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RESEARCH

Increased frequency of detection of anti-*Toxoplasma gondii* antibodies in domestic cats after outbreak of human toxoplasmosis

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Abstract

Toxoplasmosis affects various organisms, including humans. In 2018, the largest outbreak of human toxoplasmosis described so far was reported in southern Brazil, with 809 human cases reported, and water as the potentially primary source of infection. Therefore, in this study, we aimed to evaluate the seroprevalence of *Toxoplasma gondii* in naturally infected domestic cats before and after the human toxoplasmosis outbreak, as well as the potential for environmental contamination by the number of cats infected after the outbreak. We evaluated 381 serum samples from domestic cats in southern Brazil, using an indirect immunofluorescence assay, with samples considered positive at a titer of 1:20. We found that 73% (204/279) and 27% (75/279) of the samples analyzed before the outbreak were negative and positive, respectively. After the outbreak, 62% (69/112) were negative of the samples were and 38% (43/112) were positive. Notably, the proportion of positive samples before the outbreak before (27%) was significantly lower than that after the outbreak (38%; P = 0.020). Therefore, the increased seroprevalence of *T. gondii* in cats was probably correlated with the ingestion of contaminated water. Therefore, it is important to monitor animals, mainly definitive hosts, after toxoplasmosis outbreaks, considering that these animals can contaminate the environment and, consequently, humans.

Keywords Feline · Toxoplasmosis · Outbreak · Santa Maria · Antibodies · Seroprevalence

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Introduction

Toxoplasma gondii is an obligatory intracellular parasite that infects various hosts and has a heteroxenous life cycle (Dubey 2021a). Felids are the definitive hosts for this parasite that excrete non-sporulated oocysts in their feces (Dubey and Jones 2008). Various intermediate hosts, such as humans, can also become infected with this parasite (Frenkel et al. 1970).

Human infections occur through different routes, such as the ingestion of meat (specifically cured meat or uncooked foods) containing cysts with bradyzoites and vegetables or water from a source contaminated with sporulated oocysts, which account for the majority of the reported cases (Dubey 2021b). Another significant route of infection is via transplacental transmission (Dubey 2021b). Dubey (2021a) reported that the high number of cases in humans and animals may be associated with the ingestion of contaminated water.

Felids are infected in the same manner as humans (Dubey and Jones 2008). However, as definitive hosts, felids excrete non-sporulated oocysts in their feces (Dubey 2021a). The most harmful route of infection, which increases oocyst excretion via feces, is the ingestion of meat containing cysts with bradyzoites in felids. The common clinical signs observed in felids include hyperthermia, anorexia, lethargy, abdominal discomfort, neurological symptoms, and reproductive problems (Vollaire et al. 2005). However, most felid infections are subclinical (Elmore et al. 2010).

The municipality of Santa Maria, with a population of 271.735 residents, is located in Southern Brazil (IBGE 2022) (Fig. 1). On October 19, 2018, additional 2235 cases, with 902 confirmed cases were reported (PMSM 2019). Arquilla et al. (2019) reported this as the largest toxoplasmosis outbreak worldwide.

In this study, we aimed to evaluate the seroprevalence of anti-*T. gondii* IgG antibodies in domestic feline samples from Santa Maria before and after the toxoplasmosis outbreak to determine the change in infected animals post-outbreak.

Methods

Serological samples

Samples from domestic cats of the municipality of Santa Maria treated at the Hospital Veterinário Universitário of the Universidade Federal de Santa Maria (HVU-UFSM) were selected for this study. Briefly, whole blood samples were centrifuged at 2500 rpm for 10 min at the Laboratório de Análises Clínicas (LACVET) of UFSM. Serum samples were stored at –20 ºC and sent to the Laboratório de Doenças Parasitárias (LADOPAR) of UFSM for further analysis.

For analysis, 381 samples were divided into two distinct groups: pre-outbreak group including 269 samples from animals treated between March 2016 and February 2018, and post-outbreak group, including 112 samples from animals treated between March, 2018 and March, 2020. All samples were collected according to the case series, and were processed using the IFA technique.

Quantitative analysis

The parameters used to estimate the post-hoc test power were defined using the G*Power software (Faul et al. 2007). A one-tailed Z-test was used to assess the differences between the two independent proportions at a nominal significance level of 5%. By matching the observed data with

Fig. 1 Location of the municipality of Santa Maria (Latitude: 29° 41′ 2" S, Longitude: 53° 48′ 25" W), Brazil

the sample allocation, the resulting test power was estimated to be 68.5%.

