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Spatial distribution and evaluation of risk factors for bovine neosporosis in Rondônia, Brazil

[*Distribuição espacial e avaliação dos fatores de risco para neosporose bovina em Rondônia, Brasil*]

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ABSTRACT

Neospora caninum is an important worldwide parasite responsible for causing abortion in animals. Due to limited information on the occurrence of infection by this parasite in the state of Rondônia, Brazil, this study aimed to determine the seroprevalence and identify the risk factors associated with the infection in slaughtered cattle, from 19 municipalities distributed in seven microregions of the state. A total of 494 samples were obtained and subjected to anti-*N. caninum* antibodies, using the Indirect Immunofluorescence Reaction technique. Antibodies were detected in 5.06% (25/494) of the samples, in 30.30% (10/33) of farms, in nine municipalities located in four microregions of Rondônia. Of all the animals analyzed, 4.81% of the females (20/416) and 6.41% of the males (05/78) were seropositive for the parasite, with "abortion in the last 12 months" being considered an important risk factor for the occurrence of infection (OR = 9.54; p = 0.01). The present study points out the prevalence of anti-*N. caninum* antibodies in 5.06% of slaughtered animals and abortion as the main risk factor associated with infection by *N. caninum*, thus contributing to the elucidation of the epidemiology of this protozoan in Rondônia, Brazil.

Keywords: apicomplexa, abortion, *Neospora caninum*, IFAT, seroprevalence

RESUMO

Neospora caninum *é um importante parasito, responsável por causar aborto em animais e distribuído mundialmente. Devido às informações limitadas sobre a ocorrência da infecção por esse parasito no estado de Rondônia, Brasil, este estudo teve como objetivo determinar a soroprevalência e identificar os fatores de risco associados à infecção em bovinos abatidos, oriundos de 19 municípios, distribuídos em sete microrregiões do estado. Um total de 494 amostras foi obtido e submetido à pesquisa de anticorpos anti-*N. caninum, *por meio da técnica de reação de imunofluorescência indireta. Os anticorpos foram detectados em 5,06% (25/494) das amostras, em 30,30% (10/33) das propriedades rurais, de nove municípios, localizados em quatro microrregiões de Rondônia. De todos os animais analisados, 4,81% das fêmeas (20/416) e 6,41% dos machos (05/78) foram soropositivos para o parasito, sendo o "aborto nos últimos 12 meses" considerado um importante fator de risco para a ocorrência da infecção (OR = 9,54; P = 0,01). O presente estudo aponta a prevalência de anticorpos anti-*N. caninum *em 5,06% dos animais abatidos e o aborto como o principal fator de risco associado à infecção por* N. caninum, *contribuindo assim para a elucidação da epidemiologia desse protozoário em Rondônia, Brasil.*

Palavras-chave: apicomplexa, abortamento, Neospora caninum, *RIFI, seroprevalência*

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INTRODUCTION

Neospora caninum is a protozoan that causes neosporosis and has as intermediate hosts ruminants and, mainly, canines as definitive hosts. In 1989, in New Mexico (USA), this protozoan was associated with the occurrence of abortions in cattle (Thilsted and Dubey, 1989). Actually, *N. caninum* infection cause greater economic losses in North America (U$ 852.4 million /year), followed by South America (U$ 239.7 million/year), Australia (U$ 137.5 million/year) and Europe (U$ 68.7 million/year). In Brazil, the economic losses are estimated around U$ 101 million/year in beef cattle and U$ 51.3 million/year in dairy cattle (Reichel *et al*., 2013).

The diagnosis of this protozoan is based on epidemiological evidence (mainly due to the increase in the occurrence of abortions in the herd), followed by serological analysis of the animals searching for anti-*N. caninum* antibodies (Enzyme-linked Immunosorbent Assay - ELISA, Indirect Fluorescence Antibody Test - IFAT). Additionally, the presence of the parasite in host tissues can be confirmed by cytological and histological techniques (immunohistochemistry and conventional histopathological analysis), electron microscopy and molecular methods (conventional PCR, RT-PCR and nested-PCR) (Dubey *et al*., 2017; Barry *et al*., 2019).

