

Occurrence of anti-*Neospora caninum* antibodies in dogs in rural areas in Minas Gerais, Brazil

Ocorrência de anticorpos anti-*Neospora caninum* em cães de áreas rurais em Minas Gerais, Brasil

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Abstract

The aim of this study was to determine the frequency of anti-*Neospora caninum* antibodies and risk factors associated with seropositivity in 240 dogs from rural areas of the Lavras, Belo Horizonte and Nanuque regions, state of Minas Gerais, Brazil. The indirect fluorescence antibody test (IFAT) with a cutoff of 50 was used to assess the frequency of seropositive dogs. The risk factor analysis was performed using the Chi-square test (χ^2) and multiple logistic regression. The frequency of seropositivity for *N. caninum* was 15% of the dogs (36/240). Presence of dogs without defined breed ($p = 0.018$; OR = 5.9) and presence of cattle on the farm ($p = 0.053$, OR = 4.3) were associated with *N. caninum* seropositivity. Dogs in the Nanuque region had higher seropositivity for *N. caninum* (29.9%) than did those in the Lavras (6.2%) and Belo Horizonte (2.2%) regions ($p < 0.05$). The presence of seropositive dogs in rural areas of Minas Gerais demonstrates the potential risk of horizontal transmission of *N. caninum* to cattle, especially from dogs without defined breed, which were four times more likely to be seropositive than purebred dogs were.

Keywords: *Neospora caninum*, dogs, Minas Gerais, risk factors, IFAT.

Resumo

O objetivo deste estudo foi determinar a frequência de anticorpos anti-*Neospora caninum* e fatores de risco associados à soropositividade, em 240 cães de áreas rurais, nas regiões de Lavras, Belo Horizonte e Nanuque, Minas Gerais, Brasil. Foi utilizada a reação de imunofluorescência indireta (RIFI), com um ponto de corte igual a 50, para avaliar a frequência de cães soropositivos. A análise dos fatores de risco foi realizada pelo teste de Qui-quadrado (χ^2) e regressão logística múltipla. A frequência de cães soropositivos para *N. caninum* foi de 15% (36/240). A presença de cães sem raça definida (SRD) ($p = 0,018$; OR = 5,9) e bovinos na propriedade ($p = 0,053$; OR = 4,3) foi associada à soropositividade pelo *N. caninum*. A frequência de cães soropositivos foi mais elevada na região de Nanuque (29,9%), quando comparados aos das regiões de Lavras (6,2%) e Belo Horizonte (2,2%) ($p < 0,05$). A presença de cães soropositivos, em áreas rurais de Minas Gerais, demonstra o risco potencial da transmissão horizontal de *N. caninum* para os bovinos, especialmente os cães SRD, que apresentaram quatro vezes mais a chance de serem soropositivos em relação aos cães com raça definida.

Palavras-chave: *Neospora caninum*, cães, Minas Gerais, fatores de risco, RIFI.

Neosporosis is a disease caused by the protozoon *Neospora caninum* (Apicomplexa: Sarcocystidae) that may give rise to severe neuromuscular manifestations (DUBEY et al., 2007). Domestic dogs (*Canis lupus familiaris*) and wild dogs (*C. lupus latrans*, *C. lupus dingo* e *C. lupus*) can be considered to be the definitive hosts for *N. caninum*, and therefore they present a potential risk of transmission to herbivores. This parasite is an

important cause of abortion among production animals, especially cattle (DUBEY; LINDSAY, 1996; McALLISTER et al., 1998; GONDIM et al., 2004; BJÖRKMAN et al., 2010; DUBEY et al., 2011).

Although infection due to *N. caninum* is widely distributed among cattle herds in Minas Gerais (GUEDES et al., 2008), studies on canine infection due to this protozoon in rural areas of this state are still scarce (FERNANDES et al., 2004). Thus, the main objective of the present study was to carry out a serological-epidemiological study on this infection among dogs in rural areas of the state of Minas Gerais and to evaluate risk factors for this infection among these animals.

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The samples tested in this study formed part of a serum bank that had previously been used in other studies on blood parasites in dogs (COSTA-JÚNIOR et al., 2007; 2009). This serum bank was created between April and September 2004, from random collection of 240 blood samples from dogs reared on farms in six municipalities in three distinct regions of the state of Minas Gerais: region of Nanuque (97 dogs) – municipalities of Carlos Chagas and Umburatiba; region of Belo Horizonte (46 dogs) – municipality of Igarapé; and region of Lavras (97 dogs) – municipalities of Carrancas, Cruzília and São Vicente de Minas.

