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ENVIRONMENTAL RISK FACTORS FOR CANINE TOXOPLASMOSIS IN A DEPRIVED DISTRICT OF BOTUCATU, SP, BRAZIL

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ABSTRACT: Toxoplasmosis is a worldwide zoonosis caused by *Toxoplasma gondii* that can infect a large variety of animals, including humans. The present study aimed to evaluate the frequency of anti-*T.gondii* antibodies in dogs from a peripheral district of Botucatu and to establish the association among some epidemiological variables in order to evaluate risk factors for toxoplasmosis infection. Serum samples from dogs were screened using an indirect fluorescent antibody (IFA) test. Anti-*T.gondii* antibody prevalence was 56%. The highest titer was 1024 (1.79%) and the most frequent titers were 16 (57.14%) and 64 (33.93%). The chi-square (X^2) test revealed significant association among variables such as dog access to street, ingestion of raw meat and presence of synantropic animals in the domestic environment. These results demonstrate that toxoplasmosis is present in dogs from Jardim Santa Elisa district.

KEY WORDS: *Toxoplasma gondii,* dogs, IFA, risk factors.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

For its medical and veterinary importance, the *Toxoplasma gondii* has been the most intensely studied parasite among the coccidia, including aspects about its biology, biological cycle, epidemiology and pathology. It is an obligatory intracellular protozoan, with complex vital cycle involving felines as definitive hosts and a large number of intermediate hosts such as warm-blooded animals, including man.

This infection has already been registered in approximately 300 mammal species (carnivores, herbivores, insectivores, rodents and primates) and 30 bird species. Due to the wide distribution of the *T. gondii* in nature, Jacobs, in 1957, affirmed that humans live in a sea of toxoplasmic infection (11). In fact toxoplasmosis is widely distributed. It is believed that a third of the world´s human population is infected, consisting a global concern due to the increase of its occurrence and strong association with acquired immune deficiency syndrome (AIDS).

In *T. gondii´*s complex life cycle there are three infection stages: the tachyzoites that multiply quickly; the bradyzoites that multiply slowly and are usually found in the tissue cysts; and the esporozoites, found in oocysts. While the tachyzoites and bradyzoites occur in tissue of all infected animals, oocysts are only excreted in the feline feces (10), a situation partially responsible for environmental contamination, contributing extensively to the agent's great dissemination in nature.

T. gondii can be transmitted in several manners, principally transplancentally by the tachyzoite passage; by the ingestion of food or water contaminated with sporulated oocysts eliminated by the feline feces and by the ingestion of raw or undercooked meat containing tissue cysts (13). The transmission can also happen by tachyzoites contained in blood products, by organs transplants or by unpasteurized milk (24), mainly from goats.

Although *T. gondii* does not develop its enteroepithelial cycle in dogs, who are not consumed as food in most developed countries, several studies highlight their importance in the epidemiological chain of the disease, due to direct contact between humans and these animals. A risk factor to be considered is the one provoked by xenosmophilia, the habit in which they eat or roll in cat feces, because dogs may carry *T. gondii* sporulated oocysts in the fur, transmitting the infection to man (15). It is undisputed that the risk of transmission to humans, in this case, is much lower. However, this risk factor should not be ignored in the epidemiological chain of toxoplasmosis transmission to humans.

Besides this aspect, dogs can also play a role as mechanical vectors. When these animals ingest the sporulated oocysts, they can eliminate the infective form into the environment by feces, generating one more source of infection for humans. Either through coprophagia or xenosmophilia, dogs can contaminate the domestic environment, affecting humans as well as other susceptible animals (15).

Vegetables and animal products infected with oocysts, primarily swine and ovine derivatives containing cysts with bradyzoites, are most responsible for human infection. Besides food, polluted soil and infected rodents are also involved, as a consequence of dog carnivorous behavior (2).

Dogs are utilized to accomplish serological surveys with the purpose of understanding zoonosis dissemination in urban and rural environments. This type of research has great epidemiological value, because these animals are thoroughly diffused and share the same habitat as humans (12), and in many situations are considered family members.

