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Original Article

Seroprevalence and risk factors for *Neospora caninum* infection in dogs of rural areas of the Brazilian Semi-arid Region

Soroprevalência e fatores de risco para a infecção por *Neospora caninum* em cães de áreas rurais da Região Semiárida Brasileira

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ABSTRACT

Neospora caninum is a protozoan of the heteroxenous cycle, whose definitive hosts include domestic and wild canids, while intermediate hosts are herbivores. Its occurrence in dogs of rural areas deserves attention due to the risk of transmission to the animals of production, mainly cattle. The objective of this study was to determine the prevalence of *N. caninum* antibodies in dogs belonging to rural areas of the municipality of Sousa, the semi-arid region of Paraíba, Northeastern Brazil. Blood sera from dogs of six rural communities were analyzed. The indirect fluorescent antibody test was performed for the detection of anti-*N. caninum* IgG. The results were considered positive when the total peripheral fluorescence of the tachyzoites occurred at a dilution ≥1:50. The frequency of seropositivity was 9.18% (9/98 cases). Antibody titers ranged from to 200, with the 1:50 titer being predominant. Among the evaluated sites, 66.6% (4/6) showed the presence of at least one seropositive dog. The variable contact with cattle was considered a risk factor (odds ratio = 15.25) for infection by the parasite, demonstrating a higher risk of contact with contaminated tissues. It was concluded that dogs from rural areas of the municipality of Sousa were exposed to *N. caninum*, and it was suggested that contact between dogs and cattle be avoided as a control measure to prevent infection in dogs.

Palavras-chave:
Caninos
Neosporose
Sorologia

RESUMO

Neospora caninum é um protozoário de ciclo heteroxeno, cujos hospedeiros definitivos incluem canídeos domésticos e silvestres, e hospedeiros intermediários são os herbívoros. Sua ocorrência em cães de áreas rurais merece atenção devido ao risco de transmissão aos animais de produção, principalmente bovinos. O objetivo deste estudo foi determinar a prevalência de anticorpos anti-*N. caninum* em cães de zona rural do município de Sousa, Semiárido da Paraíba, Nordeste do Brasil. Foram analisados soros sanguíneos de cães de seis comunidades rurais. Para detecção de anticorpos IgG anti-*N. caninum* realizou-se a Reação de Imunofluorescência Indireta (RIFI). Os animais foram considerados positivos quando ocorria a fluorescência periférica total dos taquizoítos na diluição ≥1:50. A frequência de soropositividade encontrada foi de 9,18% (9/98). A titulação de anticorpos variou até 200, predominando o título 1:50. Dentre as localidades avaliadas, em 66,6% (4/6) havia pelo menos um cão soropositivo. A variável contato com bovinos foi considerada fator de risco (odds ratio = 15,25) para a infecção pelo parasito, demonstrando maior risco de contato com tecidos contaminados. Conclui-se que os cães de zona rural do município de Sousa estão expostos ao *N. caninum*, e sugere-se evitar o contato entre cães e bovinos como medida de controle para previnir a infecção em cães.

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INTRODUCTION

Dogs have played an important role in domestic cultures, in providing emotional support as companion animals, or for security, as guard animals (LOPES & SILVA, 2012). In rural areas, these animals are commonly used for hunting and assist in the management and protection of herds, and thus have free access to all external parts of the property (RIGG, 2001). However, these benefits can prove harmful if no attention is paid to their health. The contact between dogs and production animals increases the risk of transmission of several diseases, including leptospirosis, cenurosis and neosporosis (LOCATELLI-DITTRICH et al., 2008; TRENTIN et al., 2009; HASHIMOTO et al., 2010).

Neosporosis is caused by *Neospora caninum*, an obligate intracellular protozoan belonging to the Apicomplexa Phylum, and its definitive hosts are domestic and wild canids, which, after infection, eliminates non-sporulated oocysts in the feces. Carnivores become infected by ingesting sporulated oocysts or tissues containing bradyzoite cysts. Vertical transmission can also occur when tachyzoites cross the transplacental barrier and can infect the fetus during pregnancy (DUBEY et al., 2007). *N. caninum* presents a heteroxenous life cycle with ruminants, horses, and several species of wild animals as main intermediary hosts, which are infected through ingestion of food or water contaminated with sporulated oocysts (MONTEIRO, 2017). This parasite is considered the main cause of abortions in bovine herds around the world (REICHEL et al., 2013).

