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SEROLOGICAL ANTIBODY RESPONSE OF THE CLASSES IgM AND IgG ANTI-*Toxoplasma gondii* IN DOGS WITH OCULAR ALTERATIONS

(Resposta sorológica das classes IgM e IgG anti-Toxoplasma gondii em cães com alterações oculares)

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ABSTRACT - Toxoplasmosis is a worldwide distribution zoonosis, caused by the protozoan *Toxoplasma gondii* (*T. gondii*). In dogs, ocular toxoplasmosis is considered rare and lesions include keratoconjunctivitis sicca, uveitis, iridocyclitis, ciliary epithelial hyperplasia, retinitis, choroiditis, extraocular myositis, scleritis, episcleritis, optic neuritis, and corneal and conjunctival masses. Thus, the present study aimed to detect IgM and IgG class antibodies against *T. gondii* in 75 dogs with ophthalmic signs, treated at the Veterinary Hospital of FMVZ Unesp, Botucatu city. Serological results for the detection of IgM and IgG class antibodies were evaluated by the Indirect Immunofluorescence Reaction (RIFI) technique associated with data from a questionnaire regarding the risk factors for toxoplasmosis in the canine species administered to the animal's guardian on the day of blood collection, regarding the risk factors for toxoplasmosis in canine species. The results were 28/75 (37%) for IgG class antibodies and 34/75 (45%) for IgM class, that is, 37% of the animals were exposed to a pre-infection and 45% were in the acute phase of the disease. 65% of dogs had uveitis as a clinical sign; the others had keratoconjunctiva, ulcers, conjunctivitis, retinochoroiditis, iridocyclitis, retinitis and choroiditis. The importance of observing the ocular changes in patients was noted because it may suggest the presence of an infectious disease.

Key words - *Toxoplasma gondii*, dog, eye diseases.

RESUMO - A toxoplasmose é uma zoonose de distribuição mundial, causada pelo protozoário *Toxoplasma gondii* (*T. gondii*). Em cães, a toxoplasmose ocular é considerada rara e as lesões incluem ceratoconjuntivite seca, uveíte, iridociclite, hiperplasia epitelial ciliar, retinite, coroidite, miosite extraocular, esclerite, episclerite, neurite óptica e massas córneas e conjuntivais. Com isso, o presente estudo teve como objetivo detectar anticorpos da classe IgM e IgG anti *T. gondii* em 75 cães com sinais oftálmicos, atendidos no Hospital Veterinário da FMVZ Unesp, na cidade de Botucatu. Foram avaliados os resultados das sorologias para detecção de anticorpos da classe de IgM e IgG, pela técnica de Reação Imunofluorescência Indireta (RIFI) associado com

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dados de questionário aplicado aos tutores no dia da coleta de sangue, referente aos fatores de risco para toxoplasmose na espécie canina. Os resultados encontrados foram de 28/75 (37%) para a presença de anticorpos da classe IgG e de 34/75 (45%) para a classe IgM, ou seja, 37% dos animais foram expostos a uma pré infecção e 45% estavam na fase aguda da doença. 65% dos cães apresentaram uveíte como sinal clínico; os demais apresentavam ceratoconjuntiva, úlceras, conjuntivite, retinocoroidite, iridociclite, retinite e coroidite. Notou-se a importância de observar as alterações oculares nos pacientes porque pode sugerir a presença de alguma doença infecciosa.

Palavras-chave - *Toxoplasma gondii*, cão, alterações oculares.

INTRODUCTION

With the evolution of veterinary medicine, greater longevity of pets is possible. There is an increasing interaction between the human population and dogs, especially in large urban centers. This close and progressive interspecies coexistence requires greater efficiency of veterinary medicine to control dog diseases, especially those of a zoonotic nature. Pets have come to gaining a prominent place in the family in many situations. Thus, aspects of animal health are extremely important since there are diseases shared between animals and humans, zoonoses (Galvão et al., 2014).

