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Seroprevalence *of Neospora caninum* in dairy cattle and dogs from the Agreste region of the State of Paraíba

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ABSTRACT

Among the protozoa of veterinary importance, *Neospora caninum* is responsible for large economic and productive losses in cattle herds, with dogs being considered the definitive hosts of the parasite. The objective of this research was to study the prevalence of anti-*N. caninum* antibodies and the infection risk factors in dairy cattle and dogs in the Gado Bravo municipality of the Agreste region in Paraíba State, Brazil. Blood sera from 220 cows and 20 dogs were used, altogether obtained from a total of 21 farms. For detection of the anti-*N. caninum* IgG antibodies, the indirect immunofluorescence reaction was performed. Sera that reacted at dilutions of ≥1:200 and 1:50 for the cows and dogs, respectively, were considered positive. The analysis of risk factors was performed with the variables most associated with *N. caninum* infection, obtained by epidemiological questionnaire. The prevalence of anti-*N. caninum* antibodies was 12.27% among the cows and 57.14% on rural properties where at least one cow was positive. Among the dogs studied, a prevalence of 25% was observed. The non-vaccination of dogs against viruses, leptospirosis, or giardiasis was considered a risk factor associated with seroprevalence for *N. caninum* infection (odds ratio = 9.33). Therefore, it can be concluded that dairy cattle and dogs from rural properties in the Agreste region are very likely exposed to the infection caused by *N. caninum*.

INTRODUCTION

In Brazil, the beef and dairy production chains are two profitable segments developed by the world's second largest cattle herd, with about 200 million head (BRASIL, 2016). It is important to know the health of the herds, since parasitic diseases, including neosporosis, can cause serious economic losses for livestock, ranging from reproductive problems to the reduction of milk production (HASLER et al., 2006).

Neosporosis is a disease caused by the protozoan *Neospora caninum*, which has as its definitive hosts some wild canids and the domestic dog (DUBEY et al., 1988). Included among its intermediate hosts are cattle, goats, sheep, buffaloes, horses, and pigs. In dogs, *N. caninum* has a severe impact on the animal's neuromusculature, affecting mainly the central nervous system (BJERKAS; MOHN; PRESTHUS, 1984). This parasite causes the abortion of bovine fetuses, resulting in economic losses in the cattle breeding industry. Calves that are born infected may present with low weight, neurological signs, and the inability to stand (DUBEY, 1999). The

disease can be acquired either congenitally (considered the main route of transmission and maintenance of *N. caninum* in bovine herds) or after birth, through the ingestion of food or water contaminated with oocysts (DUBEY; SCHARES; ORTEGA-MORA, 2007).

In Brazil, the presence of anti-*N. caninum* antibodies in cattle was reported for the first time in the state of Mato Grosso do Sul (BRAUTINGAM; HIETALA; GLASS, 1996). The most recent studies report rates of 14.9% in the same state (OSHIRO et al., 2007), 19% in Pará (MINERVINO et al., 2007), 91.2% in Minas Gerais (GUEDES et al., 2008), 53.5% in Mato Grosso (BENETTI et al., 2009), 50.74% in Maranhão (TEIXEIRA et al. 2010), 13.2% in Paraná (MOURA et al., 2012), and 12.6% and 16.7% in Pernambuco and Alagoas, respectively (AMARAL et al., 2012). Recently, anti-*N. caninum* antibodies were also recorded in the state of Paraíba in other production animals, with a prevalence of 3.15% in pigs (FEITOSA et al., 2014) and 19.1% in buffaloes (BRASIL et al., 2015).

During the last decades, dairy cattle have become significantly important for livestock farming in the state of Paraíba, Northeastern Brazil. With the exception of the Zona da Mata region, where sugarcane crops predominate, small farms are common in the Agreste, Borborema, and Sertão regions. Owing to the importance of dogs in the transmission of *N. caninum* to cattle and the scarcity of studies on this protozoan in cattle in northeastern Brazil, especially in the State of Paraíba, the objective of this study was to carry out a seroepidemiological survey and to identify risk factors associated with the seroprevalence for *N. caninum* infection in dairy cattle and dogs from rural properties in the municipality of Gado Bravo, Agreste, State of Paraíba.

