SOROPREVALÊNCIA DE ANTICORPOS CONTRA *Toxoplasma gondii* EM CÃES DA GRANDE VITÓRIA, ESPÍRITO SANTO

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RESUMO

A toxoplasmose é uma zoonose de ampla distribuição mundial, e os cães, apesar dos hospedeiros intermediários, participam da cadeia epidemiológica de transmissão da doença, tanto para outros animais quanto para humanos. Como atividade de vigilância epidemiológica, foi realizada uma pesquisa sorológica para a detecção de anticorpos da classe IgG contra-*Toxoplasma gondii* em 150 cães, no período de janeiro a março de 2022, pertencentes ao banco de soro, do Laboratório Clínico Veterinário, da Faculdade de Medicina Veterinária da UVV, Vila Velha-ES. Para isso, utilizou-se a Reação de Imunofluorescência Indireta (RIFI), avaliando-se os resultados em relação aos títulos obtidos, idade e sexo. Um total de 16 (10,66%) das amostras foram positivas para *T. gondii*, com títulos variáveis entre 16 e 256, 15 (93,75%) com título 16, 11 (68,75%) 64 e três (18,75%) 256. Em relação ao sexo, dos 16 animais positivos, nove (56,25%) eram fêmeas e sete (43,75%) machos. Quanto à idade, os animais positivos variaram de três a 15 anos, e os animais com títulos mais altos tinham entre 10 e 12 anos de idade. Conclui-se que, apesar da baixa soroprevalência, a dispersão *do T. gondii ocorre* na região estudada, e que medidas de vigilância devem ser estabelecidas para o controle da infecção e da doença na população canina e humana.

Palavras-chave: cães, sentinela, sorologia, zoonose.

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EVALUATION OF CANINE TOXOPLASMOSIS SEROPREVALENCE IN ANIMALS FROM GREATER VITÓRIA-ES

ABSTRACT

Toxoplasmosis is a zoonosis of worldwide distribution, and dogs, despite intermediate hosts, participate in the epidemiological chain of transmission, both to other animals and humans. As an epidemiological surveillance activity, a serological research was carried out for antibodies of the IgG class *anti-Toxoplasma gondii* in serum samples obtained from 150 dogs, from January to March 2022, belonging to the serum bank of the Veterinary Clinical Laboratory, of the Faculty of Veterinary Medicine of UVV, Vila Velha-ES. The indirect fluorescent antibody test (IFAT-IgG) was used, evaluating the results in relation to the titers obtained, age, and gender. Sixteen (10.66%) of the samples were positive, with variable titers between 16 and 256, 15 (93.75%) with title 16, 11 (68.75%) 64, and three (18.75%) 256. Regarding gender, of the 16 positive animals, nine (56.25%) were females, and seven (43.75%) were males. Related to age, positive animals ranged from three to 15 years, and animals with higher titles were between 10 and 12 years old. It is concluded that despite the low seroprevalence, *T. gondii* dispersion occurs in the studied region and that surveillance measures should be established to control infection and disease in the canine and human populations.

Keywords: dogs, sentinel, serology, zoonosis.

EVALUACIÓN DE LA SEROPREVALENCIA DE TOXOPLASMOSIS CANINA EN ANIMALES DE GRAN VITÓRIA-ES

RESUMEN

La toxoplasmosis es una zoonosis con una amplia distribución mundial, y los perros, a pesar de sus huéspedes intermediarios, participan en la cadena epidemiológica de transmisión de la enfermedad, tanto a otros animales como a los humanos. Como actividad de vigilancia epidemiológica, se realizó una búsqueda serológica de anticuerpos de la classe *IgG* anti-*Toxoplasma gondii* en muestras de suero, obtenidas de 150 perros, en el período de enero a marzo de 2022, pertenecientes al banco de suero, del Laboratorio Clínico Veterinario, de la Facultad de Medicina Veterinaria de la UVV, Vila Velha-ES. Se utilizó el ensayo de inmunofluorescencia indirecta (IFAT) para evaluar los resultados en relación con los títulos, la edad y el sexo. 16 (10,66%) de las muestras fueron positivas, con títulos que oscilaron entre 16 y 256, 15 (93,75%) con títulos de 16, 11 (68,75%) con 64 y tres (18,75%) con 256. En cuanto al sexo, de los 16 animales positivos, nueve (56,25%) eran hembras y siete (43,75%) machos. En cuanto a la edad, los animales positivos oscilaron entre los tres y los 15 años, y los animales con los títulos más altos tuvieron entre 10 y 12 años de edad. Se concluye que, apesar de la baja seroprevalencia, la dispersión de *T. gondii* ocurre en la región estudiada, y que se deben establecer medidas de vigilancia para controlar la infección y la enfermedad en la población canina y humana.

