp. larvae can migrate widely including the liver, lungs, musculature and epidermis causing also, syndromes associated to respiratory system (LI et al., 2014; AGHAEI et al., 2018) and allergy skin disorders (MOHAMMADZADEH et al., 2018).

The severity of the disease in humans depends not only on the intensity of infection and sites of perforation but also on the intensity of inflammatory response of the

host. In infected paratenic hosts, the level of immunoglobulins IgG, IgM, and particular IgE increases, accompanied by eosinophilia (BUIJS et al 1995; ALTCHEN et al., 2003; MA et al., 2018; CHAMORRO 2021). The question of which serum antibody isotypes are most relevant still remains to be explored. Most human infections generate antibodies of the IgG1 subclass, with significant levels of both IgM and IgE (MAGNAVAL et al., 1992; SMITH, 1993; UHLÍKOVÁ et al., 1996; ALTCHEN et al., 2003).

Analyses of differential expression profiles of anti-*Toxocara* spp. antibody isotypes, studying children receiving thiabendazole therapy, found that specific IgE and eosinophilia declined within the first year, with IgA and IgG4 falling at later times (ELEFANT et al., 2006). A Western blotting (WB) assay using excretory-secretory *T. canis* antigens was implemented for monitoring IgG, IgE and IgA antibodies in 27 children with toxocariasis for 22-116 months after chemotherapy (RUBINSKY-ELEFANT et al., 2011). The WB sensitivity was: 100% for IgG antibodies detecting bands of 29-38, 48-54, 95-116, 121-162, >205 kDa. The IgA sensitivity was 65.4% detecting bands of 29-38, 48-54, 81-93 kDa. Candidates for diagnostic markers should be IgG antibodies to bands of low molecular weight (29-38 and 48-54 kDa).

There are few studies on IgA antibodies in toxocariasis even in other parasitic infections (Matsumura et al., 1983; Nunes et al., 1997; Chamorro 2022). However, they have been detected in at least two different conditions: (a) in the acute phase of the infection, disappearing afterwards, and in the chronic phase; in which IgA antibodies last for a long period of time. This process is not well understood; but this could occur when the mucosal lesion is maintained and the local secretion of IgA does not resolve the damage (Rubinsky-Elefant et al., 2011).

Western blotting is typically used as a confirmatory diagnostic test, because of its improved specificity, following a positive diagnosis by ELISA (MAGNAVAL et al., 1991; 1992). Studies in which the specificity and sensitivity of the conventional ELISA have been compared with the WB method, in both cases using excreted – secreted antigens (TES), have shown that the WB is better; both in the serodiagnosis of patients with eosinophilia (ONER et al., 2007) and in patients with ocular toxocariasis (LOGAR et al., 2004; BELLANGER et al 2010). The same authors demonstrates the need to perform Western blotting immunodiagnosis, whatever the TES–ELISA result, to improve diagnosis of human toxocariasis in patients with chronic urticaria caused by Toxocara infection.

The set of this information shows that it is still necessary to continue improving both the sensitivity and the specificity of the two methods currently most used in the serological diagnosis of toxocariasis, and that, as several authors recommend, always after a first result with ELISA should be confirmed with a WB assay (ONER et al., 2007; LOGAR et al., 2004; BELLANGER et al. 2010; Ma et al., 2018). The information analyzed here also shows that there is currently no conclusive serological method available that allows distinguishing the different clinical manifestations of toxocariasis (SMITH et al., 2009; BELLANGER et al 2010; Fillaux and Magnaval 2013; Rick M, 2013; Ma et al., 2018). In order to contribute to the knowledge of the humoral immune response in patients with different clinical pictures of toxocariasis; in the present work, using Wester blotting, we analyzed the antigens detected by different isotypes of immunoglobulins present in sera of children with different clinical manifestations of this important parasitic disease.

MATERIALS AND METHODS

LARVAL SOMATIC ANTIGEN (LS) AND EXCRETED-SECRETED ANTIGEN (ES) OF *TOXOCARA CANIS*

Worms were collected from infected dogs from different regions of the country. The method to obtained larval somatic and excreted – secreted antigens was performed according to the protocols described using PMSF protease inhibitor in phosphate saline buffer to recover excretory – secretory antigens ((BOWMAN et al. 1987 MAIZELS et al. 1987 and KOZEK VILLANUEVA, 1994).

