

Short Communication

Prevalence of antibodies against *Toxoplasma gondii* and *Neospora caninum* in dogs

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Abstract

Toxoplasmosis and neosporosis are parasitic diseases of global importance. The present study had the objective to determine the influence of age, sex and breed in the prevalence of antibodies against both diseases in dogs from Brotas city, São Paulo State, Brazil. Blood samples of 342 dogs were collected, and the age, sex and breed of each animal were recorded. The serological diagnosis for toxoplasmosis and neosporosis were performed using the immunofluorescent antibody test (IFAT). The Fischer's test was used to calculate the association probability of the variables, with $\alpha = 5\%$. For toxoplasmosis the prevalence of antibodies was 26.9% (CI 95% 22.4-31.8%), and for neosporosis 4.97% (CI 95% 3.1-7.8%). The statistical analysis revealed a higher risk of infection for *T. gondii* in dogs with more than five years.

Key words: *Toxoplasma gondii*, *Neospora caninum*, IFAT.

Toxoplasma gondii is a worldwide distributed intracellular protozoan, able to infect several species of warm-blooded animals. The infection is usually asymptomatic, but in some cases can cause severe illness and lead to death in both animals and humans. The three main routes of infection are: ingestion of oocysts excreted by cats, the definitive host; ingestion of tissues of an infected animal; and vertical transmission (Dubey, 2007). *Neospora caninum* is a parasite similar to *T. gondii* in various aspects, also affecting several species of animals, causing abortion, neurological disorders and death (Dubey and Lindsay, 1996; Reichel *et al.*, 2007). The dog has an important role in the life cycle of *N. caninum*, acting as the definitive host (Lindsay, 1999), and eliminating the oocysts in their feces. The routes of infection are the same of *T. gondii*. Thus, the prevalence of infection of *N. caninum* in dogs is related to the environmental contamination by oocysts.

Previous studies have demonstrated a wide prevalence of antibodies against both parasites in several locations of Brazil, usually up to 10.0% (Cañón-Franco *et al.*, 2003; Meireles *et al.*, 2004; Andreotti *et al.*, 2006; Collantes-Fernández *et al.*, 2008; Figueredo *et al.*, 2008; Frid-

lund-Plugge *et al.*, 2008; Locatelli-Dittrich *et al.*, 2008; Benetti *et al.*, 2009; Guimarães *et al.*, 2009; Silva *et al.*, 2010; Valadas *et al.*, 2010). In most of these studies the prevalence for *T. gondii* antibodies are higher than for *N. caninum*, indicating a higher exposure of dogs by this parasite. Some risk factors have been observed in dogs, such as the increasing infection rate according to age, and in animals with access to street, for both *T. gondii* and *N. caninum* (Cabral *et al.*, 1998; Brito *et al.*, 2002; Gennari *et al.*, 2002; Barbosa *et al.*, 2003; Cañón-Franco *et al.*, 2004; Cunha-Filho *et al.*, 2004; Fernandes *et al.*, 2004; Azevedo *et al.*, 2005). The aim of the present study was to determine the influence of age, sex and breed in the prevalence of antibodies against *T. gondii* and *N. caninum* in dogs.

Blood samples of 342 dogs were collected in the city of Brotas (22°17'12" S 48°07'35" W), São Paulo State, Brazil, from May 2005 to May 2006, and from February to May of 2009. The number of samples of each year was 189, 80 and 73, in 2005, 2006 and 2009, respectively. The animals were chosen randomly, and were from a campaign of responsible ownership of dogs, realized by local authorities. The age, sex and breed of each animal were recorded.

The exams were realized in the Zoonosis Research Nucleus (NUPEZO), UNESP, Botucatu city. For both toxoplasmosis and neosporosis diagnosis, the immunofluorescent antibody test (IFAT) was performed. Positive and negative controls were used. For toxoplasmosis, the sera was initially tested in the dilution 1:16, and samples that showed positive results were further tested in four-fold dilutions until its final titer. For neosporosis the initial dilution was 1:25, and positive samples were further tested in two-fold dilutions. The cut-off titer for toxoplasmosis was 16, and for neosporosis 25. The Fischer's test was used to calculate the association probability of the variables, with $\alpha = 5\%$.

Of the 342 animals, 65 had less than one year, 208 between one and five years, and 69 more than five years. Regarding to sex, 289 were females and 53 were males. Most of the animals were crossbred ($n = 295$), while the minority were pure breed ($n = 47$). The prevalence of infection by *T. gondii* was 26.9% (92/342; CI 95% 22.4-31.8%), with titers 16 ($n = 59$), 64 ($n = 28$), 256 ($n = 4$) and 1024 ($n = 1$). The prevalence of infection by *N. caninum* was 4.9% (17/342; CI 95% 3.1-7.8%), with titers 25 ($n = 2$), 50 ($n = 8$), 100 ($n = 2$) and 200 ($n = 2$). Eight animals had positive results for both parasites. The only statistical difference observed in the variables studied was that dogs with more than five years old had greater risk to get infected by *T.*

gondii, when compared with younger dogs. The results are summarized in Tables 1 and 2.