Living in the same habitat, dogs and humans are equally exposed to sources of common environmental infections. In spite of the different hygiene habits among diverse human populations, canine toxoplasmosis can be an important epidemic indicator of the toxoplasmosis risk for humans (17). Thus, dogs could be considered animal sentries for this illness.

Several geographically distinct works in the literature present toxoplasmosis prevalence results varying between 20 and 80%, employing different methodologies, in canine population. Table 1 summarizes the results obtained by several researchers, describing place, number of examined samples and method used.

Although *T. gondii* life cycle has been elucidated for more than three decades, little is known about the epidemiological importance of horizontal transmission. It is probable that the main transmission pathways vary among human populations, due to the differences in cultural and alimentary habits (24).

The multiple mechanisms of toxoplasmosis transmission make it one of the most predominant and propagated parasitic zoonoses on the planet (16). It is estimated that approximately one third of the worldwide human population has been exposed to *T. gondii* (13). In several studies carried out in the USA, Europe and Latin America, the seroprevalence varied in adults from 15 to 85%, with the highest prevalence being found in humid tropical areas (9).

Table 1. Anti-*T. gondii* antibody seroprevalence observed in dogs from different regions of the country (Brazil - 2002 to 2006)

Brazilian Region	Positive results (from total samples)	Seroprevalence (%)	Method	Reference
Botucatu (SP)	258/780	33.10%	IFAT[3]	Langoni *et al*. (14)
Botucatu (SP)	26/80	32.50%	IFAT	Brito *et al*. (5)
Campina Grande (PB)	129/286	45.10%	IFAT	Azevedo *et al*. (3)
Capão do Leão (RS)	10/50	20%	HIA[4]	Cademartori *et al*. (6)
Monte Negro (RO)	120/157	76.40%	IFAT	Cañón-Franco *et al*. (8)
Salvador (BA)	143/225	63.55%	IFAT	Barbosa *et al*. (4)
São Paulo (SP)[1]	24/29	82.80%	IFAT	Ortolani *et al.* (19)
São Paulo (SP)[2]	35/61	57,40%	IFAT	Ortolani *et al*. (19)
São Paulo (SP)	219/1110	19.70%	MAT[5]	Souza *et al*. (22)
São Paulo (SP)	101/200	50.50%	ELISA[6]	Meireles *et al*. (17)
Ubatuba (SP)	52/204	25.49%	IFAT	Silva *et al*. (21)
Uberlândia (MG)	59/163	36%	IFAT	Mineo *et al*. (18)

1: Krucutu indian settlement; 2: Morro da Saudade indian settlement; 3: indirect fluorescent antibody reaction; 4: hemagglutination-inhibition assay; 5: modified agglutination test; 6: enzyme-linked immunosorbent assay.

The socioeconomic impact of toxoplasmosis is enormous, especially the high costs of treatment in children that have developed mental retardation and blindness due to this disease (20). In addition to these problems, this condition still causes miscarriages and serious sequelae generated by transplacentary infection.

Public health organizations, specifically the World Health Organization (WHO), have been encouraging the careful study of *T. gondii* aiming to produce accurate epidemic data on this parasite as a zoonotic agent. Such information is essential to reveal the importance of several infection sources for humans, in order to control the disease and to avoid harming life quality. Despite this fact, few countries are regularly monitoring toxoplasmosis in humans, and even less so in animals (24).

MATERIAL AND METHODS

Epidemiological Survey

The community from the neighborhood Jardim Santa Elisa, on the periphery of the urban territorial zone of Botucatu city, Sao Paulo State, Brazil, presents some transitory rural characteristics and can be considered as a poor district. The region is intercepted by three avenues (avenue 1, 2 and 3) and six streets (streets 1, 2, 3, 4, 5 and 6) while only avenue 2 is paved with asphalt, and the others are unpaved. According to the 2002 census, Jardim Santa Elisa district presents 228 residential buildings, none of which has access to residential pavement or adequate basic sanitation. The sewerage system covers only 46.1% of the homes; the others use cesspool and concrete cesspit (84.27%), open sky sewers (13.48%) or discard the sewer in stream water (2.25%). The water supplied to the district is treated. The breeding of animals is intense, favoring promiscuous contact among them, because many are free in the streets or in improvised pastures.