In Brazil, seropositivity in dogs in urban areas is variable, with prevalence rates ranging from 1.6% in João Pessoa, Paraíba (BRASIL et al., 2018), 5.5% in Rio Grande do Sul (CUNHA FILHO, 2008), 6.5% in Natal, Rio Grande do Norte (DANTAS et al., 2013) and 7.7% in Paraíba (FERNANDES et al., 2018).

In rural dogs from Brazil, a prevalence of 16.5% were described in Rio Grande do Sul (CUNHA FILHO, 2008), while in the Agreste region of Paraíba, the seropositivity was reported in 25% of farm dogs (MELO et al. al., 2017). The contact with cattle is considered a factor related to the presence of antibodies anti-*N. caninum* (ROBBE et al., 2016).

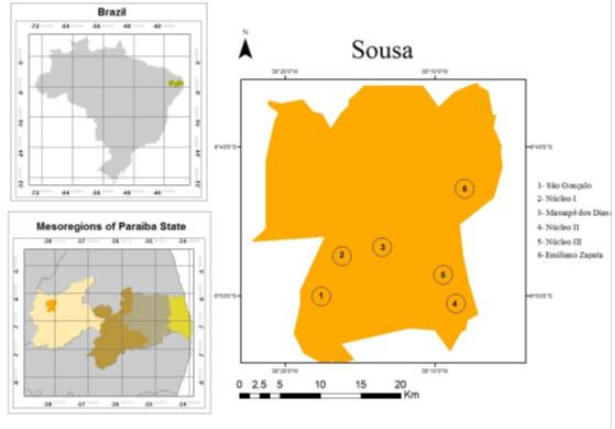
Higher prevalence of antibodies anti-*N. caninum* is observed in rural dogs when compared to urban dogs (NOGUEIRA et al., 2013). Theoretically, this is explained by the fact that animals that live isolated in environments such as the human home, come in contact with fewer pathogens, reducing the risk of protozoan infection (PAZ et al., 2019).

Although dogs play an important role in the transmission of neosporosis to production animals (mainly cattle), studies on *N. caninum* in dogs of rural areas in Northeast Brazil are lacking. Therefore, a study was necessary to demonstrate show the seroepidemiological situation in the region of Sousa, Alto Sertão of Paraíba State (PB), to clarify the possible risk factors predisposing these animals to *N. caninum* infection.

MATERIAL AND METHODS

The research was conducted in the rural area of the municipality of Sousa, PB (Lat. 06°45'33" S; Long. 38°13'41" W) that comprises of the districts of São Gonçalo, Núcleo I, Núcleo II, Núcleo III, Massapê dos Dias, and Emiliano Zapata Settlement (Figure1). This municipality has a territorial area of 738,547 km^2 and 69,161 habitants (Instituto Brasileiro de Geografia e Estatística [IBGE], 2018). The region has a semi-arid climate, with an annual mean temperature of 26.6 °C (Instituto Nacional de Meteorologia [INMET] 2010).

Figure 1. Map of the selected locations for the study in rural areas of the municipality of Sousa, PB.



We collected blood samples of 98 dogs, that were at least four months old, regardless of race and sex, from the rural area of Sousa, PB. The number of samples collected was calculated based on an expected prevalence of 50% *N. caninum* infection, since there are no previous studies of this disease in dogs in the municipality of Sousa, PB. A minimum confidence of 95% was considered (THRUSFIELD, 2007). The work was approved by the Ethics Committee on the Use of Animals by the Federal Institute of Education Science and Technology of Paraíba, Sousa Campus (Approval number, 84 01250.012779/2017-83).

During the visits, 3 mL of blood was collected by external venipuncture or cephalic venipuncture, packed into previously identified anticoagulant test tubes placed in Styrofoam with ice for transportation, and shipped to the Laboratory of Immunology and Infectious Diseases. The samples were subsequently centrifuged at 2000 rpm for five minutes to obtain serum, which was aliquoted into microtubes and stored at -20 °C until serological examination. Simultaneously, all tutors answered an investigative questionnaire previously validated by an expert, containing objective questions about sanitary management, feeding and contact of dogs with other animal species.

For the diagnosis, the indirect fluorescent antibody test was used for screening of anti-*N. caninum* IgG, using the

NC-1 strain of leaf-fixed *N. caninum* tachyzoites (DUBEY et al., 1988) and 1:50 dilution as the threshold (AZEVEDO et al., 2005). The analyses were carried out in the Laboratory of Bacterioses of the Federal Rural University of Pernambuco.

