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Frequency of anti-*Toxoplasma gondii* antibodies in dogs and cats from the metropolitan region of Vitória, Espírito Santo, Brazil

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ABSTRACT

Toxoplasmosis, an infection caused by Toxoplasma gondii, is widely distributed. Seroprevalence in pets is a reliable tool to determine environmental parasite presence and human risk of infection due to proximity to these animals. The frequency of anti-T. gondii IgG antibodies in sera of dogs and cats from Espírito Santo, Brazil and risk factors associated with the infection (sex, age, allocation, and breed) were assessed. Antibodies against T. gondii were detected in 39.4% (149/378) and 38.1% (142/373) of the dogs by enzyme-linked immunosorbent assay (ELISA) and by indirect immunofluorescence antibody test (IFAT), respectively. In cats, antibodies were detected in 15.2% (12/79) by ELISA and 7.6% (6/79) by IFAT. Canine infection was associated with stray origin and older ages. There was an agreement between techniques in the detection of antibodies in dogs (□□□□0.82) and cats (□□□ 0.63). These results indicate the parasite presence in the urban environment, suggesting the possibility of infection to humans and other animals. However, this risk is lower considering pet domiciled animals with control diet and better hygiene conditions.

1. Introduction

Toxoplasmosis caused by the protozoan *Toxoplasma gondii*, is a zoonosis with worldwide distribution that can cause miscarriages or serious abnormalities in the brain and ocular tissue of foetuses whose mothers usually become infected during pregnancy. Humans and other hosts can be infected by transplacental transmission of tachyzoites, consumption of raw or undercooked meat containing tissue cysts, ingestion of water, fruits or vegetables contaminated with oocysts, or direct contact with cat faeces in the soil (Robert- Gangneux & Dardé, 2012).

Pets, such as dogs and cats, can be used as sentinels for the environmental spread of *T. gondii* in urban areas, as they are exposed to infectious forms of the parasite (Meireles et al., 2004). The increase in the number of pets in households in recent decades and the strengthening of the human-animal relationship can facilitate the transmission of zoonosis that are important for public health, such as toxoplasmosis. Thus, information on the prevalence of infection in dogs and cats is useful to assess the risk to human health in a given area, being a public health problem, and a particular issue to be considered in the One Health studies (Jones & Dubey, 2010).

Toxoplasmosis meets the requirements as a one health disease because it has a significant impact on the health of domestic animals, wildlife, humans and ecosystems and requires consolidated and interdisciplinary approaches (Aguirre et al., 2019). Although the disease infects a diverse group of hosts, wild cats and domestic cats (*Felis catus*) are hosts capable of releasing the parasite through oocysts excreted in their faeces, infecting animals and humans, either by direct or indirect contact via the environment (Barros et al., 2022; Djurkovic-Djakovic et al., 2019; Koutsoumanis et al. 2018).

Infection in dogs, another intermediate host of the parasite, may be associated with human infection in a particular area. It has already been shown that there is a highly significant positive correlation between the distribution of antibodies in the two species, suggesting common transmission routes between them, such as carnivore feeding and the environment itself (Ulón & Marder, 1990). Human infection may also be related to the presence of cats in the environment (Souza et al., 1987; Camargo et al., 1995), specifically those capable of releasing oocysts, since acute disease outbreaks have been associated with water consumption contaminated (Balasundaram et al., 2010; Minuzzi et al., 2020). The oocysts elimination by the domestic cat is epidemiologically important. They should be investigated, because they can lead to outbreaks of acute human toxoplasmosis, and responsible for infection in pets (Tenter et al., 2000).

Detection of anti-*T. gondii* antibodies by many different methods are reported in the literature, including indirect hemagglutination (IH), the modified agglutination test (MAT), the indirect immunofluorescence test (IFAT) and the enzyme-linked immunosorbent assay (ELISA) (Dubey et al., 2012). Serological tests are important in the diagnosis of toxoplasmosis due to the common asymptomatic course of the disease. The most commonly used antibodies to indicate previous exposure to the parasite are IgM, a serological marker for the acute phase, and IgG, which remains throughout the life of the animal (Lappin, 2010).

