

SEROPREVALENCE OF *TOXOPLASMA GONDII* IN DOGS AND CATS DOMICILED IN THE WEST OF SANTA CATARINA, BRAZIL

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ABSTRACT: *Toxoplasma gondii* is a protozoan with worldwide distribution, responsible for causing toxoplasmosis, a disease that affects mammals, reptiles, amphibians and birds. The objective of this study was to evaluate *T. gondii* seroprevalence in dogs and cats domiciled in cities from West and Serrana mesoregions of Santa Catarina. A total of 338 blood samples, 211 from dogs and 127 from cats were collected and submitted to the indirect haemagglutination technique (IHT) for the detection of anti-*T. gondii* antibodies. Epidemiological questionnaire asking for information related to gender, breed, temperament, behavior, immunity, body index, diet, ectoparasites, access to the streets, hunting habit was applied to the animals owners. The results were 71.89% (243/338) of seropositive animals, being 69.19% (146/211) dogs and 76.38% (97/127) cats. The variable that presented statistical significance was commercial ration for dogs as protection factor. The present study demonstrates a high prevalence of anti-*T. gondii* antibodies in dogs and cats domiciled in West and Serrana mesoregions of Santa Catarina state, Brazil. Through the obtained results we can reinforce the importance of feeding dogs with commercial food, rather than home-made food, avoiding the consumption of raw or undercooked meat by the animals.

KEYWORDS: IHT; Risk factors; Toxoplasmosis.

SOROPREVALÊNCIA DE *TOXOPLASMA GONDII* EM CÃES E GATOS DOMICILIADOS NO OESTE DE SANTA CATARINA, BRAZIL

RESUMO: O *Toxoplasma gondii* é um protozoário de distribuição mundial, agente etiológico da toxoplasmose, uma doença que afeta mamíferos, répteis, anfíbios e aves. O objetivo deste estudo foi avaliar a soroprevalência de *T. gondii* em cães e gatos de municípios das mesorregiões Oeste e Serrana de Santa Catarina. Um total de 338 amostras de sangue, 211 de cães e 127 de gatos, foram coletados e submetidos à técnica de hemaglutinação indireta (IHT) para a detecção de anticorpos anti-*T. gondii*. Questionário epidemiológico solicitando informações relacionadas ao gênero, raça, temperamento, comportamento, imunidade, índice corporal, dieta, ectoparasitas, acesso à rua e hábito de caça foi aplicado aos proprietários dos animais. Os resultados foram 71,89% (243/338) dos animais soropositivos, sendo 69,19% (146/211) cães e 76,38% (97/127) gatos. A variável que apresentou significância estatística foi o uso ração comercial para cães como fator de proteção. O presente estudo demonstra uma alta prevalência de anticorpos anti-*T. gondii* em cães e gatos domiciliados nas mesorregiões Oeste e Serrana do estado de Santa Catarina, Brasil. Por meio dos resultados obtidos, reforçamos a importância de alimentar os cães com alimentos comerciais, em vez de alimentos caseiros, evitando o consumo de carne crua ou mal cozida pelos animais.

PALAVRAS-CHAVE: Fatores de risco; HAI; Toxoplasmose.

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INTRODUCTION

Toxoplasmosis is a disease of great relevance that affects a variety of hosts such as mammals, birds, reptiles and amphibians (DUBEY, 1998). According to Fialho, Teixeira and Araújo (2009), this is a disease of importance in public health because of the alterations it causes in human fetuses, and of importance in animal production due to economic losses such as abortion. This disease is caused by the protozoan *Toxoplasma gondii*, an obligate intracellular parasite known as the most frequent parasite in man and in homeothermic animals (DUBEY, 1998). This parasite has felids as the only definitive hosts, intermediate hosts are all mammals, birds, reptiles, amphibians. There are three infectious stages of *T. gondii*: a rapidly dividing invasive tachyzoite, a slow-dividing bradyzoite in tissue cysts, and an environmental stage, the sporozoite, protected within an oocyst (GANGNEUX; DARDÉ, 2012). The main routes of transmission are: ingestion of contaminated fruits and vegetables or water, raw or uncooked meat, unpasteurized milk and through blood transfusions, organ transplants or congenital form (GARCIA, 1999; SILVA, FIGUEIREDO; FREITAS, 2015). Vegetables can be contaminated directly from the faeces of felines (FERREIRA *et al.*, 2018) or through the source of irrigation water from the garden that can be contaminated by the rainwater flow with fecal pathogens (WELLS *et al.*, 2015).