The data obtained from the epidemiological questionnaires were analyzed in two stages for the risk factors: univariate and multivariate analyses. Initially, the univariate analysis was performed with two groups of animals, seropositive and seronegative, which were compared with the analyzed variables. The variables that presented a value of P ≤ 0.2 by the chi-square test or Fisher's exact test (ZAR, 1999) were selected for multivariate analysis using multiple logistic regression (HOSMER & LEMESHOW, 2000). The level of significance adopted in the multivariate analysis was 5%, and all analyses were performed with the SPSS 20.0 *software* for Windows.

RESULTS

Among the 98 dogs analyzed, 9.18% (9/98) were seropositive for *N. caninum*. Antibody titers ranged from 1:50 to 1:200, with seven (77.8%) samples having titers of 1:50 (Table 1).

Among the six localities evaluated, at least one seropositive animal was found in four localities (66.7%) (Table 2). This suggested a wide distribution of the seropositivity of the animals.

The results of the univariate analysis for the risk factors of *N. caninum* infection are presented in Table 3. The variables of age, contact with cattle, type of feeding, and vaccination presented P values ≤ 0.2 by the chi-square test, and were selected for the multivariate analysis using multiple logistic regression.

Table 1. Frequency of anti-*N. caninum* IgG in dogs of the rural areas of the municipality of Sousa, PB.

Titers	Positive	Frequency (%)
50	7	77,8
100	1	11,1
200	1	11,1
Total	9	100

Table 2. Frequency of dogs testing positive for anti-*N. caninum* antibodies according to the locality in the rural area of the municipality of Sousa, PB.

Localization	Number of positive animals	Frequency (%)
Assentamento Emiliano Zapata	-	-
Massapê dos Dias	-	-
Núcleo I	1	11,1
Núcleo II	1	11,1
Núcleo III	4	44,5
São Gonçalo	3	33,3
Total	9	100

Table 3. Univariate analysis for the risk factors associated with seropositivity for anti-*N. caninum* antibodies in dogs of the rural area of the municipality of Sousa, PB (P ≤ 0.2).

Variable / Category	Number of total animals	Number of positive animals	*P*-value
Age (months)			
4-12	24	0 (0)	0.192
13-36	30	4 (13.3)	
>36	44	5 (11.4)	
Contact with cattle			
No	77	3 (3.9)	0.003
Yes	21	6 (28.6)	
Food			
Ration	1	1 (100)	0.003
Homemade food	81	8 (9.9)	
Both	16	0 (0)	
Vaccination			
No	51	1 (2)	0.013
Yes	47	8 (17)	

Only the bovine contact variable (odds ratio = 15.25, 95%; confidence interval = 2.74-84.8) was identified as a risk factor by logistic regression. With respect to this variable, six out of nine seropositive animals coexisted with cattle. The final model presented a good fit (Hosmer and Lemeshow test: Chi-square = 0.000, degree of freedom = 2, P = 1).

DISCUSSION

Rural dogs are more likely to be infected with *N. caninum*, and infection may occur easily due to ingestion of raw meat, including aborted fetuses, and remains of placenta containing protozoan cysts. This is an important factor in maintaining the parasite cycle by disseminating oocysts in the environment, facilitating transmission to intermediate hosts (KING et al., 2012; MCALLISTER, 2016).

Although cattle are the most economically important intermediate host, several species of wild animals can act as intermediate *N. caninum* hosts. The agent has been detected in tissue samples from several rodents, such as *Rattus novergicus* (rat), *Microtus arvalis* (wild rat), *Arvicola terrestris* (mud rat) (HUGHES et al., 2006; FUEHRER et al., 2010). The presence of protozoan in the tissues of these animals increases the chance of parasite transmission through the wild cycle, due to the hunting habit of dogs. Such a habit may contribute to the increased prevalence of antibodies Anti-*N. caninum* in rural dogs.

Dogs can act as reservoir animals for some zoonoses caused by protozoa, for example, Leishmaniasis, in addition, they can also execute animals as sentinel animals for the presence of some protozoa, such as *T. gondii* and *N. caninum* (CONSTANTINO et al., 2016). Due to the free access of dogs to the outside area of the farms, associated with their hunting habit, there may be a greater probability of infection by N. caninum, through contact with abortion or placental remains of infected cattle and/ or wild animals.

The presence of seropositive dogs on rural properties has a positive correlation with the occurrence of anti-*N. caninum* antibodies in cattle (WOUDA et al., 1999; GUIMARÃES JÚNIOR et al., 2004). This correlation highlights the importance of this study in the evaluated area, since the Sousa microregion has a milk basin that has the highest milk production in Paraíba representing 13.6% of the total state production (SOUZA et al., 2015).