In Espírito Santo state, Brazil, there are few studies on *T. gondii* infection in humans (Abreu et al., 1998; Areal & Miranda, 2008; Buery et al., 2014) and animals (Beltrame et al., 2012; Pena et al., 2013; Acosta et al., 2016; Ferreira et al., 2018). However, in the metropolitan region of Vitória (Espírito Santo, Brazil), the infection in dogs and cats has not been previously evaluated. Thus, this study proposed to investigate the frequency of anti-*T. gondii* IgG antibodies in sera from dogs and cats and the risk factors associated with their infections. Considering the premises of the One Health initiative, this study is part of a broader study on toxoplasmosis in the state of Espírito Santo, Brazil.

2. Material and Methods

This study was carried out in the municipalities of Vitória, Vila Velha, Serra and Cariacica, located in the metropolitan region of Vitória, in the state of Espírito Santo.

Blood samples were collected from 378 dogs and 79 cats captured on public roads (wandering) or delivered by their owners (domiciled) to the Zoonosis Control Centers (CCZ) of the respective municipalities. Most animals did not present clinical symptoms of any disease at the time of collection. To identify factors associated with *T. gondii* infection, information was collected on the place of capture or residence, sex, breed and age of the dogs and cats.

Serum samples were analyzed for anti-*T. gondii* antibodies by the indirect immunofluorescence test (IFAT) (Camargo, 1978) and ELISA (Voller et al., 1976), and the frequency were evaluated.

2.1. Statistical methods

To compare frequencies among variables (allocation, sex, breed and age), the Chi-square test or, when indicated, the Fisher's exact test or Likelihood Ratio test was used. The association between variables and positive serology was evaluated by calculating the odds ratio (OR) with a 95% confidence interval. A *p*-value of less than 0.05 was considered significant. The ELISA and IFAT results were compared by McNemar's test and the kappa coefficient (κ).

3. Results

Out of the 378 dogs evaluated, 74.1% were in temporary and CCZ animal shelter in the municipalities of Vitória, Vila Velha, Serra and Cariacica, and 25.9% were from households in the same areas. Regarding sex, 56.9% were females and 39.9% males. The majority (84.4%) were mixed breed, and only 14.8% were purebred. It was not possible to identify the age of the animals in 41.0% of the samples.

From the 79 cats evaluated, 43.0% were in the CCZ or in temporary shelters, and 57.0% were from households in the studied area. Regarding sex, 54.4% were females, and 43.0% males, while two animals did not have the identification of sex. Most of the cats (87.3%) were mixed breed, and only 12.7% were purebred. Regarding age, 75.9% were at least one year old, and 20.3% were less than one year old. Of the 45 domiciled cats, most of them had access to the street (62.2%).

Sera from the 378 dogs were evaluated by ELISA and 373 by IFAT, *T. gondii* specific-IgG were found in 39.4% (95% CI = 34.7- 44.4) and 38.1% (95% CI = 33.0-43.4) dogs by ELISA and IFAT, respectively. Considering the 373 samples evaluated by both tests (Table 1), a total concordance of 91.4% (κ = 0.82; 95% CI = 0.76 - 0.88) was observed. No statistical difference was observed between the techniques ($p = 0.377$).

Out of the 79 cats evaluated, *T. gondii*-specific IgG antibodies were found in 15.2% (95% CI = 7.6-22.8) by ELISA and 7.6% (95% CI = 2.5-13.9) by IFAT (Table 1). The total concordance between the tests was 92.4% (κ = 0.63; 95% CI = 0.36 - 0.89), with a statistically significant difference between the two techniques ($p = 0.041$).

			IFAT		
			Positive	Negative	Total
Dogs	ELISA	Positive	129	19	148
		Negative	13	212	225
		Total	142	231	373
Cats		Positive	6	6	12
		Negative	0	67	67
		Total	6	73	79

Table 1 *– Results of T. gondii-specific IgG, determined by ELISA and IFAT, in sera of 373 dogs and 79 cats.*

A significant association was observed between positive serology in stray and domiciled dogs (OR = 2.030 at $p = 0.005$ for ELISA and OR = 1.777 at $p = 0.025$ for IFAT). These results show that stray dogs had approximately twice the likelihood of being positive when compared to domiciled dogs. Moreover, it was found that adult dogs present a higher risk of positivity than younger animals, with an OR = 5.780 at $p = 0.002$ for ELISA and 5.704 at $p = 0.002$ for IFAT. There was no statistically significant association between infection and sex or breed in dogs (Table 2) and no statistically significant difference between the frequency of positive dogs and the municipality of origin, by ELISA (p = 0.807) or IFAT (p = 0.186).

