

## Seroprevalence and risk factors associated with infection by *Neospora caninum* of dairy cattle in the state of Alagoas, Brazil<sup>1</sup>

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**ABSTRACT.-** Souza M.E., Porto W.J.N., Albuquerque P.P.F., Souza Neto O.L., Faria E.B., Pinheiro Júnior J.W. & Mota R.A. 2012. **Seroprevalence and risk factors associated with infection by *Neospora caninum* of dairy cattle in the state of Alagoas, Brazil.** *Pesquisa Veterinária Brasileira* 32(10):1009-1013. Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros s/n, Recife, PE 52171-900, Brazil. E-mail: rinaldo.mota@hotmail.com

The objective of this study was to investigate the prevalence of anti-*Neospora caninum* antibodies in cattle from milk producing farms of the microregion of Batalha, state of Alagoas, Brazil, as well as to identify the risk factors associated with the infection. Blood samples were collected from 1,004 cattle of 17 farms for the serological investigation regarding the presence of anti-*N. caninum* antibodies by the Indirect Immunofluorescence Reaction Technique (IMRT). From the total amount of samples analyzed, 77/1,004 (7.67%) were positive and 927/1,004 (92.33%) were negative. The logistical regression identified that cattle from farms without consortium breeding have an infection risk 6.33 ( $p < 0.001$ ; C.I. 2.89-13.10) times higher than cattle from farms with that type of breeding. Cattle from farms where the aborted fetuses are not adequately buried have an infection risk 3.04 ( $p < 0.001$ ; C.I. 1.64-5.63) times higher than cattle from farms with adequate destination of these fetuses. Infection by *N. caninum* occurs in cattle of the investigated region. The factors identified in our study can be used as risk indicators, so that control measures could be implemented to avoid infection by *N. caninum* in the herds of this region.

INDEX TERMS: *Neospora caninum*, neosporosis, bovine, epidemiology.

**RESUMO.- [Soroprevalência e fatores de risco associados à infecção por *Neospora caninum* em bovinos leiteiros no Estado de Alagoas.]** Objetivou-se com este estudo investigar a prevalência de anticorpos anti-*Neospora caninum* em bovinos procedentes de propriedades leiteiras da microrregião Batalha, Estado de Alagoas, Brasil, além de identificar os fatores de risco associados à infecção. Foram coletadas amostras

de sangue de 1004 bovinos procedentes de 17 propriedades para investigação sorológica quanto à presença de anticorpos anti-*N. caninum* através do teste de Reação de Imunofluorescência Indireta (RIFI). Do total das amostras analisadas, 77/1004 (7,67%) foram positivas e 927/1004 (92,33%) foram negativas. A regressão logística identificou que animais de propriedades sem criação consorciada têm risco 6,33 ( $p < 0,001$ ; I.C. 2,89-13,10) vezes maior de infecção do que animais de propriedades onde ocorre esse tipo de criação. Animais de propriedades onde os fetos abortados não são adequadamente enterrados têm risco 3,04 ( $p < 0,001$ ; I.C. 1,64-5,63) vezes maior de infecção do que animais de propriedades onde é feito o destino adequado dos mesmos. A infecção por *N. caninum* ocorre em bovinos na região estudada. Os fatores identificados neste estudo podem servir como indicadores de risco para que sejam implantadas medidas de controle para evitar a infecção por *N. caninum* nos rebanhos dessa região.

TERMOS DE INDEXAÇÃO: *Neospora caninum*, neosporose, bovinos, epidemiologia.

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## INTRODUCTION

*Neospora caninum* is an obligatory intracellular parasite which causes neosporosis, known as the most important cause of abortions in cattle worldwide (Dubey 2003), with reabsorption or fetal mummification, or the birth of healthy calves though chronically infected (Dubey & Schares 2006).

The infection is widely disseminated in all continents (Andreotti et al. 2003). In Brazil, serological studies revealed the presence of antibodies in Bahia (14.9% - Gondim et al. 1999), São Paulo (15.57% - Hasegawa et al. 2004), Goiás (30.4% - Melo et al. 2006), Minas Gerais (29% - Ragozo et al. 2003), Rio de Janeiro (25.74-20.38% - Munhoz et al. 2006), Rio Grande do Sul (17.8% - Corbellini et al. 2006), Mato Grosso do Sul (9.17% - Melo et al. 2008), Pernambuco (31.7% - Silva et al. 2008), Espírito Santo (17.5% - Fanti et al. 2009), and Mato Grosso (53.5% - Benetti et al. 2009).