MATERIALS AND METHODS

The research was carried out on dairy cattle properties in the municipality of Gado Bravo, Agreste, State of Paraíba, during the months of March and April 2014. The region has a semiarid climate, with a rainy season from April to July (in which more than 90% of rainfall occurs), followed by a dry season. The mean annual temperature is 23.5 °C (minimum 18 °C; maximum 29 °C), with little variation during the year (MELO et al., 2013; VILELA et al., 2008). Agreste is a microregion that includes the transition zone between the humid coast and the semiarid Sertão region, and presents characteristic vegetation of the Caatinga biome.

To calculate the minimum number of cows to be selected, the formula for random simple samples was used (THRUSFIELD, 1995), with consideration of the following parameters: (A) expected prevalence of 50% (to maximize the sample); (B) absolute error of 7%; and (c) 95% confidence level. Thus, the minimum number of cows was 196. However, 220 cows from 21 dairy farms

in the municipality of Gado Bravo were used. All the dogs present on those 21 dairy farms were included, totaling 20. Blood samples from the cows and dogs were obtained by venipuncture of the external jugular and cephalic veins, respectively. After antisepsis of the puncture site with 3% iodinated alcohol, the individual blood samples were collected into properly sterilized vacuum tubes without anticoagulant, labeled, and transported to the laboratory in isothermal boxes. The samples were centrifuged at 2000 *g* for 5 min to obtain the serum, which was then aliquoted into 1.5 mL polypropylene microtubes. The sera were kept at –20 °C until processing.

The indirect immunofluorescence (IIF) reaction was used to investigate anti-*N. caninum* antibodies, using the NC-1 strain of leaf-fixed *N. caninum* tachyzoites (DUBEY et al., 1988). Slides fixed with the tachyzoites were supplied by the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo. The cut-off point used was 1:200 (AMARAL et al., 2012) and 1:50 (AZEVEDO et al., 2005) for the cows and dogs, respectively. After three washes and drying, the slides were evaluated by ultraviolet light emission microscopy. Positive and negative controls were included.

An epidemiological questionnaire was filled out by the owners of each property with the objective of obtaining information to be used in the analysis of risk factors. The selected variables and respective categories are described in Tables 1 and 2. Risk factor analysis was conducted in two steps: univariate analysis and multivariable analysis. In the univariate analysis, each independent variable was cross-checked with the dependent variable (seropositivity), and those that presented $p \leq 0.20$ by the Chi-squared test or Fisher's exact test (ZAR, 1999) were selected for multivariate analysis, using multiple logistic regression analysis (HOSMER; LEMESHOW, 2000) with the level of significance set at 5%. All analyses were performed using SPSS 20.0 for Windows.

The present study was submitted to the Ethics Committee of the Rural Health and Technology Center of the Federal University of Campina Grande, Campus de Patos-PB, and was granted execution authorization under registration number 132/2014.

RESULTS

A 12.27% (27/220) prevalence of seropositive cows was observed, indicating that these animals had being in contact with the parasite at some point in their lives. With regard to the distribution of antibody titers among the cows, 13 (48.15%), 8 (29.63%), 5 (18.52%), and 1 (3.7%) animal presented titers of 200, 400, 800, and 1600, respectively. In 57.14% of the 21 studied properties, at least one cow was found to be seropositive for *N. caninum.*

According to the analyzed variables, none showed associations with seropositivity in cattle (p > 0.20) (Table 1).

Table 1 – Univariate analysis of possible risk factors associated with seropositivity for *N. caninum* in cattle of the municipality of Gado Bravo.

