

## Short Communication

# Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* in dogs from an urban area of North-eastern Brazil: a spatial approach

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### Abstract

**Introduction:** The present study aimed to assess the seroprevalence and spatial distribution of *Neospora caninum* and *Toxoplasma gondii* in dogs. **Methods:** Blood samples (n = 241) were collected and analyzed for the presence of anti-*N. caninum* and anti-*T. gondii* antibodies. The spatial distribution was evaluated using kernel density estimation (KDE). **Results:** Anti-*N. caninum* and anti-*T. gondii* antibodies were detected in 24.06% (58/241) and 9.54% (23/241) of samples, respectively. A heterogeneous spatial distribution of positive dogs was observed across the city. **Conclusions:** These data are pivotal for better understanding the dynamics of infection caused by these protozoa in the canine population.

**Keywords:** Neosporosis. Toxoplasmosis. Canine medicine. Spatial distribution.

Dogs are considered to be important reservoirs of several pathogens of health concern, including *Neospora caninum* and *Toxoplasma gondii*<sup>1,2</sup>. These protozoa are obligate intracellular parasites belonging to the family Sarcocystidae and are morphologically, epidemiologically, and biologically similar.

*N. caninum* has domestic and wild canids (i.e., coyotes, gray wolves and Australian dingoes) as definitive hosts, and causes a neurological syndrome that is characterized by ataxia and ascending paralysis<sup>3</sup>. In addition, this protozoon also infects livestock animals, especially bovines, causing important economic losses due to repetitive abortions<sup>4</sup>. Contrastingly, felids are the definitive hosts for *T. gondii*. This parasite has an

important medical and veterinary relevance as it infects a wide range of intermediate hosts, including dogs and human beings<sup>5</sup>.

Both of these parasitic infections occur following ingestion of fecal oocysts released by infected dogs (in the case of *N. caninum*) or cats (for *T. gondii*), or when the muscles/meat harboring cysts of intermediate hosts are consumed<sup>6</sup>. Oocyst elimination in feces occurs irregularly from natural hosts, and direct detection of oocysts in animal feces is therefore difficult. Consequently, several studies have been conducted to improve the serological detection of these pathogens using anti-*N. caninum* and anti-*T. gondii* antibodies in dogs. Based on these serological tests, the prevalence of *N. caninum* in Brazil may range from 1.16–43.1%<sup>7</sup>, while the prevalence of *T. gondii* in dogs from different Brazilian regions may range from 9.6–63.5%<sup>8</sup>.

Canine neosporosis and toxoplasmosis endanger the health of dogs and other animal species, and for their proper diagnosis, it is essential to systematically investigate the circulation of both pathogens in a given population. Despite the global importance

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**Received** 15 October 2018

**Accepted** 17 December 2018

of *N. caninum* and *T. gondii*, the majority of the epidemiological studies in dogs have been conducted only in rural areas. However, due to the increasing urban canine population, the occurrence of these parasitic infections in urban areas, where a plethora of factors may influence their circulation, should be investigated, with dogs potentially playing a role as biological indicators. Therefore, the aim of this study was to assess the seroprevalence and spatial distribution of *N. caninum* and *T. gondii* in pet dogs living in an urban area of Northeastern Brazil.

The study was conducted in the urban area of the municipality of Garanhuns (8°53'25"S and 36°29'34"W), Pernambuco, Northeastern Brazil. This region is located at 900 m above sea level and has an average temperature of 22°C, with a maximum of 24°C in January and minimum of 19°C in June, and an average relative humidity of 90%. The Ethics Committee for Animal Experimentation (ECAE) of the Universidade Federal Rural de Pernambuco approved the study (protocol number: 99/2016).

From August to December 2016, serum was extracted from the blood samples of 241 domiciled crossbreed dogs, and stored at -20 °C, until further laboratory processing. A detailed description and clinical history was recorded for each dog during serum collection. The sampling campaign was distributed over 12 neighborhoods, comprising the study area. The minimum sample size was estimated based on the domiciled population of dogs (~13,700 animals) in the municipality of Garanhuns (95% C.I.). The estimated population of dogs in the study area was determined based on WHO <sup>9</sup>.

