

Risk factor analysis associated with *Neospora caninum* in dairy cattle in Western Brazilian Amazon

Análises de fatores de risco associado a *Neospora caninum* em gado leiteiro na Amazônia Ocidental Brasileira

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Abstract

Neospora caninum is considered to be one of the main causes of abortion among cattle. The present survey was conducted in the municipality of Rolim de Moura, Rondônia State, Brazil. A questionnaire that investigates the epidemiological aspects of neosporosis was used in the analysis of risk factors associated with the animal-level and herd-level prevalence in dairy cattle. A total of 416 bovine blood samples were collected from 30 farms, and *N. caninum* antibody levels were measured by Indirect Fluorescent Antibody Test (IFAT). Analysis of dairy cattle serum samples revealed the presence of anti-*N. caninum* antibodies to be 47.36% (n = 197). Risk factors associated with *N. caninum* infection were the management system and access locations of dogs. The results of the present survey indicated that infection of dairy cattle with *N. caninum* is widespread in the studied region of Western Amazon, which has implications for prevention and control of neosporosis in this region. Therefore, integrated control strategies and measures are recommended to prevent and control *N. caninum* infection in dairy cattle. In addition, direct contact between dairy cattle, dogs and wild animals, which can influence the epidemiology of neosporosis, should be investigated further.

Keywords: Risk factors, prevalence, neosporosis, Western Amazon, Brazil.

Resumo

A infecção por *Neospora caninum* é considerada uma das principais causas de aborto entre bovinos. Esta pesquisa foi realizada no município de Rolim de Moura, estado de Rondônia, Brasil. Um questionário que investiga os aspectos epidemiológicos da neosporose foi utilizado na análise dos fatores de risco associados à prevalência em animais e em rebanhos. Um total de 416 amostras de sangue bovino foi colhido em 30 fazendas, e os níveis de anticorpos de *N. caninum* foram mensurados pela reação de Imunofluorescência Indireta (RIFI). A análise das amostras mostrou prevalência de anticorpos contra *N. caninum* de 47,36% (n = 197). Os fatores de risco associados à infecção por *N. caninum* foram o sistema de manejo e os locais de acesso dos cães. Os resultados da presente pesquisa indicam que a infecção de bovinos leiteiros com *N. caninum* está disseminada na região estudada da Amazônia Ocidental, o que tem implicações para a prevenção e controle da neosporose nessa região. Portanto, estratégias e medidas de controle integrado são recomendadas para prevenir e controlar a infecção por *N. caninum* em gado leiteiro. Além disso, o contato íntimo entre gado leiteiro, cães e animais selvagens, pode influenciar a epidemiologia da neosporose e deve ser investigada mais detalhadamente.

Palavras-chave: Fatores de risco, prevalência, neosporose, Amazônia Ocidental, Brasil.

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Introduction

Neosporosis is a parasitic disease caused by *Neospora caninum*, a protozoan misdiagnosed because of their morphological similarities (Dubey et al., 1988). *Neospora caninum* is considered to be one of the main causes of abortion among cattle (Almería et al., 2017; Dubey & Schares, 2011; Maldonado Rivera et al., 2020; Reichel et al., 2013). Although this parasite has not been demonstrated as a zoonosis, antibodies to *N. caninum* have been reported in humans (Duarte et al., 2020; Lobato et al., 2006).

In pregnant cows, infection with *N. caninum* can lead to several outcomes, including early foetal death and re-absorption; abortion, stillbirth or parturition of a deformed calf; and birth of clinically normal but infected offspring (Dubey et al., 2006; Favero et al., 2017). Neosporosis is of great economic importance, resulting in huge economic losses for farmers.