SERA

From patients diagnosed in Parasitology Unit of Luis Calvo Mackenna Hospital, by ELISA using ES/IgG (JACQUIER et al. 1991), were grouped as follows:

a) 101 sera positive for *T.canis* from children with an average age of seven years, divided according to clinical diagnosis:

- 32 sera from patients with asymptomatic or covert toxocariasis, ie with no specific symptoms but with positive serology.
- 42 sera from patients with systemic manifestation, with divided according to clinical symptoms: 23 sera from patients with clinical symptoms in the lungs, 5 sera from patients with cutaneous symptoms, 1 patient serum with clinical manifestation in the liver, 11 sera of patients with CNS, 2 sera from patients with clinical heart symptoms.
- 27 sera from patients with ocular toxocariasis.

b) Controls: 10 sera from patients after being treated for toxocariasis that showed negative serology by ELISA

- 10 sera from apparently healthy patients with negative serology by ELISA were used as controls.

c) 24 patients with other parasitic infections, as follows: 6 sera of patients with Chagas' disease, 6 sera from patients with toxoplasmosis, 7 hydatid disease patient sera, 5 sera from patients with cysticercosis. All sera were used at 1:40 dilution in TBS with 1% skim milk.

The protein concentration determined by Bradford, (1976) method was in the extract LS (15 ug / ul) and the extract ES (10 ug / ul).

WESTERN BLOTTING TECHNIQUE

The electrophoretic process was performed in 7.5% minigels polyacrylamide in the presence of SDS (LAEMMLI, 1970), using the technique of ZINGALES (1984). Molecular weight standard (MWS) were included in each run (western Chemichrome control C 4236 SIGMA). The blotting technique described by Godin and Handman (1984) was performed. Nitrocellulose membranes were incubated with anti-IgG or anti-human IgA (SIGMA) conjugated to alkaline phosphatase. The Estimation of the molecular weights (MW) were done using colored markers (Chemichrome controlling western SIGMA C 4236) whose MW were 220, 100, 60, 45, 30, 20, 12 kDa.

RESULTS

Analysis by Western blotting was performed with larval somatic (LS) and excretory – secretory (ES) antigens from larvae of *Toxocara canis* that were recognized by specific IgG and IgA antibodies present in the sera of patients with different clinical manifestations of the disease.