The 9.18% prevalence of seropositive dogs found is similar to studies conducted in other Brazilian states on dogs from rural areas. Studies in the regions of Minas Gerais (NOGUEIRA et al., 2013), Espírito Santo (ACOSTA et al., 2016), and Paraná (SNAK et al., 2018) showed *N. caninum* seropositivity in 13,78%, 11.76%, and 11.66% of the animals analyzed, respectively. On the other hand, few studies showed higher percentages, for example, Teixeira (2008) detected seropositivity in 62.37% of dogs in the milk producing properties of Maranhão, and Benetti et al. (2009) reported positivity in 66.7% of the dogs inhabiting dairy farms in Mato Grosso. In contrast with the results of this study, Brasil et al. (2018) reported a frequency of 1.6% anti-*N. caninum* IgG in dogs of the urban areas of João Pessoa, PB. When compared to the frequency of anti-*N. caninum* antibodies in dogs of urban areas, higher prevalence of antibodies in rural dogs was observed.

Regarding antibody titers, the results were similar to those described in the Espírito Santo region, with the 1:50 titer being predominant (NOGUEIRA et al., 2013). Animals with subclinical infection usually have low titers of anti-*N. caninum* antibodies (<800), while animals with clinical infection have titers >800 (BARBER & TREES, 1998). In this study, the highest titer was 200, and no clinical signs or neurological disorders were observed in the evaluated dogs that would characterize the occurrence of subclinical infection.

A higher prevalence of anti-*N. caninum* antibodies was also observed in animals aged between one and three years (13.3% [4/30]). On the other hand, no positive result was observed in animals aged less than one year. In contrast, Cunha Filho et al. (2008), when evaluating 339 dog samples, observed a statistically significant difference (P = 0.0004) in *N. caninum* infection in animals aged three years and above. In this study, age may not have been identified as a risk factor for *N. caninum* infection because the sample was lower than that observed in other studies. Although the results differ in the age group with the highest prevalence of antibodies, the non-occurrence in animals under one year of age suggests later exposure to the agent, attributed to hunting habits, marking of territory in search of females in the case of adult males, and restricted access to the outside of the property.

In some studies, the diet of dogs was considered a risk factor for infection, and a higher prevalence of anti-*N. caninum* antibodies was demonstrated in dogs consuming mixed feed (homemade food + feed) as compared to animals consuming a diet based on homemade food (TEXEIRA et al., 2012; IGARASHI et al., 2015). Despite the statistical difference, a higher prevalence of anti-*N. caninum* antibodies was demonstrated in the animals fed exclusively with ration in this study; however, this result is not reliable, because the sample number of animals belonging to this category was insufficient. Although the habit of vaccinating the dogs reflects greater care of them by the tutors, dogs with a history of vaccination showed a higher prevalence of anti-*N. caninum* antibodies than unvaccinated dogs in this study (P = 0.013). This result differs from that of Melo et al. (2017), who identified the habit of not vaccinating dogs as a risk factor for anti-*N. caninum* antibodies, and these animals were 9.33 times more likely to be seropositive for this protozoan. This variability can be attributed to a spurious association between vaccination and infection by *N. caninum*, because other factors such as access of the animals to the whole property and contact with cattle may have influenced the seropositivity of the dogs.

Although other studies showed higher seropositivity rates in dogs that had contact with cattle than those that did not, this condition was not identified as a risk factor

for *N. caninum* infection (MARTINS et al., 2012; FERNANDES et al., 2018). This study observed that dogs that had contact with cattle were 15.25 times more likely to present with anti-*N. caninum* antibodies. Bruhn et al. (2012) demonstrated that the conviviality of dogs with cattle was associated with the positivity of the protozoan (P = 0.053; odds ratio = 4.3). The result of this work can be attributed to acess to external parts of the property, increasing the chances of contact with tissues infected with the protozoan or even with intermediary hosts of the parasite, such as birds and wild animals. The cohabitation of cattle and dogs predisposes the contact and ingestion of biological samples from aborted fetuses and placental remnants by dogs, as well as increases the potential risk of horizontal transmission to cattle, and is an important factor in the maintenance of the *N. caninum* life cycle.

CONCLUSIONS

The present findings confirmed that the canine rural population of the municipality of Sousa, semi-arid region of PB, was exposed to *N. caninum*, and contact with cattle was a risk factor for infection. It is suggested that control measures be adopted to prevent the occurrence of infection, such as restricting the dogs' access area on the property, avoiding their contact with placental remains, aborted bovine fetuses and avoiding the consumption of raw meat.

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