The State of Rondônia is located in the Western Amazon and has the second largest cattle herd stock in the North Region, which accounts for the third largest herd in Brazil, with 32 million animals (IBGE, 2016). In Brazil, the presence of *N. caninum* antibodies in cattle has been studied in several areas (Cerqueira-Cézar et al., 2017; Gennari, 2004), but only five of these reports was related to the Amazon region (Aguiar et al., 2006; Benetti et al., 2009; Boas et al., 2015; Silva et al., 2017; Minervino et al., 2008). Of the studies conducted in cattle in the Amazon region, only two were conducted in Rondônia (Aguiar et al., 2006; Boas et al., 2015).

The study of Aguiar et al. (2006) took place in the rural area of Monte Negro, Rondônia, and a *N. caninum* prevalence of 10.4% and 12.6% was detected in cattle and farm dogs, respectively.

Boas et al. (2015) confirmed the presence of anti-*N. caninum* antibodies (10.6%) in cattle from Rondônia State, suggesting the necessity to further investigate the epidemiology of *N. caninum* in the Amazon region.

Cañón-Franco et al. (2003) reported a seroprevalence of 8.3% for *N. caninum* in the urban dog population of Monte Negro municipality, state of Rondônia, suggesting the presence of the agent infecting cattle and other species in the region.

The possibility of transmission of *N. caninum* among wild and domestic animals has been widely discussed. The existence of a so-called wild cycle of neosporosis that may occur between canids and wild herbivores could influence the epidemiology of the disease in domestic livestock (Gondim et al., 2004; Rosypal & Lindsay, 2005).

In the Legal Amazon, the raising of livestock is the activity most strongly correlated with deforestation. Currently, livestock is present both at the consolidated border and in the expansion zones of forest occupation, popularly known as capoeira areas (Rivero et al., 2009). In Rondônia, this intimate contact between dairy cattle, dogs and wild animals could influence the epidemiology of neosporosis.

In this context, the present study aimed to evaluate the current status of the prevalence of *N. caninum* in dairy cattle from the state of Rondônia, Brazil and correlate possible variables associated with seropositivity for these protozoan.

Methods

Ethics statement

All procedures using animals complied with the Ethical Principles in Animal Research adopted by the College of Animal Experimentation and were approved (protocol number PP010/2014) by the Ethical Committee for Animal Welfare, UNIR, Rolim de Moura, Rondônia, Brazil.

Study region

The survey was conducted in Rolim de Moura (11°48'13"S, 61°48'12"W), which extends over 402 km² in Rondônia State, North Brazil (IBGE, 2016). The properties evaluated were classified according to the size: into small (<10 hectares), medium (11-100 hectares) and large (> 100 hectares). The climate is Aw of the Köppen classification (Peel et al., 2007), which is characterized as equatorial with changes to the hot and humid tropical with a well-defined dry season from March to September, minimum temperature of 24 °C and a maximum of 32 °C, with precipitation between high and moderately high (2000 to 2250 mm) and 85% relative air humidity.

The georeferencing (Figure 1) of the visited properties was carried out with a GPS device. The data were then transported to the QGIS Desktop (3.12.3) program (QGIS Development Team, 2020), where they were placed on the Rolim de Moura/RO map. The database modelling and chart plotting stages were carried out at the Institute of Agrarian Sciences, Federal University of the Jequitinhonha and Mucuri Valleys.