According to Moura *et al.* (2015), in cats the most common clinical signs are anorexia, fever, lethargy, vomiting, weight loss, abortion, nervous alterations, ocular dysfunctions, cardiac symptoms and, eventually, sudden death. In dogs, toxoplasmosis appears more severe in puppies. The most common clinical signs described are ataxia, diarrhea, and respiratory alteration, such as dyspnoea and cough that may progress to pneumonia, especially in canine distemper virus coinfections, as seen from the opportunistic effect of *T. gondii* as previously mentioned.

Parasitological tests in animals present many difficulties and limitations such as laboratory capacity, cost with examinations, owner's adhesion, lack of

awareness of the diagnostic importance. Serological methods are the most used, which consist of the search for IgM and IgG class antibodies. Among the main ones: indirect immunofluorescence test (IFAT), enzyme-linked immunosorbent assay (ELISA), but in addition, the indirect hemagglutination technique (IHT), agglutination by immunoabsorption (ISAGA), and molecular biology reactions can be performed (Bresciani *et al.*, 2008). The IHT is a practical, low cost test, does not require sophisticated equipment and is considered a good method for the screening of toxoplasmosis (COSTA *et al.*, 2007).

The prevalence of antibodies to *T. gondii* is high in most regions, however, the diagnosis is often not performed to quantify them, so the aim of this study was to evaluate the seroprevalence of anti-*T. gondii* antibodies in dogs and cats domiciled in West and Serrana mesoregions of Santa Catarina state, Brazil.

2 MATERIALS AND METHODS

2.1 STUDY AREA

The state of Santa Catarina is in the southern region of Brazil composed of 95 thousand square kilometers, distributed among 295 municipalities, and the social indexes of this state are among the best in the country. Thirteen cities from West and Serrana mesorregions (Figure 1) were selected to carry out the research project due to the location of the Veterinary Care

2.2 SAMPLE COLLECTION

The collection of the samples, the authorization of the use of the material for study purposes and the completion of an epidemiological record occurred from March to December 2018. Blood collection was collected from jugular vein puncture with needle 40 mm x 12 mm in a collection tube without anticoagulant. After that, the serum was obtained by centrifugation at 2,000 rpm for 10 minutes, being aliquoted and conditioned at -10°C for further analysis.