Antibodies in the serum samples were investigated by means of the indirect immunofluorescence antibody test (IFAT), using tachyzoites from *N. caninum* fixed on glass slides (Imunodot Laboratory, Jaboticabal, SP, Brazil) and canine anti-IgG conjugate (Sigma Chemical Co., St. Louis, Missouri, USA). Serum samples in which the tachyzoites presented fluorescence at a dilution of 1:50 were considered to be positive (DUBEY et al., 1988). At the time of blood sample collection, each dog was identified and the owner was interviewed with the aim of gathering information about the farm property: rearing of domestic poultry and/or cattle (without distinction between different breeds or the rearing methods used for these animals) and any presence of wild canids of any type, in contact with the domestic dogs, as well as individual data on the dogs analyzed (rearing methods, breed, sex, age and size).

Associations between seropositivity for *N. caninum* and the variables studied were evaluated by means of the Chi-square (χ^2) or Fisher exact test. The latter was used when the number of observations in at least one cell of the test was less than five. For the significant variables ($p < 0.05$), odds ratios (OR) were calculated with their 95% confidence intervals. Variables that presented $p < 0.20$ in the χ^2 or Fisher exact test were selected for constructing the multiple model, by means of logistic regression, with the aim of ascertaining the adjusted odds ratios. For this, the dependent variable “seropositivity for *N. caninum*” was transformed into a dichotomous variable (0 = negative; 1 = positive). All the analyses were performed using the PASW 18.0 statistical software.

Out of the 240 dog serum samples tested, 15% (36) presented anti-*N. caninum* antibodies. This percentage was lower than what was reported by Fernandes et al. (2004) and Romanelli et al. (2007), respectively, among dogs in rural areas of Uberlândia, MG (21.7%), and Guarapuava, PR (29%); and it was higher than the rate observed by Aguiar et al. (2006) among dogs in rural areas of Monte Negro, Rondônia (12.6%). Compared with urban dogs (GENNARI et al., 2002; GUIMARÃES et al., 2009), those from rural areas tend to present greater seropositivity for *N. caninum*, since they are at greater risk of exposure to the parasite, through ingestion of remains of placentas, aborted fetuses or intermediate hosts such as birds and small mammals (FERNANDES et al., 2004).

The frequencies of seropositive dogs in the Nanuque, Lavras and Belo Horizonte regions were 29.9% (29/97), 6.2% (6/97) and 2.2% (1/46), respectively. Dogs on farms in the Nanuque region presented a 19.2-times greater chance (CI = 2.5-145.9; $p < 0.01$) of seropositivity for *N. caninum* than shown by the dogs in the Belo Horizonte region and a 6.5 times greater chance (CI = 2.5-16.5; $p < 0.01$) than shown by the dogs in the Lavras region. In the Nanuque region, beef cattle farms with extensive rearing schemes and low levels of technification predominated,

thus differing from the Lavras and Belo Horizonte regions, where dairy cattle predominated and there was better zoosanitary control over the herds. This is probably why the dogs on these farms were less infected by *N. caninum*. Aguiar et al. (2006) did not find any significant difference ($p > 0.05$) between the type of farming practiced (dairy, beef or both) and the prevalence of dogs that were seropositive for *N. caninum* in cattle herds in the state of Rondônia. However, because of the large differences in cattle epidemiology and rearing in different regions of Brazil, caution is required in making this type of comparison.

Table 1 presents the results from univariate analysis on the variables and their associations with occurrences of anti-*N. caninum* antibodies. Among the variables tested, the presence of domestic poultry showed associations ($p < 0.05$) with positivity for *N. caninum* (odds ratio = 3.4; 95% CI = 0.99-11.5) and with the variable of no defined breed for the dog ($p < 0.05$) (odds ratio = 5.4; 95% CI = 1.2-23.1). The variables of no defined dog breed, presence of poultry and presence of cattle were included in the multiple logistic regression model, and no defined dog breed ($p = 0.018$; OR = 5.9) and presence of cattle on the farm ($p = 0.053$; OR = 4.3) remained statistically significant in the final model. Cunha Filho et al. (2008) did not find any association ($p > 0.05$) between breed and positivity for *N. caninum* among dogs in rural areas of Pelotas, state of Rio Grande do Sul.

