Short Communication

Risk factors and anti-*Toxoplasma gondii* and *Neospora caninum* antibody occurrence in dogs in João Pessoa, Paraíba state, Northeastern Brazil

Fatores de risco e ocorrência de anticorpos anti-*Toxoplasma gondii* e *Neospora caninum* em cães de João Pessoa, Estado da Paraíba, Nordeste do Brasil

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Abstract

The aim of this study was to determine the occurrence of infections due to *Toxoplasma gondii* and *Neospora caninum* and corresponding risk factors among dogs attended at veterinary clinics in the city of João Pessoa, Paraíba, northeastern Brazil. Blood samples were collected from 384 dogs that were attended at 34 veterinary clinics between April 2015 and May 2016. These two agents were diagnosed through the indirect immunofluorescence reaction (IFAT). Among the 384 animals evaluated, 37 (9.6%) were positive for *T. gondii*, with titers ranging from 16 to 512. Six dogs (1.6%) were positive for *N. caninum*, with titers of 50 to 200. Access to the streets (OR = 4.60; 95% CI = 1.74-12.20) and environments close to forested areas (OR = 2.79; 95% CI = 1.32-5.93) were found to be risk factors for *T. gondii* infection. The dogs attended at veterinary clinics in João Pessoa are exposed to infections caused by *T. gondii* and *N. caninum*. Dog owners should avoid having access to the street or contact with forest environments.

Keywords: Toxoplasmosis, neosporosis, domiciled dogs, Northeastern Brazil.

Resumo

O objetivo deste trabalho foi determinar a ocorrência e os fatores de risco das infecções por *Toxoplasma gondii* e *Neospora caninum* em cães atendidos em clínicas veterinárias da cidade de João Pessoa, Paraíba, Nordeste do Brasil. Foram colhidas 384 amostras de sangue cães procedentes de atendimento de 34 clínicas veterinárias, no período de abril de 2015 a maio de 2016. O diagnóstico para os dois agentes foi realizado pela reação de imunofluorescência indireta (RIFI). Dos 384 animais avaliados 37 (9,6%) foram positivos para *T. gondii* com títulos variando de 16 a 512. Para *N. caninum* seis (1,6%) cães foram positivos com títulos de 50 a 200. Foram constatados como fatores de risco para *T. gondii* as variáveis acesso à rua (OR = 4,60; IC 95% = 1,74-12,20) e ambiente próximo a matas (OR= 2,79; IC 95% = 1,32-5,93). Os cães atendidos em clínicas veterinárias em João Pessoa estão expostos às infecções por *T. gondii* e *N. caninum*. Os proprietários devem evitar o acesso dos animais à rua ou o contato com ambientes florestais.

Palavras-chave: Toxoplasmose, neosporose, cães domiciliados, Nordeste do Brasil.

Introduction

The interrelations among animals, humans and the environment have intensified over recent decades, which has been causing a variety of ecological imbalances. From a public health point of view, these changes have taken on great importance, given that there is a real possibility of increased transmission of zoonoses (ZINSSTAG et al., 2011). Thus, pets such as dogs, which are increasingly incorporated into family groups as members of the family, may play a major role in maintaining and transmitting infectious agents. They may also act as sentinels for their respective diseases (ULLMANN et al., 2008). For this reason, monitoring of diseases like toxoplasmosis and neosporosis is needed and their risks towards the ecosystem need to be assessed.

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Toxoplasmosis and neosporosis are caused by *Toxoplasma gondii* and *Neospora caninum*, respectively, which are obligate intracellular coccidian protozoa belonging to the phylum Apicomplexa. Cats and dogs are the definitive hosts of *T. gondii* and *N. caninum*, respectively. Transmission of these parasites takes place through ingestion of oocysts that are eliminated from the feces of the definitive hosts; through ingestion of tissue cysts that are present in the musculature of infected animals; and through the transplacental route (DUBEY, 2003, 2010). *T. gondii* is considered to be a zoonotic agent (DUBEY, 2010). On the other hand, there is no evidence of zoonotic characteristics regarding *N. caninum*, although some studies have demonstrated that humans can present antibodies against this protozoon (OSHIRO et al., 2015). Even though dogs are not definitive hosts for *T. gondii*, they have an important role in mechanical dissemination of the agent (LINDSAY et al., 1997).

