

# Factors associated with the seroprevalence of *Neospora caninum* (Apicomplexa: Toxoplasmatinae) in sheep from the State of Sergipe, Brazil

Fatores associados com a soroprevalência de *Neospora caninum* (Apicomplexa: Toxoplasmatinae) em ovinos no Estado de Sergipe, Brasil

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## Abstract

The aim of this study was to determine the prevalence and factors associated with the occurrence of antibodies against *Neospora caninum* in sheep from Sergipe, northeastern Brazil. A total of 932 sheep serum samples from 54 properties in 19 municipalities from the State of Sergipe, Brazil were collected and assayed using an indirect fluorescent antibody test (IFAT) to assess antibodies against *N. caninum*. A cut-off point of 1:50 was adopted and results showed that 12.45% (116/932) of sheep were serum-reactive. Based on an unconditional logistic regression, the presence of dogs on the property was associated with protection (OR= 0.323), whereas the use of exchanged or borrowed breeding males was associated with infection (OR= 22.287). These results indicate that the occurrence of antibodies against *N. caninum* is endemic in the State municipalities.

**Keywords:** epidemiology, natural infection, flock, neosporosis.

## Resumo

A prevalência e os fatores associados à ocorrência de anticorpos contra *Neospora caninum* em ovinos de Sergipe, Nordeste brasileiro, foram estudados. Para tanto, foram coletadas 932 amostras de soros de ovinos, procedentes de 54 propriedades de 19 municípios do Estado. Para determinação de animais sororreagentes foi utilizado o teste de técnica de imunofluorescência indireta (TIFI) para determinação de anticorpos contra *N. caninum*, com ponto de corte de 1:50. Observou-se que 12,45% (116/932) dos ovinos foram sororreagentes. Na regressão logística não condicional a presença de cães na propriedade foi fator associado à proteção (OR= 0,323); enquanto que a troca ou empréstimo de machos reprodutores foi identificado como fator associado à infecção (OR= 22,287). A prevalência encontrada indicou que a ocorrência de anticorpos contra *N. caninum* foi endêmica no estado de Sergipe.

**Palavras-chave:** epidemiologia, infecção natural, rebanho ovino, neosporose.

## Introduction

Neosporosis is significant in sheep production because it triggers disorders related to reproduction, and, consequently, economic loss related to abortion, neonatal mortality and the birth of weak lambs (Faria et al., 2010; Pinto et al., 2012). The prevalence of infection in Brazilian sheep flocks varies from 1.8% in Rio Grande do Norte (Soares et al., 2009) to 64.2% in Pernambuco (Tembue et al., 2011).



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The risk factors for this species are not widely known, but some studies have identified a relationship between seropositivity and property size, ingestion of backwater, animal slaughter on the property, viscera exposed to dogs and the presence of reproductive problems (Faria et al., 2010; Machado et al., 2011; Munhóz et al., 2010). Considering the relevance of this disease and the scarcity of epidemiological information in Sergipe, the aim of this study was to identify the main factors related to the frequency of antibodies against *N. caninum* in a sheep flock from the State of Sergipe.

## Material and methods

This study was conducted in northeastern Brazil (latitude between 9°30'49" and 11°34'05" and longitude between 36°23'40" and 38°15'00"), in the State that comprises 247,703 sheep and is divided into three mesoregions: Sergipe Backlands, which holds 41.19% of the population, Agreste of Sergipe, which comprises 44.12%, and Eastern Sergipe, which holds 14.69% of the flock (Sergipe, 2007).

Producers were selected by means of non-probabilistic convenience sampling; due to the absence of a representative list of sheep producers in the state, random sampling would have been impractical. In order to determine the sample population, municipalities that held the largest sheep flocks of each region of the State were selected (Figure 1). In total, 19 were selected: six are located in the mesoregion of the Sergipe Backlands (83,728 animals), six in Agreste of Sergipe (89,688 animals) and seven in Eastern Sergipe (20,373), comprising 193,789 animals, or 78.23% of the sheep population of the State (247,703).

