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ORIGINAL ARTICLE

Neospora caninum antibodies in dairy cattle of Lages Municipality, Santa Catarina State, Brazil

Anticuerpos séricos contra *Neospora caninum* en rebaños lecheros de la ciudad de Lages, Estado de Santa Catarina, Brasil

AB Moura*, AP Souza, AA Sartor, V Bellato, EB Teixeira

Departamento de Medicina Veterinária, Centro de Ciências Agroveterinárias, Universidade do Estado de Santa Catarina, Brasil.

RESUMEN

Los bovinos son los principales hospederos intermediarios del coccidio *Neospora caninum*. En esta especie se concentran la mayoría de los daños reproductivos. A fin de determinar la prevalencia de *N. caninum* e identificar factores de riesgo para la infección en el ganado lechero de la ciudad de Lages, Estado de Santa Catarina, se tomaron muestras de sangre de bovinos (n = 373) y los perros (n = 33) en 19 propiedades. La detección de anticuerpos contra *N. caninum* fue realizada por medio de IFA. Fueron consideradas positivas las muestras con títulos ≥ 200 (ganado bovino) y ≥ 50 (perros). Informaciones relacionadas con la categoría de los animales, la raza, la frecuencia y tipo de trastornos de la reproducción, la producción de leche y la presencia de los perros fueron obtenidos mediante un cuestionario. Los datos fueron analizados con la prueba exacta de Fisher y χ^2 ($P \leq 0,05$). De las muestras de ganado y canino evaluados, 86 (23,1%) y 7 (21,2%) fueron positivas a *N. caninum*, respectivamente. Se encontró correlación entre los animales con baja producción y la seropositividad a *N. caninum* ($P = 0,003$). La presencia de perros positivos en las propiedades no representa un factor de riesgo de positividad en el ganado vacuno. No se observó una asociación estadísticamente significativa entre trastornos reproductivos con la aparición de anticuerpos contra *N. caninum* en bovinos evaluados. Los resultados obtenidos indican que *N. caninum* está presente en el ganado lechero de la ciudad de Lages, Santa Catarina.

Key words: *Neospora caninum*, dairy cattle, risk factors, Santa Catarina State.

Palabras clave: *Neospora caninum*, bovinos lecheros, factores de riesgo, Santa Catarina.

INTRODUCTION

Bovine are the main intermediate host of *Neospora caninum*, a coccidian protozoan. This protozoan parasite is a major cause of bovine abortions and neonatal mortality worldwide (Thilsted and Dubey 1989). Infection occurs following ingestion of sporulated oocysts or introduction by the transplacental route (Dubey and Lindsay 1996). Calves congenitally infected can develop the clinical disease or maintain the infection in the herd through vertical transmission, which is the main route of transmission of *N. caninum* in dairy cattle (Davison *et al* 1999[a]).

Research has demonstrated that neosporosis is the major cause of abortions in bovine (Sadrebazzaz *et al* 2007), with abortions being a common clinical sign of infection. Nevertheless, in some countries or regions, other causes of abortion (infectious or not) may be more important than *N. caninum* infection. Flausino *et al* (2006) observed a strong association among abortions and seropositivity for *N. caninum* in dairy herds in Rio de Janeiro State. Recurrent abortions in the same cow are an indication of possible *N. caninum* infection (Locatelli-Dittrich *et al* 2004). Aborted fetuses and calves congenitally infected can present viable cysts of the parasite in their tissues, and become a source of infection for definitive hosts such as dogs, that may feed on infected carcasses. In addition to reproductive disorders, *N. caninum* is implicated as a factor in reduced milk production (Thurmond and Hietala 1997, Hernandez *et al* 2001, Hobson *et al* 2002) and lower concentrations of fat and protein in milk (Tiwari *et al* 2007). Cruz *et al* (2011) demonstrated the importance of neosporosis on health herd in dairy cattle.

There is no evidence that *N. caninum* infects humans, however, the prevalence of antibodies against this agent in humans has been reported (Almeida 2004, Lobato *et al* 2006). Experimental inoculations in primates demonstrated congenital transmission of the protozoa and showed that clinical neosporosis in primates was similar to congenital toxoplasmosis in humans (Baar *et al* 1994).

