

An overview of *Toxocara cati* infection in stray cats in the metropolitan region of Tehran, Iran, and a comparison of two diagnostic methods

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Abstract

Toxocara cati is one of the most important and widespread of the helminth zoonosis. In the present study, helminthosis due to *Toxocara cati* in 55 stray cats in Tehran was studied by necropsy. In addition, two different diagnostic methods, including serological and coprological tests, for infection with this parasite were compared. The dot-ELISA assay used *Toxocara cati* crude antigens to evaluate the presence of serum antibodies against the mature nematode. The coprological sedimentation method was carried out to assess the output of eggs. In autopsied cats, 52.7% were infected with *T. cati*. Seropositive cases were detected in 53.8% of examined cats, whereas the prevalence in feces was 40%. The sensitivity and specificity for dot-ELISA method was 65.5% and 60.9% respectively. In sedimentation method the sensitivity was 69%, and the specificity was 92.3%. The positive and negative predictive values for dot-ELISA and sedimentation were 67.9%, 58.3% and 90.9%, 72.2% respectively. These results suggest that coprological methods for diagnostic and control programs of toxocarosis in cats are the optimal investigations.

Introduction

In Iran and other parts of the world, toxocarosis due to *Toxocara cati* and *T. canis* in wild and domestic carnivores is considered to be one of the most important public health problems. Stray cats are reservoirs of helminthic parasites. The feces of cats deliver hundreds or even thousands of worm eggs; these eggs can survive for a long time in the environment, and the major way of transmission to humans is by contact with the eggs.

A number of ascarids, such as *Toxascaris leonine*, not only infect both cats and dogs, but can also cause mixed infections with *T. cati* and *T. canis* (Antolova *et al.*, 2004 and Arango, 1998). Although such infections usually pass unnoticed, they are capable of producing unpleasant and dangerous complications. The larvae can lodge in the liver, lungs, muscles, eyes, heart or brain. In rare cases, these infections can lead to paralysis and blindness (Beaver, 1969; Schantz and Glickman, 1981; Schantz, 1989). The potential sources of *T. cati* infection include not only kittens, which are practically all infected, but also stray cats, which can

shed large number of eggs into the environment.

Although only small studies have been carried out previously in Iran with regards to the prevalence of parasitic infections in cats, a high frequency of infection has been demonstrated in some areas. These investigations were performed based on fecal examination or necropsy (Mirzayans, 1971; Sadjjadi *et al.*, 1998; Jamshidi *et al.*, 2002 and Bahadori *et al.*, 2004). Microscopic examination of feces has a low sensitivity for the diagnosis of infection. In the studies that use serodiagnosis, seropositivity was often lower in developed than developing countries. The prevalence of *T. cati* infection is not only different in various countries, but it may also be different between the various parts of the country. Magnaval *et al.* (1994) showed that 2-5% of apparently healthy human adults from urban areas in France were seropositive for *Toxocara* infection compared to 14-37% in rural areas. Additionally, a rate of *Toxocara* seropositivity of 20% was reported in Malaysia (Lokman Hakim *et al.*, 1993) and of 63.2% in Indonesia (Chomel *et al.*, 1993).

The main objective of the present work was to investigate the epidemiology of toxocarosis due to *T.*

cati in stray cats that live in the metropolitan region of Tehran, Iran. Furthermore, as the etiological confirmation of this nematode depends on the availability of efficient diagnostic techniques, *T. cati* antigens were evaluated in this study by dot-ELISA and the results were compared with those from necropsy and fecal examinations.

Materials and Methods

Animals

A total of 55 stray cats (28 females and 27 males) were captured at different locations in Tehran between September and December, 2006. The cats were collected by trapping and sacrificed with high doses of anesthetic drugs (sodium thiopental). Carcasses were then sealed in plastic bags and examined within 24 h. The adult *T. cati* worms were isolated and fixed in 70% ethanol.