Serological test

To detect the IgG antibodies, multi-post slides were pre-sensitized with *T. gondii* tachyzoites (obtained from VERO cell culture). Briefly, feline serum samples (with primary antibodies) were diluted in 1X phosphate-buffered saline (PBS) solution (pH 7.4) at a ratio of 1:20, as previously described (Dubey 2021a; Hornok et al. 2008). After vortex mixing, 8 µL of the diluted sample was placed in each well of the multipost slides and incubated at 37 ºC for 1 h at approximately 80% humidity.

Next, the samples were washed with 1X PBS and distilled water, incubated with the fluorescein-conjugated IgG antibod, at a ratio 1:100 (Sigma-Aldrich Inc., St. Louis, MO, USA), and washed.

Finally, a solution (glycerol + PBS in a 50:50 ratio) was added, and the slide was covered with a coverslip. The samples were visualized at 400 × magnification using an OptiPhase microscope (INV403). Feline and *T. gondii* serum samples were used as positive and negative controls for *T. gondii*, respectively. Samples were considered positive when the entire surface of the *T. gondii* tachyzoites exhibited fluorescence. Samples with partial reactions were considered apical.

Results

Detection rates of positive and negative samples before and after the outbreak and Anti-IgG *T. gondii* serological results of the feline samples are shown in Table 1.

Notably, the proportion of cases in the pre-outbreak group (27%) was significantly lower than that in the post-outbreak group (38%; P = 0.020).

Table 1 Detection of anti-IgG *Toxoplasma gondii* antibodies in domestic cats before (March 2016 – February 2018) and after (March 2018 – March 2020) an outbreak of human toxoplasmosis, in Santa Maria, Brazil

City/Country	Detection of anti-IgG *Toxoplasma gondii*	Pre-outbreak (March 2016—February 2018)	Post-outbreak (March 2018 – March 2020)
Santa Maria, Brazil	Sample negative	73% (204/279)	62% (69/112)
	Sample Positive	27% (75/279)	38% (43/112)

Discussion

Here, we observed a significant increase in anti-*T. gondii* antibody levels in serum samples of domestic felines after an human toxoplasmosis outbreak.

Minuzzi et al. (2021) suggested the water distribution in the city of Santa Maria as a possible source of infection of the outbreak. As humans and felines share the same water source, our study aimed to evaluate the increase in the number of infected felines after the outbreak to determine whether the source of infection was same. We found an increase in the rate of positive detection in animals post-outbreak, suggesting that the source of the infection was potentially the same.

Increased seroprevalence in *T. gondii*-infected felines may be associated with an increase in environmental contamination by oocysts (Jones and Dubey 2010; Montazeri et al. 2020). Notably, domestic cats with chronic *T. gondii* infection re-excrete oocysts in their feces when exposed to the feline leukemia virus (FeLV) (Dubey 2021a). Dubey (2021a) revealed that felines exposed to this virus excrete up to one million oocysts.

Other outbreaks involving the contamination of water sources by feces of felines possibly infected with *T. gondii* have already been described. The toxoplasmosis outbreak that occurred in Canada in 1977 suggested the contamination of the municipality's water reservoir, which received water from two springs to which domestic and wild cats had free access (Dubey 2021a; Isaac-Renton et al. 1998). In this study, 4/7 domestic cats that were evaluated had antibodies, while 11/12 cougars (*Felis concolor*) had antibodies, with 12.5 million viable *T. gondii* oocysts isolated from a single cougar (Dubey 2021a; Dubey et al. 2008).

In 2004, the highest number of reported cases of human toxoplasmosis in Coimbatore, India was reported. In this case, the water distribution from a reservoir, which was accessible to felines, was also associated with a contamination source (Dubey 2021a). In Brazil, the municipalities São José dos Campos (Dubey 2021a; Magaldi et al. 1969), São Luís (Dubey 2021a; Silva et al. 2002), Santa Isabel do Ivaí (Dubey 2021a; Vaudaux et al. 2010), and Montes Claros de Goiás (Da Costa et al. 2020; Dubey 2021a) also had water contaminated with *T. gondii* oocysts as a source of infection in human cases. This suggests the importance of controlling feline access to water resources to prevent environmental contamination as well as the infection of humans and other animals using the same source.

Mortari et al. (2023) assessed the levels of anti-*T. gondii* IgG antibodies in 2245 serological samples from dogs in the municipality of Santa Maria, Brazil. They analyzed 1159 samples collected before the outbreak and 1086 samples collected after the outbreak. The results revealed that 16%

(185/1159) of pre-outbreak samples and 43% (466/1086) of post-outbreak samples tested positive. These findings indicated that other animal species were also infected during the human toxoplasmosis outbreak in Santa Maria.

