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Neospora caninum seroprevalence and risk factors for ewes from Santa Catarina Plateau, Brazil

Soroprevalência de *Neospora caninume* e fatores de risco para ovelhas da região Serrana de Santa Catarina, Brasil

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Abstract

Neospora caninum can cause reproductive problems and economic losses in sheep flock. In order to evaluate *N. canium* seroprevalence and to identify risk factors, blood samples were collected from 1,308 ewes at 92 properties in 30 municipalities of Santa Catarina Plateau, southern Brazil. The antibodies against *N. caninum* were detected by Indirect Immunofluorescence Antibody Test (IFAT, ≥1:50) and the seropositive were titrated in serial twofold up to a maximum dilution. Data concerning animal management and information about reproductive problems were obtained from questionnaires. The results were tabulated and analyzed using the Fisher's exact and Chi-Square tests (P<0.05). Among the 1,308 animals evaluated, 92 (7%) were seropositive to *N. caninum*. The titers observed varied from 1:50 to 1:3200. In 39 (42.4%) properties there was at least one infected animal. Breed (P=0.0249) and contact with cattle (P=0.0491) showed correlation with the prevalence of *N. caninum*. No association was found between *N. caninum* serology and reproductive disorders.
Key words: *Neospora caninum*, ewes, seroprevalence, risk factors

Resumo

A infecção por *Neospora caninum* em ovelhas pode resultar em problemas reprodutivos. Para avaliar a prevalência da infecção por *N. caninum*, identificar fatores de risco e a existência de correlação entre neosporose ovina e desordens reprodutivas, sangue de 1308 ovelhas em idade reprodutiva foram colhidos em 92 propriedades de 30 municípios do Planalto Catarinense. A detecção de anticorpos contra *N. caninum* foi realizada por meio da Reação de Imunofluorescência Indireta (RIFI, ≥1:50) e as amostras positivas foram analisadas em diluições seriadas de base dois até a titulação máxima reativa. Dados referentes ao manejo dos animais e informações acerca de problemas de ordem reprodutiva foram obtidos por meio da aplicação de questionário. Os resultados foram tabulados e analisados estatisticamente por meio dos testes exato de Fisher e de qui-quadrado (P<0,05). Dos 1308 animais avaliados, 92 (7%) foram sororreagentes a *N. caninum*. Os títulos observados variaram de 1:50 a 1:3200. Em 39 (42,4%) propriedades foram identificados ao menos um animal positivo. Raça (P=0,0249) e contato com bovinos (P=0,0491) apresentaram correlação com a prevalência para *N. caninum*. Não foi verificada associação entre sorologia para *N. caninum* e desordens reprodutivas.
Palavras-chave: *Neospora caninum*, ovelhas, soroprevalência, fatores de risco

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Introduction

N. caninum is an obligate intracellular protozoan belonging to the subfamily Toxoplasmatinae, family Sarcocystidae in the phylum Apicomplexa (MUGRIDGE et al., 1999) which has a heteroxenous life cycle. Its definitive hosts are dogs, coyotes, dingoes and wolves (McALLISTER et al., 1998; GONDIN et al., 2004; KING et al., 2010; DUBEY et al., 2011). Cattle, sheep, goats, horses and deer were found among infected intermediate hosts.

The first report of neosporosis in naturally infected sheep was performed by Dubey et al. (1990). Abortion was observed by Dubey and Lindsay (1990) in experimentally infected sheep with *N. caninum* and McAllister et al. (1996) reported a clinical picture similar to sheep toxoplasmosis and bovine neosporosis. The same was described by Buxton et al. (1997) who found IgM and IgG antibodies in the fetuses of sheep inoculated. Buxton et al. (1998) reported fetal death and reabsorption, abortion, stillbirth and the birth of live but infected lambs among sheep inoculated with NC-1 strain *N. caninum* at days 45, 65 and 90 of gestation. Koyama et al. (2001) isolated *N. caninum* from the brain of a pregnant sheep. Bishop et al. (2010) found *N. caninum* DNA in the brain and spinal cord of a sheep with severe lesions (multifocal vasculitis and gliosis). In Brazil, Pena et al. (2007) isolated *N. caninum* from the brain of an infected sheep.

Buxton et al. (2001) showed that primary infection in pregnant sheep resulted in loss of all fetuses while inoculation of sheep before pregnancy did not cause mortality and also conferred protection against subsequent challenge during pregnancy.

Otter et al. (1997) and Helmick et al. (2002) found no association or rare involvement of *N. caninum* abortions in sheep, respectively. Spilovska and Reiterova (2008) concluded that the sporadic occurrence of neosporosis in sheep had low impact on economic and reproductive losses. In Brazil, Romanelli et al. (2007) and Soares et al. (2009) found no relationship between seroprevalence of neosporosis and reproductive problems in sheep.