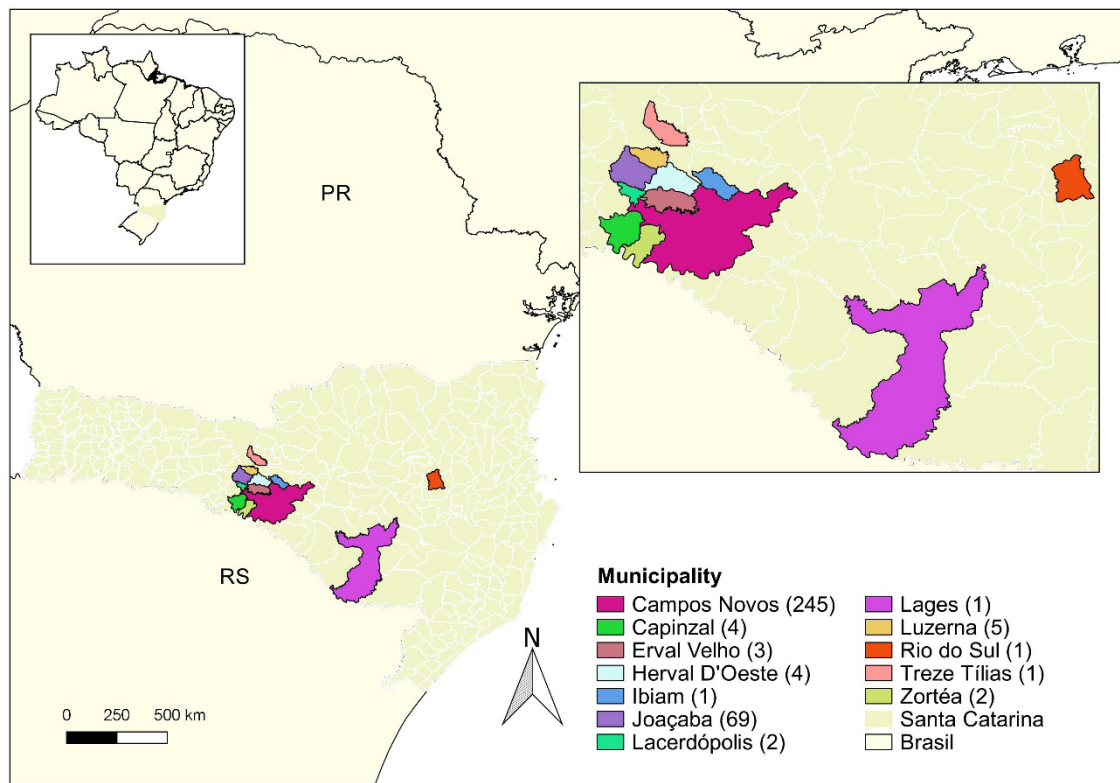


Figure 1. Representative map of the cities of origin of the animals used in the present survey of anti-*T. gondii* antibodies and the respective number of animals per city.

2.3 EPIDEMIOLOGICAL QUESTIONNAIRE

The epidemiological record was a questionnaire answered by the animal guardian and with clinical aspects performed by the veterinarian. The questions evaluated were: species, sex, breed and weight; immunity, such as: vaccinated, dewormed; alternatives related to temperament, such as: docile, fearful or aggressive; behavior, such as: excited, alert, normal, apathetic or comatose; body index: obese, overweight, normal, lean or cachectic; diet: commercial, homemade food or both; presence or absence of ectoparasites, among them: fleas, lice, ticks; with or without access to the street; and hunting habit.

2.4 SEROLOGICAL TESTS

Serum samples were tested by IHT for the detection of anti-*T. gondii* antibodies from dogs and cats using Toxotest HAI® (Wiener Laboratory), following

the manufacturer's recommendations and performing the qualitative and quantitative test. Samples with a titer of 16 or higher were considered as reagents.

2.5 STATISTICAL ANALYSIS

From the test results, the epidemiological data were computed and analyzed through EpiInfo. The statistical significance of the observed and expected results were analyzed by chi-square (2) and Fisher's exact tests, at a significance level of 5.0%. The measure of association was determined by odds ratio (OR) at a 95% confidence interval (IC).

2.6 ANIMAL RESEARCH ETHICS COMMITTEE

This study was approved by the Animal Research Ethics Committee of the University of the West of Santa Catarina under Process N° 51/2018 and complied with biosafety and ethical standards.

3 RESULTS

A total of 338 samples were collected, 211 from dogs and 127 from cats. The seropositivity of the animals and their relation to the studied characteristics are visualized in Table 1.

The variable that presented statistical significance was commercial ration for dogs as a protection factor ($p=0.0218$). Of the dogs fed exclusively on commercial diet, 46.43% (13/28) were positive, while 88.89% (8/9) from those fed exclusively on homemade food were positive (OR=9.23; IC96%= 3.46–24.59) and 72.34% (34/47) from those fed with both were positive (OR=3.01; IC96%= 1.13–8.03).

The serological titulation for anti-*T. gondii* antibodies ranged from 16 to 262,144, 16 (15.68%) being the most frequent, followed by 64 (11.83%) and 512 (9.47%) as shown in Table 2.