Variable / category	Total number of cows	No. of seropositive cows (%)	p Value
Sex			
Male	1	0 (0,0)	
Female	219	27 (12.3)	1.000
Age (months)			
2–12	12	1 (8.3)	
13–24	5	0 (0.0)	
25–48	43	3 (7.0)	0.449
>48	160	23 (14.4)	
Type of power supply			
Voluminous	6	1 (16.7)	
Concentrated and bulky	214	26 (12.1)	0.549
Type of creation			
Intensive	3	0 (0.0)	
Semi-intensive	217	27 (12.4)	1.000
Some vaccine			
No	1	0 (0.0)	
Yes	219	27 (12.3)	1.000
Presence of wild canids			
No	30	3 (10)	
Yes	190	24 (12.6)	1.000
Abortions on property			
No	163	17 (10.4)	
Yes	57	10 (17.5)	0.240
Repetition of heat			
No	207	26 (15.5)	
Yes	13	1 (7.7)	1.000
Abortion at some stage of bovine reproductive life			
No	216	27 (12.5)	
Yes	4	0 (0.0)	1.000
Cleaning the environment			
Weekly	24	2 (8.3)	
Monthly	196	25 (12.8)	0.746
Access to dams			
No	7	1 (14.3)	
Yes	213	26 (12.2)	1.000

There was a higher prevalence of seropositive cows older than 48 months (14.4% (23/160)) than of other age groups. The presence of dogs was verified in all the studied properties, and therefore this variable was not submitted to statistical analysis. The variable abortions on the property also stood out, representing 17.5% (10/57) of the seropositive animals compared with the properties without this occurrence.

The prevalence of anti-*N. caninum* IgG antibodies in dogs was 25% (5/20). Among the 12 properties that presented at least one seropositive cow, 41.66% (5/12) showed dogs also seropositive for *N. caninum.*

The results of the test of association between the variables of the questionnaire and the seropositivity for *N. caninum* in dogs are shown in Table 2.

Table 2 – Univariate analysis of possible risk factors associated with seropositivity for *N. caninum* in dogs from the municipality of Gado Bravo.

Variable / category	Total number of dogs	No. of seropositive dogs (%)	p Value
Sex			
Male	13	3 (23.1)	1.000
Female	7	2 (28.6)	
Age (months)			
2–12	2	0 (0.0)	
13–24	3	1 (33.3)	0.792
25–48	6	2 (33.3)	
>48	9	2 (22.2)	
Breed			
Undefined	19	5 (26.3)	1.000
Other	1	0 (0.0)	
Environment			
Domiciled	1	0 (0.0)	1.000
Semi-domiciled	19	5 (26.3)	
Some vaccine (health concern)			
No	6	4 (66.7)	0.014
Yes	14	1 (7.1)	
Other dogs			
No	3	1 (33.3)	1.000
Yes	17	4 (23.5)	
Access to dams			
No	1	0 (0.0)	1.000
Yes	19	5 (26.3)	
Acquisition			
Young	16	3 (18.8)	0.249
Adult	4	2 (50)	
From			
Same town	17	5 (29.4)	0.539
Other	3	0 (0.0)	

The non-vaccination variable was the only one that showed association with seropositivity in dogs and was considered a risk factor for *N. caninum* infection (odds ratio = 9.33; 95% CI = 1.30–67.03; p = 0.014) (Table 2). However, it was found that all seropositive dogs had access to dams and lived in a semi-domiciled environment.

DISCUSSION

The prevalence of anti-*N. caninum* IgG antibodies found in our study was relatively low compared with that found in other studies in different regions of Brazil. Using the IIF reaction, high seropositivity rates were found by Guedes et al. (2008), who verified a prevalence of 91.7% in dairy cattle in the microregion of Lavras, Minas Gerais. Teixeira et al. (2010) verified a prevalence of 50.74% in cattle of rural properties in three microregions of Maranhenses State. Oshiro et al. (2007)

obtained a prevalence of 14.9% in cattle in Mato Grosso do Sul. Minervino et al. (2007) found an animal prevalence of 18.7% and a herd level of 80% in the state of Pará. In the state of Mato Grosso, Benetti et al. (2009) obtained a 53% prevalence in the animals and 100% in the herd. On the other hand, in 57.14% of the properties we studied, there was at least one seropositive cow. This concurred with the supposition of Teixeira et al. (2010) that a high prevalence in the herds is possible due to the presence of dogs in all the properties studied.

