SEROPOSITIVITY FOR *Neospora caninum* AS FACTOR ASSOCIATED WITH INFECTIOUS MASTITIS IN CROSSBRED COWS IN NORTHEASTERN BRAZIL*

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ABSTRACT. de Oliveira U.V., Galvão G. da S., de Magalhães V.C.S., Costa S.C.L., Andrioli J.L., Ribeiro A.R. da P. & Munhoz A.D. **Seropositivity for *Neospora caninum* as factor associated with infectious mastitis in crossbred cows in Northeastern Brazil.** [Soropositividade para *Neospora caninum* como fator associado à mastite infecciosa em vacas mestiças no nordeste brasileiro]. *Revista Brasileira de Medicina Veterinária 34(4):270-274, 2012.* Laboratório de Análises Clínicas Veterinária, Departamento de Ciências Agrárias e Ambientais, Universidade Estadual de Santa Cruz, Campus Soane Nazaré de Andrade, Rodovia Ilhéus-Itabuna Km 16, Ilhéus, BA 45662-900, Brazil. E-mail: munhoz@uesc.br

The aim of this study was to verify the relation of absence of infectious mastitis in cows exposed to *Neospora caninum*. From 10 dairy farms located in northeastern Brazil 20% of lactating cows were selected, totaling 203 animals. The blood of each cow was obtained for serology; *California Mastitis Test* (CMT) was performed for measurement of somatic cell count. Milk was collected only from teats CMT reagent for bacterial isolation. Serology was performed by indirect immunofluorescent antibody test using cut-off point of 1:200. A structured interview was carried out about animals' management, with variables that could influence mastitis cases, such as: presence of calf during milking, use of milking machine, veterinary care, somatic cell count routinely measured, age of animals, culling of cows in the farms, cleanliness of milker before milking and disinfection of teats before and after milking. Unconditional logistic regression was performed where it was observed that the presence of the calf during milking (p<0.0001) and cows seropositive for *N. caninum* (p=0.0311) were protection associated factors. It was concluded that crossbred cows exposed to *N. caninum* with no history of abortion were less susceptible to mastitis.

KEY WORDS. Cattle, neosporosis, mastitis.

RESUMO. Para verificar a relação da ausência da mastite infecciosa em vacas expostas a *Neospora caninum*, foram selecionadas 20% das vacas em lactação de 10 propriedades leiteiras localizadas no Nordeste do Brasil, totalizando 203 animais. O sangue de cada vaca foi colhido para sorologia; O *California Mastitis testis* (CMT) foi utilizado para a mensuração da contagem de células somáticas. O leite foi colhido apenas dos tetos reagentes ao CMT, para o isolamento bacteriano. A sorologia foi realizada através da reação de imunofluorescência indireta, utilizando ponto de corte de 1:200. A entrevista estruturada foi realizada sobre o manejo dos animais, com variáveis que poderiam influenciar casos

de mastite, tais como: presença de bezerro durante a ordenha, uso de equipamento de ordenha, cuidados veterinários, contagens de células somáticas mensuradas rotineiramente, idade dos animais, abate de vacas em fazendas, higienização do ordenhador antes da ordenha, e desinfecção dos tetos antes e após a ordenha. Regressão logística não condicional foi utilizada, onde foi observado que a presença do bezerro durante a ordenha (p<0,0001) e vacas soropositivas para *N. caninum* (p= 0,0311) foram fatores de proteção associados. Conclui-se que vacas mestiças expostas *N. caninum* sem histórico de aborto foram menos susceptíveis a mastite

PALAVRAS-CHAVE. Bovinos, neosporose, mastite.

INTRODUCTION

Neospora caninum is a coccidian parasite with worldwide distribution, accounting for losses in dairy cattle due to induction of abortion (Dubey 1999). Evidence shows that cattle exposed to *N. caninum* can develop some degree of protective immunity against abortion and vertical transmission, suggesting that immunoprophylaxis can be a viable alternative (Dubey et al. 2007).

The subclinical infectious mastitis is that positive to *California Mastitis Test* (CMT), confirmed by microbial growth (Bradley 2002). Infectious mastitis is triggered by invading pathogens and involves leukocyte migration toward the mammary gland (Paape et al. 2003); as infection persists, the number of somatic cells found in milk increases (Leitner et al. 2000, Zhao & Lacasse 2007), indicating inflammation of the gland.