Residential visits were accomplished during July of 2006. A questionnaire for epidemiological evaluation of the residents was applied and blood samples were collected from 100 dogs (48 males and 52 females), including 37 young animals (0 to 5 years old) and 63 senior animals (above 5 years old). According to the municipal government, there are approximately 250 dogs in the district, in agreement with the data referring to the animals vaccinated in 2005. This district has been monitored for some years by the Zoonosis Laboratory of the São Paulo State University with extension works for health education, but no previous study accomplished seroepidemiological evaluation of the district´s animals for toxoplasmosis.

The food supplied to the animals was divided in three categories: exclusively commercial (several available brands on the market); homemade food (surpluses of human food with varied composition, predominantly rice and sometimes meat); and processed and homemade food.

The animals' access to the street is free, without the owners' surveillance, due to the precarious conditions of housing infrastructures that don't possess physical barriers such as walls and gates that would impede the free movement of these animals. Thereby, allowing the constant contact between dogs and cats in the same environment.

The owners signed a consent form, clarifying the study objectives for them. The project was approved by the Ethics Committee for Animal Research of the School of Veterinary Medicine and Animal Husbandry, UNESP, protocol n. 145/2006-CEEA, following the Ethical Principles in Animal Experimentation. The risk factors evaluated were linked to canine feeding habits, street access, residential location and presence of rodents and felines.

Blood Sample Collection

Blood samples between 2 to 5 mL were taken from each animal by venous puncture (jugular or cephalic), with disposable 5 mL syringe and 30 x 7 mm needle. The blood was transferred immediately to a 15 mL tube, without anticoagulant. In the laboratory of the Center for Zoonosis Research, NUPEZO, of the Department of Veterinary Hygiene and Public Health, UNESP, the samples were centrifuged at 3,000 rpm for 10 minutes, and then the serum was removed and transferred to 1,5 mL polystyrene tubes, identified and stored at –20°C, until the analysis.

Indirect Fluorescent Antibody (IFA) Test

IFA test was employed to detect anti-*T. gondii* antibodies, according to the standard technique described by Camargo (7).

The serum samples were diluted 1:16, 1:64, 1:256, 1:1,024, 1:4,096 in flat-bottom microplates and screened by IFA, for the detection of IgG antibodies. Conjugated anti-IgG was employed for the species studied. Peritoneal exudates of mice (*Mus musculus*), infected with *Toxoplasma gondii* tachyzoites of the RH strain, were used as antigen. Positive and negative control sera (previously known) were included on each plate. Readings were carried out in a fluorescent Zeiss SH 250 microscope,

considering the condition in which the serum reacting at dilutions equal or higher than 1:16 as positive, when impregnated tachyzoite antigen showed total peripheral fluorescence. Absence of fluorescence, or its presence only in the parasite extremities (polar fluorescence), was considered a negative reaction.

Statistical Analysis

The chi-square test was used to determine statistical association between seroprevalence for toxoplasmosis in dogs and the risk factors evaluated by the questionnaire, with statistical significance of 95%, $p \leq 0.05$ (23).

RESULTS

Of the 100 samples, 56 dogs showed anti-*T. gondii* antibodies (IgG). Among the positive results, 32 presented a titer of 16 (57.14%); in 19 of them the titer was 64 (33.93%); followed by four showing 256 titer (7.14%) and one of 1.024 titer (1.79%) (Table 2).

Table 2. Anti-*T. gondii* IgG antibody titer distribution in canine serum samples of Jardim Santa Elisa, Botucatu city, Sao Paulo State, Brazil, 2006.

Antibody titer	**Reactive dogs**	
(UI)	**Number**	**Percentage (%)**
16	32	57.14
64	19	33.93
256	4	7.14
1024	1	1.79
Total	**56**	**100**

Seropositivity for toxoplasmosis, according to the risk factors evaluated and to the occurrence probability (p), is presented in Table 3.