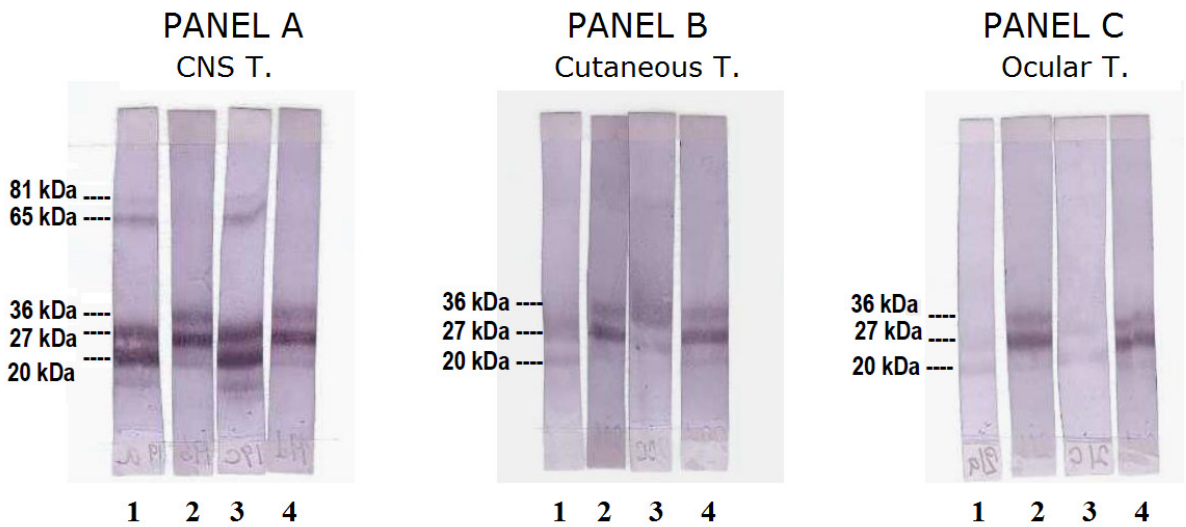

Figure 1- Antigens detected by antibodies against *Toxocara canis* in serum samples from patients with different manifestations of human toxocarosis detected with four methods: 1) Somatic antigens (SA) and IgA antibody (Sa/IgA); 2) Somatic antigens (SA) and IgG antibody (SA/IgG), 3) Excretory - secretory antigens (ES) and IgA antibody (ES/IgA), and 4) Excretory - secretory antigens (ES) and IgG antibody (ES/IgG). Panel A: central nervous system toxocariasis, panel B: cutaneous toxocariasis, and panel C: ocular toxocariasis), using the western blot technique.

Figure 1 shows that the main difference observed in the detected antigens is due to the antibody class analyzed. Thus, for example, with IgA antigens of 20, 27, 65 and 81 kDa are detected; with IgG, antigens of 27 and 36 kDa are detected (Panel A). With the cutaneous (panel B) and ocular (panel C) toxocariasis sera, only with IgG, two antigens of the 27 and 36 kDa were detected. This shows that, at least with these sera, the greatest difference is due to the class of the immunoglobulin detected, and not mainly to the type of antigen used: somatic or excreted-secreted.

When analyzing 101 sera with human toxocariasis, without being classified by clinical manifestation, it was observed that under the four conditions it was not possible to detect all of them as positive (Table 1). The maximum detection using one detection system was 81% that was obtained using LS /IgG and the minimum (19%) was obtained with ES/IgA antibody. Nineteen percent of sera were positive using the system ES/IgA, indicating acute infection that is consistent with acute clinical records.

ANALYSIS OF SERA CLASSIFIED ACCORDING TO CLINICAL MANIFESTATION

Serum samples from patients with pulmonary toxocariasis (P) (n = 23): the maximum positive sera (74%) was achieved when using the LS / IgG and minimal with the ES / IgA (17%). The panel of antigens (kDa) that is required to detect the largest percentage of the sera that were positive for each of the four test conditions is shown in Table 2. They include antigens that are recognized by IgA and IgG with LS (27 kDa) and ES (48 and 20 kDa).

Ag/Ab system	Kind of toxocariasis:			
	Pulmonary (Set/WB; WB/E; %ELISA)	**Cutaneous (Set/WB; WB/E; %ELISA)**	**Neurotoxocariasis (Set/WB; WB/E; %ELISA)**	**Ocular (Set/WB; WB/E; %ELISA)**
LS/IgA	20, 27, 65 (8/8; 8/23; 35%)	27 (4/4; 4/5; 80%)	27 (3/3; 3/11; 27%)	20, 27 65, 81 (11/12; 12/27; 44%)
LS/IgG	27, 52, 87 (17/17; 17/23; 74%)	27 (5/5; 5/5; 100%)	27, 81 (8/8; 8/11; 73%)	27, 36, 52, 65, 101 (22/23; 23/27; 85%)
ES/IgA	20, 48 (4/4; 4/23; 17%)	27 (4/4; 4/5; 80%)	20 (1/1; 1/11; 9%)	20, 40 (2/2;2/27; 7%)
ES/IgG	20, 45, 48 (13/13; 13/23; 57%)	27, 75 (5/5; 5/5; 100%)	27, 48 (3/3;3/11; 27%)	27, 36, 45 (12/12;12/27; 44%)

Set = number of sera which detected the corresponding set of antigens using WB.

WB =number of total sera detected by western-blot.

WB/E =number of sera detected by western blot / number of sera detected by ELISA.

%ELISA =percentage of positive sera detected by western-blot in relation to the total sera detected by ELISA.

Table 1- Sets of antigens detected by sera from patients with different kind of toxocariasis.