T. gondii belongs to the phylum *Apicomplexa*, class *Sporozoa*, subclass *Coccidia*, family *Sarcocystidae*, genus *Toxoplasma*. It has three infectious forms, that is, the tachyzoites that are important in transplacental infection; bradyzoites that are present in the muscles of intermediate hosts, mainly sheep, goats and pigs, in addition to other mammals and birds; and sporozoites that are also called oocysts and are eliminated in the feces of their definitive hosts, the felines (Silva and Silva, 2016).

One of the ways to evaluate the dispersion of the agent in the environment is the study of seroprevalence in animals that are considered as sentinels, since they are exposed to risks similar to humans for toxoplasmic infection. In the urban area, dogs are considered an excellent model for studies as sentinel animals, frequently used in seroepidemiological surveys (Moura et al., 2009).

In dogs, the clinical signs of the disease are variable such as anorexia, fever and depression, pneumonia, diarrhea and neurological manifestations. Clinical disease is uncommon in this species and ocular manifestation is less frequent than in cats, in which 75% of cases of uveitis are related to seropositivity for *T. gondii* (Azevedo, 2017).

In dogs, it is common to associate this disease with immunosuppressive diseases such as distemper and ehrlichiosis, with greater severity in puppies. In most cases, the disease in these animals is linked to immunosuppression and the absence of vaccination against canine distemper virus. Thus, neurological disease can be observed, with signs of seizures, cranial nerve deficits, tremors, ataxia and paresis or paralysis in

encephalomyelitis; paraparesis and tetraparesis; noise sensitivity; myositis; loss of muscle mass and stiffness; eye disease described as necrotizing conjunctivitis, anterior uveitis, endophthalmitis and chorioretinitis (Calero-Bernal and Gennari, 2019).

A study carried out with dogs with experimental ocular toxoplasmosis found changes in the fundus of the eye, such as areas of hyperreflectivity, papilledema, perivascular edema and vitreitis (Abreu et al., 2001). Ocular toxoplasmosis in dogs is considered rare and lesions include dry keratoconjunctivitis, uveitis, iridocyclitis, ciliary epithelial hyperplasia, retinitis, choroiditis, extraocular myositis, scleritis, episcleritis, optic neuritis and corneal and conjunctival masses (D´Ark-Moretti, 2002; Swinger 2009).

Serological diagnosis of the infection can be performed by the indirect immunofluorescence reaction (RIFI) for presenting good specificity and sensitivity. This test allows the identification of antibodies according to the classes of immunoglobulins IgM or IgG (Costa et al., 2008). It is a simple and economically viable technique (Bresciani, 2009) and it is, also, considered a gold standard test (Dubey et al., 2020).

As ocular toxoplasmosis in dogs is considered rare and, according to the literature, ophthalmic signs may indicate the first clinical symptoms of toxoplasmosis. The present study aimed to serologically evaluate by the means of indirect immunofluorescence animals with ophthalmic changes treated at the Veterinary Hospital of the Faculty of Veterinary Medicine and Zootechnics of UNESP, campus of Botucatu, Department of Surgery and Anesthesiology and Veterinary Ophthalmology, for the research of antibodies of IgM and IgG class anti-*T. gondii* and correlate the results with the evaluated epidemiological variables.

MATERIAL AND METHODS

Ethics Committee

The project was approved by the animal use ethics committee of Faculty of Veterinary Medicine and Zootechnics at UNESP in Botucatu, São Paulo, under protocol number 069/2019.

Sample size

The sample size was based on the estimation of a proportion with the precision desired by the OpenEpi software, version 3.1 (Sullivan and Soe, 2006). The number of samples required was 75 based on an anticipated frequency of 9% by the software for

the presence of *T. gondii* in dogs with ocular alterations and an absolute precision of 5% with a 95% confidence interval (Franco, 2013).

It was established that the animals were divided into two groups by age, young 44 dogs (1 to 6 years old) and Senior 31 dogs (7 to 16 years old).