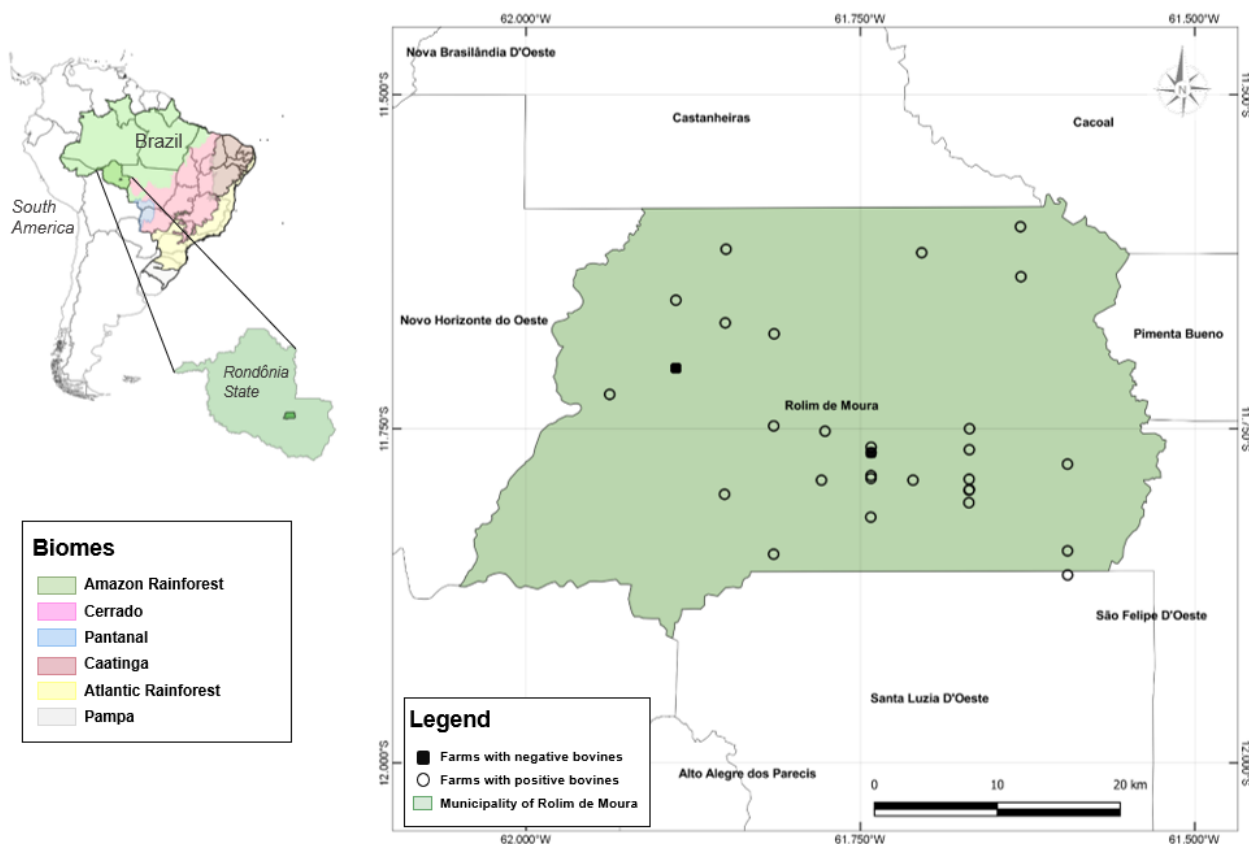


Figure 1. Point locations of dairy herds in the studied subpopulation.

Epidemiological survey

A comprehensive questionnaire investigating the epidemiological aspects of neosporosis was used in the analysis of risk factors associated with the animal-level and herd-level prevalence. The analysed variables and respective categories were as follows: sex, age, animal category, management system (intensive, semi-intensive and extensive), herd size (small: < 100 animals, medium: 100-150 animals and large: > 150 animals), technical monitoring (yes or no), stock up facility for food (yes or no), surface type of milking shed (wooden clapboard or cemented), farm hygienic status (not clean or clean), water source (dam or drinking fountain), feeder (wooden or cemented), presence of wild animals (yes or no), presence of dogs (yes or no), access locations of dogs (pasture or barn), abortion in the last 12 months and stage of gestation that abortions occurred (first, second or third trimester). The main classification criteria for the management system was animal stocking rate (SR: animal unit ha⁻¹, in which an animal unit = 450 kg of body weight): extensive: with pasture support capacity of up to 1.9 AU/ha; semi-intensive: with support capacity between 2 and 3.5 AU/ha and intensive: with support capacity between 3.6 and 7 AU/ha. Furthermore, in the extensive system the animals to lived predominantly pasture feeding management and in the semi-intensive, food management based on grazing, salt mineral and feed supplementation. Intensive system was characterized by a significant investment in buildings, machinery (such as milking parlour with automatic milking facilities) and private pastures (which are cultured and fenced).