The minimum number of samples to be tested (383) was calculated using the statistical software EPI INFO version 3.5.1 (Centers for Disease Control and Prevention, 2017), considering an expected prevalence of 50%, sampling error of 5% and confidence level of 95%, for a population of 193,789 animals. The number of properties visited was estimated according to the minimum amount of samples collected in each municipality, which was determined in proportion to the flock, whereas an average of 15 samples was established for each property.

Sample collection was approved by the Ethics Committee on Animal Use, pursuant to file number (CEUA/UESC-39/2009). Blood samples were collected by means of external jugular vein puncture. Such samples were centrifuged at 1600 x g for 10 minutes, whereas sera were separated, inserted into microtubes and frozen at -20 °C. During the visit, a semi-structured interview was conducted to collect data related to the animal, producer, property, production system, and health and feeding management.

A total of 932 samples from 54 properties, distributed in 19 municipalities, were collected, of which 38.20% (356/932) originated from Sergipe Backlands, 44.85% (418/932) from Agreste of Sergipe and 16.95% (158/932) from Eastern Sergipe. Among the sampled animals, 192 (20.60%)



**Figure 1.** Map of Sergipe and the mesoregions: Sergipe Backlands, Eastern Sergipe and Agreste of Sergipe. The sampled municipalities are demonstrated.

were males, of which 90 were breeding males, and 740 (79.40%) were females, of which 545 were brood ewes. With respect to age stratification, 298 were young sheep (31.97%), ranging from 6 months to 1 year of age, 303 (32.51%) were young adults, ranging from 1 to 3 years of age, and 331 (35.51%) were over 3 years of age.

The indirect immunofluorescence technique was used to determine antibodies against *N. caninum*. Slides suitable for indirect immunofluorescence were used. They were impregnated with antigens of the *N. caninum* NcBA strain of positive control serum, obtained by means of blood collection from a six-month-old lamb, 30 days after the inoculation of *N. Caninum* tachyzoites ( $1 \times 10^6$ ), and a negative control serum for *N. caninum*. The initial dilution consisted of 1:50 (Figliuolo et al., 2004), and positive samples underwent sequential dilutions with a base of two until the reaction result was negative. The analysis was performed with an epifluorescence microscope Binocular BX 51 (Olympus™). Samples in which the microscopic field presented more than 50% tachyzoites with total peripheral fluorescence were considered positive.

To identify risk factors associated with *N. caninum* infection, a bivariate analysis was performed with a Chi-squared test and Fisher's exact test, with a significance level of 5%, using the statistical software EPI INFO version 3.5.1 (Centers for Disease Control and Prevention, 2017). All variables with  $p \leq 0.2$  in the bivariate analysis underwent a collinear analysis determined by a Spearman's test, using the software BioEstat 5.0 (Ayres et al., 2007). Subsequently, an unconditional logistic regression multivariate analysis was conducted using the software EPI INFO version 3.5.1.

## Results and discussion

The prevalence in the present study was higher than that from other studies conducted in the northeastern region of Brazil, where the largest population of sheep is located (55.55%). Antibodies against *N. caninum* were found in 116/932 (12.45%) animals, with a mass fraction consisting of 1:50 (18/932; 1.93%), 1:100 (38/932; 4.08%), 1:200 (13/932; 1.40%), 1:400 (22/932; 2.36%), 1:800 (10/932; 1.07%), 1:1600 (08/392; 0.86%) and 1:3200 (06/932; 0.64%). Considering each mesoregion separately, 57/356 (16.01%) animals from Sergipe Backlands, 47/418 (11.24%) from Agreste of Sergipe and 12/158 (7.59%) from Eastern Sergipe had positive results. Out of the assessed municipalities, 78.94% (15/19) were exposed to *N. caninum*, and 75.93% (41/54) of the assessed properties presented at least one animal with a positive result, with a seroprevalence ranging from 4.76 to 58.33%. Among the analysed municipalities, Gararú, located in the Sergipe Backlands, had a larger number of positive animals (25.81%) and the municipalities of Salgado, Itaporanga D'ajuda and Arauá, located in Eastern Sergipe, and Monte Alegre, in the Sergipe Backlands, had no animals with positive results (Table 1).