Antigen preparation

Adult *T. cati* male and female worms were obtained at necropsy from cats that had been infected naturally. After washing three times for 5 min in phosphate buffered saline (PBS; 0.01 M, pH 7.2), the worms were homogenized and sonicated in PBS (pH=7.4) that contained 0.05 mM phenylmethylsulphonyl fluoride (PMSF) as an antiprotease. The protein concentration was measured according to Bradford's method (Bradford, 1976). The prepared antigens were stored at -70°C until use.

Serological examinations

Blood samples were collected from all of the animals from the saphenous vein into tubes that lacked anticoagulants. The blood samples were centrifuged, and serums were stored at -20°C until they were used for serological examinations. Specimens were tested by dot-ELISA using a technique that was based on those described previously by Arriaga de Morilla *et al.* (1989) with slight modifications. In brief, the optimum concentrations of antigen and serum were determined by checkerboard titration. Nitrocellulose papers were cut into 0.5 cm diameter strips and 1 µl of soluble antigen was added directly onto each paper strip. The *Eimeria tenella* antigen was used as the control. The discs were dried at room temperature for 15 min. Nonspecific binding sites on the disks were blocked by adding 200 µl nonfat dried milk that contained PBS-Tween 20 at 0.5% concentration to each well. After shaking the plate at room temperature for 60 min. the discs were washed three times (10 min each) with 0.5% PBS-Tween 20. Then, 200 µl of the serum samples, which was diluted to 1:100 in 0.5% PBS-Tween 20, was added to each plate and incubated at room temperature for 60 min.

After washing the disks in 0.5% PBS-Tween 20, a

200 µl/well anti-ovine antibody conjugate (Sigma, Company) was added. It was diluted to 1:1000 in 0.5% PBS-Tween 20 and incubated for 60 min. Then it was washed three times in PBS as described before and incubated in DAB (3, 3'-diaminobenzidine, Sigma) substrate with 10 µl of 30% hydrogen peroxide. Bands were visible within 10 min and further development was stopped by removing the substrate with distilled water at room temperature.

Coprological examination

Fecal samples were collected directly from the rectum of each cat following necropsy. The samples were concentrated by the sedimentation technique (Gaspard *et al.*, 1995). Samples were designated as positive if one or more *T. cati* ova were detected.

Intestinal examination

The cats were euthanized by high doses of anesthetics and the intestinal contents were inspected for helminthic parasites.

Statistical analysis

Statistical analysis using the χ^2 test was applied on the data to evaluate the relationship between age and sex of cats with *T. cati* infection.

Results

In the intestinal examination, the presence of mature *T. cati* was detected in 29 cases (52.7%). No significant differences were observed with regards to age and sex between infected and non-infected groups (Table 1). In 52 blood samples, 28 cases (53.8%) had positive serological tests. The presence of *T. cati* ova in 55 fecal samples was also diagnosed in 22 cases (40%; Table 2). According to our results, the sensitivity and specificity of the serological tests were 65.5% and 60.9%, respectively. In the fecal examination, the sensitivity and specificity of the test were 69% and 92.3%, respectively (Table 3).

Discussion

Toxocara cati is still the most prevalent species of infectious worm in the feline. In the present study, the rate of infection with *T. cati* in stray cats living within the metropolitan region of Tehran, Iran, was determined by necropsy. The results were compared with two methods: dot-ELISA assay for the detection of antibodies in the serum, and a coprological test for the detection of *T. cati* eggs. The prevalence rate of infection in this study (52.7%) was much higher than other studies carried out in different parts of Iran by Mirzayans (1971) and Jamshidi *et al.*, (2002) with prevalence rates recorded of 31.4% and 13%, respectively. Based on a report by Labarthe *et al.*

Table 1: Frequency and relative frequency of *T. cati* infection in cats based on gender and age.

