Seroprevalence and factors associated with *Neospora caninum* infection in sheep from southeastern Bahia, Brazil*

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ABSTRACT. Rocha D.S., Guimarães L.A., Bezerra R.A., Mendonça C.E.D., Dórea T.G., Munhoz A.D. & Albuquerque G.R. [**Seroprevalence and factors associated to *Neospora caninum* infection in sheep from Southeastern Bahia, Brazil.**] Soroprevalência e fatores associados à infecção de *Neospora caninum* em ovinos no sudeste da Bahia, Brasil. *Revista Brasileira de Medicina Veterinária, 36(4):443-447, 2014*. Departamento de Ciências Agrárias e Ambientais, Universidade Estadual de Santa Cruz, BA 415Km 16, Salobrinho, Ilhéus, BA 45662-000, Brasil. E-mail: gralbu@uesc.br

This study aimed at identifying the main factors related to *Neospora caninum* seroprevalence in sheep. The Indirect Immunofluorescence Reaction (RIFI) was used to identify antibodies in 795 samples of sheep serum, from 31 farms distributed in nine municipalities of the Southeastern Bahia. The prevalence was 13.2% (105/795), with titers varying between 50 (12.4%), 100 (26.7%), 200 (14.3%), 400 (19%), 800 (18%), 1600 (4.8%) and 3200 (4.8%). In the statistical model of logistic regression the following factors were found: presence of sheepfold (p= 0.009), use of hay (p=0.045), capacity rate (p=0.003) and the presence of dogs (p= 0.010) as protection factors associated to the infection by *N. caninum*. It may be concluded that the sheep flock from Southeastern Bahia, is exposed to the infection due to *N. caninum*.

KEY WORDS. Neosporosis, epidemiology, serology, sheep.

RESUMO. O objetivo do presente estudo foi identificar os principais fatores asssociados à soroprevalência de *Neospora caninum* em ovinos. Foi utilizada a Reação de Imunofluorescência Indireta (RIFI) para detecção de anticorpos em 795 amostras de soro ovino, de 31 propriedades distribuídas em nove municípios do sudoeste da Bahia. A prevalência encontrada foi de 13,2% (105/795), com títulos variando entre 50 (12,4%), 100 (26,7%), 200 (14,3%), 400 (19%), 800 (18%), 1600 (4,8%) e 3200 (4,8%). No modelo estatístico de regressão logística foram encontrados como fatores : presença de aprisco (p= 0,009), uso de feno (p=0,045), taxa de lotação (p=0,003) e a presença de cães (p= 0,010) como fatores de proteção associados à infecção por *N. caninum*. Conclui-se que o rebanho ovino do sudoeste da Bahia estão expostos à infecção causada por *N. caninum*.

PALAVRAS-CHAVES. Neosporose, IFI, ocorrência, ovinos, sorologia.

INTRODUCTION

Neosporosis is a disease whose etiologic agent is the protozoa *Neospora caninum*. Dogs (McAllister et al. 1998), coyotes (Gondim et al. 2004), Australian dingoes (King et al. 2010) and gray wolves

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(Dubey et al. 2011) are considered definitive hosts and, therefore, they can play an important role in the environment maintenance and dissemination of this agent.

This parasite has a heteroxenous life cycle and can infect many animals, such as sheep (Dubey et al. 1990), goats (Dubey et al. 1992), bovines (Dijkstra et al. 2001) and chicken (Costa et al. 2008). Although the presence of antibodies anti-*N. caninum* has been observed in human beings (Lobato et al. 2006), its zoonotic potential has not been confirmed yet (Dubey et al. 2007). The more common transmission routes are: the transplacental route (Buxton et al. 1998); the ingestion of cysts present in tissues, by carnivores (Bergeron et al. 2001); the ingestion of oocysts eliminated (McAllister et al. 1998) by the definitive hosts in the water or contaminated foods.

Sheep raising is an economic activity globally explored, present at different areas under the most diverse bioclimatological characteristics (Milk 2004). The actual Brazilian sheep flock is of 17,662,201 animals, being 57.2% in the Northeastern region, that present capacity for the exploration of domestic ruminants, mainly sheep, for its availability of natural vegetation. Bahia is the second largest national producer with 3,072,176 sheep (IBGE 2011).