Sample collection

From May to September 2019, 75 blood samples were collected from dogs treated at the Veterinary Hospital of the Faculty of Veterinary Medicine and Zootechnics (FMVZ) at UNESP, Botucatu-SP, with ocular alterations on clinical examination. The owners signed the Informed Consent Form (ICF).

At the time of blood collection through the jugular or cephalic vein, an epidemiological questionnaire was administered to the animal's guardian containing questions related to age, sex, food and the environment in which the animal lived in order to assess the possible associations with the serological results.

The samples were collected in a minimum volume of 1 ml of animals with some ocular alteration and stored in a collection tube without EDTA, sent to the Diagnostic Service of Zoonoses of the Veterinary Hospital of UNESP, Botucatu campus, where they were centrifuged at 3,000 rpm for 10 minutes. The obtained serum was stored in 1.5 ml microtube identified with animal data and kept under freezing temperature at -20 ° C until processing.

Serological diagnosis

The Indirect Immunofluorescence Reaction (RIFI) was performed according to for the detection of antibodies of the IgM and IgG anti-*T. gondii* (Camargo, 1964). Tachyzoites from the RH strain of *T. gondii* were used as an antigen. 0.5 ml of serum from each sample was used, initially performing the screening test and then, in case of positivity, the titration to evaluate the titre until its extinction (Sabin, 1941).

It started with the titration of 16 IU (international unit) and the highest titer obtained was 256. The conjugates used for IgG and IgM were the Goat ant Dog IgM and Ig: FITC, branded Bio-Rad.

Statistical analysis

The prevalence of interpretations of the results of the serological test was calculated by the total number of dogs with ocular alterations as a result of the serological test (RIFI) during the months of May to September 2019, correlations between

the studied variants were performed. The result was presented as a percentage and a 95% confidence interval (CI). To calculate P, the Kruskasl Wallis statistical method (Nonparametric ANOVOA) was used. To evaluate the correlation between the variants for the diagnosis of toxoplasmosis was performed using Pearson correlation test with a significance level of 5%.

RESULTS

Of the total of 75 samples analyzed, 39 (52%) were positive for both classes of antibodies, 28 (37%) with anti-*T. gondii* titre for IgG class antibodies and 34 (45%) for IgM. The highest antibody titre obtained for IgG and IgM was 256 IU and was found for both classes of antibodies. The 16 IU titre (47% IgM and 50% IgG) was the most frequent among the processed samples followed by the 64 IU titre (44% for IgM e 29% for IgG) and 256 IU (9% for IgM e 21% for IgG).

Of the animals evaluated, 65% presented uveitis as a clinical sign; the others presented 8% keratoconjunctive, 5% ulcers, 12% conjunctivitis, 6% retinochoroiditis, 1% iridocyclitis, 2% retinitis and 1% choroiditis.

According to the age of the animals, two groups were established: young (1 to 6 years old) and senior (7 to 16 years old). In the group of 44 young animals, 22 had IgM, and 22 for IgG. Of the 31 animals classified as senior, 10 had IgM titres, and 6 for IgG (Table 1).

Table 1 - Distribution of anti-*Toxoplasma gondii* antibody titres of the IgM and IgG classes according to the age of the dogs. Botucatu-SP. 2019.

Titre	Young IgM N (%)	Young IgG N (%)	Senior IgM N (%)	Senior IgG N (%)
1:16	10/22 (45,5%)	11/22 (50%)	5/10 (50%)	3/6 (50%)
1:64	10/22 (45,5%)	7/22 (32%)	4/10 (40%)	1/6 (17%)
1:256	2/22 (9%)	4/22 (18%)	1/10 (10%)	2/6 (33%)

The variables sex, food and environment were statistically compared and it was not obtained (Table 2).

Table 2 - Researched variables and statistical results obtained for positive dogs in the serological exam for anti-*Toxoplasma gondii* antibodies of the IgM and IgG classes. Botucatu- SP. 2019.