In Brazil, earlier studies indicated wide range in *N. caninum* seroprevalence according to the region and the herd type, being the lowest seroprevalence (2.45%) recorded at the state of Mato Grosso and the highest (97.2%) at Minas Gerais (Aguiar *et al*., 2016; Cerqueira-Cézar *et al*., 2017). To our knowledge, in the literature consulted, three studies were registered in the state of Rondônia regarding the occurrence of *N. caninum*. The first one involving beef cattle, dairy, and beef/dairy (Aguiar *et al*., 2006), the second involving only the dairy cattle (Boas *et al*., 2015), and another only dairy cattle (Venturoso *et al*., 2021). Among the infection by *N. caninum* in cattle, the risk factors commonly reported are the presence of canids in the environment, access of these animals to cattle, herd fitness, animal management system, in addition to carcasses, fetuses and fetal attachments of animals left behind to pasture (Fort *et al*., 2015; Ribeiro *et al*., 2019; Snak *et al*., 2018; Spiti *et al*., 2018; Venturoso *et al*., 2021). Due to the few reports in the different microregions of the state of Rondônia and given the importance of the parasite as a cause of abortion and economic losses in the cattle herd, this study was conducted to detect the occurrence of anti-*Neospora caninum* antibodies in cattle and the possible risk factors attributed to the infection caused by this protozoan.

MATERIAL AND METHODS

This study received approval from the Animal Use Ethics Committee of the Federal University of Goiás, Brazil and was conducted in compliance with the ethical principles governing animal experimentation of the Brazilian National Animal Experimentation Control Council (CONCEA).

The state of Rondônia is in the western Brazilian Amazon region, has an area of 237,576 km², predominant tropical climate and average annual temperature between 24-26ºC, characterized by hot and rainy weather. Therefore, to facilitate the interpretation of the spatial distribution results of the registered cases the data were grouped in seven microregions: i. Alvorada d'Oeste (Nova Brasilândia do Oeste), ii. Guajará-Mirim (São Francisco do Guapoé), iii. Cacoal (Castanheiras, Ministro Andreazza, Rolim de Moura, Cacoal), iv. Porto Velho (Buritis, Campo Novo de Rondônia), v. Ariquemes (Ariquemes, Cacaulândia, Machadinho do Oeste), vi. Colorado do Oeste (Colorado do Oeste), vii. Ji-Paraná (Jaru, Nova União, Vale do Paraíso, Theobroma, Ji-Paraná, Ouro Preto do Oeste, Presidente Médici) (Table 1 and Fig. 1).

Table 1. Microregions of the state of Rondônia, their respective county, properties of bovine origin, number of animals and the representativeness in the total sample

Microregions	Counties	Identification of the rural property	N of animals	Representativeness of the total sampled (%)
Alvorada d'Oeste	Nova Brasilândia do Oeste	1	02	0.40
Guajará-Mirim	São Francisco do Guapoé	2	02	0.40
Cacoal	Castanheiras	3	03	0.61
	Ministro Andreazza	4	03	0.61
	Rolim de Moura	5	05	1.01
		6	03	0.61
		7	12	2.43
		8	1	0.20
		9	7	1.42
	Cacoal	10	2	0.40
		11	14	2.83
Porto Velho	Buritis	12	14	2.83
		13	1	0.20
	Campo Novo de Rondônia	14	6	1.21
		15	2	0.40
Ariquemes	Ariquemes	16	01	0.20
	Cacaulândia	17	01	0.20
		18	16	3.24
	Machadinho do Oeste	19	157	31.78
		20	20	4.05
		21	20	4.05
		22	6	1.21
Colorado do Oeste	Colorado do Oeste	23	37	7.49
Ji-Paraná	Jaru	24	02	0.40
	Nova União	25	15	3.04
	Vale do Paraíso	26	07	1.42
		27	02	0.40
	Theobroma	28	03	0.61
		29	02	0.40
	Ji-Paraná	30	56	11.34
	Ouro Preto do Oeste	31	37	7.49
	Presidente Médici	32	03	0.61
		33	32	6.48
Total			494	100