Risk factors reported in papers from around the world have been associated with infection by *N. caninum* in cattle, such as: feed for adult cattle with moist corn silage during the Summer (OR 6.58), presence of birds (OR 4.6-10.42) (Bartels et al. 1999), calf feed with "pool" of colostrum (OR 2.07) (Corbellini 2005), presence of dogs (OR 4.2) on the farms, size of the farms (OR 0.90) (Corbellini et al. 2006), and age (Fanti et al. 2009).

The importance of investigating the herds regarding neosporosis is justified by the great economic losses reported worldwide (Hernández et al. 2001), and also by the still limited knowledge of the prevalence of the agent in some states of Brazil. Thus, the objective of this study was to investigate the prevalence of anti-*N. caninum* antibodies in cattle of milk producing farms in the microregion of Batalha, state of Alagoas, Brazil, as well as to identify the risk factors associated with the infection.

## MATERIALS AND METHODS

The state of Alagoas is located in the Central-Eastern portion of the Northeast of Brazil, between the parallels 8°48'12" and 10°30'12" of the Southern latitude and the meridians 35°09'36" and 38°13'54" of the Western longitude. It is divided into three mesoregions: Alagoan Eastern Region, Alagoan Agreste Region and the Alagoan Sertao Region (UFAL-GEM 1994, Assis et al. 2007).

The microregion of Batalha (Milk producing basin), part of the Mesoregion of the Alagoan Sertao Region (Fig.1), is composed of the municipalities of Batalha, Belo Monte, Jacare dos Homens, Jaramataia, Major Izidoro, Monteirópolis, Olho D'Água das Flores and Olivença (IBGE 2005).

To compose the sample for the prevalence study, a total of 96,034 cattle heads were considered, as well as an expected prevalence of 30%, obtained through the average of articles published in Brazil, with a confidence level of 95% and statistical error of 5% (Thrusfield 2004). Thus, the determined minimum sample was of 323 animals. However, it was decided that the study would involve 1,004 samples obtained from 17 farms of 8 municipalities. The choice of the farms and the animals was carried out according to the accessibility and availability of the producers. Blood samples were also collected from the dogs present on the farms at the moment of collection from the cattle.

For the anti-*N. caninum* antibodies study, the Indirect Immunofluorescence Reaction Technique (IMRT) was used. The serum samples were submitted to selection, having as the cut point the dilution of 1:200 (for cattle) and of 1:50 (for dogs) in blades previously sensibilized with tachyzoites of *N. caninum* cultivated in Vero cells. The anti-IgG-bovine and anti-IgG-canine antibodies conjugated with the isotiocyanate of fluoresceine of the Sigma-Chemical brand were used as secondary antibodies. Previously known standard, positive and negative serums were included in all blades (Dubey 1988).

The reactions were considered positive when the tachyzoites presented total periferic fluorescence (Dubey & Lindsay 1996). The positive serums in the dilution 1:200 (cattle) and 1:50 (dogs), were submitted to sequential dilutions to determine the title of the antibodies.