In relation to street access, the percentage of seropositive dogs was statistically higher ($p = 0.018$) than the one observed in the negative group for toxoplasmosis.

Of the seropositive dogs that feed on raw meat, the percentages observed in the infected group are greater ($p = 0.027$) than in the seronegative group. On the other hand, there was no association among dogs that receive only commercial ration,

those fed exclusively homemade food and the ones fed both type, with p = 0.658, p = 0.107 and p = 0.207, respectively.

Concerning young animals, the proportion that reacted was statistically less (p = 0.020) than the non-reactive group. Regarding contact with cats, there was no statistically significant difference between dogs that reacted or not to toxoplasmosis (p = 0.207).

The risk evaluation in relation to the habits of hunting and coprophagy or xenosmophilia, in the present study, did not display significant association, with p = 0.691 and p = 0.184.

When verifying the percentage of seropositive and sinantropic dogs, an association was found since the number of reactive dogs was statistically greater (p = 0.015) than non-reactive ones.

No significant association was found for infection with regard to location in the neighborhood, specifically, among avenues 1, 2, 3 and the other locations; p = 0.797, p = 0.935, p = 0.262 and p = 0.443, respectively.

Table 3. Distribution of dogs that presented anti-*T. gondii* IgG antibodies, accordingly to epidemiological inquiry carried out in Jardim Santa Elisa, Botucatu city, 2006

Variables	Reactive dogs (number)	Non-reactive dogs (number)	Total (number)	P
Access to street				
Yes	45	27	72	0.018
No	11	17	28	
Age (years)				
0-5	14	23	37	0.020
> 5	42	21	63	
Raw meat ingestion				
Yes	21	9	30	0.027
No	35	35	70	
Exclusive feeding of commercial dog food	18	16	34	0.658
Feeding of homemade food	25	12	37	0.107
Feeding of commercial and homemade food	13	16	29	0.218
Frequent contact with felines				
Yes	15	17	32	0.207
No	41	27	68	
Hunting habit				
Yes	17	15	32	0.691
No	39	29	68	
Coprophagia/xenosmophilia behavior				
Yes	12	5	17	0.184
No	44	39	83	
Presence of synanthropic animals				
Yes	42	24	66	0.015
No	14	20	34	
District distribution				
Avenue 1	14	12	26	0.797
Avenue 2	25	20	45	0.935
Avenue 3	11	5	16	0.262
Streets	6	7	13	0.443

Statistic: chi-square test.

DISCUSSION

The literature consulted showed results varying between 20.0% (6) and 82.80% (19) for canine toxoplasmosis seropositivity. The 56.0% prevalence of toxoplasmic infection in the dog population from the Jardim Santa Elisa district may be considered a high result. This data does not necessarily signify that animals have toxoplasmosis. It only denotes that these dogs had contact with the pathogenic agent, and thereby the acquired infection. Since these animals share common infection sources with their owners or other residents of the neighborhood, they constitute a risk factor for the local human population (12).

The results from the current study are similar to the ones obtained by Ortolani *et al*. (19), who found 57.40% positivity in dogs from the indigenous village Morro da Saudade, in São Paulo city. They are also comparable to the outcomes obtained by Barbosa *et al.* (4) and Meirelles *et al.* (17) that, respectively, observed 63.55% and 50.50% positivity; rates lower, however, than the results achieved by Ortolani *et al.* (19) in their study on dogs of another indigenous village, Krucutu, where they encountered 82.80% of dogs with anti-*T.gondii* antibodies.

On the other hand, the levels found in the present study are higher than follow-ups performed by Mineo *et al.* (18), Brito *et al.* (5), Silva *et al.* (21), Souza *et al.* (22), Cademartori *et al.* (6), Azevedo *et al.* (3) and Langoni *et al.* (14), who verified, respectively, 3.0%, 32.5%, 25.49%, 19.70%, 20.0%, 45.10% and 33.10%.

Conclusions from Cañón-Franco *et al.* (8) and Ortolani *et al.* (19) presented numbers above the 56.9% positivity verified in the Jardim Santa Elisa district; these two groups found respective positivities of 76.40% and 82.80%.