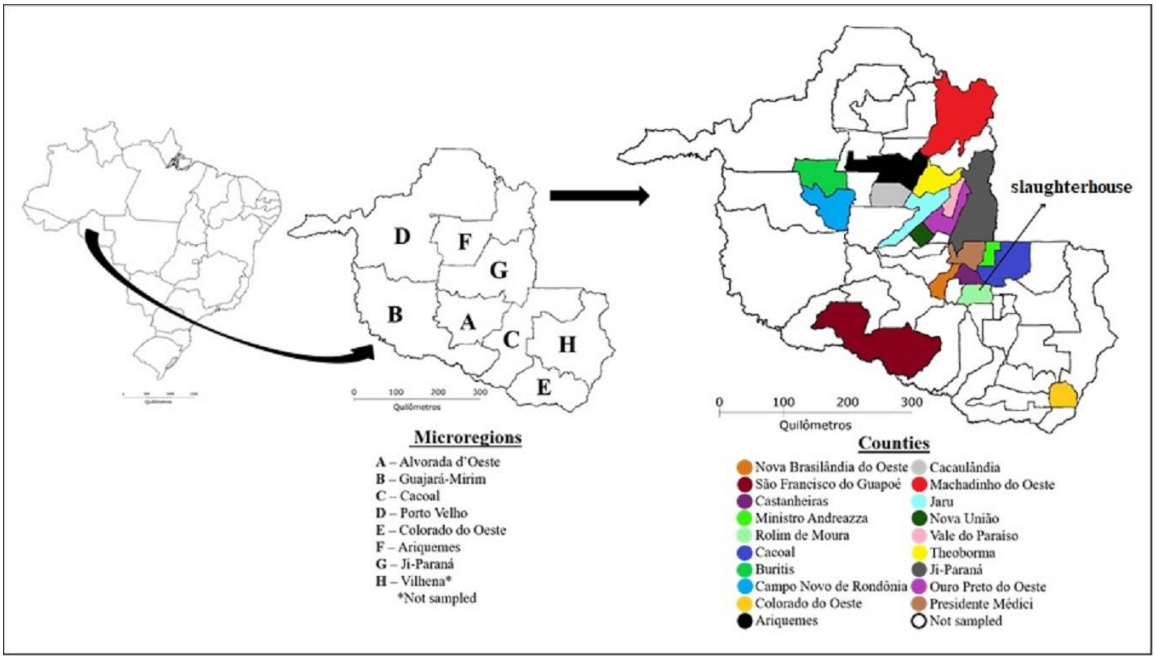

Figure 1. Spatial location of the state of Rondônia, Brazil. Microregions and county of origin of the sampled cattle and slaughterhouse.

The sampling of the present study was estimated through the calculation for infinite sample, using the following parameters: total size of the local population (13510913 cattle); 95% confidence interval and 5% sampling error. In this way, we observed that the minimum sampling of animals necessary to carry out this study was 385 individuals. However, due to the greater availability of samples, we used blood serum from 494 cattle (beef or beef / milk cattle), 78 males and 416 females, randomly selected at the time of slaughter in a slaughterhouse located in the municipality of Rolim de Moura (Fig. 1). The animals came from 33 rural properties within 19 municipalities distributed according to the microregions above mentioned.

A questionnaire was administered to the owner of the selected animals with the following questions: 1) presence of dogs in the property (yes or no); 2) access of the dogs to bovines (yes or no) 3) breeding system (Cow/calf, stocker and feedlot); 4) farm activity (beef, dairy or mixed); 5) bovine gender (female or male); 6) if animals are slaughtered on the property (yes or no); and 7) spontaneous abortion cases in the last 12 months (yes or no), question answered with the aid of reproductive data of the animals stored in each rural property. Data regarding the age, gender and origin of such animals were collected directly from the slaughterhouse.