However, many other researchers have observed a correlation between neosporosis and reproductive disorders in sheep (JOLLEY et al., 1999; KOBAYASHI et al., 2001; HASSIG et al., 2003; WEST et al., 2006; HOWE et al., 2008; ASADPOUR et al., 2013).

There are few published data on the seroprevalence of *N. caninum* in sheep. In Brazil, Aguiar et al. (2004) described 29% of seropositive animals and 60% of the properties had at least one infected animal. Figliuolo et al. (2004) reported 9.2% seropositivity. In 86.6% of the properties, at least one animal was diagnosed positive for *N. caninum*. Vogel et al. (2006) and Romanelli et al. (2007) found 3.2% and 9.5% seropositive animals, respectively. Soares et al. (2009) found at least one animal infected with *N. caninum* in 17.1% of the properties. The seroprevalence was 1.8%. Ueno et al. (2009) observed 8.81% of infected sheep with *N. caninum*. Munhoz et al. (2010) observed 13.91% seropositivity among sheep from 11 properties.

Although some survey regarding neosporosis in sheep have been carried out in Brazil, the state of Santa Catarina lacks epidemiological information about this disease, which should be assessed in sheep flocks, as it is an important activity in the State. The objective of this study was to determine the seroprevalence of *Neospora caninum* between ewes, on reproductive age, from Santa Catarina Plateau, Brazil.

Material and Methods

The sheep flock from Santa Catarina Plateau consists of 74,195 animals (IBGE, 2011). The sampling consisted of 1,308 animals, with an expected prevalence of 12%, 2.5% error and 95% confidence level (EPI-INFO 6.0, 1996).

From april to october of 2012, blood samples were collected from ewes of breeding age (≥18 months) in the plateau of the state of Santa

Catarina. The plateau is located in mountain region of the state of Santa Catarina, in southern Brazil at an approximate altitude of 916 m and with a mean annual temperature of 14.3ºC. It is the most central mesorregion of the state of Santa Catarina, borderline all other mesorregions and consists of thirty municipalities. The samples were tested by IFAT using NC1 strain tachyzoites of *N. caninum* as antigen (DUBEY et al., 1988), according to Conrad et al. (1993) and Paré et al. (1995). Samples that showed titers ≥ 50 were considered positive (JOLLEY et al., 1999) and these were titrated 2-fold up to a maximum dilution. Positive and negative sera were used as controls.

Information about the animals was obtained through questionnaires to the owners concerning animal management, frequency and type of reproductive disorders in the last 12 months, diet and exposure to dogs, cattle and/or other animals. Data was analyzed by Fisher's exact (R DEVELOPMENT CORE TEAM, 2009) and Chi-Square tests ($P<0.05$) in order to correlate the results with the risk factors and/or reproductive disorders.

Results and Discussion

Among 1,308 animals evaluated in this study, 92 (7%) were infected with *N. caninum*. The titers observed were 50 (20), 100 (27), 200 (15), 400 (15), 800 (10), 1600 (3) and 3200 (2). In 39 (42.4%) of the 92 properties assessed at least one animal was positive for *N. caninum* (Table 1), with prevalence rates ranging from zero to 40%, which shows the widespread occurrence of coccidiosis among animals at the Plateau of Santa Catarina. Al-Majali et al. (2008), Aguiar et al. (2004), Figliuolo et al. (2004), Soares et al. (2009), Faria et al. (2010) and Salaberry et al. (2010) found 45.8%, 60%, 86.6%, 17.1%, 53.8% and 83.3% of the properties with animals that tested positive for *N. caninum*, respectively.

The prevalence of *N. caninum* infection observed in this study (7%) was similar to results from other surveys conducted in Brazil (FIGLIUOLO et al., 2004; ROMANELLI et al., 2007; UENO et al., 2009; SALABERRY et al., 2010; MUNHOZ et al., 2010; FARIA et al., 2010; LANGONI et al., 2011; MACHADO et al., 2011; ANDRADE et al., 2012) and in other countries, such as China (HUI, 2008), Czech Republic (BARTOVA et al., 2009) and Spain (PANADERO et al., 2010), with reported seropositivity rates for *N. caninum* ranging from 8.0 to 13.91% in the sheep samples analyzed using ELISA or IFAT.

Lower values were reported by Al-Majali et al. (2008), in Jordan (4.3%, ELISA); Spilovska and Reiterova (2008) in Slovakia (3.7%); Vogel et al. (2006) in Rio Grande do Sul (3.2%, ELISA); Soares et al. (2009) in Mossoró, RN (1.8%, IFAT, 1:50); Suzuki et al. (2011) in Uruguay (0.7%, ELISA); Moraes et al. (2011) in Maranhão (4.69%, IFAT) and Rosa et al. (2011) in Lages, SC (5.83%, IFAT, 1:50).