The detection of anti-*N. caninum* and anti-*T. gondii* antibodies was performed with an immunofluorescence antibody test (IFAT). For *N. caninum*, the cut-off was set at 1:50 and the technique was performed as previously described<sup>10</sup>. The NC-1 strain from *N. caninum* cultured in bovine monocytes was used as an antigen. For *T. gondii*, the cut-off was set at 1:16 and the reaction was carried out as previously described<sup>11</sup>. The RH-strain from *T. gondii* tachyzoites recovered from mice was used as an antigen. In both tests, positive and negative serum samples were used as controls.

The chi-square test with Yates correction ( $\chi^2$ ) was used to compare the presence of the pathogens with gender. In addition, a chi-square test with partitioning was used to assess the differences between the presence of the pathogens and the age (< 1 year; from 1 to 3 years old; > 3 years old) of animals. The significance level was set at 5%. All analyses were performed using the statistical software BioEstat version 5.0.

The plane coordinates obtained by Global Positioning System (GPS) were used for the development of the spatial map of the municipality of Garanhuns. The georeferenced data were entered into the QGIS version 2.8 and the spatial distribution of the seropositive dogs was evaluated using the kernel density estimation (KDE). KDE is a non-parametric technique that generates a continuous density surface from point data. In addition, the KDE provides a simple alternative for analysis of focal patterns, where outputs are easily readable and understood. The color gradient represents the density of cases, ranging from green (lowest prevalence) to red (highest prevalence).

Out of all animals enrolled (n = 241), 49.79% (120/241) were female and 50.21% (121/241) male, with ages ranging from two months to 18 years. No animals presented clinical signs suggestive of *N. caninum* and/or *T. gondii* infection.

Serologically, anti-*N. caninum* and anti-*T. gondii* antibodies were detected in 24.06% (58/241) and 9.54% (23/241) of samples, respectively. Statistically significant differences were observed between the presence of the pathogens ( $\chi^2 = 14.422$ ;  $p = 0.001$ ), but not between genders ( $\chi^2 = 0.005$ ;  $p = 0.9442$ ) and ages ( $\chi^2 = 0.8417$ ;  $p = 0.8395$ ).

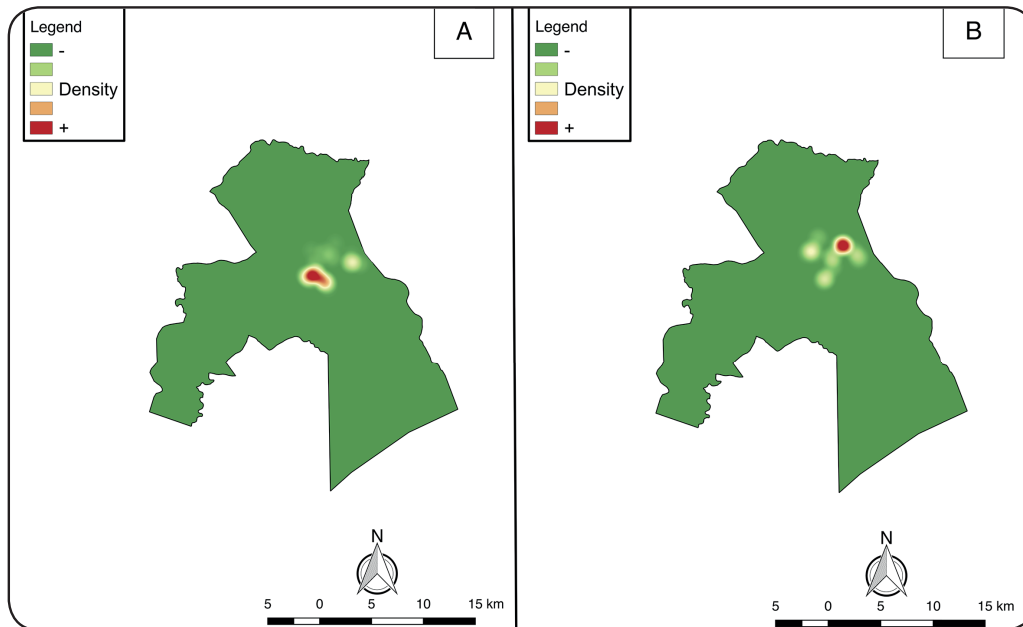
The kernel map (**Figure 1**) showed a heterogeneous spatial distribution of dogs positive for both pathogens over the city, with a hotspot in the northeast region, more precisely in the Brasilia neighborhood. Positive samples were also observed in six other neighborhoods (Boa Vista, Cohab 2, Cohab 3, Vale do Mundaú, Magano and João da Mata).