Serum samples

The number of samples was calculated assuming that the prevalence of *N. caninum* is approximately 50% to maximize the sample size, obtain a minimal confidence interval (CI) of 95%, and maintain the statistical error

under 1%. Calculations were executed using an EpiInfo program (CDC, version 7.2.0.1), resulting in a sample size of 416 bovines collected from the 30 farms (Thrusfield, 2005). Cattle from each farm were selected randomly using a table of random digits. In the case of cattle, approximately ten percent of animals from each farm were sampled. The sampled animals were crossbred cows, usually derived from the crossing of animals of a purebred European origin (Holstein) with animals of one of the zebu breeds (Gyr) in various degrees of blood. All the animals sampled were clinically healthy. A total of 416 crossbred Holstein cattle blood samples (10 mL) were collected by venocentesis, using identified vacuum tubes, and centrifuged at 1000g for 10 min. The serum was separated and stored at -20°C until analysis.

Serological tests

Neospora caninum antibody levels were measured by Indirect Fluorescent Antibody Test (IFAT), using rabbit anti-bovine IgG conjugate labelled with fluorescein isothiocyanate (Sigma, St. Louis, MO, USA) according to Dubey et al. (1988), with a cut-off value of 1:200 (Benetti et al., 2009; Gennari, 2004). *Neospora caninum* tachyzoites (NC-1) maintained in Vero cell cultures were used as antigens. Positive and negative control sera were added to each slide, and all the positive samples were retested using a two-fold serial dilution.

Determination of prevalence and statistical analyses

Statistical tests were performed using STATA, version 14.1, software (Stata Corp LP, College Station, Texas, USA). The research variables were presented as percentages and 95% CIs for proportion. For inferential statistics, the seroprevalences of *N. caninum* infection were considered as the dependent variables and other factors were considered as the explanatory variables.

Pearson's Chi square (χ^2) or Fisher exact tests were executed to evaluate the differences between groups. To investigate the independent risk factors of each explanatory variable, all variables that showed a p value ≤ 0.25 in the univariate analysis were offered to the multivariate logistic regression model as suggested by (Bendel & Afifi, 1977; Bursac et al., 2008). It is advised to use an initial screening p value cut-off point of 0.25, as more traditional levels such as 0.05 can fail to recognize variables known to be important. The occurrence probability ratio [odds ratio (OR)] and the corresponding 95% CI were calculated by using univariate and multiple logistic regression. A p value < 0.05 was considered as the level of statistical significance for all tests.

Results

Analysis of crossbred Holstein cattle serum samples revealed the prevalence percentages of anti-*N. caninum* antibodies to be 47.36%. Herd-level seroprevalence was of the order of 93.33%. Seroprevalence results of *N. caninum* are summarized by herds in Table 1.

Table 2 shows data according to the animal category. Seroprevalences for *N. caninum* were 77.78% (95% CI: 39.99–97.19) in bulls, 53.06% (95% CI: 38.27–67.47) in heifers, 47.28% (95% CI: 41.46–53.16) in cows and 39.06% (95% CI: 27.10–52.07) in calves. There was no statistically significant association between groups and subgroups of the animal categories ($p > 0.05$).

All the evaluated herds showed the presence of wild animals, domestic dogs and abortion in the last 12 months; therefore, these explanatory variables were not analysed statistically.

Regarding the sex, age, stock up facility for food, water source and stage of gestation that abortions occurred, there were no significant differences ($p > 0.05$) in the seropositivity for neosporosis and no were included in the logistic regression model (Table 3).