The prevalence of antibodies against *N. caninum* in sheep in Brazil ranges from 1.8% in Rio Grande do Norte (Soares et al., 2009) to 64.2% in Pernambuco (Tembue et al., 2011). Among the studies conducted in Brazil with identical techniques and cut-off points, similar results were found by Faria et al. (2010) in Alagoas (9.6%) and Munhóz et al. (2010) in São Paulo (13.9%). However, the prevalence verified in this study was higher than the rates identified by Salaberry et al. (2010) in Minas Gerais (8.1%) and Figliuolo et al. (2004) in São Paulo (9.2%), and lower than the rates found by Andreotti et al. (2009) in farms in the city of Campo Grande, central-western Brazil (30.8%), by Rossi et al. (2011) in Minas Gerais (47.1%) and by Tembue et al. (2011) in Pernambuco (64.2%). Variations by location might be related to differences in the region, climate, animal age, sample size (Dubey et al., 2011), production systems (Melo et al., 2001), or even pathogenic synergism (Melo et al., 2004).

The variables "age", "presence of bovines on the property", "exchange/borrowing of breeders", "frequent deworming", "contact with dogs", "ingestion of water straight from the source", "compacted soil stall", "natural mating" and "presence of a sheep caretaker" presented p-values lower than 0.20 (Table 2). Based on collinearity, the "presence of a sheep caretaker" and "exchange/borrowing of breeders" presented a p-value higher than 0.8, such that "presence of a sheep caretaker" was excluded from the initial model of the unconditional logistic regression. The final logistic regression model demonstrated that "contact with dogs" acted as a protective factor (OR= 0.323,  $p < 0.001$ ), whereas "exchange/borrowing of breeders" acted as a risk factor for infection by *N. caninum* (OR= 22.287,  $p < 0.001$ ).

**Table 1.** Detection of antibodies against *Neospora caninum* in sheep in Sergipe, Brazil, and their distribution by titration in the assessed municipalities.

Municipalities	Dilutions								Total	Positive (%)
	Negative	1:50	1:100	1:200	1:400	1:800	1:1600	1:3200		
<b>Eastern Sergipe</b>	<b>146</b>	<b>3</b>	<b>4</b>	<b>0</b>	<b>1</b>	<b>3</b>	<b>0</b>	<b>1</b>	<b>158</b>	<b>7.59</b>
Itabaianinha	22	1	2	0	0	1	0	0	26	<b>15.38</b>
Tomar do Geru	20	0	1	0	0	0	0	0	21	<b>4.76</b>
Estância	32	2	0	0	0	2	0	1	37	<b>13.51</b>
São Cristovão	14	0	1	0	1	0	0	0	16	<b>12.50</b>
Others <sup>1</sup>	58	0	0	0	0	0	0	0	58	<b>0.00</b>
<b>Agreste of Sergipe</b>	<b>371</b>	<b>8</b>	<b>17</b>	<b>5</b>	<b>10</b>	<b>4</b>	<b>2</b>	<b>1</b>	<b>418</b>	<b>11.24</b>
Tobias Barreto	93	3	4	2	6	2	0	1	111	<b>16.22</b>
Itabaiana	35	0	2	0	0	0	1	0	38	<b>7.89</b>
Poço Verde	58	1	3	1	0	1	0	0	64	<b>9.38</b>
Simão Dias	26	1	0	2	1	0	0	0	30	<b>13.33</b>
Campo do Brito	21	0	2	0	1	0	0	0	24	<b>12.50</b>
Lagarto	138	3	6	0	2	1	1	0	151	<b>8.61</b>
<b>Sergipe Backlands</b>	<b>299</b>	<b>8</b>	<b>17</b>	<b>10</b>	<b>10</b>	<b>2</b>	<b>6</b>	<b>4</b>	<b>356</b>	<b>16.01</b>
Nossa Senhora da Glória	109	4	13	5	7	1	1	2	142	<b>23.24</b>
Monte Alegre	32	0	0	0	0	0	0	0	32	<b>0.00</b>
Canindé do São Francisco	42	2	2	1	1	0	1	0	49	<b>14.29</b>
Poço Redondo	44	2	0	0	0	1	1	1	49	<b>10.20</b>
Porto da Folha	49	0	2	1	0	0	0	1	53	<b>7.55</b>
Gararu	23	0	0	3	2	0	3	0	31	<b>25.81</b>
<b>TOTAL</b>	<b>816</b>	<b>19</b>	<b>38</b>	<b>15</b>	<b>21</b>	<b>9</b>	<b>8</b>	<b>6</b>	<b>932</b>	<b>12.45</b>

<sup>1</sup>Municipalities of Salgado, Itaporanga D'ajuda and Arauá.