The differences among several research results should be appraised in terms of numbers of samples evaluated and the methodology used because these factors can interfere in the antibody titer obtained, and not only in toxoplasmosis cases. Another aspect to be highlighted is the inter-technician variation, besides characteristics of reagents, antigens and conjugates that are from different laboratories.

Apart from these factors, results diverge among the same researchers when animal samples come from different places. This fact confirms the importance of the environment over agent maintenance and, consequently, over toxoplasmosis transmission to animals, including humans (4).

In developing countries, namely Brazil, the toxoplasmosis incidence is high and its transmission is related to hygiene habits, poor sanitation, feline presence and

climatic factors. These aspects contribute to the process of parasite development process and transmission. Its prevalence varies among areas and depends on the existence of risk factors, justifying the results found in several studies (8).

The titer most frequently observed in this study was 16 (57.14%), which agrees with the one observed by Langoni *et al.* (14) in the same district, who employed the same serologic test. Findings like the anti-*T. gondii* antibody titer 16 in a sample can simply confirm that the animal had contact with the agent. A second serology, screening only IgG antibodies, after a couple of weeks is necessary to differentiate a chronic infection from an active current infection.

The variable street access exerted some influence on the results obtained in the current study. This agrees with several authors, such as Ali *et al.* (1) that found in Trinidad and Tobago a prevalence of 51% for toxoplasmosis in dogs that had access to street and 14.7% in animals maintained strictly in domestic environment, a difference that was statistically significant ($p = 0.004$). A similar result was observed in Monte Negro, Rondônia, where Cañón-Franco *et al.* (8) encountered positive association between dogs that had unrestricted street access (84.9% prevalence) and the ones kept on the domestic property (58.8%) ($p \leq 0.001$).

It is suggested that dogs that can move freely through neighborhood are more susceptible to infection by *T. gondii* oocysts from water and food. In addition, street access can favor hunting chances, inherent to the behavior of a carnivorous animal (5).

A positive correlation between raw meat supply and seropositivity for toxoplasmosis was found in dogs. Thus, raw meat is considered an important source of transmission to humans and other animals. Garcia *et al.* (12) also demonstrated a positive correlation between the antibody titer distribution ratio for the human and canine species, confirming that the man-dog relationship was epidemically relevant, possibly due to the same transmission pathways, i.e., raw food.

No substantial differences were detected for seropositivity distribution among animals fed exclusively commercial food, only homemade food or both together. This conclusion is in agreement with Ali *et al.* (1), who despite finding a higher prevalence in dogs that received homemade food, did not conclude that this difference was significant when compared to the percentage of animals that were fed commercial ration or both types jointly ($p = 0.05$). Cañón-Franco *et al.* (8) confirmed this, when they compared commercial diet with a homemade one ($p = 0.086$).

Our current results on the risk factor due to frequent contact with cats were similar to those obtained by Ali *et al.* (1), who also did not observe statistical differences. These outcomes suggest that environmental exposure to *T. gondii* can be epidemiologically more important than direct contact with cats. In this case, cats assume an important role not by direct contact, but by oocyst elimination and environmental contamination, thus posing greater risk for human and others animal populations. A study by Azevedo *et al.* (3) revealed important association between seropositivity and domestic presence of cats.

The percentage of young animals that reacted was statistically lower ($p = 0.020$) than the one observed in the non-reactive group, reinforcing the results found by Ali *et al.* (1), Cañón-Franco *et al.* (8) and Garcia *et al.* (12), who all corroborated on the higher probability of adult animals having had previous contact with the parasite.

Contrary to what was expected, canine hunting habits did not increase toxoplasmosis seroprevalence; which can be explained, partially, by the fact that the present study did not evaluate if the dogs ate the hunted animals. Besides, since these animals have free access to the street, their owners could not say wether the dogs were hunting or not.

Positive association was observed between seropositivity and presence of sinantropic animals, contrary to Azevedo *et al.* (3) who did not find significant disparity between the presence of rodents and other preys and the presence of titers for toxoplasmosis in dogs.

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