In Brazil, the prevalence of antibodies against *N. caninum* in cattle ranges from 6.8% to 67.8% in the different regions of the country (Gennari 2004). Melo *et al* (2001) analyzed the frequency of *N. caninum* infection in 18 dairy herds in the state of Minas Gerais and reported a cattle seroprevalence ranging from 0% to 72.73%. Table 1 displays the importance and the presence of *N. caninum* infection in bovine of several regions of Brazil. Although an outbreak of abortions due to *N. caninum* was confirmed in the Santa Catarina State (Corbellini *et al* 2001), no epidemiologic studies were done to establish the

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* Av. Luís de Camões, 2090, Lages, SC, Brazil; a2abm@cav.udesc.br

Table 1. Prevalence of antibodies against *Neospora caninum* in bovine in Brazil, technique employed and reference.
Prevalencia de anticuerpos contra *Neospora caninum* en bovinos en Brasil, técnica empleada y referencia.

Reference	County	Samples evaluated	Occurrence (%)	Technique (Cut off)
Hasegawa *et al* (2004)	SP	777	15.57	IFAT (1:200)
Locatelli-Dittrich *et al* (2004)	PR	172	34.8	**
Ogawa *et al* (2005)	PR	385	12	IFAT (1:200)
Rodrigues and Cury (2005)	MG	245	4.48	IFAT internet
Vogel *et al* (2006)	RS	798	11.4	ELISA (**)
Munhoz *et al* (2006)	RJ*	303	25.7	ELISA (**)
	RJ*	260	20.4	ELISA (**)
Mineo *et al* (2006)	MG*	174	20	ELISA (1:100)
	MG*	69	10	ELISA (1:100)
Melo *et al* (2006)	GO	930	30.4	IFAT (1:250)
Guimarães *et al* (2006)	MG	362	90	IFAT (1:200)
Juliano *et al* (2006)	GO and TO	468	38.24	ELISA (**)
Oshiro *et al* (2007)	MS	2,448	14.9	IFAT (1:50)
Guedes *et al* (2008)	MG*	559	91.2	IFAT (1:200)
	MG*	575	97.2	IFAT (1:200)
Locatelli-Dittrich *et al* (2008)	PR	1,263	33	ELISA (**)
Minervino *et al* (2008)	PA	160	19	IFAT (1:100)
Mello *et al* (2008)	MS	392	9.17	IFAT (1:50)

ELISA = Enzyme-linked immunosorbent assay.
IFAT = Indirect immunofluorescent antibody test (Técnica de inmunofluorescencia indirecta).
* Municipality or herds distinct.
** Not Described.

* Ciudades o rebaños separadas.
** No se describe.

seroprevalence of *N. caninum.* This study was designed to examine seroprevalence of *N. caninum* in dairy cattle of Lages municipality, Santa Catarina State and risk factors for neosporosis infection.

MATERIAL AND METHODS

Between August 2007 and February 2008, blood was collected from 373 bovines on 19 dairy farms in Lages Municipality, situated in the mountain region of the Santa Catarina State, southern Brazil (27º48'57"S, 50º20'33"W) at an altitude of 916 m, with a mean annual temperature of 14.3ºC. The size sample considered a prevalence of 20%, error of 2.5% and a confidence level of 95% (EPI-INFO 6.0). Blood samples were randomly collected from calves, heifers, lactating cows, dry cows and bulls. Nearly 84% of the animals were lactating cows randomly selected among the adult female cattle. The occurrence of abortions was not used as a criterion to select the farms or the animals. Sera were obtained by centrifugation and kept at -20°C until assayed.

An indirect immunofluorescent antibody test (IFAT) was performed according to Conrad *et al* (1993) using tachyzoites of the NC1 strain of *N. caninum* (Dubey *et al* 1988[b]), and a commercial fluorescein-labeled whole rabbit anti-bovine IgG (Sigma-Aldrich Co.) was used as the secondary antibody. Complete tachyzoite peripheral fluorescence (Paré *et al* 1995) at a dilution of 1:200 was considered positive for *N. caninum* antibodies in cattle (Dubey and Lindsay 1996). Negative and positive control sera were used on each slide. Positive sera were diluted two-fold up to the maximum titer.

Additional blood samples were gathered from dogs (n = 33) that coexisted with bovine on the evaluated properties. Dog blood was analyzed for *N. caninum* antibodies using the IFAT method (cut-off ≥ 50, Silva *et al* 2007).

Herd data (breed, management, frequency and type of reproductive or neurologic disorders in the last 12 months, production of milk, diet and presence of dogs) was obtained from the owners.

STATISTICAL ANALYSIS

The data were tabulated and analyzed statistically by using Fisher's exact[1] and qui-square tests ($P \leq 0.05$) to correlate the results of serology with potencial risk factors.

[1] R Development Core Team. 2009. R: A language and environment for statistical computing R Foundation for Statistical Computing, Vienna, Austria ISBN 3-900051-07-0, URL http://wwwR-projectorg.