**Table 2.** Variables with biological plausibility and p-values lower than 20%, inserted in the initial unconditional logistic regression model, after a Chi-squared analysis between the variable and the presence of antibodies against *Neospora caninum*.

Variables	Positive		Negative		Odds Ratio (CI 95%)	p-value
	Sheep		Sheep			
	n	%	n	%		
Age						
Under one year of age	44	14.8	254	85.2	1	
Over one year of age	72	11.4	562	88.6	1.35 (0.90-2.02)	0.1726
Presence of bovines in the property						
Yes	102	13.5	652	86.5	1	
No	14	7.9	164	92.1	1.83 (1.02-3.29)	0.0533
Exchange/borrowing of breeders						
Yes	32	20.4	125	79.6	1	
No	84	10.8	691	89.2	2.11 (1.34-3.30)	0.0015

n = Number of samples; CI = Confidence interval.

Table 2. Continued...

Variables	Positive		Negative		Odds Ratio (CI 95%)	p-value
	Sheep		Sheep			
	n	%	n	%		
Frequent deworming						
Yes	72	11.2	570	88.884.8	1	
No	44	15.2	246		0.721 (0.47-1.06)	0.1125
Contact with dogs						
Yes	84	10.4	723	89.6	1	
No	32	25.6	93	74.4	0.34 (0.21-0.53)	0.0000
Ingestion of water straight from the source						
Yes	65	16.1	339	83.9	1	
No	51	9.7	477	90.3	1.79 (1.21-2.65)	0.0044
Compacted soil stall						
Yes	35	9.5	332	90.5	1	
No	81	14.3	484	85.7	0.63 (0.41-0.96)	0.0387
Presence of a sheep caretaker						
Yes	38	10.4	327	89.6	1	
No	78	13.8	489	86.2	0.73 (0.48-1.10)	0.1589

n = Number of samples; CI = Confidence interval.

Recent studies on occurrence and risk factors have demonstrated that *N. caninum* infects sheep in several other regions of the world. In Italy, Gazzonis et al. (2016) found an occurrence of 19.3%, and demonstrated the well-known variables that affect the presence of *N. caninum* in sheep herds, such as the rearing system and farm size; small and family-run farms were at a higher risk, and outcomes from the survey mainly indicated that these farms should implement proper sanitary measures and monitor the spread of the infection among small ruminants. In China, Nie et al. (2018) found a seroprevalence of 8.4%, and observed that the seroprevalence of *N. caninum* in male Tibetan sheep (10.8%) was significantly higher than in females (7.4%;  $p < 0.01$ ); this is probably related to the different hormone levels between males and females. According to Azevedo Filho et al. (2017), vertical transmission (11%) was clearly detected in a sheep flock.

The presence of dogs on the property was a significant factor ( $p < 0.001$ ), whereby positive rates were lower with their presence, so dogs were considered a protective factor in this study. The function of the definitive host is known in the epidemiology of infection by *N. caninum*; however, the presence of dogs might limit the entrance of stray and/or wild dogs, reducing the contamination of the environment with oocysts (Barling et al., 2001). Moreover, the idea of the congenital transmission of this parasite is sustained, since only two of the assessed properties applied artificial reproduction methods and the majority reported reproductive problems, such as the birth of weak lambs, and, mainly, abortions (Pinto et al., 2012). The results also indicate a dissemination of *N. caninum* in the sheep flocks of Sergipe and restraining the exchange/borrowing of breeders between flocks is suggested based on the possibility of *N. caninum* dissemination.

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