The titers of anti-*N. caninum* IgG antibodies in this study resembled the results described by Teixeira et al. (2010). However, our results differed from the values cited by Aguiar et al. (2006), who also using the IIF reaction and found a higher prevalence of the titers between 1:50 and 1:800 in the state of Rondônia. Dubey (1999) and Lindsay; Upton; Dubey (1999) have shown that IgG antibody titers ≥1:800 are suggestive of clinical

neosporosis. In this study, the cows with titers ≥1:800 had no history of abortion (Table 2), which is usually considered a clinical sign of protozoal infection. Therefore, there was no relationship between a titer of ≥1:800 and clinical signs such as abortion.

The seroprevalence of 12.27% in cattle and the frequency of 57.14% in the examined rural properties that presented at least one seropositive animal resemble the study done by Moura et al. (2012), which found 13.2% and 40% of *N. caninum* seropositive samples in beef cattle and properties, respectively, in the microregion of Paraná State. These authors also used the IIF reaction, considered a gold standard technique because cross-reactions are infrequent (BJÖRKAN; UGGLA, 1999; TREES et al., 1993). Our results differed from those obtained by Melo et al. (2001), who used the enzyme-linked immunosorbent assay and found a prevalence of *N. caninum* infection in two systems of milk production from 18 bovine herds in Minas Gerais, Brazil. In 8 of the 18 dairy herds, the prevalence of seropositivity ranged from 0 to 72.73%, whereas in the other 10 herds, the prevalence ranged from 3.7% to 25%. The variation of these results may be related to the different technique used for serological analysis. The presence of dogs in all rural farms studied is a risk factor for transmission of the etiological agent (VANLEEUWEN et al., 2010). However, Dubey; Schares; Ortega-Mora (2007) stated that other factors can increase the risk of neosporosis in the herd, such as size and density of the herd and, especially, incorrect management practices. In addition, the lack of adequate sanitary control, coupled with other management failures of the owners, contributed to the seropositivity of the animals under study.

Although abortions on the farms did not have statistical associations in our study, other authors such as Guedes et al. (2008) and Amaral et al. (2012) have observed female cattle to present a higher probability of seropositivity for *N. caninum* when abortion is observed in the property. Although the presence of anti-*N. caninum* antibodies in bovine sera does not confirm the diagnosis of neosporosis, it may help to clarify the cause of abortions in serologically negative animals for other abortive diseases, such as brucellosis and leptospirosis (DUBEY; SCHARES; ORTEGA-MORA, 2007).

The 25% prevalence of anti-*N. caninum* IgG antibodies in the dogs in this study is considered low when compared with the 62.37% rate found by Teixeira (2008) in three microregions of the state of Maranhão and the 67.6% rate found by Benetti et al. (2009) in Cuiabá, MT, both studies using the IIF reaction on samples from dogs on rural farms. Our prevalence is considered high, however, when compared with the 8.4%, 8.46%, and 7.5% rates obtained by Azevedo et al. (2005), Balthazar et al. (2013), and Dantas et al. (2014) in samples from a larger number of dogs in the cities of Campina Grande (PB), Rio de Janeiro (RJ), and Patos (PB), respectively, using the IIF reaction.

Being inhabitants of rural areas, the dogs in this study are more likely to be seropositive for *N. caninum*. Several authors have confirmed that dogs from rural areas or in frequent contact with this environment present a higher prevalence of antibodies than that found in dogs living in urban areas (AGUIAR et al., 2006; AZEVEDO et al., 2005; BALTHAZAR et al., 2013; DANTAS et al., 2014).

The habit of vaccinating the animals is a characteristic that reflects greater care by the owners, and results in a low prevalence of seropositivity for *N. caninum*, similar to that seen in animals that receive ambulatory care (DANTAS et al., 2014).

CONCLUSIONS

It is concluded that dairy cattle and dogs from rural properties in the municipality of Gado Bravo are exposed to infection caused by *Neospora caninum*. In order to minimize economic losses to cattle farmers, further studies should be conducted to elucidate the possible causes of protozoan infection in cattle herds in the region.