Blood samples from all animals were obtained during slaughter at the time of bleeding. For this purpose, 10mL of blood were collected from the jugular veins and carotid arteries and placed in sterile plastic tubes (15mL) without the presence of anticoagulant. At a laboratory, to obtain the serological samples, each tube was subjected to centrifugation at 1000g for 10 minutes, after clot retraction. Subsequently, the blood sera were stored in polypropylene tubes and frozen at -20ºC until the moment of the investigation of anti-*N. caninum* antibodies by IFAT.

The serological analyzes were carried out at the Veterinary Parasitology Laboratory of the State University of Londrina, according to the methodology proposed by (Conrad *et al*., 1993). Serum samples were analyzed using IFAT for the detection of IgG class anti- *N. caninum* antibodies, using the cattle anti-IgG conjugate (Sigma Chemical F7887 - FITC) produced in rabbits. All reactions included previously known standard serum samples, both positive and negative. The reading was conducted under a fluorescent microscope and positivity of samples was confirmed when titers ≥100 were obtained. Serum samples considered positive in the initial

dilution (1: 100), were successively diluted, in the ratio of two, to obtain the final titer, being considered positive serological samples in which the tachyzoite emitted total peripheral fluorescence and titers equal to or greater than 100 (Dubey and Lindsay, 1996).

Data were analyzed using Pearson's chi-square test to assess possible risk factors for the occurrence of *N. caninum* in bovines, in case of $p \leq 0.05$ values, odds ratio (OR) estimates were obtained with a confidence interval equal to 95% (IC 95%). The risk factors were calculated considering the risk for herd / property, except for the sex of the animal (calculated from the risk for each bovine), using the R software (R Core Team, 2019).

RESULTS

Data regarding the occurrence of anti-*N. caninum* antibodies and risk factors evaluated are shown in Table 2. Of the 494 serological samples analyzed, anti-*N. caninum* antibodies were detected in 5.06% (25/494) of cattle, with titers 100, 200 and 400, respectively observed in 72% (18 / 25), 20% (5/25) and 8% (2/25) of the positive samples. In 30.30% (10/33) of the sampled rural properties, the presence of seropositive animals for *N. caninum* was detected. Referring to state microregions, in 57.14% (04/07: Porto Velho, Ariquemes, Colorado do Oeste and Ji-Paraná) of the microregions positive animals were observed. Among the 19 municipalities where the bovines originated, 47.36% (9/19) had at least one positive animal. (Fig. 2). In 10 sampled municipalities all bovine sera evaluated were negative for the presence of anti-*N. caninum* antibodies: Ariquemes, Jaru, Nova Brasilândia do Oeste, São Francisco do Guapoé and Theobroma.

Table 2. Rural properties where bovines seropositive for *Neospora caninum* were diagnosed, their respective occurrence and risk factors investigated

Microregions	County	Rural property identification	Occurrence (%)	Presence of dogs	Dog access to cattle	Breeding system	Livestock activity	Sex	Slaughter on the property	Abortion in the last 12 months prior to the study
Porto Velho	Buritis	26	7.14 (01/14)	Yes	Yes	CBF	Meat/milk	Female	Yes	Yes
Ariquemes	Cacaulândia	25	6.25 (01/16)	Yes	Yes	CBF	Meat/milk	Female	Yes	Not
	Machadinho do Oeste	08	6.37 (10/157)	Yes	Yes	CBF	Meat/milk	Female	Not	Yes
		20	10 (02/20)	Yes	Yes	CBF	Meat/milk	Female	Yes	Not
Colorado do Oeste	Colorado do Oeste	15	10.81 (04/37)	Yes	Yes	CBF	Meat	Male	Yes	Not
Ji-Paraná	Nova União	23	6.67 (01/15)	Yes	Yes	CBF	Meat/milk	Female	Yes	Yes
	Vale do Paraíso	31	50 (01/02)	Yes	Yes	CBF	Meat	Male	Yes	Not
	Ji-Paraná	09	1.79 (01/56)	Not	Not	CBF	Meat/milk	Female	Not	Not
	Ouro Preto do Oeste	11	5.41 (02/37)	Yes	Yes	CBF	Meat/milk	Female	Yes	Yes
	Presidente Médici	12	6.25 (02/32)	Yes	Yes	CBF	Meat/milk	Female	Yes	Yes