The neosporosis is important for the sheep species because it causes disorders related to reproduction, and consequent economic losses due to abortions, neonatal mortality, and birth of weak lambs (Faria et al. 2010, Pinto et al. 2012). The infection has a cosmopolitan distribution (Dubey & Schares 2011). In Brazil the prevalence of the infection in sheep flocks varies from 1.8% in Rio Grande do Norte (Soares et al. 2009) to 64.2% in Pernambuco (Tembue et al. 2011).

Sheep have been used as experimental model for infection by *N. caninum* in bovines (Dubey & Schares 2011) and in this species there are few studies about risks factors. However, some studies carried out have identified a relationship between the frequency and size of the property, ingestion of water from dams, slaughter of animals in the property, dogs with access to the visceras, and presence of reproductive problems in the property (Faria et al. 2010, Munhoz et al. 2010, Machado et al. 2011). Considering the importance of the disease and few epidemiologic information in Bahia, this work aimed to identify the main factors related to the frequency of antibodies against *N. caninum* in sheep flocks in the Southeastern Bahia.

MATERIAL AND METHODS

Study area and period of collection

The study was carried out from February 2011 through August 2012. The area selected for the collection of samples was the microregion Ilhéus-Itabuna in the State of Bahia (8º 32' south latitude to 39º22' west longitude). Bahia is the third largest Brazilian state with a total area of 564,839,850km²; it is composed by 417 cities divided into 6 mesoregions. The microregion of Ilhéus-Itabuna has 40 Municipalities, and the samples were collected at the nine of them with the largest production. The climate is predominantly humid tropical with average temperature of 24ºC and an average rainfall of 1750mm per year.

Study population and blood collection

The study involved 795 animals selected for convenience, in 31 properties enrolled with the Cooperative of Sheep and Goats Producers of Southern Bahia (COOPERVINO), distributed in nine cities. The sample size was calculated in 650 animals with the statistical program EPI-INFO version 3.5.1 (www.cdc.gov), considering a prevalence estimate of 50%, with sampling error of 5% and confidence interval of 99%, for a population of 33,000 animals (IBGE 2009, 2011). The collection from a larger number of animals is possible.

The flock was basically composed by Santa Inês or crossbred animals. The age of the animals was estimated through dental chronology and the animals were classified into two age groups: above and below 36 months of age. Blood samples were not collected from lambs younger than six months of age due to the eventual presence of colostral antibodies.

The blood was collected by jugular vein puncture; each serum was separated after centrifuging and stored at -20ºC until the moment of use. This study was approved by the ethics committee (CEUA-UESC 031/09).

Epidemiologic questionnaire

A structured interview with objective questions was prepared for the determination of the risk factors, concerning the general characteristics of the property such as: total area (≥100 hectares or <100 hectares); farming system (extensive or intensive); type of production (familiar or commercial); size of the flock (≥100 animals or <100 animals); capacity rate (≥01 animal/ha or <01 animal/ha); presence of dogs in the property (yes or no); transit of dogs between neighboring properties (yes or no); amount of dogs (<03 dogs or ≥03 dogs); water source (trough or river/dam); presence of sheepfold (yes or no); dogs with access to the place of food storage and/or water source of the animals (yes or no); use of mostly fresh food - grass (yes or no); as well as data related to gender (male or female); age (≥ of 36 months or < of 36 months); and breed of each animal (crossbred and purebred). The variable transit of dogs between properties refers to the presence of dogs that do not belong to the property (living in the property), coming from neighboring properties, or nomads.

Detention of antibodies

The Indirect Immunofluorescence (RIFI) was performed to research anti-*N. caninum* antibodies. Appropriate blades for RIFI were used, containing antigens of the strain NcBA of *N. caninum,* positive control serum – obtained through the inoculation of N. caninum ($1x10^6$) in a lamb of 6 months of age and negative to N. caninum – and anti-sheep antibody conjugated with Fluorescein Isothiocyanate (F-7634, Sigma, USA). The initial dilution was 1:50 (Figliuolo et al. 2004), and the positive controls were submitted to sequential dilutions in base two up to negativation.

Statistical Analysis

To identify the factors related to the infection by *N. caninum*, a bivariate analysis was carried out using the Qui-square Test and the Fisher's Exact Test, with significance level of 5%, using the statistical program EPI-INFO version 3.5.1. All variables with p ≤0.2 in the bivariate analysis were submitted to a collinearity analysis determined by the Spearman Test, in the program BioEstat 5.0; later, a multivariate analysis of unconditional logistic regression was performed in the program EPIINFO version 3.5.1.