Palabras clave: perros, centinela, serología, zoonosis.

INTRODUCTION

Toxoplasmosis is a zoonosis of wide distribution in the world, and the occurrence of infection in both animals and humans is variable. Several risk factors are associated with the

infection, such as the presence of cats at home and the positivity for toxoplasmosis. Its agent is *Toxoplasma gondii* (*T. gondii*), a protozoan of the phylum Apicomplex. The maintenance of this parasite occurs through various sources of infection, very widespread in nature.

If the infection occurs primarily during pregnancy, *T. gondii* is transmitted vertically by the tachyzoites, which cause fetal transplacental infection (1). Horizontally, infection occurs from sporulate oocysts present in the environment, contaminating soil and water. Another possibility is the ingestion of cysts containing bradyzoites, found in the raw or undercooked meat of different mammals and birds. This fact is relevant for the one health, and researchers highlights the large participation of pigs and sheep in this form of transmission (2,3).

There are other forms of transmission, such as the tachyzoites present in blood, plasma, unpasteurized milk, and in cases of transplants (1). Transplacental transmission of this infecting form to pregnant women is emphasized, with relevant from the point of view of the one health. Epidemiologically, it is important to know which transmission routes are most important for planning educational actions aimed at the human and animal population.

The animals of the family *Felidae* are definitive hosts of the parasite, and in the urban area, cats stand out for the elimination of oocysts, which are released by feces after sexual multiplication, in the enteroepithelial cycle, contaminating the environment after sporulation. On the other hand, dogs are considered sentinel animals because they roll in the sand (xenosmophilia) contaminating the fur, with sporulate oocysts, with risks to other animals and humans (4). This species is widely used in seroepidemiological surveys, evaluating the degree of dispersion of *T. gondii* in a given region (5,6).

The literature reports the seroprevalence of canine infection worldwide, as well as the occurrence of the disease in dogs (7–21). In many situations, toxoplasmosis occurs concomitantly with immunosuppressive diseases such as distemper and ehrlichiosis (11,12,17,22,23).

The diagnosis of toxoplasmosis is mainly based on the immunological response of the animals to *T. gondii* by stimulating the production of immunoglobulins of the classes IgG, IgM, IgA, and IgE (24). Several serological tests are used for diagnosis. Sabin-Feldman's reaction was the first to be used; however, currently, the technique is less used by the need for living antigens (25). According to Dubey (26), it is very sensitivitye and specific, not occurring cross-reactions, but due to the practicality of the technique, was being replaced by others, also of good specificity sensitivity.

Widely used is the indirect fluorescent antibody test (IFAT), allows the detection of antibodies of IgM and IgG classes, of acute and chronic phase, respectively, immunoenzymatic ELISA test with various modalities, and also the direct agglutination test (MAT), which can also be used for both IgG and IgM research (26,27). The indirect hemagglutination test (HAI) is comparable to IFAT in terms of sensitivity and specificity (28). Its advantage is the practicality of use.

There are also latex agglutination tests, however, of lower sensitivity for animal serum samples (29) and the complement fixation test. Both were less used in the diagnostic routine. Silva *et al.* (27) compared IFAT and MAT in the detection of *T. gondii* antibodies in serum samples from sheep, goats, canines, felines, and the results revealed positive reactions, with titers ranging from 16 to 256, validating the technique, with high sensitivity and specificity, in the diagnosis of toxoplasmosis.

Although with a variable seroprevalence, the dispersion of *T. gondii* in dogs is great, demonstrating its role as a sentinel for this infection (5,30). Seroprevalence is generally higher in older animals, males and in rural environments, in contact with small animals such as passerines, rodents and other animals. It is also higher in dogs fed with homemade food containing remnants of raw or undercooked meat (10).