The feline seroprevalence of *T. gondii* varies worldwide. According to Montazeri et al. (2020), an analysis conducted from 1967 to 2017 revealed that the seroprevalence in South America ranged from 29 to 45%, in North America from 28 to 43%, in Europe from 38 to 48%, in Africa from 20 to 81%, in Asia from 24 to 30%, in Australia from 15 to 89%, and in Antarctica from 45 to 75%. The disparity in data may be attributed to the limited number of studies conducted in certain regions, such as Asia (Montazeri et al. 2020). Additionally, it is necessary to consider that the studies included in this meta-analysis used different serological techniques, which can result in variations in test sensitivities and specificities.

Seroprevalence of *T. gondii* in Brazil differs from that reported by Montazeri et al. (2020). For example, in Fernando de Noronha, 72% (18/25) of evaluated samples demonstrated the presence of antibodies via indirect immunofluorescence assay (IFA) (Costa et al. 2012). Cavalcante et al. (2006) detected a positivity rate of 87.3% (55/63) in the Amazon region using a modified agglutination test (MAT) and IFA.

Dubey et al. (2004) assessed the seroprevalence in felines during a human toxoplasmosis outbreak in Santa Isabel do Ivaí, Paraná, Brazil. Similar to Santa Maria, this outbreak was associated with the water distribution as a source of infection (Bowie et al. 1997; Minuzzi et al. 2021). In this study, 58 felines within a 3 km area, were examined using a Modified Agglutination Test (MAT). They found that 84.4% (49/58) of animals harbored antibodies against the parasite.

Variations in seroprevalence may be related to several factors, including the characteristics of the evaluated samples, place of residence, age of patient, and exposure to the protozoan. In the study conducted in Santa Isabel do Ivaí, seroprevalence was assessed considering the relationship between the number of animals with antibodies and infected humans in the same household. In our study, it was not possible to carry out this assessment as there was no data related to the infection of the owners these animals as well as georeferencing data of the owners' residences in the municipality.

In a study conducted in São Paulo using MAT, Pena et al. (2006) detected antibodies against *T. gondii* in 35.4% (84/237) of all samples. In a semi-arid region of Brazil, 53.4% (55/103) of animals infected with the feline immunodeficiency virus (FIV) and 75% (18/24) of animals co-infected with FIV and *T. gondii* tested positive for the parasite, suggesting co-infections as potential risk factors (Feitosa et al. 2021). Coinfection with *T. gondii* has also been described in association with FeLV and the parasite, *Neospora caninum* (Munhoz et al. 2017). Munhoz et al. (2017) reported that 45.45% (105/231) of the evaluated samples had antibodies against *T. gondii*.

In Guarulhos, SP, antibodies against *T. gondii* were detected in 26.3% (132/502) of the animals using MAT (Silva et al. 2002). In Araçatuba, São Paulo, Cardia et al. (2013) reported a positivity rate of 16.3% (63/386) by IFA. Cruz et al. (2011) identified 16.3% (46/282) of positive samples using IFA. In Lages, Santa Catarina, Rosa et al. (2010) found that 14.33% (43/300) of cats tested positive by IFA. Bolais et al. (2017) detected 9.67% (36/372) positive animal samples from Rio de Janeiro using MAT. This study aimed to establish the seroprevalence of anti-*T. gondii* in the domestic cat population in Rio de Janeiro using the indirect hemagglutination test –HAI (Bastos et al. 2014). These studies have demonstrated a lower detection rate of anti-*T. gondii* antibodies compared with the pre-outbreak rate of human toxoplasmosis observed in Santa Maria.

Pena et al. (2006) and Bastos et al. (2014) detected antibodies against *T. gondii* using the MAT and HAI techniques, respectively. Different techniques from those used in the present study (IFA).

Feitosa et al. (2021) and Munhoz et al. (2017) used IFA, but with different cutoff points, 1:16 and 1:64 respectively, in the present manuscript was 1:20.

Increase in the number of feline samples with anti-*T. gondii* antibodies after the toxoplasmosis outbreak suggests that the animals were infected by the same source as humans, the water distribution of the city. Mortari et al. (2023) also reported that dogs wereinfected by this source of infection. But cats should be considered with more attention because they are defenitive hosts to *T. gondii*, therefore they necessarily participate in the parasite life cycle and epidemiology contributing directly with toxoplasmosis dissemination among humans and animals.

Conclusions

The 2018 human toxoplasmosis outbreak in Santa Maria has attracted worldwide attention because of its magnitude. Here, we observed an increase in cats seroprevalence post-outbreak, indicating that the domestic cats were also infected with *T. gondii*. Our findings suggest that felines acquired the infection from the same source as humans, namely the water distribution of the city. Additionally, infection in definitive host animals can potentially initiate excretion of oocysts in their feces and possibly environmental contamination, being relevant the restriction of feline access to water resources and water reservoirs for human consumption. Therefore, other animals may have been affected by the toxoplasmosis outbreak, warranting further investigations.

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Declarations

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