This study aimed to assess the presence of anti-*N. caninum* and anti-*T. gondii* antibodies in domiciled dogs and investigated, for the first time, the spatial distribution of both infections in the studied area. The seroprevalence recorded for *N. caninum* (24.06%) was lower than that obtained in a previous study performed in the same area 10 years ago, in which a seroprevalence of 34.5% was reported<sup>12</sup>. The infection of dogs by *N. caninum* has been extensively reported in rural areas and is associated with close contact with bovines. Conversely, in urban areas the most common route of transmission is through ingestion of homemade meals containing raw or undercooked meat that harbors cysts<sup>13</sup>. This fact might explain the decrease in seroprevalence observed in the present study compared to ten years ago<sup>12</sup>. It has now been established that the consumption of commercial food by dogs has increased worldwide in recent years, which has contributed to a reduction in the transmission of some pathogens. Similarly, the frequency of infection by *T. gondii* (9.54%) was lower than previously reported in the same area (54.4%)<sup>12</sup> and in other studies conducted elsewhere<sup>14</sup>.

No difference was observed in the seroprevalence of pathogens between males and females. Although older animals were considered to have been more exposed to both protozoa, no difference was observed in the seroprevalence of different age groups.

Seropositive animals for both pathogens were heterogeneously distributed over the city, but with a hotspot in the northeast region, more precisely in the Brasilia neighborhood. Interestingly, a very large meat market is located in this area, where dogs are frequently fed raw meat waste. The other six neighborhoods where seropositive animals were diagnosed (i.e., Boa Vista, Cohab 2, Cohab 3, Vale do Mundaú, Magano, and João da Mata) are peripheral areas close to remnants of the Atlantic Forest. These areas are affected by the presence of other animal species, including wildlife, which may have an important role in the epidemiology of the infection. In addition, residents of these peripheral neighborhoods have complained of environmental problems and the absence of basic sanitation, which might influence overall circulation of these pathogens.

It is important to remark that the spreading of both pathogens throughout the urban area accounts for their



**FIGURE 1:** Kernel estimation map of seroprevalence of *Neospora caninum* (A) and *Toxoplasma gondii* (B) in dogs of the municipality of Garanhuns, Pernambuco, Brazil.

facilitated dissemination among susceptible animals<sup>15</sup>. From an epidemiological point of view, this finding is interesting, especially considering the zoonotic role of *T. gondii*. Conversely, while dogs are not considered a source of infection for humans, they do act as important indicators of environmental contamination by this protozoon<sup>6</sup>.

Finally, this study assessed for the first time the spatial distribution of *N. caninum* and *T. gondii* seropositive dogs in the municipality of Garanhuns. Data reported in the present study are pivotal for better understanding the spread of these pathogens in canine populations. In addition, these results should sound an alarm bell for veterinarians and health professionals, warning them of the potential risks presented by these neglected parasites.

**Acknowledgments:** Authors would like to thank Alessio Giannelli (Poulpharm Bvba, Belgium) for his critical suggestions on the manuscript.

**Conflict of Interest:** The authors declare no conflict of interest.

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