RESULTS

Anti-*N. caninum* antibodies were found in 105/795 (13.2%) animals, with titers varying between 50 (12.4%), 100 (26.7%), 200 (14.3%), 400 (19%), 800 (18%), 1600 (4.8%) and 3200 (4.8%). All nine cities analyzed had properties with at least one seropositive animal; of the 31 properties studied, 25 presented positive animals, with seropositivity varying from 2.2% to 40%. Besides, all properties used the extensive farming system and did not use artificial reproduction methods. Among the analyzed Municipalities, Ilhéus presented the largest frequency of positive animals (20.1%) and Itaju do Colônia the lower positivity ones (1.6%) (Table 1).

The related factors identified by the bivariate and multivariate analyses are distributed, respectively, in Tables 2, 3, 4 and 5. Among the analyzed factors, there was a statistical relation between the seropositivity and the area of the property, use of hay, presence of sheepfold, presence of dogs, and capacity rate. After the performance of the Spearman and logistic regression tests, the presence of sheepfold, use of hay, presence of dogs, and capacity rate, were identified as protection factors to *N. caninum* seroprevalence in the sheep of this study.

As regards the variables related to the structure and the handling of the properties, those properties that did not have a sheepfold presented an average of 19.4% of positive animals, and properties with sheepfolds presented an average of 11.5% of positive animals. Properties that did not offer hay presented 14.7% of seropositive animals, while properties that offered it presented 9.1% of seropo-

Table 1. Detection and distribution of anti-*Neospora caninum* antibodies in sheep in some municipalities from Southeastern Bahia.

Municipalities	Reactive to *N. caninum*		No reactive to *N. caninum*		Total	
	N	%	N	%	N	%
Camacan	4	7,8	47	92,2	51	6,4
Canavieiras	14	8,8	145	91,2	159	20
Ibicaraí	5	18,5	22	81,5	27	3,4
Ilhéus	36	20,1	143	79,9	179	22,5
Itabuna	2	7,7	24	92,3	26	3,3
Itacaré	10	10,1	89	89,9	99	12,5
Itaju do Colônia	1	1,6	60	98,4	61	7,7
Itapé	22	18,3	98	81,7	120	15,1
Pau Brasil	11	15,1	62	84,9	73	9,2
Total	105	13,2	690	86,8	795	100

Table 2. Bivariate analysis of factors associated with the presence of *Neospora caninum* in sheep flocks of Southeastern Bahia.

Variables	Animals		X^2	P value	OR	CI 95%
	Positive	Negative				
Age group			0,63	0,423	1,23	0,78 – 1,96
≥03 years	75	462				
<03 years	30	228				
Breed			2,02	0,155	0,73	0,47 – 1,12
Purebred	47	364				
Crossbred	58	326				
Gender			0,01	0,898	1,01	0,51 – 1,88
Male	12	78				
Female	93	612				

Table 3. Bivariate analisys of factors associated with the presence of *Neospora caninum* analyzed, relating to property in sheep flocks from Southeastern Bahia.

Variables	Animals		X^2	P value	OR	CI 95%
	Positive	Negative				
Area property			7,62	0,005	1,89	1,19 – 3,02
<100 hectares	73	377				
≥100 hectares	32	313				
Use of hay			3,71	0,05	0,58	0,33 – 0,96
Yes	19	190				
No	86	500				
Water source			0,00	0,992	1,02	0,67 – 1,58
Trough	51	331				
River, dam	54	359				
Presence de Pen			6,58	0,01	0,54	0,34 – 0,85
Yes	72	553				
No	33	137				
Ration			0,42	0,51	0,84	0,55 – 1,30
Yes	64	447				
No	41	243				
Production system			0,02	0,887	0,92	0,52 – 1,57
Familiar	18	126				
Commercial	87	564				
Size of the herd			0,63	0,427	1,21	0,78 – 1,89
<100	65	395				
≥100	40	295				
Stocking rate			5,17	0,02	0,60	0,39 – 0,91
<1	63	328				
≥1	42	362				

Table 4. Bivariate analysis of factors associated with presence of *Neospora caninum* analyzed, related to the definitive host, in sheep flocks from Southeastern Bahia.