CBF: cow-calf, background-stocker and finishing operations.

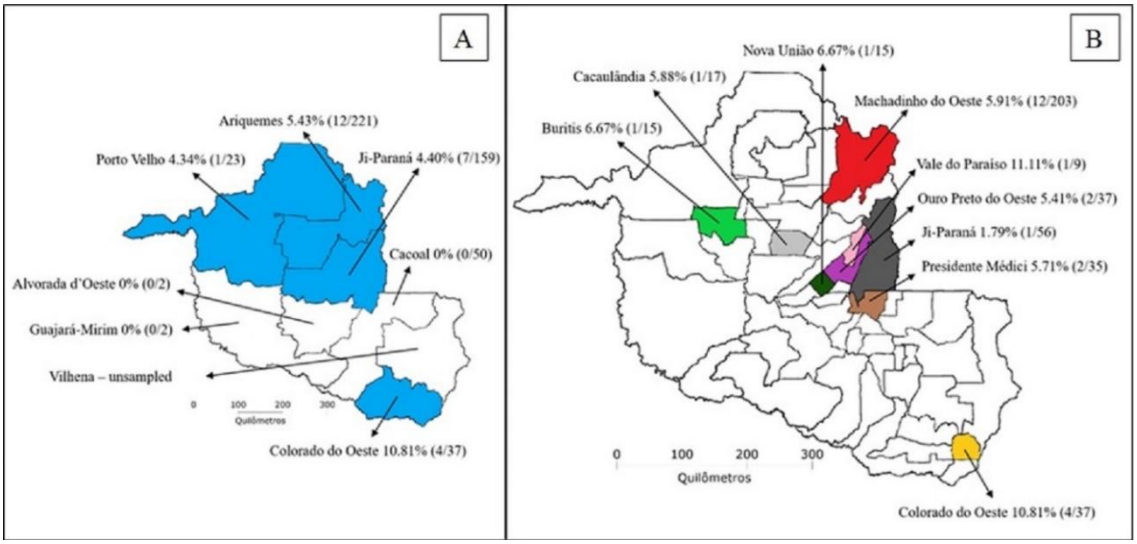

Figure 1**.** Spatial distribution of the occurrence of seropositive cattle for *Neospora caninum* in the microregions (A) and counties (B) of the state of Rondônia, Brazil.

Of the total blood serum analyzed, 84.21% (416/494) were females and 15.79% (78/494) males, of which, 4.81% (20/416) were females and 6.41% (05/78) of males positive for anti-*N. caninum* antibodies. Regarding livestock type, 6.67% (05/75) of animals of beef cattle and 4.77% (20/419) of animals of mixed cattle (beef and milk) were serologically positive for the protozoan. Regarding the herd / property, 12.5% (02/16) of the beef cattle and 47.06% (08/17) of beef/dairy cattle showed seropositive bovines.