Variables	ELISA		IFAT	
	Positive	*p* value	Positive	p value
	n (%)	OR (95% IC)	n (%)	OR (95% IC)
Allocation		*p* = 0.005*		*p* = 0.025*
Domiciliation	27 (27.6)	2.030 (1.229 – 3.355)	27 (28.4)	1.777 (1.072 – 2.946)
Stray	122 (43.6)		115 (41.4)	
Sex		*p* = 0.459		*p* = 0.768
Male	56 (37.1)	1.176 (0.766 – 1.803)	56 (37.3)	1.067 (0.693 – 1.642)
Female	88 (40.9)		82 (38.9)	
Breed		*p* = 0.543		*p* = 0.772
MB	123 (38.6)	1.195 (0.672 – 2.125)	119 (37.5)	1.092 (0.602 – 1.981)
PB	24 (42.9)		21 (39.6)	
Age		*p* = 0.002*		*p* = 0.002*
< 1 year	3 (12.5)	5.780 (1.670 – 20.003)	3 (12.5)	5.704 (1.647 –19.750)
≥ 1 year	90 (45.2)		88 (44.9)	

Table 2 *– Frequency of anti-T. gondii specific IgG antibodies in the sera of 378 dogs as determined by ELISA and IFAT, according to allocation, sex, breed and age. The symbol "*" stands for significant statistical correlation ($p < 0.05$) with the analyzed parameter, at the Chi-square test or, when indicated, the Fisher's exact test or Likelihood Ratio statistical test. MB: mixed breed; PB: pure bred.*

Out of the 79 cats evaluated, 43.0% were in the CCZ or in temporary shelters, and 57.0% were from households in the studied area. Regarding sex, 54.4% were females, and 43.0% males, while two animals did not have the identification of sex. Most of the cats (87.3%) were mixed. There was no statistically significant difference between the frequency of positive cats and the municipality of origin, by ELISA (p = 0.410) or by IFAT (p = 0.757),

According to ELISA, a significant association was observed between positive anti-*T. gondii* antibodies and gender, with an OR value of 11.344 ($p = 0.007$), indicating that the chance of female cats being positive is approximately 11 times higher than for males. There was no statistically significant difference between infection and allocation, breed, or age. Regarding IFAT results, there was no association between toxoplasmosis and the variables (Table 3).

Variables	ELISA		IFAT	
	Positive n (%)	*p* value OR (95% IC)	Positive n (%)	*p* value OR (95% IC)
Allocation		*p* = 0.597		*p* = 0.394
Domiciliation	6 (13,3)	1.393 (0.407 – 4.772)	2 (4,4)	2,867 (0.493 – 16.667)
Stray	6 (17,6)		4 (11,8)	
Sex		*p* = 0.007*		*p* = 0.220
Male	1 (2.9)	11.344 (1.383 – 93.017)	1 (2.9)	4.342 (0.482 – 39.076)
Female	11 (25.6)		5 (11.6)	
Breed		*p* = 0,644		*p* = 1.000
MB	10 (14.5)	1.475 (0.273 – 7.980)	6 (8.7)	-
PB	2 (20.0)		0	
Age		*p* = 0,060		*p* = 0.333
< 1 year	0 (0)	-	0	-
≥ 1 year	12 (20.0)		6 (10.0)	

Table 3 *– Frequency of anti-T. gondii specific IgG antibodies in the sera of 79 cats as determined by ELISA and IFAT, according to allocation, sex, breed and age. The symbol "*" stands for significant statistical correlation (p < 0.05) with the analyzed parameter, at the Chi-square test or, when indicated, the Fisher's exact test or Likelihood Ratio statistical test. MB: mixed breed; PB: pure breed.*

4. Discussion

Seroepidemiological studies for detection of anti-*T. gondii* antibodies in dogs and cats are useful for assessing environmental contamination by the parasite and, consequently, the potential risk of human infection in the evaluated site. The proximity between domesticated animals and man contribute to the disease transmission. For this reason, pets may act as sentinels in urban area (Meireles et al., 2004; Jones & Dubey, 2010; Brasil et al., 2018).