RESULTS

Neospora caninum antibodies were found in 23.1% (86 positive/373 tested) of cattle and 21.2% (7/33) of dogs. The titers detected in bovine serum samples were 1:200 (18), 1:400 (23), 1:800 (29), 1:1600 (14) and 1:3200 (2). Serological evidence of neosporosis was found on all farms included in the study (table 2), suggesting widespread occurrence of the protozoan among dairies of Lages, Santa Catarina.

Infection was primarily found in animals with low milk production (P = 0.008). More than half (55.6%) of the cows with low production (< 10 liters of milk per day) were *N. caninum* seropositive (table 3).

Table 2. Positive dairy cattle (IFAT, ≥1:200) for *Neospora caninum*, per farm, from the municipality of Lages, Santa Catarina State, for analyzed variable and total. 2008.

Ganado lechero positivo (IFAT ≥1:200) por *Neospora caninum,* por granja, del municipio de Lages, Estado de Santa Catarina, para variables analizadas y total. 2008.

Farm	Bovines Evaluated	Bovines Positive (%)	
01	19	03	(15.8)
02	19	03	(15.8)
03	33	07	(21.2)
04	37	07	(18.9)
05	24	04	(16.7)
06	12	01	(8.3)
07	24	05	(20.8)
08	15	05	(33.3)
09	35	15	(42.9)
10	25	05	(20)
11	13	09	(69.2)
12	13	04	(30.8)
13	26	02	(7.7)
14	18	02	(11.1)
15	05	01	(20)
16	06	03	(50)
17	16	05	(31.3)
18	12	01	(8.3)
19	21	04	(19.1)
Total	373	86	(23.1)

DISCUSSION

In the present study, *N. caninum* seropositivity was similar to the 19% prevalence reported by Minervino *et al* (2008) in Santarém, Pará State, 23.6% described by Ragozo *et al* (2003) and 26% related by Romero-Salas *et al* (2010). Alternatively, Ogawa *et al* (2005), Vogel *et al* (2006), Corbellini *et al* (2006) and Oshiro *et al* (2007) reported lower seropositive values of 12%, 11.4%, 17.8% and 14.9%, respectively. In contrast, Boaventura *et al* (2006), Guimarães *et al* (2006), Locatelli-Dittrich *et al* (2008) and Benetti *et al* (2009), found 40.7%, 90%, 33% and 53.5% of positive animals had antibodies against *N. caninum*. Ragozo *et al* (2003) and Guimarães Junior *et al* (2004) both reported the highest rates of seropositivity were from older cows. A similar predominance was observed in this study, were 84% of positive are lactating cows, but the difference was not significant (table 3). Horizontal transmission within dairies endemically infected normally shows a predominance of infection in older cattle (Davison *et al* 1999[a]). Therefore, the results suggested infection in the herds in this study occurred by horizontal transmission, which has been reported by others (Davison *et al* 1999[a], Dyer *et al* 2000).

The low productivity cows, with bigger seropositive rates, were from two farms with less technological systems, suggesting management practices could have contributed to a higher prevalence of *N. caninum* antibodies in these herds. The same was observed by Corbellini *et al* (2006) and Wang *et al* (2010)

The prevalence of neosporosis in bovines is higher in aborting cows when compared to normally calving cattle (Davison *et al* 1999[b], De Meerschman *et al* 2002, Ghalmi *et al* 2007, Almería *et al* 2009), although Oshiro *et al* (2007) found a positive correlation between low titers of bovine *N. caninum* antibodies and abortions. In this study, no significant association was observed between the prevalence of antibodies against *N. caninum* and reproductive disorders in bovine, in agreement with findings of Aguiar *et al* (2006). Similarly, Paz *et al* (2007) found no association between *N. caninum* seropositivity and pregnancy rates in cows belonging to a surrogate herd submitted to embryo transfer technology.

Although Basso *et al* (2010) observed the horizontal infection route determines the occurrence of epidemic abortions, the abortion rate was not increased in a dairy herd infected by horizontal transmission (Dijkstra *et al* 2002), which suggested the existence of low virulent strains of the protozoan, such as observed for *T. gondii*. The identification and characterization of *N. caninum* strains of low virulence could explain the lack of an association between seroprevalence for neosporosis and reproductive disorders in the dairies included in the present study (Dubey *et al* 2006, Rojo-Montejo *et al* 2009[a], Rojo-Montejo *et al* 2009[b]).

At least one dog positive for neosporosis was detected in six (31.6%) of 19 evaluated dairies. However, we found no correlation between *N. caninum* seroprevalence in cattle and the presence of infected dogs. Similarly, Aguiar *et al* (2006), Locatelli-Dittrich *et al* (2008) and Mello *et al* (2008) did not observe any correlation between presence of dogs and seropositivity for *N. caninum* in cows. In contrast, Guimarães Junior *et al* (2004) reported a positive correlation between the presence of dogs and the prevalence

Table 3. Frequency of positive dairy cattle (IFAT, ≥1:200) for *Neospora caninum* from the municipality of Lages, Santa Catarina State, for analyzed variable and total. 2008.