We evaluated six (20.0%) small-sized properties, 16 (53.3%) medium-sized properties and eight (26.7%) large-sized properties, with a prevalence rate of 44.4%, 49.6% and 51.1%, respectively, and there was no statistically significant difference ($p > 0.05$).

The data from the univariate analysis is summarized in Table 3, and we can see that the statistically significant ($p < 0.05$) explanatory variables were the management system (OR: 1.68, $p = 0.01$), farm hygienic status (OR: 1.84, $p = 0.01$) and access locations of dogs (OR: 1.92, $p < 0.01$).

Table 1. Detection of anti-*N. caninum* antibodies (IFAT-IgG) in dairy cattle herds from Western Amazon, Brazil.

Herds	Seroprevalence		Total animals	95% CI ^a		
	%	Number of seropositive animals				
1	69.23	9	13	38.60	-	90.9
2	37.04	10	27	19.40	-	57.63
3	42.31	11	26	23.35	-	63.08
4	66.67	16	24	44.67	-	84.37
5	33.33	3	9	7.49	-	70.07
6	88.89	8	9	51.75	-	99.72
7	38.10	8	21	18.11	-	61.57
8	40.00	8	20	19.12	-	63.65
9	60.87	14	23	38.54	-	80.29
10	47.83	11	23	26.82	-	69.41
11	56.25	9	16	29.88	-	80.25
12	69.23	9	13	38.57	-	90.91
13	43.48	10	23	23.19	-	65.50
14	8.33	1	12	0.21	-	38.48
15	50.00	5	10	18.71	-	81.29
16	50.00	7	14	23.04	-	76.96
17	45.45	5	11	16.75	-	76.62
18	42.86	6	14	17.66	-	71.14
19	75.00	6	8	34.91	-	96.81
20	100.00	8	8	63.06	-	100.00
21	58.82	10	17	32.93	-	81.56
22	14.29	1	7	0.36	-	57.87
23	14.29	1	7	0.36	-	57.87
24	11.11	1	9	0.28	-	48.25
25	54.55	6	11	23.38	-	83.25
26	87.50	7	8	47.35	-	99.68
27	66.67	4	6	22.28	-	95.67
28	0.00	0	8	0.00	-	0.00
29	30.00	3	10	6.67	-	65.25
30	0.00	0	9	0.00	-	0.00
TOTAL	47.36	197	416	42.47	-	52.28

^a CI: confidence interval.

The multivariable logistic regression model (Table 3) of the predictors of *N. caninum* seroprevalence was performed in all variables (management system, herd size, technical monitoring, surface type of milking shed, farm hygienic status, feeder and access locations of dogs) with p value ≤ 0.25 in the univariate analysis.

Applying the multivariate logistic regression model, tests of the association between the seroprevalence of *N. caninum* infection and potential predictors showed that the management system (OR: 2.05, 95% CI: 1.19-3.53), access locations of dogs (OR: 2.24, 95% CI: 1.43-3.49), surface type of milking shed (OR: 0.54, 95% CI: 0.31-0.94) and farm hygienic status (OR: 0.54, 95% CI: 0.33-0.89) were significant ($p < 0.05$) independent predictors for *N. caninum* infection (Table 3).

Table 2. Seroprevalence of *N. caninum* in crossbred Holstein cattle from Western Amazon by animal category.

Animal category	Seroprevalence		Total		95% CI ^a	
	%	Number				
Cows*	47.28	139	294	41.46	-	53.16
Pregnant cows [†]	33.33	4	12	9.93	-	65.11
Dry cows [‡]	64.29	9	14	35.14	-	87.24
Lactating dairy cows [†]	46.15	120	260	39.98	-	52.42
Calved cows [†]	75.00	6	8	34.91	-	96.82
Calves*	39.06	25	64	27.10	-	52.07
Female [‡]	47.62	20	42	32.00	-	63.58
Male [‡]	22.73	5	22	7.82	-	45.37
Heifers*	53.06	26	49	38.27	-	67.47
Bull*	77.78	7	9	39.99	-	97.19
Total	47.36	197	416	42.47	-	52.28

^a CI: confidence interval; * Fisher's Exact Test ($\chi^2_{(3)} = 5.61$; $p = 0.13$); [†] Fisher's Exact Test ($\chi^2_{(1)} = 4.97$; $p = 0.17$); [‡] $\chi^2_{(1)} = 3.76$; $p = 0.06$.