In Table 1 is shown the different sets of antigens detected by the four Ag/Ab systems grouped according to the kind of toxocariasis were the sera came from. Thus, as it is observed, with the LS/IgA system and sera from pulmonary toxocariasis are detected three antigens of 27, 52, 87 kDa. In this case, 8 sera detected this set of antigens out of the 8 positive sera with western-blot; corresponding to the 35% of the total number of 23 positive sera from pulmonary toxocariasis detected by ELISA.

Analyzing sera from pulmonary toxocariasis (n = 23) and comparing the LS with ES source of antigens is observed that an antigen of 27 kDa is recognized only on the LS antigens. In contrast, only with the ES antigens is detected an antigen of 48 kDa. The antigen of 20 kDa is not specific of ES source because also is detected with the LS/IgA system. No is observed any specific antigen depending of immunoglobulin classes; but, the higher percentage of sera were detected with the IgG, either with LS or ES source of antigens.

Analyzing sera from cutaneous toxocariasis (n = 5) it is observed that the same antigen of 27 kDa is detected with the four Ig/Ab systems, and only with the ES/IgG system was detected a second antigen of 75 kDa. Comparing the immunoglobulin classes, the higher percentage of sera were detected with the IgG (100%).

Sera from patients with neuro toxocarosis (n = 11): the maximum positive sera (73%) was achieved when using the LS / IgG and minimum ES / IgA (9%). 27 kDa antigen is present in both antigen extracts, but is not recognized by IgA antibodies in ES.

Serum samples from patients with human ocular (O) toxocariasis (n = 27): the maximum positive sera (85%) was achieved when using the LS / IgG and minimal the ES / IgA (7%). Comparing the LS with ES antigens extracts, it is observed that an antigen of 65 kDa was only detected with the LS. Similarly, antigens of 40 or 45 kD are only present in ES extract. Comparing the immunoglobulin classes, it is observed that an antigen of 20 kDa is only

detected by IgA; on the contrary, an antigen of 36 kDa was only detected with IgG.

In general comparing the different kind of toxocariasis, along same Ig/Ab system, the Table 2 shows that almost all these systems (with the exception of LS/IgA) detected different panel of antigens that can differentiate these different forms of toxocariasis. It is true that various molecular weights of antigens are repeated in various forms of toxocariasis; such as the 27 KDa antigen that is detected in the four forms of toxocariasis with the LS/IgC system. It is likely that it is the same molecule detected in the two antigen extracts; but the difference is that the sets of antigens are different. In some cases, there are even antigens that with the same Ig/Ab system were only detected in a single form of toxocariasis; such as the 81 kDa antigen detected in ocular toxocariasis with the LS/IgA system; or the 87 kDa antigen detected in pulmonary toxocariasis with the LS/IgG system; or the 48 kDa antigen detected in pulmonary toxocariasis with the ES/IgA system; or the 75 kDa antigen detected in cutaneous toxocariasis with the ES/IgG system. Even neurotoxocariasis could be differentiated from the other three forms of this disease, since with three Ig/Ab systems different profiles or antigen panels are detected; with the exception of the LS/IgA system that detects an antigen of 27 kDa in this form of toxocariasis and in the skin.

Other toxocariasis, analyzed with these four Ag/Ab systems, were hepatic toxocariasis and cardiac toxocarosis, respectively (not shown). In the first case only one serum sample was provided to us which was positive with the four Ag/Ab systems; detecting an antigen of 20 kDa on both antigenic extracts and with both antibody classes (not shown). On the second case, only two sera from patients with cardiac toxocarosis were analyzed; detecting an antigen of 27 kDa with the four Ag/Ab systems. In this last case, LS / IgA and LS / IgG recognized an antigen of 59 kDa and ES / IgG detected a 20 kDa one.

Serum samples of patients with asymptomatic human toxocariasis (n = 32): the maximum positive sera (81%) was achieved when using the LS / IgG and minimal to ES / IgA (28%) (Table 2). The panel of antigens is required to detect 100% of the sera that were positive for each of the four test conditions is shown in Table 2.

A 27 kDa antigen is present in both LA and ES extractsc and is recognized by both classes immunoglobulins, whereas the 65 kDa is present only in the LS extract and is recognized by both classes of immunoglobulins. Something similar happens with the 40 kDa antigen present only in type ES antigen extract and is also recognized by the 2 classes of immunoglobulins.