Table 1. Relation of the seropositive dogs and cats according to the evaluated variables

Variables	Dogs	Cats
Seropositive	69.19% (146/211)	76.38% (97/127)
Gender		
Males	48.82% (103/211)	31.50% (40/127)
Females	51.18% (108/211)	68.50% (87/127)
Breed		
Defined breed	29.38% (62/211)	1.57% (2/127)
Without a defined breed	70.62% (149/211)	98.43% (125/127)
Temperament		
Docile	86.90% (73/84)	43.56% (44/101)
Fearful	11.90% (10/84)	18.81% (19/101)
Aggressive	1.19% (1/84)	37.62% (38/101)
Animal behavior		
Excited	8.33% (7/84)	0.00 (0/101)
Alert	20.24% (17/84)	77.23% (78/101)
Normal	70.24% (59/84)	22.77% (23/101)
Apathetic	1.19% (1/84)	0.00 (0/101)
Body score		
Obese	3.57 % (3/84)	0.00 (0/101)
Overweight	3.57% (3/84)	3.96% (4/101)

Normal weight	85.71% (72/84)	78.22% (79/101)
Lean	7.14% (6/84)	16.83% (17/101)
Cachectic	0.00 (0/84)	0.99% (1/101)
Food		
Commercial ration	33.33% (28/84)	83.17% (84/101)
Homemade food	10.71% (9/84)	1.98% (2/101)
Ration and homemade food	55.95 % (47/84)	14.85% (15/101)
Clinical examinations		
Ectoparasites	57.14% (48/84)	82.18% (83/101)
Fleas	53.57% (45/84)	82.18% (83/101)
Fleas and ticks	3.57% (3/84)	0.00 (0/101)
External conditions		
Access to the street	67.86% (57/84)	18.81% (19/101)
Residence	32.14% (27/84)	81.19% (82/101)
Habit		
Hunt	10.71% (9/84)	25.74% (26/101)

Table 2. Indices of dogs and cats seropositive for anti-*T. gondii* antibodies according to their titrations. Dilutions up to 1: 262,144 were performed

Titulation	Dogs	Cats
16	22.60 (33/146)	20.62% (20/97)
32	8.22% (12/146)	5.15% (5/97)
64	19.18% (28/146)	12.37% (12/97)
128	11.64% (17/146)	3.09% (3/97)
256	6.85% (10/146)	10.31% (10/97)
512	12.33% (18/146)	14.43% (14/97)
1,024	4.11% (6/146)	13.40% (13/97)
2,048	5.48% (8/146)	7.22% (7/97)
4,096	4,79% (7/146)	11.34% (11/97)
8,192	2.05% (3/146)	0.00 (0/97)
16,384	0.68% (1/146)	0.00 (0/97)
32,768	1.37% (2/146)	1.03% (1/97)
65,536	0.68% (1/146)	0.00 (0/97)
262,144	0.00 (0/97)	1.03% (1/97)

4 DISCUSSION

The seroprevalence for *T. gondii* was 69.19% (146/211) in dogs, this prevalence is considered high when compared to other studies where seropositivity ranged from 45.1%, 57.4%, 60.7%, 76.4% using the IFAT method, in the states of Paraíba, Tocantins, Minas Gerais and Roraima respectively (AZEVEDO *et al.*, 2005; RAIMUNDO *et al.*, 2015; GUIMARÃES *et al.*, 2009; CAÑÓN-FRANCO *et al.*, 2004). Lower prevalences or close to those were found by other authors through the HAI method, with 23.1% and 52.7% of seropositivity, in the cities of Araçatuba (São Paulo state) and Uberlândia (Minas Gerais state), respectively (BRESCIANI *et al.*, 2008; CABRAL, SILVA, MINEO, FERREIRA; DURAN, 1998).

Of the male dogs evaluated, 66.02% (68/103) were seropositive for *T. gondii*, regarding to females, 72.22% (78/108) were positive. The sex was not statistically associated to seropositivity, and they could be equally infected, as already mentioned (GERMANO; ERBOLATO; ISHIZUKA, 1985; CABRAL *et al.*, 1998; GAIO, SALINA, MENOZZI; LANGONI, 2014), as well as the breeds, fact also observed by other authors (AZEVEDO *et al.*, 2005; BRESCIANI *et al.*, 2008).