Table 3. Univariable analysis and multivariable logistic regression model of the predictors of *N. caninum* seroprevalence among dairy cattle in the Western Amazon, Brazil.

Risk factors	IFAT - IgG		Total = n (%)	Univariable analysis		Multivariable logistic regression	
	Reagent n (%)	Non-Reagent n (%)		OR ^a (95% CI [†])	P-values	AOR ^b (95% CI [†])	P-values
Sex							
female	185 (48)	200 (52)	385 (93)	1.46 (0.65<OR<3.40)	0.32		
male	12 (39)	19 (61)	31 (7)				
Age							
Adult	146 (48)	157 (52)	303 (73)	1.13 (0.72<OR<1.79)	0.58		
Young	51 (45)	62 (55)	113 (27)				
Management system							
Extensive	124 (53)	110 (47)	234 (56)	1.68 (1.11<OR<2.54)	0.01*	2.05 (1.19<OR<3.53)	0.01*
Semi-intensive	73 (40)	109 (60)	182 (44)				
Herd size							
Medium	119 (51)	115 (49)	234 (72)	1.35 (0.81<OR<2.28)	0.23	1.00 (0.60<OR<1.67)	0.99
Large	39 (43)	51 (57)	90 (28)				
Technical monitoring							
No	170 (49)	179 (51)	349 (84)	1.41 (0.81<OR<2.50)	0.21	1.13 (0.63<OR<2.03)	0.67
Yes	27 (40)	40 (60)	67 (16)				
Stock up facility for Food							
No	6 (54)	5 (45)	11 (3)	1.34 (0.34<OR<5.66)	0.76		
Yes	191 (47)	214 (53)	405 (97)				
Surface type of Milking Shed							
Wooden clapboard	14 (61)	9 (39)	23 (12)	1.95 (0.80<OR<4.75)	0.14	0.54 (0.31<OR<0.94)	0.03*
Cemented	75 (44)	94 (56)	169 (88)				
Farm hygienic status							
Not clean	73 (55)	61 (45)	134 (45)	1.84 (1.13<OR<3.00)	0.01*	0.54 (0.33<OR<0.89)	0.02*

^a OR: odds ratio. ^b AOR: adjusted odds ratio. [†] CI: confidence interval. * Significant predictor ($p < 0.05$).

Table 3. Continued...

Risk factors	IFAT - IgG		Total = n (%)	Univariable analysis		Multivariable logistic regression	
	Reagent n (%)	Non-Reagent n (%)		OR ^a (95% CI [†])	P-values	AOR ^b (95% CI [†])	P-values
Clean	65 (39)	100 (61)	165 (55)				
Water source							
Dam	73 (49)	75 (51)	148 (73)	1.30 (0.67<OR<2.54)	0.41		
Drinking fountain	24 (43)	32 (57)	56 (27)				
Feeder							
Wooden	84 (51)	82 (49)	166 (88)	1.79 (0.66<OR<5.20)	0.21	1.62 (0.63<OR<4.22)	0.32
Cemented	8 (36)	14 (64)	22 (12)				
Access locations of dogs							
Pasture	140 (53)	123 (47)	263 (63)	1.92 (1.25<OR<2.94)	< 0.01*	2.24 (1.43<OR<3.49)	< 0.01*
Barn	57 (37)	96 (63)	153 (37)				
Stage of gestation that abortions occurred							
Third third	74 (50)	74 (50)	148 (89)	1.38 (0.47<OR<4.17)	0.52		
Second third	8 (42)	11 (58)	19 (11)				

^a OR: odds ratio. ^b AOR: adjusted odds ratio. [†] CI: confidence interval. * Significant predictor (p < 0.05).