Ag/Ac system	Most frequent antigens (kDa)	SERA + / n + (WB) (%)	Samples + / n (E) n = 32
LS/IgA	15, 27, 65, 75	16/16 (100)	16/32 (50)
LS/IgG	27, 36, 65	26/26 (100)	26/32 (81)
ES/IgA	27, 40, 93	9/9 (100)	9/32 (28)
ES/IgG	27, 40, 52	17/17 (100)	17/32 (53)

LS = somatic antigen, ES= excreted-secreted antigen Ag/Ab= antigen/antibody system

n+ (WB) = total number of positive samples by Westhern blot.

n+ (E) = total number of positive samples by ELISA

Table 2- Most frequent antigens detected in sera from patients with asymtomatic human toxocarosis.

DISCUSSION

Identify specific antigens of *T. canis* is an important task to develop as it would achieve better diagnostic techniques and evaluation of the efficacy of chemotherapeutic treatment (Morales et al, 2002). One technique to address this problem is Western blot. With respect to the preparation of the antigens used in

western blot, Robertson et al (1989) described proteolytic enzymes that are secreted by in vitro culture of larval *T. canis* and suggested that some species of nematodes, including *Toxocara* spp, have protease activity in their products ES. For this reason in the present study were processed the antigens used in the presence of protease inhibitors.

The most commonly used serological test for diagnosis is the ELISA assay using ES antigen (JACQUIER et al, 1991). A positive ELISA *Toxocara* can be confirmed by western blot because is sensitive and highly specific detecting low MW bands of 24 to 35 kDa (MAGNAVAL et al, 1991). The sera tested in this study were positive by ELISA for toxocariasis using ES / IgG. The results in this study show that under our conditions, the western blot technique allowed us to detect up to 81% of sera, which was obtained when using the LS antigen recognized by IgG antibodies.

The western blot sensitivity was lower compared to the ELISA test. One explanation could be that there is a significant percentage of antibodies in sera from patients which are directed against native antigens and are not detected by the technique of western blot; due that the antigens used were denatured with detergent (SDS) and reduced with β-mercaptoethanol. However, 10 sera from patients who had received specific treatment for human toxocariasis showing negative ELISA, were positive in the Western blot system LS / IgG. A 27 kDa antigen was detected in all positive samples, suggesting that it is an antigen that would not evidence active infection.

Santillán (2000) conducted a study in human toxocarosis in which specific bands detected by Western blot using ES extract recognized antigens corresponded to 30, 32, 55, 70 and 120 kDa; in which the approximately 120 kDa triplet is responsible for cross-reactivity with other parasites. On European studies, antigens of 24, 25, 26, 30, 37, 50, 70, 120 and 150 kDa were detected; the authors conclude that when observing high MW bands (50, 70.120, and 150 kDa) can be treated a recent infection or cross-react with other helminths (MAGANAVAL et al, 1991, COURTADE et al, 1995).

Morales et al (2002) conducted a study in rabbits in which a correlation between the pattern of bands by western blot and the state of infection was observed. Thus, within the first month, after infection, specific antigens of 35, 92, 116 and kDa were observed; whereas antigens of 28, 31, 45, 66, 80 and 92 kDa appear later. Antigens of 35 and 92 kDa were maintained throughout the course of the disease. Moreover, the results showed concordance between ELISA and western blot. Similarly Magnaval et al (1991) - in a study on the application of western blot to the diagnosis of human toxocariasis- showed a correlation between these two immunological methods.

To our knowledge, till now it has not been reported in the literature studies that used the four systems analyzed in this paper. To compare the antigens used (LS and ES), most detection is achieved, with LS independent of the class of antibody (IgG and IgA). Moreover, if the presence of IgA determines the acute phase of infection, our results would show that the maximum acute 43% would be detected when using the LS antigen and a minimum of 18% with ES. These percentages are consistent with those reported in literature (approximately 30%) (MINVIELLE et al, 1999). Because, almost the all studies reported in literature, using western blot, correspond to ES/IgG, here we only performed comparisons with respect to this system. In this sense, our results are consistent with those of other authors, who point out that the low MW antigens would be specific for toxocariasis (Magnaval et al,

1991). Low MW bands that were mainly detected were of 20, 27, 36, 40 and 45 kDa.