Interactions between *N. caninum* and the host immune system can aid in the protection of the host, the parasite, or both; thus, it is critical to understand the consequences of inducing specific immune responses in the host (Innes et al. 2007). Systemic immunity in cattle is closely associated with the immune response of the udder (Mallard et al. 1998), and seropositive cattle without a history of abortion due to *N. caninum* are more efficient at producing a protective response within the mammary gland (Peregrine et al. 2004). However, few studies on immune response in the udder and *N. caninum* have used animals of purely European origin (*Bos taurus taurus*) (Ould-Amrouche et al. 1999, Peregrine et al. 2004). This study was motivated by the absence of information regarding crossbred cattle for dairy production (European x zebu) and aims to determine the association between exposure to *N.caninum* and infectious mastitis in crossbred cattle.

MATERIALS AND METHODS

The study was conducted in the northeastern region of Brazil (Altitude 47m; 14°70" S and 39°03"W) in 10 dairy farms, from July 2008 to July 2010. Presence of seropositive animals to *N. caninum* (Galvão et al. 2011) and identical production system (semi-extensive system) were the criteria for selection of herds. The herds had an average daily production of 353 kg of milk in the rainy season, and 261.8 kg during the dry season; in addition, the group was composed of crossbred animals (European x zebu) with varied degrees of consanguinity. At least twenty 20% of the lactating cows were selected in each herd, comprising the 203 animals from which blood samples were collected. The selection criterion was to use the cows that were in the milking parlor at the time of the visit. CMT was then performed on the samples collected from all teats. The collected blood was centrifuged at 350*g* for 10 minutes and, after separation of serum, it was placed in a cryotube, labeled, and kept at -20ºC until the conduction of serology. The CMT reagent used was 0.2% bromocresol purple.

Approximately 10mL of milk were collected and put in sterile test tube with screw cap, identified and kept under refrigeration until processing of teats that presented positive reaction by CMT. An aliquot of 20µL of milk was inoculated in 5% defibrinated sheep blood agar and subsequently incubated in a bacteriological incubator at 37°C for 24 to 48 hours. The isolation and identification of bacteria was carried out according to their morphology, staining and biochemical characteristics as proposed by Quinn et al. (2005), characterizing the animal as positive or negative for infectious mastitis.

A structured interview was performed with owners or managers of the farms to obtain information about the management of animals that could influence the cases of mastitis, such as: presence of calf during milking, use of milking machine, veterinary care, somatic cell count (SCC) routinely measured, age of animals, culling of cows in the farms, cleanliness of milker before milking and disinfection of teats before and after milking. The age of the animals was categorized by the determination of the median.

Detection of anti-*N. caninum* antibodies was performed by indirect immunofluorescent antibody test (IFAT) (Yamane et al. 1998), using tachyzoites of the Nc-Bahia strain (Gondim et al. 2001) and a *cutoff* point of 1:200. Database and statistics sof-

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Table 1. Factors assessed in order to verify association with infectious mastitis in cattle from Northeastern Brazil

Factors	Category	Cattle				Odds Ratio	P*
		Positive		Negative		(CI 95%)	
		n	%	n	%		
Presence of calf during milking	Yes	43	27.90	111	72.10	0.15 (0.07-0.31)	<0.000001
	No	35	71.40	14	28.60	**	
Seropositive for *Neospora caninum*	Yes	03	15	17	85	0.25 (0.07-0.89)	0.04
	No	75	40.98	108	59.02	**	
Use of milking machine	Yes	50	45.00	61	55.00	1.87 (1.04-3.34)	0.05
	No	28	30.40	64	69.60	**	
Veterinary care	Yes	28	28.30	71	71.70	0.42 (0.23-0.76)	0.006
	No	50	48.10	54	51.90	**	
Somatic cell count routinely measured	Yes	16	27.58	42	72.41	0.51 (0.26-0.98)	0.06
	No	62	42.80	83	57.20	**	
Cleanliness of milkers before milking	Yes	28	34.60	53	65.40	0.76 (0.42-1.36)	0.44
	No	50	41.00	72	59.00	**	
Disinfection of teats before and after milking	Yes	42	37.90	66	62.10	1.04 (0.59-1.83)	0.99
	No	36	38.90	59	61.10	**	
> 6 years old	Yes	47	41.60	66	58.40	1.35 (0.76-2.41)	0.37
	No	31	34.40	59	65.60	**	

*Using Chi Square test.
** Reference category.