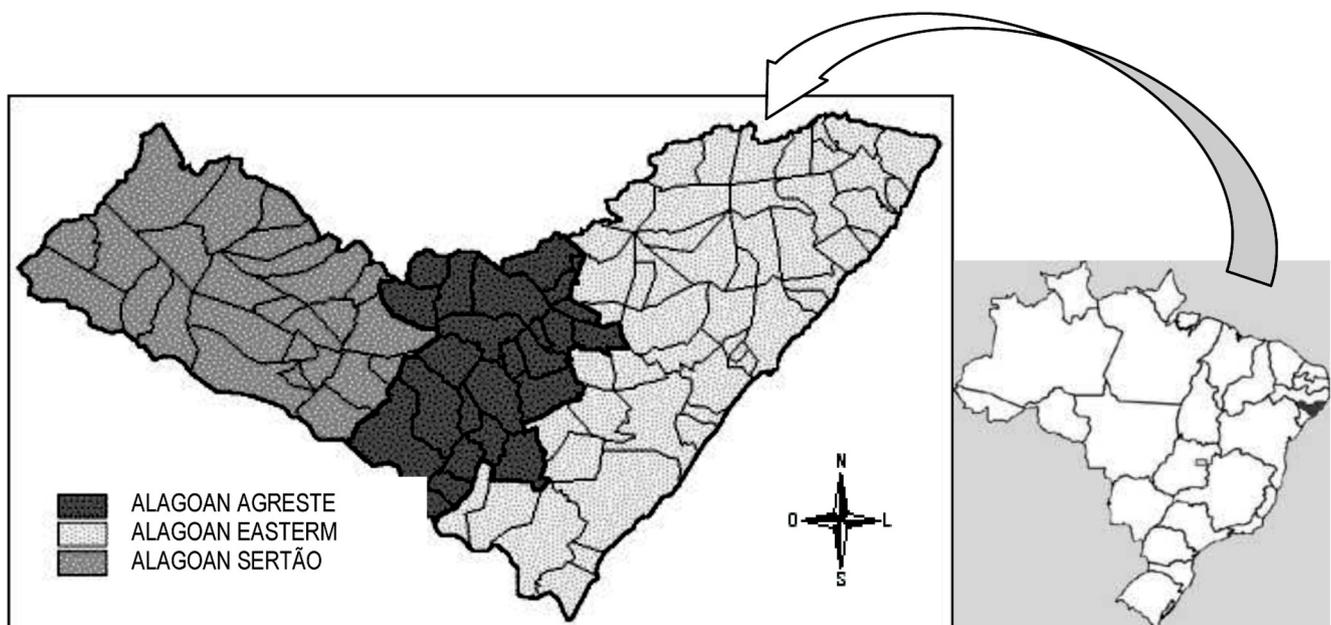


Fig.1. Mesoregions of the state of Alagoas. (Source: [http://www.zonasuldemaceio.com.br/alagoas/imagens/250px-Alagoas\\_Meso-MicroMunicip.svg.png](http://www.zonasuldemaceio.com.br/alagoas/imagens/250px-Alagoas_Meso-MicroMunicip.svg.png)).

For the study of risk factors, investigative questionnaires were applied with objective questions about the general characteristics of the farm and of the herd, and the nutritional, reproductive and hygienic-sanitary handling.

To identify the risk factors associated with infection by *N. caninum*, an univariate analysis of the interest variables was carried out using the Pearson's chi-square test. Later, a multivariate analysis using the logistical regression model was carried out, considering as a dependent variable the serological status of the animal (positive or negative) for *N. caninum*. All variables that presented a value of  $p < 0.10$  in the univariate analysis were included in this model. The significance level adopted was 0.05. To execute the statistical calculations, the program SPSS for Windows, version 180 - Statistical Package for Social Science, was used.

**RESULTS**

The results of this study reveal that from the 1,004 analyzed samples, 77 (7.67%) were positive and 927 (92.33%) negative. From the 17 farms investigated, 15 had at least one cow that was positive (Table 1).

In the univariate analysis, the variables of sex, destination of aborted fetuses, consortium breeding with other species, presence of forested areas in the surroundings of the farms, presence of domestic dogs and wild animals, water used to wash the stables and the origin of the drinking water for the cattle, presented a value of  $P < 0.05$ , and therefore were selected for the multivariate analysis (Table 2). From these variables, only the adequate destination of aborted fetuses (OR=3.04) and non-consortium breeding (OR=6.33) were considered risk factors associated with infection by *Neospora caninum* in the herds studied (Table 3).

With respect to the sex variable, there was a greater number of seropositives amongst females (8.2%) when compared with males (0.0%).

The cattle of dairy farms where there was no consortium breeding with other species, presented a risk about 6.33 times greater (OR=6.33;  $P < 0.001$ ) of infection by *N. caninum* than cattle of farms with consortium breeding (Table 3).