Class IgM				
		N (%)	**P value**	**Pearson's correlation**
Sex	Male	14/34 (41%)	0,925	0,100215
	Female	20/34 (59%)		
Food	Ration	23/34 (68%)	0,3853	0,043741
	Natural	11/34 (32%)		
Environment	Rural	5/34 (15%)	0,5455	-0,07342
	Urban	29/34 (85%)		
Class IgG				
Sex	Male	6/28 (21%)	0,235	0,213109
	Female	22/28 (79%)		
Food	Ration	21/28 (75%)	0,5019	-0,06419
	Natural	7/28 (25%)		
Environment	Rural	5/28 (18%)	0,0245	-0,12314
	Urban	23/28 (82%)		

The frequency of reactive animals in the RIFI for anti-*T. gondii* antibody of the IgM was 45% and for IgG 37%. The fact of finding a response to anti-*T. gondii* IgM reveals that these animals were probably in the acute phase of infection and IgG responses are due to previous exposure to the agent.

DISCUSSION

In the present study, 52% (39/75) of dogs were found to be reactive for IgM and IgG immunoglobulins. It is noteworthy that RIFI was used in both surveys and perhaps this difference is due to the difference in the number of animals used in the two surveys. Uveitis is common in toxoplasmosis and is usually induced by cytolysis resulting from the replication of tachyzoites and deposition of intraocular immunocomplexes (Chavikin et al., 1994). The results of this present study disagree with Ferreira et al. (2016) that found uveitis only in 8.03% (48/598) of the animals reactive for *T. gondi*. In the study, 65% (49/75) of dogs were found to be ophthalmic sign uveitis.

The compared variables, sex, food and environment, did not obtain proven statistical results for the variables. There was no statistically significant difference between the variables of sex, race and environment, which corroborates with Varandas et al.

(2012). Age influenced the results found, with a higher frequency of seropositive individuals in young dogs, probably due to the greater number of animals in this age group. This result disagrees with another (Ferreira et al., 2016) that obtained a higher number of seropositive individuals in senior dogs, a fact that can be explained by the greater chances of contact with the agent, being usually higher seropositivity in older animals (Navarro et al., 1997).

The majority of antibody titres ranged between 16 (50%) and 64 (29%) for IgG immunoglobulin, similar results from other studies that detected 31.4% and 38.4% response for titres 16 and 64 (Garcia et al., 1999; Abreu et al., 2012). In the present study, 21% of the animals showed a serological response to the 256 IgG immunoglobulin titre. IgG titres probably represent previous exposure to one of the parasite's infectious forms, such as ingesting water and food contaminated with oocysts or eating meat containing bradyzoites (Dubey et al., 2020). High IgM values confirm the acute phase of the disease (Fábrega et al., 2020).

The variation in the frequencies of *T. gondii* reported in dogs may be related to behavioral, socioeconomic, cultural, climatic and health differences. Dogs can serve as environmental indicators of pathogen circulation and of the appropriate ecological conditions for the maintenance of the parasite in dogs and human infections (Cunha et al., 2020).

Therefore, due to the difficulty in finding studies regarding ocular toxoplasmosis in dogs in the literature, this study suggests the continuity of studies in this line of research in order to assess the actual participation of this pathogen in ocular diseases in dogs, mainly from the research of anti-*Toxoplasma gondii* IgM, which is related to the acute phase of the infection. Another suggestion is to include routine examinations in dogs for ocular toxoplasmosis for surveillance of this epidemiology.

CONCLUSION

Dogs with ophthalmic alterations seen at the Veterinary Hospital of the Faculdade de Veterinária de Botucatu have high levels of anti-*T. gondii* for IgM and IgG. The main clinical symptoms were uveitis with a higher number of cases, keratoconjunctiva, ulcers, conjunctivitis, retinochoroiditis, iridocyclitis, retinitis and choroiditis.

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