tware Epi Info version 3.5.1 (Centers for Disease Control and Prevention) were used to perform the analysis of the association between the explanatory variables, seropositivity to *N. caninum* and mastitis. The bivariate analysis was made using the χ^2 test, or Fisher exact test, whenever necessary. Before further analysis, Biostat Program for Windows version 5.0 was employed to perform Spearman's correlation matrix, in order to verify the collinearity among the independent variables ($p \geq 0.80$). Variables with $p \leq 0.20$ at the bivariate analysis and $p<0.80$ at the correlation analysis were included in the multivariable analysis by logistic regression, which was employed to confirm the association between factors and mastitis. The variables introduced in the model were selected through the backward elimination procedure based on the likelihood-ratio test. The required level of significance for a factor to be considered as associated in the final model was set at 5%.

RESULTS AND DISCUSSION

Of the tested animals without infectious mastitis 59.02% (108/183) were seronegative, and 85% (17/20) were seropositive for *N.caninum*, thereby showing a significant association (p=0.043) between seropositivity and lack of mastitis (Table 1). All the seropositive cattle had no history of abortion, as reported by producers.

In bivariate analysis, besides of seropositivity to *N. caninum*, the variables: presence of calf during milking, use of milking machine in the property, ve-

terinary care, somatic cell count routinely measured obtained p<0.2 (Table 1), did not show collinearity with each other and thus formed the initial model of logistic regression. The final model showed the presence of the calf during milking and seropositivity for *N. caninum* as a protective factor associated with mastitis (Table 2). As all farms culling old animals, this variable cannot be included in the analysis.

The results of this study corroborate the work of Ould-Amrouche et al. (1999), who noted an association between seropositivity and low SCC count, and Peregrine et al. (2004), who verified that cattle seropositive to *N. caninum* were less likely to show infectious mastitis when compared to seronegative cattle.

An important aspect to consider is that the animals used in this study were crossbred (European x zebu), whereas previous studies have used animals of purely European origin; hence, the mechanisms of resistance to mastitis associated with exposure to *N. caninum* may be similar between *B. t. indicus* and *B. t. taurus*. It is likely that these mechanisms of resistance are related to Th1-type responses observed in cattle that were experimentally infected with

Table 2. Final multivariable logistic regression model for infectious mastitis in cattle from Northeastern Brazil

Factors	Odds Ratio	95% CI	p
Presence of calf during milking (Yes/No)	0.1494	0.0719-0.3104	<0.0001
Seropositive for *Neospora caninum* (Yes/No)	0.2260	0.0585-0.8738	0.0311

Likelihood <0.0001.

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N. caninum (Marks et al. 1998, Lunden et al. 1998, De Marez et al. 1999, Williams et al. 2000, Andrianarivo et al. 2001, Tress et al. 2002). The Th1-type response stimulates production of cytokines, such as interferon gamma (Quiroga et al. 1993), tumor necrosis factor (Rewinski & Yang 1994), granulocyte-macrophage colony stimulating factor (Sordillo et al. 1992, Sordillo & Streicher 2002) and nitric oxide (Goof et al. 1996) these factors are related to the immune defense mechanisms of the host.

Once these cytokines have been employed in the defense of the mammary gland (Daley et al. 1993), it is possible that *N. caninum* seropositive cattle can develop a cross-protective immunity in the udder due to an increase in the Th1 response components, making them more resistant to infections. This hypothesis is consistent with the findings of Hassig & Gottstein (2002) that herds with abortion problems caused by *N. caninum*, and likely an inefficient Th1 response, had high mastitis rates and decreased milk production. Thus, the protective mechanisms of the host, which aid in preventing abortion, can also help to protect the udder. However, further studies are needed to confirm this hypothesis and to determine whether the breed standard can contribute to absence to mastitis.

Although old cows are culled off as prophylactic control, it is believed that culling reaches the same proportion of *N. caninum* seropositive and seronegative animals, as the characteristics of the farms of this study resemble the controls in the study by Hobson et al. (2005), who did not observe a higher culling rate for seropositive animals. Finally, the presence of the calf during milking in *B. t. indicus* had a positive effect, also observed in other studies, either by reducing cases of mastitis (Oliveira et al. 2011), probably by the depletion of the gland, or increase in milk production (Combelles et al. 2003).

CONCLUSION

The exposure to *N. caninum* is a factor associated with absence of infectious mastitis in crossbred cattle in the conditions of this study.

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