In Brazil, recent studies on the seroprevalence of *T. gondii* in dogs demonstrated variation from 11.5% to 70.8% (DANTAS et al., 2013; LANGONI et al., 2014), while for *N. caninum* this variation was from 4.2% to 43.1% (SOUSA et al., 2012; RAIMUNDO et al., 2015). The main risk factors reported in relation to *T. gondii* were the following: contact with cats (RODRIGUES et al., 2016); male sex (LANGONI et al., 2014; RODRIGUES et al., 2016); and greater age and access to the streets (AZEVEDO et al., 2005; DANTAS et al., 2013; RAIMUNDO et al., 2015). In relation to *N. caninum*, the risk factors were homemade diets, rural environment, greater age, cleansing of the environment only done weekly and presence of rats (BRESCIANI et al., 2008, PARADIES et. al., 2007, DANTAS et al., 2013).

Epidemiological surveys on toxoplasmosis and neosporosis among dogs are of strategic public health importance, because they provide an overview of the dynamics of these diseases in a given region. Such knowledge is of major importance in places like the city of João Pessoa, Paraíba, northeastern Brazil, which has a climate and environment that are favorable for parasite development, as well as there is a lack of epidemiological data on these infections in this region. In this light, the aim of this study was to determine the anti-*T. gondii* and *N. caninum* antibody occurrence and the risk factors associated with infections in dogs attended at veterinary clinics in João Pessoa.

Materials and Methods

Ethics

All the experimental procedures were approved by the Committee of Ethics and Animal Experimentation of Federal University of Campina Grande (Protocol CEP nº 010-2016).

Study area

The survey was conducted in the municipality of João Pessoa, capital city of the state of Paraíba, which has about 811 thousand inhabitants (IBGE, 2017), distributed in 59 boroughs, and with an area of preserved Atlantic Forest in the center of the urban spot and bordered by mangroves (PARAÍBA, 2014). Its climate is humid tropical, with annual average temperatures of 23°C and annual rainfall rate above 1,906 mm (PEREIRA, 2014).

Sampling

The selection of the veterinary clinics was based on data from the Regional Council of Veterinary Medicine (CRMV-PB), which has informed the existence of 40 registered clinics, however, in the on-the-spot verification four establishments had closed and in two others the owners preferred not to participate. Then, 34 veterinary clinics in the municipality of João Pessoa distributed in the four zones of the city (north, south, east and west) were used.

The minimum number of animals was determined by the formula for simple random sampling (THRUSFIELD, 2004):

$$n = \frac{Z^2 \times P\,(1-P)}{d^2} \qquad (1)$$

Where:
n = number of sampled animals
Z = value of the normal distribution for the confidence level of 95%
P = expected prevalence of 50% (sampling maximization)
d = error of 5%

In total, 384 apparently healthy dogs, unvaccinated or vaccinated over six months against leptospirosis were selected. Females in the periparturient and lactation periods were excluded from the study. The collections were carried out in the period from April 2015 to May 2016. Samples were collected by venipuncture of the cephalic or jugular vein, using vacuum tubes of 5 mL, and sera were placed into microtubes and frozen until the serologic tests were performed.

Serological diagnosis of infections due to T. gondii and N. caninum

To detect anti-*T. gondii* and anti-*N. caninum* antibodies, the indirect immunofluorescence reaction (IFAT) was used. For *T. gondii*, the dilution of 1:16 was taken to be the cutoff point (LANGONI et al., 2014) and the technique was performed in accordance with the methodology described by Camargo (1964), using RH-strain tachyzoites recovered from mice. For *N. caninum*, the cutoff point was the dilution of 1:50 (ACOSTA et al., 2016) and the technique was performed in accordance with Paré et al. (1995), using the NC-1 strain from *N. caninum* cultured in bovine monocytes as the antigen. The slides for both tests were produced in the Laboratory of Bacterioses of Domestic Animals of the Federal Rural University of Pernambuco. In both tests, both positive and negative serum samples were used as controls. Samples with tachyzoites that demonstrated complete peripheral fluorescence were considered positive and subjected to serial dilutions, with the endpoint titer defined as the reciprocal of the last dilution showing a positive result.