Discussion

In the present study, the animal-level and herd-level seroprevalence of *N. caninum* in dairy cattle was 47.36% and 93.33%, respectively. In Brazil, the seroprevalence of anti-*N. caninum* antibodies in cattle varies from 6.7% to 97.2% (Cerqueira-Cézar et al., 2017). Regarding seroepidemiological studies in dairy cattle carried out in the Amazon region, the prevalence found in this research was higher than the results found by Minervino et al. (2008) in Pará (17.5%; cut-off 1:50); Aguiar et al. (2006) in Rondônia (11.2%; cut-off 1:25); Boas et al. (2015) in Rondônia (10.2%; cut-off 1:100); and similar to those described by Benetti et al. (2009) in Mato Grosso (53.5%; cut-off 1:200). However, these values are not entirely comparable since different cut-offs were applied and IFAT reading is partially subjective (Álvarez-García et al., 2003; Campero et al., 2018; Von Blumröder et al., 2004; Wapenaar et al., 2007).

We observed that *N. caninum* is widely spread in the studied region, with only two farms obtaining negative results; however, we could not exclude the presence of this protozoan in these properties due to the number of samples collected.

The high infection at the herd-level and the similarity with the seroprevalence reported by Cerqueira-Cézar et al. (2017) could indicate that *N. caninum* is endemic in the country. This prevalence may be explained by the hot and humid climate of the Amazon region and high levels of precipitation that average 2000 mm annually, which may contribute to higher viability among oocysts in the environment (Dubey et al., 2007; Moloney et al., 2017).

Milk production in Rondônia (8th place in the ranking of the largest national producers) is an economic activity composed of approximately 32,000 families, the majority of which are small producers (IBGE, 2019). Dairy farming has great social relevance, being one of the rural activities that employs most of the labour among the productive chains in family farming.

Higher *N. caninum* prevalence was detected in bulls in relation to cows. This difference could be related with the constant renewal of the cow herds, while the bulls remained in the herd for a longer period of time than the females. These results are in agreement with those reported by Guimarães et al. (2004).

In this study, we found a prevalence of 39.06% for calves, which can be explained by vertical and horizontal transmissions. For heifers, the prevalence was 53.06%, which could have occurred due to the maintenance of horizontal infection.

Vertical transmission is considered effective in bovines, since it is responsible for maintaining the infection in the herd (Dubey et al., 2006). However, the infection could not be sustained in the herd only with vertical transmission,

since its efficiency is not 100%. Therefore, horizontal transmission also plays a key role in perpetuating the disease in the herds. Without it, the infection levels would tend to reduce over time (French et al., 1999).

All the herds evaluated showed the presence of wild animals and domestic dogs, and these explanatory variables were not analysed statistically. In agreement with several researchers (Cerqueira-Cézar et al., 2017; Corbellini et al., 2006; Hobson et al., 2005; Macchi et al., 2020; Otranto et al., 2003; Ribeiro et al., 2019; Schares et al., 2003, 2004a), an association with the number of dogs, there was no report of differences between the presence and absence of this species.

The prevalence increment association with rise in herd size could be linked to compromised hygienic measures in maintaining adequate waste disposal practices, increased likelihood of more dogs in the vicinity of the farm and/or a tendency towards raising replacement heifers from their own infected stock (Dubey et al., 2007).

The differences in prevalence could be influenced by many factors, such as the method and cut-off used; the number of cows examined; and their health status, age, gender and breed (Bártová et al., 2015). However, it can be influenced by the type of trough, barn floor, cleaning of facilities, and the presence of dogs on agricultural property (Cerqueira-Cézar et al., 2017).