The statistical analysis of the epidemiological questionnaire indicated commercial diet as a protection factor for dogs. Dogs fed only on homemade feed were 9.23 times more likely to be positive for *T. gondii* when compared to animals fed commercial feed. Dogs with home feed and commercial feed were 3.01 times more likely to be positive for *T. gondii* when compared to those fed only with feed. In general, the commercial feed is a balanced food, with better quality and free of contaminants, improve immunity and reduces the animal access to the raw or undercooked meat. However, there are authors who did not observe statistical difference in relation to this variable (BRESCIANI *et al.*, 2008; CAÑÓN-FRANCO *et al.*, 2004). A study published by Benitez *et al.* (2017), evaluated the prevalence of humans and their respective dogs and obtained higher statistical seroprevalence in owners (41.54%) compared to their own dogs (16.32%),

which can be explained by contamination transmitted by food since the exposure to foods such as raw and undercooked meat to dogs was much lower compared to the guardian.

The titers of the seropositive dogs varied between 16 to 65,536 where chronic, or animals in the beginning of the disease, predominated. Similar indexes were obtained in a study conducted by Dantas *et al.* (2013) in Natal, Rio Grande do Norte, state; they studied the occurrence and risk factors associated with *T. gondii* in 476 dogs, where 11.5% (55/478) were seropositive, of these 47.2% (26/55), 22% (12/55), 18% (10/55), 11% (6/55) and 1.8% (1/55) seropositive animals with titers 64, 128, 256, 512, and 1024 respectively. In another study, conducted by Ferreira *et al.* (2016), many antibody titers in canines were between 16 (37.45%) and 64 (34.75%).

In felines, the seroprevalence of anti-*T. gondii* antibodies was 76.38% (97/127), results higher than those observed in some studies where seroprevalences ranged from 28.07% (48/171), 29% (29/100) 37.9% (93/245) by the IFAT method in the cities of Cascavel, Palotina and Porto Alegre, respectively (ANDRADE *et al.*, 2015; SOUZA *et al.*, 2017; PINTO, ARAÚJO, STOB; MARQUES, 2009). Pinto *et al.* (2009), used the HAI method, where 26.9% (66/245) of the cats were seropositive.

Lucas, Hagiwara, Loureiro and Birgel (1999), evaluated 248 domestic cats treated at the Veterinary Hospital of the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo, using IFAT, they observed 17.7% (44/248) of serum reagents, where 4.8% (12/250), 5.7% (14/250), 4.8% (12/250), 1.2% (3/250), 1.2% (3/250), presented titers 16, 64, 256, 1024, 4096, respectively.

Depending on the host and the via, *T. gondii* can be infective at any stage of evolution, significantly increasing the risk of infection for domestic animals and humans. There are many prevention methods to reduce cases of toxoplasmosis in both humans and animals, the main ones are: washing hands before meals, incinerating cat feces, protecting pregnant women from contamination, washing well fruits and

vegetables and, as pointed by this work, avoiding the consumption of raw or undercooked meat (FIALHO *et al.*, 2009). According to Galvão, Vaconcellos, Navarro and Bresciani (2014), preventive methods are the best resources in relation to cases of toxoplasmosis. It is also recommended to avoid or try to abolish the habit of coprophagia observed in some animals, as well as the control of insects and rodents in the environment since they may be carriers of the disease. Don't consume and provide animals with raw meat or raw goat's milk, and the use of available commercial products or foods is indicated.

5 CONCLUSION

The present study demonstrates a high prevalence of anti-*T. gondii* antibodies in dogs and cats domiciled in West and Serrana mesoregions of Santa Catarina State, Brazil. Through the obtained results we can reinforce the importance of feeding dogs with commercial food, rather than home-made food, avoiding the consumption of raw or undercooked meat by the animals.

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