Risk factor analysis

Epidemiological questionnaires were applied to dog owners in order to obtain data to be used in the risk factor analysis. The analyzed variables and their respective categories were: educational level of the owner (illiterate, incomplete primary school, complete primary school, incomplete high school, complete high school, incomplete university degree, university degree), gender (female, male), breed

(pure-bred, crossbred), age (up to 48 months, 49 to 72 months, above 72 months), access to street (no, yes), feed (commercial food, homemade food, food scraps, raw meat), access to treated water (no, yes), reason for keeping the dog (company, guard, other), contact with dogs (no, yes), contact with bovine (no, yes), contact with equine (no, yes), contact with wild animals (no, yes), contact with cats (no, yes), contact with goats (no, yes), contact with sheep (no, yes), contact with swine (no, yes), type of housing (masonry, shacks, stilts, mud huts), where the animal is kept (soil, cement, soil/cement), environment where the animal lives (rural or urban), environment near woodlands area (no, yes), regular garbage collection (no, yes), cleaning and disinfection of the environment (daily, weekly/fortnightly, monthly), contact with flooded areas (no, yes), presence of rodents (no, yes), occurrence of abortions (no, yes), occurrence of stillbirths (no, yes), presence of ticks (no, yes).

An univariable exploratory analysis of the data was carried out for the selection of variables with $P \leq 0.2$ using the chi-square or Fischer exact tests. Subsequently the significant variables passed to a multivariable analysis using the multiple logistic regression with significant level of 5% (HOSMER & LEMESHOW, 2000). The adjustment of the final model was verified with the Hosmer and Lemeshow test, by which a value of $P \geq 0.05$ indicates a good fit. The collinearity between independent variables was verified by correlation analysis; for the variables with strong collinearity (correlation coefficient > 0.9), one of the two variables was excluded from the multiple analysis according to the biological plausibility (DOHOO et al., 1997). Confounding was evaluated by the monitoring the alterations in the model's parameters (>20%) when adding new variables. Data analyses were carried out using the SPSS 23.0 for Windows software.

Results

Among the 384 animals analyzed, 37 (9.6%) were seropositive for *T. gondii*, with the following respective antibody titers and frequencies: 16 (35.1%), 32 (18.9%), 64 (8.1%), 128 (13.5%), 256 (16.3%) and 512 (8.1%). For *N. caninum*, six dogs (1.6%) were seropositive, with titers and frequencies of 50 (66.8%), 100 (16.6%) and 200 (16.6%), respectively. One animal was positive for both parasites. Figure 1 presents the geographical locations of the homes of the dogs that were positive and negative for *T. gondii* (Figure 1A) and *N. caninum* (Figure 1B) in João Pessoa.

In univariable analysis on the risk factors associated with *T. gondii* (Table 1), the following variables were selected: type of rearing, access to treated water, aim of rearing, contact with dogs, contact with wild animals, environment in which the animal lived, locality, frequency of cleansing of the environment and contact with rats. Through multivariable analysis (Table 2), the variables of access to the streets (OR = 4,60; 95% CI = 1.74-12.20) and environment close to forested areas (OR = 2.79; 95% CI = 1.32-5.93) were identified as risk factors for *T. gondii*. Final model presented good fit (Hosmer and Lemeshow test: chi-square = 0,102; degrees of freedom = 1; $P = 0,749$). There were no risk factors for *N. caninum*.