**Table 1. Distribution of samples tested for anti-*Neospora caninum* antibodies in the municipalities and properties of the microregion of Batalha, state of Alagoas, Brazil**

Property	Municipality	Nº of samples tested	Nº positive samples
Farm 1	Batalha	53	1
Farm 2	Batalha	47	7
Farm 3	Major Izidoro	51	2
Farm 4	Major Izidoro	28	7
Farm 5	Olivença	67	1
Farm 6	Belo Monte	125	7
Farm 7	Monteirópolis	89	10
Farm 8	Batalha	57	3
Farm 9	Jacaré dos Homens	54	8
Farm 10	Jacaré dos Homens	69	0
Farm 11	Olho D'água das Flores	96	3
Farm 12	Olho D'água das Flores	84	12
Farm 13	Jaramataia	62	13
Farm 14	Jaramataia	11	1
Farm 15	Jaramataia	26	1
Farm 16	Jaramataia	13	0
Farm 17	Major Izidoro	72	1
TOTAL		1,004	77

**Table 2. Univariate analysis for risk factors associated or not to infection by *Neospora caninum* in bovines of the state of Alagoas, Brazil**

Variates	N	Univariate analysis		P
		<i>N. caninum</i> RIFI (%)	OR (CI 95%)	
<b>Sex</b>				
Female	944	77 (8,2%)		0,011*
Male	60	0 (0,0%)	-	
<b>Destination of placentas</b>				
Did not have abortion	26	1 (3,8%)	1,0	0,817
Bary	373	27 (7,2%)	1,95 (0,25-4,96)	
Cremate	57	3 (5,3%)	1,39 (0,14-14,02)	
Remains exposed	548	46 (8,4%)	2,29 (0,30-17, 29)	
<b>Consortium breeding</b>				
Yes	205	3 (1,5%)	1,0	<0,001*
No	799	74 (9,3%)	6,87 (2,14-22,02)	
<b>Slaughterhouse in the surrounding area of the farm</b>				
Yes	401	21 (5,2%)	1,0	0,018*
No	603	56 (9,3%)	1,85 (1,10-3,11)	
<b>Origino of the drinking water for the cattle</b>				
Public	288	12 (4,2%)	1,0	<0,001*
Natural source	146	25 (17,1%)	4,75 (2,31-9,77)	
Dam	89	10 (11,2%)	2,91 (1,21-6,98)	
More than one source	481	30 (6,2%)	1,53 (0,77-3,04)	
<b>Residual water used to wash the stables spreads through the corrals</b>				
Yes	302	16 (5,3%)	1,0	0,001*
No	343	18 (5,2%)	0,99 (0,49-1,98)	
It does not wash	359	43 (12,0%)	2,43 (1,34-4,41)	
<b>Presence of wild animals</b>				
No	80	1 (1,3%)	1,0	<0,001*
Yes	924	76 (8,2%)	5,92(2,37-14,81)	
<b>Presence of domestic dogs</b>				
No	275	5 (1,8%)	1,0	<0,001*
Yes	729	72(9,9%)	6,49 (2,57-16,36)	
<b>Aborted fetuses are adequately buried</b>				
Yes	298	13 (4,4%)	1,0	0,011*
No	706	64 (9,1%)	2,18 (1,18-4,03)	
<b>The animals are serologically or pathologically tested against the most common aborting agents</b>				
Yes	583	37 (6,3%)	1,0	0,094
No	319	30 (9,4%)	1,53 (0,93-2,53)	

Base = 1,004 cattle. N = Total number of animals for each variable; RIFI = Reaction of Indirect Imunofluorescence; OR = odds ratio; CI = Confidence Interval; \* statistically significant.

**Table 3. Multivariate analysis for risk factors associated or not to infection by *Neospora caninum* in cattle of the microregion of Batalha, state of Alagoas, Brazil**

Independent Variable	OR	CI 95%	p
Non-consortium breeding	6,33	2,89 - 13,10	<0,001
Aborted fetuses inadequately buried	3,04	1,64 - 5,63	<0,001

OR = Odds ratio; CI = Confidence Interval 95%.

The cattle that lived on dairy farms where the destination of aborted fetuses was not adequate also had a 3.04 times greater chance (OR=3.04;  $P < 0.001$ ) of infection by *N. caninum* than cattle from farms where the aborted fetuses were incinerated or buried (Table 3).