Variables	Animals		X^2	P value	OR	CI 95%
	Positive	Negative				
Dogs with access to the water source			3,27	0,07	0,59	0,35- 1,02
Yes	140	315				
No	53	103				
Presence of dogs			4,67	0,03	0,45	0,24 – 0,91
Yes	92	648				
No	13	42				
Transit of stray dogs or wild canids			0,45	0,498	0,84	0,56 – 1,28
Yes	54	383				
No	51	307				

Table 5. Multivariate analysis of risk factors associated with the presence of *Neospora caninum* in sheep flocks from Southeastern Bahia.

Variable	OR	CI 95%	P value
Presence de Pen			
Yes			
No	0,51	0,31 – 0,85	0,009
Use of hay			
Yes			
No	0,57	0,33 – 0,98	0,045
Presence of dogs			
Yes			
No	0,36	0,17 – 0,79	0,010
Stocking rate			
<1			
≥1	0,48	0,30 – 0,78	0,003

sitive animals. The capacity rate showed that when there was less than one animal grazing per hectare the positivity was 16.1%, in comparison with the percentage of 10.4% when this number was higher or equal to one animal.

In relation to the variables related to dogs, it was observed that the presence of dogs was a significant factor; however, the properties with dogs had less positive sheep (12.4%) when compared to properties where there were no dogs (23.6%).

DISCUSSION

In Brazil the prevalence of anti-*N. Caninum* antibodies in sheep varies from 1.8% in Rio Grande do Norte Soares et al. 2009) to 64.2% in Pernambuco (Tembue et al. 2011). Of the works carried out in Brazil using identical techniques and cut points, similar results were found by Faria et al. (2010) in Alagoas (9.6%) and Munhoz et al. (2010) in São Paulo (13.9%). However, those results were higher than those found by Salaberry al. (2010) in Minas Gerais (8.1%), Ueno et al. (2009) in the Federal District (8.8%) and Fiugliuolo et al. (2004) in São Paulo (9.2%); and lower than those found by Andreotti et al. (2009) in Mato Grosso (30.8%), Rossi et al. (2011) in Minas Gerais (47.1%) and Tembue et al. (2011) in Pernambuco (64.2%). The observed variations may be related to regional or climatic differences, age of the animals, and samples sizes (Dubey et al. 2011).

There were seropositivity differences (Table 1) among the analyzed cities. Differences among cities of the same microregion may be justified by the particularity of the climatic conditions that interfere in the viability of oocysts present in the environment. Ilhéus has an Atlantic Forest due to the cocoa exploration, what provides an average temperature of 24ºC and relative air humidity of 83%, whereas Itaju do Colônia had its cocoa exploration activity replaced by cattle raising, increasing the temperature (average of 27ºC), reducing humidity (average of 72%) and allowing the direct incidence of the sun as a result of loss of the forest, which is not favorable to the maintenance of viable oocysts.

Through logistic regression statistical analysis it was possible to observe that the properties that did not have sheepfold and did not supply hay presented higher positivity (p=0.009 and p=0.045, respectively) when compared to the properties with sheepfold and hay supply. There are no studies reporting the significance of these factors; however, it is understood that when the sheepfold is not used as shelter during the day, and when animals are not supplemented with hay, they remain more time grazing, and in environments contaminated with oocysts the possibility of infection by *N. caninum* becomes high.

The variable capacity rate demonstrated showed that when there is less than of one animal grazing per hectare the positivity is higher (p=0.003), when compared to the capacity rate of one animal per hectare. This result shows that, although the environment contaminated with oocysts favors the horizontal transmission with *N. caninum*, this may not be the only route of contamination in the studied properties, that is, the vertical transmission may also be present (Buxton et al. 1998).

The presence of dogs in the properties was a significant factor (p=0.010), being that when there were dogs in the property the positivity was lower than on those properties that had no dogs. In this case, this may be considered a protection factor in this study. The function of the definitive host is known in the epidemiology of the infection by *N. caninum*; however, the presence of dogs in the properties may restrict the transit of wandering and/or wild dogs, diminishing the contamination of the environment with oocysts (Barling et al. 2001).

It is possible to assume that the vertical transmission with *N. caninum* is more efficient than the horizontal transmission, as it is not environment--dependent, and Pinto et al. (2012) have already reported disorders related to the reproduction in the sheep. The studied properties did not use artificial reproduction methods and the majority of them reported the presence of reproductive problems, such as birth of weak lambs and, mainly, abortions.

Sheep from Southeastern Bahia is exposed to the infection caused by *N. caninum*. The main factors associated to the protection against the infection are: presence of sheepfold, use of hay, presence of dogs, and capacity rate.

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