Coiro *et al*. (31) found 20.8% seropositivity in 302 canine serum samples in Botucatu-SP. Dantas *et al*. (32), working with 476 dogs, obtained 11.59% positivity. In Rondônia, Cañón-Franco *et al.* (33) and Azevedo *et al*. (34), in Paraíba found 76.4% and 45.1% of reactive animals, respectively.

Gaio *et al*. (35) determined the frequency of anti-T. *gondii* IgG antibodies in 689 serum samples from healthy dogs in the rural area of Botucatu-SP, randomly selected, being 298 (43.3%) females and 391 (56.7%) males, by IFAT. They obtained 144 (21%) positivity. The authors found a statistical correlation between antibody titers and age, which did not occur in relation to gender and race.

Gava *et al*. (36), conducted a retrospective study analyzing the results of serological tests for antibody research of the IgG anti-T. *gondii* class, in dogs, considering dilution 1:16 as a cutoff point. The study covered the period from January 2016 to September 2020. Of the total samples evaluated, 985 (79.62%) were non-reactive, and 252 (25.58%) serorreactive. The prevalent titer was 16 (8.40%), followed by 64 (7.51%), 256 (2.42%), 1024 (1.21%), and 4096 (0.80%). The authors concluded by a low seroprevalence, which can be explained by good health education and responsible guarding, reducing the exposure of these animals to external environments.

Olbera *et al*. (37) evaluated the cumulative incidence and spatial distribution of dogs exposed to *T. gondii*. Of the 576 animals, blood samples were evaluated at the initial moment, 180 days, and 360 days later, being second and third samples, respectively. Three hundred fifty animals were seronegative on the first day, and 39.2% were seropositive for toxoplasmosis.

Working with 1043 serum samples from dogs from five urban centers of the State of Paraíba, with the objective of determining the frequency of seropositive animals for toxoplasmosis by IFAT, and other pathogens, Fernandes *et al.* (38) obtained 22.1% positivity, having found as a risk factor age greater than 48 months. Paz *et al*. (39), studying 269 serum samples from dogs in Belem-Pará, obtained 38.9% positivity, with titles ranging from 16 to 64. Mantovan *et al*. (40) in 181 dogs found 9.39% of serum reagents in the municipality of Pardinho, Botucatu region, with variable titles from 16 to 4096.

Costa *et al*. (41) evaluated the occurrence of *T. gondii* infection in dogs, in five regions, in the urban area of Botucatu-SP, by modified agglutination test (MAT) in 670 serum samples, and they found 58.6% females and 41.4% males positive for infection. Among infected dogs, 4.6% were less than one year old, and 95.4% were older. The titers were: 16 (69.8%), 64 (13.8%), 256 (15.5%) and 1024 (0.9%).

Studying the prevalence of antibodies anti-*T. gondii* in 342 dogs by IFAT, in the municipality of Brotas-SP, Langoni *et al*. (42) found 26.9% of seropositive dogs, with titers16 (n= 59), 64 (n= 28), 256 (n= 4) and 1024 (n= 1). Of the variables statistically evaluated, such as gender, age, and breed, only for age, a difference was found, and in dogs older than five years, the risk of infection was higher.

Researching serum samples also by IFAT, in 205 canine serum samples in the city of Ubatuba-SP, as well the risk factors for toxoplasmic infection, Silva *et al.* (43) obtained 25.4% of positive samples, with titles ranging from 16 to 256. The results showed a significant association with the access of dogs to the street and also with the consumption of homemade food. The authors conclude the importance of toxoplasmosis as a public health problem in the region studied and suggest sanitary measures for infection control.

Considering the importance of toxoplasmosis in the context of One Health and for public health, the objective of this study was to investigate the seroprevalence of *T. gondii* in dogs attended at the Veterinary Hospital of Vila Velha University-UVV-ES, and evaluate the variables associated with the infection.

MATERIAL AND METHODS

Serum samples from 150 dogs were obtained from January to March 2022. These samples were stroed at -20°C and randomly chosen at the Clinical Pathology Laboratory of the University, where they were collected. After selecting the number of samples established for the study, the breed, gender, and age were verified in the registration records of the respective animals. This is a convenience study, taking advantage of sera samples previously used in other studies.

The experimental part of the research was carried out at the Zoonosis Diagnosis Service of the Department of Animal Production and Preventive Veterinary Medicine of the Faculty of Veterinary Medicine and Animal Science, Unesp, Botucatu-SP.