Gender		Nematode				Total		
		Positive		Negative		Fr	%	
		Fr	%	Fr	%			
Female	Age(year)	<1	4	33.3	8	66.7	12	100
		1	1	33.3	2	66.7	3	100
		2-3	4	50.0	4	50.0	8	100
		3<	3	60.0	2	40.0	5	100
	Total		12	42.9	16	57.1	28	100
Male	Age(year)	<1	2	25.0	6	75.0	8	100
		1	3	50.0	3	50.0	6	100
		2-3	3	75.0	1	25.0	4	100
		3<	6	66.7	3	33.3	9	100
	Total		14	51.9	13	48.1	27	100

Table 2: The results of infection with *T. cati* in stray cats using three different methods.

Method	Positive (n)	Negative (n)	Total (n)
Necropsy	29	26	55
Dot-ELISA	28	24	52
Sedimentation	22	33	55

Table 3: Evaluation of Dot-ELISA and fecal examination with necropsy in the diagnosis of *T. cati* infection.*

Method	Sensitivity (%)	Specificity (%)	Positive predictive value	Negative predictive value
Dot-ELISA	65.5	60.9	67.9	58.3
Sedimentation	69	92.3	90.0	72.7

*Parameters evaluated using necropsy as the standard test

(2004) in Brazil, 25.2% of cats were infected with *T. cati*, but this was lower than the prevalence found in Spain (55.2%) by Calvet *et al.* (1998) and in England (53.3%) by Nichol *et al.* (1981). The prevalence of *T. cati* infection can vary based on the presence of veterinary care, habits of the local animal populations, the season of the year and the geographic region (Labarthe, 2004). In this study, the high prevalence of *Toxocara* infection (52.7%) in cats may be related the ability of stray cats to roam within a contaminated environment, the long life span and resistance of helminth eggs in the environment, contact with paratenic hosts, particularly mice, and the lactogenic transmission of *T. cati* in natural conditions.

The accurate detection of *T. cati* infection in the population of stray cats is important in epidemiological surveys. The fecal examination as a standard method in diagnosis of *T. cati* eggs has some limitations because

of sampling errors and certain difficulties in the identification of eggs among the fecal debris. A number of serological techniques (Noordin *et al.*, 2005 and Hummer *et al.*, 1992) have been developed as promising alternative diagnostic tools. The enzyme-linked immunosorbent assay (ELISA) for the detection of serum antibodies has been already used in several countries for clinical and epidemiological purposes with regards to human toxocariasis and currently represents the only specific means for the serological diagnosis of this parasitic disease.

A comparison of IgG-ELISA and IgG4-ELISA for the serodiagnosis of *Toxocara* showed that although both can be used, IgG4 would be superior because of secondary screening of antigen clones in an effort to develop improved serological tests (Noordin *et al.*, 2005). Because of the high prevalence of *T. cati* infection and its zoonotic potential, attention should be given to find an effective means of control of this infection. The recommendations by the Companion Animal Parasite Council (CAPC) and the Centers for Disease Control and Prevention (CDC) should also be used in the control of this infection in both human and animals (Blagburn *et al.*, 2004).

The immunodot has the advantage of being more rapid, cheaper and more suitable under conditions in the field. The low sensitivity and specificity of the dot-ELISA method in our study may be related to the presence of a large proportion of peptides that were considered to be non-specific. Common antigenic components may be present between *Toxocara* and phylogenetically related helminthes. Patients with these infections cannot be discriminated by ELISA because of false-positive results. Furthermore, *Toxocara* antibodies in humans remain positive for many years (Ree *et al.*, 1984), and this is not a useful tool for the measurement of treatment success. Previous contact should also be considered responsible for false-positive dot-ELISA results. Further studies that will select and purify those specific immunodominant peptides should be managed. Using immunoblots with glycoprotein fractions of *T. cati*, purified by affinity chromatography would lead to a better specificity and sensitivity when tested on serum samples. Nevertheless, the main drawback of this methodology is its difficult and time-consuming methodology. Efforts should be strengthened to develop highly sensitive and specific assays for the serodiagnosis of toxocariasis.

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