Discussion

The results found in the present study regarding the occurrence of *T. gondii* are close to those found by Dantas et al. (2013) in Natal, Rio Grande do Norte, who observed that 11.5% of the dogs were seropositive; and by Dantas et al. (2014) in Patos, Paraíba, who observed that 15.6% were seropositive. Both of those
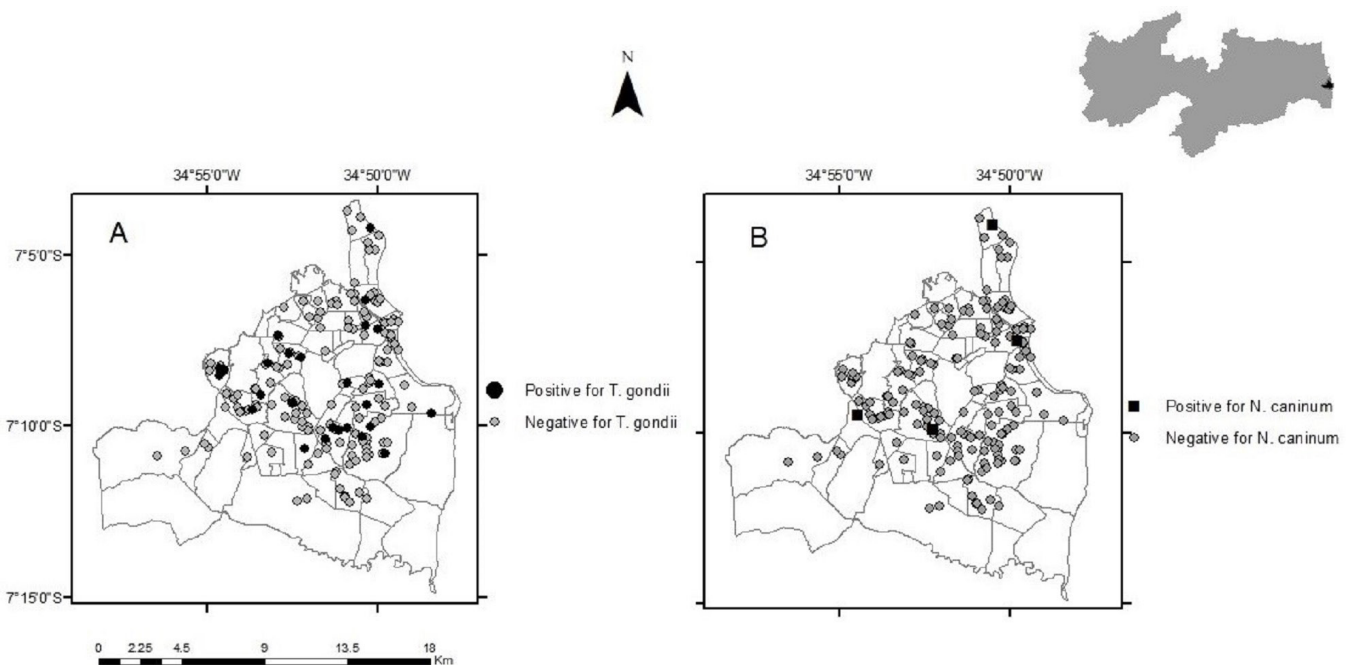

Figure 1. Geographic location of dogs attended at veterinary clinics in the city of Joao Pessoa, Paraíba, Northeastern Brazil, from April 2015 to May 2016, according to the serological condition for *T. gondii* (A) and *N. caninum* (B). The detail shows the city of João Pessoa in the state of Paraíba.

Table 1. Univariable analysis ($P \leq 0.20$) of the risk factors associated with seropositivity for *T. gondii* in dogs attended at veterinary clinics in João Pessoa, Paraíba, Northeastern Brazil, from April 2015 to May 2016.

Variables	Categories	Total nº of animals	Nº seropositives animals (%)	P*
Access to street	No	358	30 (8.4)	0.002
	Yes	26	7 (26.9)	
Access to treated water	Não	7	2 (28.6)	0.139
	Sim	377	5 (9.3)	
Reason for keeping the dog	Company	269	21 (7.8)	0.115
	Guard	68	8 (11.8)	
	Other	47	8 (17.0)	
Contact with dogs	No	68	10 (14.7)	0.171
	Yes	316	27 (8.5)	
Contact with wild animals	No	368	33 (9.0)	0.057
	Yes	16	4 (25.0)	
Where the animal is kept	Cement	243	19 (7.8)	0.032
	Soil/Cement	116	12 (10.3)	
	Soil	25	6 (24.0)	
Environment where the animal lives	Rural	30	5 (16.7)	0.191
	Urban	354	32 (9.0)	
Environment near woodlands areas	No	192	11 (5.7)	0.009
	Yes	192	26 (13.5)	
Cleaning and disinfection of the environment	Daily	287	23 (8.0)	0.138
	Weekly/ fortnightly	93	13 (14.0)	
	Monthly	4	1 (25.0)	
Presence of rodents	No	206	15 (7.3)	0.093
	Yes	178	22 (12.4)	

*P-value.

Table 2. Risk factors associated with seropositivity for *T. gondii* in dogs attended at veterinary clinics in João Pessoa, Paraíba, Northeastern Brazil, from April 2015 to May 2016.