In the present study, it was found that 21% of the dogs belonged to defined breeds (50/240), and Pinscher (60.4%) and Fila Brasileiro (10.4%) were the commonest breeds. The results indicate that in the areas studied, dogs of no defined breed constituted a risk factor for infection by *N. caninum*, since these dogs presented a chance of being seropositive that was almost six times greater than shown by dogs of defined breeds.

The variable of presence of domestic poultry was not significant in the final multiple logistic regression model because of its association with the factor of dog breed, which was seen univariate analysis using the χ^2 test ($p < 0.046$). Hence, the presence of poultry was a confounding variable that was more associated with the presence of dogs of no defined breed than with positivity for *N. caninum*. It is likely that these two variables would characterize smallholdings or family producers, where stray dogs would have easier access and would have a greater possibility of living together with reared hens, which they might eat because of the carnivorous and hunting habits inherent to domestic dogs. Domestic poultry may act as intermediate hosts for *N. caninum* (COSTA et al., 2008; MARTINS et al., 2011) and can easily become infected through their habit of frequently pecking at the ground where they live, thus ingesting sporulated oocysts of this coccid. It is possible that dogs become infected through ingesting tissue from these birds that are infected with the parasite (HEMPHILL; GOTTSTEIN, 2000). However, further studies are needed in order to define the intensity of this association.

The presence of cattle on farms, which did not present any significant association ($p > 0.05$) with the frequency of dogs that were seropositive for *N. caninum* in the univariate model, started to have an association in the multiple model. This was probably because the presence of dogs with no defined breed was related more to the stray habits of these animals, in which they might invade other farms and feed on reproductive material from cattle

Table 1. Occurrences of anti-*Neospora caninum* antibodies (IFAT $\geq 1:50$), according to the variables analyzed, among dogs from rural areas in the Belo Horizonte, Lavras and Nanuque regions, state of Minas Gerais, Brazil, 2004.

Variables		No. of dogs			p value ¹	OR ²
		N	+	%		
Presence of domestic poultry	Yes	189	33	17.5	0.046	3.4
	No	51	3	5.9		(0.99-11.5)
Presence of cattle	Yes	201	34	16.9	0.083	-
	No	39	2	5.1		
Contact between dogs and wild canids	Yes	69	8	11.6	0.348	-
	No	171	28	16.4		
Dog rearing method	Unconfined	225	35	15.6	0.706	-
	Confined	15	1	6.7		
Defined breed	Yes	51	2	3.9	0.013	5.4
	No	189	34	18.0		(1.2-23.1)
Sex	Male	175	29	16.6	0.263	-
	Female	65	7	10.8		
Age	≤ 2 years	119	16	13.4	0.504	-
	> 2 years	121	20	16.5		
Size	Small/medium	176	27	15.3	0.806	-
	Large	64	9	14.1		
Region	Belo Horizonte	46	1	2.2	-	1
	Lavras ³	97	6	6.2	0.429	-
	Nanuque ³	97	29	29.9	0.001	19.2
						(2.5-145.9)

¹Chi-square test; ²Odds ratio (OR) with 95% confidence interval; N = number of dogs analyzed; + = number of positive dogs; ³P_{Lavras-Nanuque} = 0.001 (OR = 6.5; 95% CI = 2.5-16.5).

(placental remains and/or aborted fetuses) on neighboring farms. In other words, dogs of no defined breed could become infected with *N. caninum*, independent of the presence of cattle on the farm where they were in fact reared. This would diminish the effect of the presence of cattle on farms where dogs were diagnosed as seropositive for *N. caninum*. However, after adjustment of the odds ratio by means of the multiple model, “presence of cattle on the farm” became significant, thereby showing that independent of the “presence of dogs of no defined breed” (i.e. probably strays), places where cattle rearing took place presented a higher chance of having dogs infected with *N. caninum*.

The presence of seropositive dogs in rural areas of the state of Minas Gerais demonstrates the potential risk of horizontal transmission of *N. caninum* to cattle reared on farms in the Lavras, Belo Horizonte and Nanuque regions.

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