Comparing ELISA and IFAT, we observed agreement with the detection of *T. gondii* antibodies in dogs and cats (Silva et al., 1997; Macri et al., 2009; Zhu et al., 2012). However, ELISA was able to identify more positive results than IFAT when considering the feline population. Although, IFAT is an excellent standard for the serodiagnosis of toxoplasmosis due to its high specificity, ELISA show many advantages, such as higher sensitivity and automated procedures (Higa et al., 2000). The frequency of anti-*T. gondii* IgG antibodies were evaluated in dogs by ELISA and IFAT suggesting environmental contamination by oocysts and high infection risk (Silva et al., 2007; Acosta et al., 2016;). For cats, the frequencies of anti-*T. gondii* IgG antibodies were lower than other studies in Brazil, showing that they are mostly domiciliated (Meireles et al., 2004; Pereira et al., 2018; Pinto et al., 2009). These features directly influence in the food quality for the animals, since domiciled ones have access to industrialized food and non-domiciled may be exposed to uncontrolled food sources in the streets. Afonso et al. (2006) reported that regular feeding of animals and the absence or low density of prey could limit predation. It can explained the low prevalence observed in this study. The more selective habits of cats, in general, added to a lower intake of food and water, can restrict exposure to infection by *T. gondii,* as already described by Meireles et al. (2004).

A higher seropositivity was detected among stray dogs, justified by the higher susceptibility to risk factors, such as the ingestion of food found in human waste as well as contact with cat faeces. At the same time, the lower number of infections in domiciled dogs might be related to the ingestion of industrialized food and less contact with the external environment. Our results are similar with the findings of Mineo et al. (2004), showing a higher prevalence in animals from CCZs than in veterinary clinics and veterinary hospital dogs. This results suggesting that the origin and condition of the animals influences the rate of infection by *T. gondii.* Moura et al. (2009), also described that dogs with access to the streets are more susceptible to infection than dogs living into the house. No significant difference in the presence of specific antibodies was found between stray and domiciled cats, these findings agrees with DeFeo et al. (2002). Unlike, Miró et al. (2004) observed higher prevalence among cats from rural areas and stray cats when compared to domiciled cats.

The association of canine infection by *T. gondii* with the adult age can be justified by a longer exposure to the risk factors (Azevedo et al., 2005). In contrast, among cats, positive serology was only observed in adults, and age was not associated with the infection, similarly to the findings of Jackson, Hutchison & Siim (1987), that identified no significant difference between the infection of felines aged less or more than six months.

Sex was not associated with canine infection by *T. gondii*, which might be due to the exposure to similar risk factors by males and females (Cabral et al., 1998; Cañon-Franco et al., 2004). ELISA showed a higher frequency of positivity among female cats, like the study of Besné-Mérida et al. (2008), which suggested that genetic or endocrine reasons could explain this difference. However, there might be some unidentified element disturbing the association that was not found in other studies (Garcia et al., 1999; Salant & Spira, 2004).

The fact, dogs or cats being purebred or without defined breed did not influence the presence of specific antibodies, as corroborated by other studies (Azevedo et al., 2005; Pinto et al., 2009), may be because the owners similarly care for animals without defined breed and purebred.

5. Conclusion

Based on our data, a clear correlation in the frequencies of infection was found while comparing domiciled and non-domiciled animals. However, lower infection rates were found in domiciled cats and this increased with age. Those data show the occurrence of *T. gondii,* environment and risk of human infection by pet animals, although this phenomenon is likely to be less dangerous by those fully domiciled animals. The large number of susceptible dogs suggests the requirement to adopt preventive measures to contain the biological chain of this pathogen, such as restricting the animals' access to public areas and feeding them with industrialized food. It is necessary an integrative research that approach various aspects of the transmission chain of the parasite to assess the risk, develop control methods, and guide future actions, such as the research of oocysts in soil and water, detecting possible sources of infection.

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