The indirect fluorescent antibody test (IFAT) was used, according to Camargo *et al*. (28). Initially, screening tests were performed, and in the case of reagent samples, the titration of the respective sample was subsequently performed for evaluation of the final title for immunoglobulin, of the IgG class anti-*T. gondii*, using the conjugate batch CCZ 184, produced and kindly donated by the Zoonosis Control Center of São Paulo-SP. The cutoff established was the titer 16.

The limiting factor of the research is the number of samples evaluated, which could be higher, but considering the moment of obtaining samples, in which we were in the full Covid pandemic 19, there was a decrease in the care of animals routinely examined in the clinic of small animals.

RESULTS AND DISCUSSION

Of the 150 samples, 87 (58.0%) were females and 63 (42%) were males. In total, 16 (10.66%) were serum reagents with titers ranging between 16 and 256. Of the positive samples, 15 (93.75%) presented title 16, 11 (68.75%) 64 and three (18.75%) title 256.

Regarding gender, of the 16 positive animals, nine (56.25%) were females, and seven (43.75%) were males. Of the positive females, 7 (77.8%) were without defined breed and the other two were poodle. Of the seven males, only two (28.57%) had no defined breed and the others (71.43%) were golden retriever, Siberian husky, poodle, basset hound and bulldog. The ages of the reagent animals ranged from three to 15 years. The animals with the highest titles were between 10 and 12 years old.

Knowledge of the epidemiological situation of a disease facilitates the establishment of strategies for its control. For example, it may be possible to use prophylactic vaccines, or treatment of infected animals. It all depends on the infectious agent involved and the scientific and technological advances related to the disease. Thus, the importance of seroepidemiological surveys is highlighted to know the dispersion of the infectious agent in the animal population and the environment, and when it is a zoonosis such as toxoplasmosis, the risks to the human population, and its implication in the concept of one health.

The results of the present study revealed a seroprevalence of 10.66% in dogs of both sexes with variable titers from 16 to 256. Although low, there was previous exposure to *T. gondii* with infection of the animal. The largest response with detectable IgG class antibodies was in females (56.25%). Regarding age, the frequency of positive animals was higher in the older ones (10 -12 years), which is expected by the higher chances of contact and exposure with other animals, and with the various risk factors for infection (35,38,41,42,44).

Regarding sex, previous study showed that infection is more frequent in males due to greater exposure to the infecting forms of the parasite, since in many situations, they accompany bitches in heat, having higher chances and consequently higher risk of infection, probably by the ingestion of sporulated oocysts present in water and the environment (5). On the other hand,

Silva *et al.* (43), Langoni *et al.* (42) and Gaio *et al.* (35) found no significant difference in this variable. The association between gender and frequency of IFAT reagents was statistically evaluated, which revealed no association, with a chi-square value of 0.023 and P = 0.5422.

Regarding infection rates, there is a very large variation; however, the number of animals evaluated and the diagnostic test used that can interfere with the final results of the studies and it should be taken into account (33,35,36).

Dantas *et al*. (32) found 11.5% seroprevalence, similar to the results of the present study with 10.66% of serum reagent animals. A result similar to that of the present study was also found by Mantovan *et al*. (40) with 9.39% seropositivity, obtained with the examination of 181 dogs. Other studies reveal variable seroprevalence between 20 and 30% among the animals studied, in studies conducted in different regions, including other states of the Federation (35,38,39).

Cañón-Franco *et al*. (33) found 76.4% of sero reactive animals in the State of Rondônia and Azevedo *et al*.(34) 45.1% in Paraíba, and Gava *et al*. (36) in Botucatu-SP, results higher than in the present study. Environmental factors can influence the results since variables such as temperature and humidity favor or hinder the maintenance of viable oocysts in the environment. Another aspect of being considered is the number of animals evaluated in the different studies.

CONCLUSIONS

The results obtained allow us to conclude that the seroprevalence of toxoplasmosis in dogs in greater Vitória was 10.66%, with titles ranging from 16 to 256, and that in older animals, aged between 10 and 12 years, seroprevalence was higher.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The experiment were conducted in accordance with laws and regulations and approved by the Ethics Committee on the Use of Animals (CEUA/UVV), protocol number 634/2022.

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