According to the literature, the most common antigens in all clinical presentations were those of low MW; which in our study corresponded to 20 and 27 kDa, detected in the four systems used. Higher MW antigens were detected in the systems LS /IgA and LS/ IgE, which have been mentioned in literature to be responsible for cross-reactions (Smith et al, 1982, Vergara, 1991).

Magnaval et al (1991), using the western blot technique for immunodiagnosis of human toxocariasis, classified into two groups seven antigens detected: a group of high MW (132, 147 and 200 kDa) and a low MW (24, 28, 30 and 35 kDa), they suggest that the high MW group may be due to cross-reactivity, while the low MW appear to be more specific Toxocara spp.

Jacob et al (1995) observed four bands with MW between 29 and 210 kDa and noticed that 66 kDa were also identified in some patients whose sera were used as negative controls. Nunes et al (1997) detected numerous bands between the next molecular weight ranges: 29 – 35, 50 – 55, 97 – 116 and about 205 kDa. Consistent with other studies, no cross-reactivity with low MW antigens was observed with other antigens of ascarids.

In general, no reported studies are found regarding the possible association between antitoxocara specific antibodies and various clinical manifestations of toxocariasis using Western blot method.

The percentage of toxocariasis cases in children reported in our country by hospitals varies with the clinical manifestation of the disease and the different prevalences observed; ocular toxocariasis in particular is difficult to diagnose. For example, the serological diagnosis of human ocular toxocariasis using ELISA with ES / IgG system, is less sensitive than for other manifestation (GLICKMAN et al, 1986). Another problem could be the cutoff used for the diagnosis of human toxocarosis. Various studies have shown that, in case of ocular syndrome, by lowering the ELISA cutoff (1:8 or 1:2) increases the probability of detection of cases. Moreover, a test negative does not exclude the possibility of human ocular toxocariasis (GILLESPIC et al, 1993).

The main objective of this study was to find the possible association of clinical manifestations of human toxocariasis and recognition of specific antigens unique to each presentation. In general, as it was mentioned in results, with almost all Ig/Ab systems it was found different antigen sets depending of toxocariasis form. Even in several cases and using the same Ag/Ab system it is found a unique antigen. For instance, with the LS/ IgA an antigen of 81 kDa was detected only with ocular toxocariasis sera. The same was observed with other systems, such LS/IgG, ES/IgA and ES/IgG in which a 87, 48 and 75 kDa antigens were detected with pulmonary, pulmonary and cutaneous toxocariasis sera. Among other case are: an antigen of 81 kDa with LS/IgG system detected by neorotoxocariasis, and an antigen of 101 with LS / IgG detected bu ocular sera. When analyzed 24 serum samples from patients with other parasitic and human toxocarosis negative by ELISA. Cross-recognition was observed mainly PM antigen greater than 50 kDa (106, 101, 97, 93, 81, 65, 56 and 52 kDa), this is consistent with that reported in literature by other authors (Smith et al, 1982) (appointments), which supports the use of the western blot technique to differentiate the antigens that are recognized by the antibodies. The similar was found with sera from asymptomatic toxocariasis, in which an antigen of 40kDa with ES / IgA system was detected. This fact is important because this

antigen would be detected during the acute phase of infection in individuals without clinical symptoms.

CONCLUSION

In this work is shown that with four serological methods using either somatic or excretory-secretory source of antigens, or detecting the IgA or IgG immunoglobulins four forms of toxocariasis can be differentiated. To mention: pulmonary toxocariasis, cutaneous, neurotoxocariasis and ocular toxocariasis. However, it is necessary to increase the number of patients studied with these different clinical pictures; particularly with a greater number of cases of neurotoxocariasis and ocular toxocariasis, where a very small number of patients with both forms of this disease were studied.

It is also very important to highlight that a 40 kDa antigen was detected in the sera of asymptomatic patients, which could be useful to detect patients who were exposed to the parasite.

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