The Western Amazon of Brazil has prevalence values within the range found in other important regions of cattle production in Brazil. In the present study, the management system (OR 1.68, 95% CI: 1.11-2.54), farm hygienic status (OR: 1.84, 95% CI: 1.13-3.00) and access locations of dogs (OR 1.92, 95% CI: 1.25-2.94) were identified as risk factors associated ($p < 0.05$) with the prevalence of *N. caninum* infection in the univariable analysis.

In our study, the hygienic status of the farms was assessed according to floor condition, ventilation, drainage, and waste disposal routines. All these hygienic factors may contribute to maintaining the *N. caninum* cycle by providing canids access to tissue cysts through feeding with infected meat or organs and providing cattle access to oocysts through contamination of feed and water with dog faeces (Dubey et al., 2007; Schares et al., 2004b).

Applying multivariate logistic regression analysis (Table 3), tests of the association between the seroprevalence of *N. caninum* infection and potential predictors revealed a strong association with the management system (OR 2.05, 95% CI: 1.19-3.53) and access locations of dogs (OR 2.24, 95% CI: 1.43-3.49).

Farms with an extensive management system (Table 3) were more than 2.05 (95% CI: 1.19-3.53) at risk of acquiring the infection compared to the farms with a semi-intensive management system. This can be explained by the greater chances of contact with oocysts in the environment when the extensive management system is used. Animals with access to pasture have greater opportunity to infect themselves horizontally, when compared to those raised in the semi-intensive system, that the animals remain part of the day free in pastures and part of the day confined. Thus, an effective management mode is of extreme importance to control the spread of disease. These results are similar to those cited by Macchi et al. (2020), Ragozo et al. (2003), Ribeiro et al. (2019), Silva et al. (2008).

The access of dogs to the pasture presented a significant risk factor (OR: 2.24, 95% CI: 1.43-3.49), influencing the high seroprevalence. This result agrees with Nematollahi et al. (2011) who found that the presence of domestic dogs or dingoes can directly reflect the high rates of neosporosis in these locations. Aguiar et al. (2006) showed contact with forest areas and the presence of dogs were not associated with the coccidian infection.

The presence of dogs on cattle farms has been considered an important risk for cattle infection and abortion by *N. caninum* in different countries (Bartels et al., 1999; Corbellini et al., 2006; Mainar-Jaime et al., 1999; Martins et al., 2012; Paré et al., 1998; Wouda et al., 1999). In addition, Barling et al. (2000) found a significant association between *N. caninum* infection in cattle and the presence of coyotes and grey foxes (*Urocyon cinereoargenteus*) in farms in the United States.

Thus, observation of a higher seroprevalence associated with the access of wild carnivores to cattle ranches in Rondônia suggests that they may be involved in maintaining a wild cycle in this scenario. The apparently higher risk of exposure in the herds with wild carnivore access corroborates reports from Brazil (Martins et al., 2012).

A possible way of infection could be the consumption of water contaminated with *N. caninum* oocysts from the faeces of infected wild or domestic canids, because they may come to the water source region to drink and at the same time defecate in or near it (Sun et al., 2015). Thus, a strict detection system for the drinking water of cattle should be implemented in the study regions.

It is concluded that neosporosis can be one of the possible causes of abortion in dairy cattle in Rolim de Moura, Rondônia. Regarding the distribution in dogs as definitive hosts for the parasite, further studies in dog and cattle are recommended. Neosporosis is of great economic importance, resulting in huge economic losses for farmers.

Conclusion

The results of the present survey indicate that infection of dairy cattle with *N. caninum* is widespread in Rolim de Moura, Western Amazon, which has implications for the prevention and control of neosporosis in this region. Therefore, integrated control strategies and measures are recommended to prevent and control *N. caninum* infections in dairy cattle. In Rondônia, this intimate contact between dairy cattle, dogs and wild animals could influence the epidemiology of neosporosis.

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