REFERENCES

AGUIAR, D. M. et al. M. Prevalence of anti-*Neospora caninum* antibodies in cattle and dogs from Western Amazon, Brazil, in association with some possible risk factors. **Veterinary Parasitology**, Amsterdam, v. 142, n.1-2, p.71-77, 2006.

AMARAL, R. L. G. et al. Neospora caninum em bovinos em matadouros de Pernambuco e Alagoas. **Pesquisa Veterinária Brasileira**, Seropédica, v. 32, n. 10, 963-966, 2012.

AZEVEDO, S. S. et al. Seroepidemiology of *Toxoplasma gondii* and *Neospora caninum* in dogs from the State of Paraíba, Northeast region of Brazil. **Research in Veterinary Science**, v. 79, n.1, p. 51- 56, 2005.

BALTHAZAR, L. M. C. et al. Cães sororreagentes a *Neospora caninum* (Apicomplexa: Toxoplasmatinae) atendidos em uma clínica veterinária na cidade do Rio de Janeiro, RJ. **Revista Brasileira de Medicina Veterinária**, Rio de Janeiro, v. 35 n. 2, p. 48-51, 2013.

BENETTI, A. H. et al. Pesquisa de anticorpos anti-*Neospora caninum* em bovinos leiteiros, cães e trabalhadores rurais. **Revista Brasileira de Parasitologia Veterinária**, Jaboticabal, v. 18, n.1, p. 29-33, 2009.

BJERKAS, I.; MOHN, S.F.; PRESTHUS, J. Unidentified cyst-forming sporozoan causing encephalomyelitis and myositis in dogs. **Zeitschrift fur Parasitenkunde**, v.70, p.271-274, 1984.

BJÖRKAN, C.; UGGLA, A. Serological diagnosis of Neospora caninum infection. **International Journal for Parasitology**, v. 29, p. 1497-1507, 1999.

BRASIL, A. W. L. et al. Risk factors for *Toxoplasma gondii* and *Neospora caninum* seropositivity in buffaloes in Paraiba State, Brazil. **Revista Brasileira de Parasitologia Veterinária**, Jaboticabal, v. 24, n.4, p. 459-463. 2015.

BRASIL. Ministério da Agricultura Pecuária e Abastecimento (MAPA). Pecuária.   Disponível   em:<

http://www.agricultura.gov.br/animal/especies/bovinos-e-bubalinos>. Acessado em 15 de abril de 2016.

BRAUTINGAM, F. E.; HIETALA, S. K.; GLASS, R. Resultados de levantamentos sorológicos para espécie *Neospora* em bovinos de corte e leite. In: CONGRESSO PANAMERICANO DE CIÊNCIAS VETERINÁRIAS, 15. 1996, Campo Grande. **Anais...**Campo Grande: PANVET, 1996. p. 284.

DANTAS, S. B. A. et al. Fatores de risco para a ocorrência de anticorpos contra *Toxoplasma gondii* e *Neospora caninum* em cães domiciliados no Nordeste do Brasil. **Semina: Ciências Agrárias**, Londrina, v. 35, n. 2, p. 875-882, mar./abr. 2014.

DUBEY, J. P. et al. Newly recognized fatal protozoan disease of dogs. **Journal of American Veterinary Medical Association**, New York, v. 192, n.9, p. 1269–1285, 1988.

DUBEY, J. P. Recent advances in *Neospora* and neosporosis. **Veterinary Parasitology**, Amsterdam, v.8, n.4. p. 349-67, 1999.

DUBEY, J. P.; SCHARES, G.; ORTEGA-MORA, L. M. Epidemiology and control of Neoporosis and *Neospora caninum*. **Clinical Microbiology Reviews**, v. 20, n. 2, p. 323-367, 2007.

FEITOSA, T. F. et al. *Toxoplasma gondii* and *Neospora caninum* in slaughtered pigs from Northeast, Brazil. **Veterinary Parasitology**, v. 202, p. 305–309, 2014.