The high prevalence of antibodies against *T. gondii* confirms previous studies performed in Brazil, demonstrating the wide distribution of the parasite in the animal population, especially dogs. As we observed in the present study, older animals have greater risk to get infected by *T. gondii*, (Brito *et al.*, 2002; Cañón-Franco *et al.*, 2004; Azevedo *et al.*, 2005; Silva *et al.*, 2010) due to the chronic pattern of the disease, which increases the chances of infection during the life time. The breed and sex did not show significant differences of infection, as observed by others authors (Cabral *et al.*, 1998; Ali *et al.*, 2003; Azevedo *et al.*, 2005).

The low prevalence of antibodies against *N. caninum* in dogs is also in accordance with previous studies. However, because the dog is the definitive host, it can eliminate thousands of oocysts in the environment. Thus, environmental contamination by *N. caninum* can be considerable. As observed by other authors (Cheadle *et al.*, 1999; Ferroglio *et al.*, 2007; Collantes-Fernández *et al.*, 2008), the sex does not influence the infection by *N. caninum*. In relation to age, no statistical difference was observed. However, that disagree with other studies (Wouda *et al.*, 1999; Fernandes *et al.*, 2004; Ferroglio *et al.*, 2007), where older animals presented a greater risk to get infected, probably by

Table 1 - Statistical association between the studied variables and serological results to *Neospora caninum* in dogs from Brotas, São Paulo State, Brazil.

| Variable | N ^a | IFAT ^b | Percentage; IC95 ^c | OR (IC95) ^d | p ^e | |
|-------------|----------------|-------------------|-------------------------------|------------------------|----------------|------|
| Sex | Male | 53 | 2 | 3.8; 1.2-12.7 | 1.4 (0.3-6.3) | 0.49 |
| | Female | 289 | 15 | 5.2; 3.2-8.4 | | |
| Age (years) | < 1 | 65 | 2 | 3.1; 0.9-10.5 | - | 0.73 |
| | 1.1 - 5 | 208 | 11 | 5.3; 3.0-9.2 | | |
| | > 5.1 | 69 | 4 | 5.8; 2.4-14.9 | | |
| Breed | Crossbred | 295 | 15 | 5.1; 3.1-8.2 | 0.8 (0.2-3.8) | 0.58 |
| | Pure | 47 | 2 | 4.2; 1.3-14.2 | | |

^aN: number of animals tested. ^bIFAT: imunofluorescent antibody test. ^cFrequency of positive animals based in the variable (confidence interval = 95%). ^dOR: Odds ratio (confidence interval = 95%). ^ep: p value to $\alpha = 0.05$.

Table 2 - Statistical association between the studied variables and serological results to *Toxoplasma gondii* in dogs from Brotas, São Paulo State, Brazil.

| Variable | N ^a | IFAT ^b | Percentage; IC95 ^c | OR (IC95) ^d | p ^e | |
|-------------|----------------|-------------------|-------------------------------|------------------------|----------------|------|
| Sex | Male | 53 | 14 | 26.4; 16.5-39.6 | 1.0 (0.5-2.0) | 0.54 |
| | Female | 289 | 78 | 27.0; 22.2-32.4 | | |
| Age (years) | < 1 | 65 | 5 | 7.7; 3.4-16.8 | - | 0.00 |
| | 1.1 - 5 | 208 | 61 | 29.3; 23.6-35.9 | | |
| | > 5.1 | 69 | 26 | 37.7; 27.2-49.5 | | |
| Breed | Crossbred | 295 | 83 | 28.1; 23.3-33.5 | 0.6 (0.3-1.3) | 0.13 |
| | Pure | 47 | 9 | 19.1; 10.5-32.6 | | |

^aN: number of animals tested. ^bIFAT: imunofluorescent antibody test. ^cFrequency of positive animals based in the variable (confidence interval = 95%). ^dOR: Odds ratio (confidence interval = 95%). ^ep: p value to $\alpha = 0.05$.

the same reasons that *T. gondii*. We did not observe statistical difference related to breed. Capelli *et al.* (2004) detected a higher prevalence in pure breed dogs, and suggested a genetic factor that predispose to infection, or a more efficient vertical transmission. Collantes-Fernandez *et al.* (2008) found a higher prevalence in crossbreed dogs, but affirmed that this result was probably biased to the large sample size. Fernandes *et al.* (2004) found similar results. The role of breeds in the epidemiology of canine neosporosis is not well established.

The present study shows that dogs from Brotas city are widely infected by *T. gondii* and *N. caninum*, corroborating previous studies realized in other Brazilian cities. Dogs with more than five years have greater risk of get infected by *T. gondii*. The sex appears not to influence in the infection by both parasites. More studies are needed to understand the role of different breeds in the pattern of infection by *N. caninum*.

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