Aguiar et al. (2004), in Monte Negro–RO, reported 29% of seropositivity (IFAT, 1:50); Andreotti et al. (2009), comparing the ELISA (based on recombinant protein NcSRS2) and IFAT (1:50), found an occurrence of 32% and 30.8%, respectively in a flock of Campo Grande, MS; Abo-Shehada and Abu-Halaweh (2010), in Jordan, identified a prevalence of 63% using ELISA; Rossi et al. (2011), observed a prevalence of 23% by immunoblot among sheep in Uberlândia, MG; Nasir et al. (2012), in Pakistan, found 27.7% of infected animals (ELISA) and Tembue et al. (2011), in Pernambuco, found 64.2% of infected sheep (IFAT, ≥ 1:50), rates which are significantly higher than those usually observed.

Table 1. Seropositivity for *Neospora caninum* (IFAT ≥ 50) in ewes by the municipalities of Santa Catarina Plateau. Lages, SC, 2012.

Municipality	Properties n	Positive Properties n (%)	Samples by municipality	Samples Positive n (%)
Abdon Batista	1	0	4	0
Anita Garibaldi	2	0	29	0
Brunópolis	3	2 (66.6)	38	3 (7.9)
Bocaina do Sul	2	1 (50)	28	1 (3.6)
Bom Jardim da Serra	3	2 (66.6)	38	4 (10.5)
Bom Retiro	3	3(100)	62	6 (9.7)
Capão Alto	3	1 (33.3)	56	2 (3.6)
Campo Belo do Sul	4	2 (50)	61	6 (9.8)
Campos Novos	6	4 (66.6)	112	19 (17)
Celso Ramos	1	0	5	0
Cerro Negro	2	1 (50)	23	1 (4.3)
Correia Pinto	3	1 (33.3)	35	1 (2.9)
Curitibanos	7	4 (57.1)	141	12 (8.5)
Frei Rogério	2	2 (100)	25	3 (12)
Lages	12	3 (25)	198	10 (5.1)
Monte Carlo	1	0	9	0
Otacílio Costa	2	0	5	0
Painel	4	2 (50)	62	4 (6.5)
Palmeira	2	0	20	0
Ponte Alta	3	0	26	0
Ponte Alta do Norte	2	1 (50)	20	1 (5)
Rio Rufino	1	1 (100)	15	6 (40)
Santa Cecília	4	0	48	0
São Cristóvão do Sul	2	0	24	0
São Joaquim	5	4 (80)	95	8 (8.4)
São José do Cerrito	5	2 (40)	70	2 (2.9)
Urubici	3	1 (33.3)	26	1 (3.8)
Urupema	2	0	15	0
Vargem	1	1 (100)	8	1 (12.5)
Zortéa	1	1 (100)	10	1 (10)
Total	**92**	**39 (42.4)**	**1308**	**92 (7)**

An association between the prevalence of *N. caninum* and crossbreed animals (P=0.0249) was observed. Among positives, 64.1% were crossbred and these ewes showed seroprevalence 1.65 times greater (9.9 versus 6,0%) than the animals breed (Table 2). Crossbred sheep totaled 974 samples (74.5%) and they predominate in the area studied, where most of the properties are small family farms and the sheep industry, which is not the main farm activity, is concurrent with other activities. Faria et al. (2010), in the state of Alagoas, found that properties size ≤ 30 ha were identified as a risk factor for infection by *N. caninum* in sheep. In this study, the lowest infection rates were observed in flocks belonging to professional farmers, where they raise purebred animals and the sheep industry plays an important economic role (Table 2). In these properties, animal management is more

appropriate, reducing the contact with sources of *N. caninum* infection. However, Salaberry et al. (2010) found no correlation between breed and *N. caninum* infection and Nasir et al. (2012), on the contrary, found higher infection prevalence in purebred animals.

Table 2. Prevalence for *Neospora caninum* (IFAT, ≥ 50) in ewes from Santa Catarina Plateau. Lages, SC, 2012.