Varible	Logistic regression coefficient	Standard error	Wald	Degrees of freedom	Odds Ratio	CI 95%	P*
Access to street	1.527	0.497	9.421	1	4.60	[1.74-12.20]	0.002
Environment near woodlands areas	1.028	0.384	7.166	1	2.79	[1.32-5.93]	0.007

Hosmer and Lemeshow test: chi-square = 0.102; degrees of freedom = 1; P = 0.749; *P-value.

populations consisted of dogs attended at veterinary clinics, as well as the IFAT was used as diagnostic test, with titers ranging from 64 to 1,024.

However, in an evaluation on dogs during a vaccination campaign in Campina Grande, Paraíba, Azevedo et al. (2005) found a prevalence of 45.1% using the IFAT. In this context, since the serological diagnostic method used was the same in these studies, it is believed that the characteristics of the canine population may have influenced the seroprevalence of toxoplasmosis. The dogs of the present study were attended at veterinary clinics, which leads us to believe that they were receiving better care from their owners regarding hygiene and health than were dogs that were vaccinated through public campaigns.

Although dogs that are regularly attended at veterinary clinics have received better care regarding hygiene and health (DANTAS et al., 2013), these animals may nonetheless acquire oocysts of *T. gondii* from the environment or through contact with the feces of infected cats. These oocysts may adhere to the animals'

fur, which gives rise to the possibility that humans might become infected through contact with these animals. One important point is that dogs may act as sentinels for toxoplasmosis. Thus, epidemiological monitoring of the behavior of this zoonosis in these animals is immensely important (NAVA, 2008).

Dogs play a fundamental role in transmission of neosporosis to other animals, especially to cattle; however, the presence of seropositive dogs does not indicate oocyst elimination in feces (BANDINI et al. 2011). Neosporosis in cattle causes economic losses relating to abortions of the order of US$ 1.2 billion worldwide (REICHEL et al., 2013). High prevalences of anti-*N. caninum* antibodies in dogs are generally correlated with living in rural areas (ROBBE et al., 2016), which may explain the low frequency of seropositive dogs in the present study, given that all the dogs investigated originated from the urban zone of João Pessoa.

Access to the streets among these dogs was a risk factor for infection with *T. gondii* in the present study. Similar results were described by Lopes et al. (2011) in Portugal, such that dogs with

access to the streets had a higher chance of presenting anti-*T. gondii* antibodies. Dantas et al. (2013) stated that dogs that had access to the streets were 4.6 times more likely to be seropositive for *T. gondii*. Access to the streets provides greater possibilities for dogs to eat intermediate hosts such as birds and rodents, which are common sources of *T. gondii* infection (SVOBODA & SVOBODOVÁ, 1987), and for them to consume sporulated oocysts that are present in water and in the environment.

Gennari et al. (2015) evaluated wild rodents and marsupials of the Atlantic Forest in the state of São Paulo and found that some individuals were seropositive for *T. gondii*. This suggested that these animals might be sources of infection for the fauna of the region and for domestic animals. In the present study, it was observed that dogs living in homes that were close to forested areas presented greater risk of infection with *T. gondii*. In the municipality of João Pessoa, there is an area of preserved Atlantic Forest (Figure 1) covering an area of more than 515 hectares that borders 25 districts of the city (CAVALCANTI, 2013). Moreover, there are several other areas with thick plant cover. Because of this proximity between city districts and forested areas, it is believed that this facilitates contact with a wide variety of sources of *T. gondii* infection, which increases the possibility of disease transmission.

The occurrence of toxoplasmosis and the risk factors for this disease found among the dogs of this study emphasize the importance of monitoring not only this disease in the canine population, but also various other zoonotic diseases of importance for public health. The changes that have stemmed from urbanization and deforestation of previously unexploited areas have altered the behavior and speed of propagation and appearance of these diseases. Thus, an in-depth approach addressing the ecosystem, based on multidisciplinary concepts of public health, is needed (KARESH et al., 2012; MWANGI et al., 2016).

Conclusion

The dogs attended at veterinary clinics in João Pessoa are exposed to infections caused by *T. gondii* and *N. caninum*. Dog owners should avoid having access to the street or contact with forest environments.

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