**DISCUSSION**

The results of this study showed a frequency considered low when compared with results obtained in other studies carried out in other states of Brazil: 17.5% in Espírito San-

to (Fanti et al. 2009), 30.4% in Goiás (Melo et al. 2006), 31.7% in Pernambuco (Silva et al. 2008), 33.0% in Parana (Locatelli-Dittrich et al. 2008), 50.74% in Maranhão (Teixeira et al. 2010), 53.54% in Mato Grosso (Benetti 2006), and 91.2% in Minas Gerais (Guedes et al. 2008).

The fact that there was a greater frequency of cattle that were seropositive amongst the females could be explained by the fact that the research study was carried out on milk producing dairy cattle herds which are predominantly females. However, in this study, as well as in the study of Teixeira et al. (2010), there was no significant association for this variable.

The high risk observed in this study for non-consorted cattle breeding with other species can be attributed to a higher concentration of cattle on the farms that breed this species exclusively, and also to the possibility of these animals having been introduced into the herd already seropositive for *Neospora caninum*, as previously reported by Fortunato (2010). Although the "animal density" variable was not investigated in this study, it is widely known that the number of animals in the herds is directly related to prevalence of infection (Barling et al. 2001). Dubey et al. (2007) also reported that normally, in these situations, more concentrates are used for the animal feed, and that the storing locations where this type of feed is kept attracts rodents which are potential prey for the definitive hosts of *N. caninum* (Gondim 2006).

Not giving adequate destination for aborted fetuses was also a confirmed risk factor in the multivariate analysis. This finding is very important epidemiologically, since dogs or wild canids can have access to infected tissues exposed in the surrounding fields of farms, increasing the chances of these definitive hosts to become infected (Dijkstra et al. 2001, Cunha-Filho et al. 2008). In one of the studied farms which presented cattle that were seropositive for *N. caninum*, two dogs were identified as being positive from the existent nine. According to the investigation questionnaire applied on this farm, the dogs had access to the corrals where the cattle were handled and probably where the fetuses were aborted, resulting in inadequate exposure of the cattle to the dogs. These results reinforce the necessity to destroy the aborted fetuses on the farm, to reduce the infection risk of canids and the prevalence of infection of the cattle. About this specific aspect Cunha-Filho et al. (2008) also observed that the adequate destination of placentas and carcasses on farms with neosporosis was considered a protecting factor for dogs. Fernandes et al. (2004) also reported that in rural areas, dogs can get infected more easily when they have access to carcasses, aborted fetuses and rests of placentas.

The greater frequency of seropositive animals in farms where they were not serologically or pathologically tested for the more common agents of abortion constitutes an important piece of data which indicates the necessity of qualified technical assistance in the efforts to avoid maintenance of the agent in the herds (Silva et al. 2008).

The farms that did not have forests near by or in the surrounding areas, presented a higher frequency of seropositive animals, even without having statistical significan-

ce for this variable, indicating the importance of domestic definitive hosts on these farms (Santos et al. 2009).

The greater prevalence of infection by *N. caninum* in cattle has been related to the presence of dogs and wild animals on the farms. In spite of the fact that this study did not find any significant statistical association in the multivariate analysis for these variables, the frequency of seropositive cattle was considerably higher in the farms where these animals were present, than in those where the presence of these animals was not reported. A similar result was also observed by Santos et al. (2009) who studied the frequency of anti-*N. caninum* antibodies in cattle in Minas Gerais, Brazil. On the other hand, Schares et al. (2004), Corbellini (2005) and Corbellini et al. (2006) found significant association between seropositivity and the cited variables, reinforcing the importance of the control of these animals on the farms to avoid horizontal transmission in the herd.

In the interviews, the presence of dogs or people from farms or neighboring communities, who frequented the facilities of the researched farms – even without dogs – was reported. Based on this fact, it is important to consider the possibility of the occurrence of seropositive cattle, even on farms without seropositive dogs.

## CONCLUSIONS

Infection by *Neospora caninum* occurs in cattle of the studied region.

The factors identified in this study could be used as risk indicators for the implementation of control measures to avoid infection by *N. caninum* in the cattle herds of this region.

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