Variable		Animals		Positive[1]		Positive[2]		P
		n	%	n	%	n	%	
Breed	No defined	974	74.5	59	6.0	59	64.1	0.0249
	Defined	334	25.5	33	9.9	33	35.9	
Diet	Pasture	1050	80.3	72	6.8	72	78.2	0.5887
	Supplementation	258	19.7	20	7.8	20	21.7	
Reproductive problems	Yes	381	29.1	15	4.0	15	16.3	0.0041
	No	917	70.1	77	8.4	77	83.7	
	Uninformed	10	0.8	-	-	-	-	
Contact with dogs	Yes	1219	93.2	84	6.9	84	91.3	0.59
	No	89	6.8	8	9.0	8	8.7	
Contact with cats	Yes	999	76.4	76	7.6	76	82.6	0.228
	No	299	22.,8	16	5.3	16	17.4	
	Uninformed	10	0.8	-	-	-	-	
Contact with cattle	Yes	1134	86.7	74	6.5	74	80.4	0.0491
	No	164	12.5	18	11.0	18	19.6	
	Uninformed	10	0.8	-	-	-	-	
Total		**1308**	**100**	**92**	**-**	**92**	**100**	

[1] Relationship between the total number of positive animals per variable and the total number of animals from each variable.
[2] Relationship between total positive animals among each variable and the total number of positive animals.
P: Level descriptive test $\chi 2$.

Although the contact with cattle, which is the natural intermediate host for *N. caninum*, does not necessarily reflect the risk for *N. caninum* infection in sheep, it also showed a correlation with the prevalence of infection (P=0.0491). Presumably, this correlation can also be attributed to management conditions. Most of the seropositive animals (80.4%) were observed in the properties where there was contact between sheep and cattle, which represented 86.7% of the total flocks assessed (Table 2).

No association was found between serology for *N. caninum* and reproductive disorders. Among the 92 seropositive animals, 77 (83.7%) showed no reproductive problems (Table 2). Among animals with reproductive problems (n=381), only 15 (4%) were infected with *N. caninum*. However, infected animals by *N. caninum*, with prevalence rates ranging from 3.3% to 30%, were found in eight of the 24 properties in which reproductive disorders have been reported. Romanelli et al. (2007) and Soares et al. (2009) also found no relationship

between serology for *N. caninum* and reproductive disorders. Salaberry et al. (2010), in Uberlândia, MG found statistically significant differences in flocks with abortions associated with the occurrence of antibodies against *N. caninum*. The same was observed by Machado et al. (2011) in the state of São Paulo. Pinto et al. (2012), in Brazil, observed the involvement of the *N. caninum* in sheep abortion by immunohistochemistry. Abortion in small ruminants is normally associated with *Toxoplasma gondii* and the role of *N. caninum* is uncertain. According to Howe et al. (2012), the role of *N. caninum* in reproductive disorders in sheep seems to depend on a multifactorial approach involving serology and fetal tissues and fluids studies for conclusive diagnosis. The same was observed by Moreno et al. (2012).

There were no statistically significant differences between the 30 municipalities of the Santa Catarina Plateau where samples were taken from or between the properties. However, the highest titres (800 to 3200) were observed in concentrated form in five farms from four municipalities. These properties also showed high seropositivity among animals sampled (22.2% to 40%). However, only one of the five properties had the occurrence of abortions and/or stillbirths reported in the 12 months prior to blood collection.

The contact with dogs showed no association with *N. caninum* seropositive, suggesting that vertical transmission might be more common in the flocks assessed. As in the present study only females of breeding age (≥18 months) were assessed, a higher occurrence of vertical transmission can be inferred but not proven. In goats, the congenital infection perhaps is one of the main routes of *N. caninum* transmission (MESQUITA et al., 2013). Figliuolo et al. (2004), in the state of São Paulo; Soares et al. (2009), in Mossoró, RN; Salaberry et al. (2010), in Uberlândia, MG; Romanelli et al. (2007), in Guarapuava, PR and Moraes et al. (2011), in Maranhão, also did not identify contact with dogs as a risk factor for infection by *N. caninum*. In contrast, Munhoz et al. (2010) found an association between the access of dogs to the viscera of sheep and the slaughter of animals within the property with the seropositivity of the flock. Al-Majali et al. (2008), in Jordan, have identified properties with more than one dog as a risk factor, but this association was not observed in this study even though it was common to all properties assessed. Abo-Shehada and Abu-Halaweh (2010) and Machado et al. (2011) identified the presence of dogs in the flock as a risk factor for infection.

Diet was not associated with *N. caninum* infection, even in animals that received some type of supplementation (corn grain, animal feed and corn silage) which are more susceptible to fecal contamination by dogs. Romanelli et al. (2007) did not find an association with the type of diet, but Moraes et al. (2011), in Maranhão, observed a correlation between seropositivity to *N. caninum* in sheep and diet supplementation.

Conclusions

The present study indicates the presence of *N. caninum* in the sheep flocks of Santa Catarina Plateau and the need to identify the agent in fetal tissues and fluids responsible for ewe abortions to elucidate the role of *N. caninum* in reproductive disorders in this species.

Observations

This work was assessed and approved by the Ethical Committee leading Research with Human (CEPSH) from UDESC, protocol n° 244/2009 on December 17, 2009 and the Ethics Committee in Animal Experimentation (CETEA) from CAV/UDESC protocol n° 1.35/09 on September 29, 2009.

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