GUEDES, M. H. P. et al. Frequência de anticorpos anti-*Neospora caninum* em vacas e fetos provenientes de municípios do sul de Minas Gerais. **Revista Brasileira de Parasitologia Veterinária,** Jaboticabal, v. 17, n. 4, p. 189-194, 2008.

HASLER, B. et al. Simulating the impact of four control strategies on the population dynamics of *Neospora caninum* infection in Suiss dairy cattle. **Preventive Veterinay Medicine,** v. 77, p. 254-283, 2006.

HOSMER, D. W.; LEMESHOW, S. **Applied logistic regression**. New York: John Wiley & Sons, 2000, 375 p.

LINDSAY, D. S.; UPTON, S. J.; DUBEY, J. P. A structural study of the *Neospora caninum* oocyst. **Internacional Journal of Parasitology,** v. 29, n.10, p.1521-23, 1999.

MELO, C. B. et al. Freqüência de infecção por *Neospora caninum* em dois diferentes sistemas de produção de leite e fatores predisponentes à infecção em bovinos em Minas Gerais. **Revista Brasileira de Parasitologia Veterinária**, Jaboticabal, v. 10, n. 2, p. 67-74, 2001.

MELO, L. R. B. et al. Resistência anti-helmíntica em pequenos ruminantes do semiárido da Paraíba, Brasil**. ARS VETERINÁRIA**, Jaboticabal, SP, v. 29, n.2, p.104-108, 2013.

MINERVINO, A. H. H. et al. Prevalence of Neospora caninum antibodies in cattle from Santarém, Pará, Brazil. **Research in Veterinary Science**, London, v.84, n.2, p. 254-256, 2007.

MOURA, A. B. et al**.** Occurrence of anti-*Neospora caninum* antibodies in beef cattle of microrregion of Guarapuava, Paraná state, Brazil. **Arquivos do Instituto Biológico**, São Paulo, v.79, n.3, p.419-422, 2012.

OSHIRO, L. M. et al. Prevalence of anti-*Neospora caninum* antibodies in cattle from the state of Mato Grosso do Sul, Brazil. **Revista Brasileira de Parastologia Veterinária**, Jaboticabal, v. 16, n. 3, p. 133-138, 2007.

TEIXEIRA, W. C. et al. Prevalência de anticorpos anti- *Neospora caninum* (Apicomplexa: Sarcocystidae) em bovinos leiteiros de propriedades rurais em três microrregiões no estado do Maranhão. **Pesquisa Veterinária Brasileira**, Seropédica, v.30, n.9, p. 729-734, 2010.

TEIXEIRA, W. C. **Prevalência de anticorpos IgG anti- *Neospora caninum* e anti- *Toxoplasma gondii* em bovinos e caninos das mesorregiões norte e centro maranhense, Maranhão, Brasil.** Recife: UFRPE, 2008. Dissertação (Mestrado) Pro- reitoria de Pesquisa e Pós- graduação, Programa de Pós- graduação em Ciências Veterinárias, Universidade Federal Rural de Pernambuco, Recife, 2008.

TREES A. J. et al. Prevalence of antibodies to *Neospora caninum* in population of urban dogs in England. **Veterinary Record**, v. 132, p.125-126, 1993.

THRUSFIELD, M. **Veterinary Epidemiology**. 2ª ed. Cambridge: Blackwell Science, p. 479, 1995.

VANLEEUWEN, J. A. et al. Risk factors associated with *Neospora caninum* seropositivity in randomly dairy cows herds. **Preventive Veterinary Medicine**, v. 93, p. 129-138, 2010.

VILELA, V. L. R. et al. Ensaios preliminares para validação do método FAMACHA© em condições de semi-árido paraibano. **Revista Brasileira de Parasitologia Veterinária**, Jaboticabal, v. 17, v. 1, p.154-157, 2008.

ZAR, J. H. **Biostatistical analysis**. 4. ed. Upper Saddle River: Prentice Hall, 1999. 663 p.