Frecuencia de ganado lechero positivo (IFAT ≥1:200) por *Neospora caninum* del municipio de Lages, Estado de Santa Catarina, para variables analizadas y total. 2008.

Variable	Category	Animals		Positives[1]		Positives[2]		P
		n	%	n	%	n	%	
Category Group	Calf	4	1.1	1	25.0	1	1	0.985
	Heifer	33	8.8	7	21.2	7	8	
	Lactating Cow	314	84.2	72	22.9	72	84	
	Dry Cow	19	5.1	5	26.3	5	6	
	Bull	3	0.8	1	33.3	1	1	
Breed	Holstein	164	44.0	38	23.2	38	44	0.3207
	Jersey	179	48.0	37	20.7	37	43	
	Flamenga	5	1.3	1	20.0	1	1	
	Cross Breed	7	1.9	4	57.1	4	5	
	Gir	4	1.1	2	50.0	2	2	
	Mini-Jersey	12	3.2	4	33.3	4	5	
	Lageano	1	0.3	0	0.0	0	0	
	Norman	1	0.3	0	0.0	0	0	
Milk Production	High	221	59.2	45	20.4	45	52	0.008
	Middle	73	19.6	17	23.3	17	20	
	Low	18	4.8	10	55.6	10	12	
	No lactation	61	16.4	14	23.0	14	16	
Reproductive and Neurologic Disorders	No Problems	291	78.0	65	22.3	65	76	0.528
	Abortions	21	5.6	5	23.8	5	6	
	Return Season	53	14.2	13	24.5	13	15	
	Stillborn	6	1.6	2	33.3	2	2	
	Mummified	1	0.3	1	100.0	1	1	
	Neurologic	1	0.3	0	0.0	0	0	
Total		373	100	86	–	86	100	

Positive[1] = Relation among the positive animals within a category and the total of animals of its category.
Positive[2] = Relation among the positive animals within a category and the total of positive animals.
P = descriptive level of the χ^2 test.

Positives[1] = Relación entre los animales positivos dentro de la categoría y el número total de animales de su categoría.
Positives[2] = Relación entre los animales positivos dentro de la categoría y el total de animales positivos.
P = Nivel descriptivo de la prueba χ^2.

of *N. caninum* antibodies in cattle and Vega *et al* (2010) suggest that a close relationship between definitive and intermediate host, can facilitate the horizontal transmission. Interestingly, Barling *et al* (2001) suggested the presence of a dog among the cattle provided a protective factor against *N. caninum* infection. Perhaps the presence of a cattle-working dog prevented contamination of feed and water sources by other canids (stray and wild dogs) that might be more important sources of infection than tame dogs.

This study showed that *N. caninum* is present in dairy herds of Lages Municipality, Santa Catarina State with a prevalence of 23.1%, which is within the range found in other important regions of cattle production in Brazil. Although age did not affect the presence of the protozoan ($P > 0.05$), the high percentage of positive cows suggest horizontal transmission of the agent inside the evaluated herds. The prevalence of antibodies against *N. caninum* was not associated with the existence of bovine reproductive disorders and the presence of dogs positive for *N. caninum*

did not increase the risk factor for bovine neosporosis in the evaluated dairies.

SUMMARY

Bovines are the main intermediary host of the protozoan *Neospora caninum,* which is a major cause of bovine abortions and neonatal mortality worldwide. Sera were collected from 373 dairy cattle and 33 dogs on 19 dairy farms in Lages city, Santa Catarina State, Brazil, to determine the prevalence of *N. caninum* antibodies and risk factors. Tests for *N. caninum* antibodies were done using an indirect immunofluorescent antibody test (IFAT). Positive reactions with titers ≥1:200 (cattle) and ≥1:50 (dogs) were found in 86 (23.1%) and seven (21.2%) bovines and dogs, respectively. Of the bovines, four (1.1%) were calves, 33 (8.8%) were heifers, 314 (84.2%) were lactating cows, 19 (5.1%) were dry cows and three (0.8%) were bulls. Infection was primarily found in animals with low milk production (P = 0.008). The presence of seropositive dogs on the properties did not affect the prevalence of bovine infection. No correlation between bovine reproductive disorders and the prevalence of *N. caninum* antibodies was observed. Our results showed that *N. caninum* infection is widespread among dairy cattle of Lages, Santa Catarina.

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