Table 3. Risk factors analyzed for the occurrence of *Neospora caninum* in rural properties sampled in the state of Rondônia, Brazil

Risk factors		Total Properties	Property with seropositive animals for *N. caninum*	Property without seropositive animals for *N. caninum*	p* value	Odds ratio	IC (95%)	p** value
Presence of dogs	Yes	31	09	22	1.00	-	-	-
	Not	02	01	01				
Dog access to cattle	Yes	31	09	22	1.00	-	-	-
	Not	02	01	01				
Breeding system	CBF	27	10	17	0.19	-	-	-
	BF	06	0	06				
Livestock activity	Beef	16	02	14	0.07	-	-	-
	Beef/dairy	17	08	09				
Bovine sex	Female	416	20	396	0.75	-	-	-
	Male	78	05	73				
Slaughter on the property	Yes	23	08	15	0.66	-	-	-
	Not	10	02	08				
Abortion in the last 12 months	Yes	07	05	02	0.02	9.54	1.16 - 128.57	0.01
	Not	26	05	21				

*: Pearson's Chi-square test (significance level p≤0.05). **: *Odds ratio* (significance level p≤0.05).
CBF: *c*ow-calf, background-stocker and finishing operations; BF: background-stocker and finishing operations.

When evaluating the risk factors only the variable abortion in the last 12 months was statistically significant (OR = 9.54; CI = 1.16-128-57; p = 0.01) (Fig. 3). The other variables such as presence of dogs in the property, access of the dogs to bovines, breeding system, farm activity and if animals are slaughtered on the property were not a significant risk factor (p > 0.05) for the occurrence of *N. caninum.*

DISCUSSION

The present study provides additional data on the distribution and risk factors for the occurrence of *N. caninum* in the state of Rondônia, northern Brazil. The seroprevalence of *N. caninum* obtained was 5.06% and in four of the seven microregions the presence of animals with antibodies against *N. caninum* was detected, indicating the presence of this parasite in the state.

Earlier studies in Rondônia state verified a higher seroprevalence of *N. caninum* in cattle herd than the obtained in the present study. At the municipality of Ji-Paraná (Boas *et al*., 2015) the seroprevalence was 10.62% (66/621) in dairy cattle, at the municipality of Monte Negro was 10.4% (220/2109) in beef cattle, milk, and dual aptitude (Aguiar *et al*., 2006), and 47,36% (197/416) at the municipality of Rolim de Moura in dairy cattle (Venturoso *et al*., 2021). It is important to highlight that the difference between the seropositivity observed in the present study and those performed in the region can be explained by the different cut-off points adopted in each analysis. Another important factor to be considered is that regardless of the smaller number of animals sampled, the present study differs from these previously reported since it covered a larger number of municipalities and microregions.

Among the variables analyzed, only the occurrence of abortions in the last 12 months was the statistically significant risk factor for the occurrence of *N. caninum*. The other variables analyzed, such as animal origin, sex, presence of dogs on the property, access of dogs to livestock, breeding system, livestock activity, slaughter of animals on the property were not statistically significant in this study.

The presence of abortions in the last 12 months expressed a significant association (OR = 9.54; CI = 1.16-128-57; p = 0.01) with the occurrence of neosporosis, corroborating a previous study carried out in the municipality of Monte Negro, Rondônia (Aguiar *et al*., 2006). *N. caninum* is known to be one of the main causes of abortion, repeat estrus, and births of weak or persistently infected calves (Dubey and Lindsay, 1996; Antoniassi *et al*., 2013). In addition, herds with a history of abortion are more likely to have coccidia seropositive cattle, reinforcing their importance as a cause of abortion (Sun *et al*., 2015; Schmidt *et al*., 2018). This fact was evidenced in the present study, in which rural properties with a history of abortion in the last 12 months prior to the study had a 9.54 times greater risk of having animals positive for anti-*N. caninum* antibodies. It is important to report that infected bovine females do not always present reproductive problems, these animals can produce apparently healthy calves, however infected by the parasite, which become the main responsible for maintaining the agent in the herd (Dubey, 2003; Mazuz *et al*., 2014).

As observed in previous studies, the variable "sex" was not statistically significant (Padilha *et al*., 2017; Udonsom *et al*., 2018; Liu *et al*., 2018). Regarding "livestock activity", properties with beef/dairy herds had a higher occurrence of seropositive cattle than properties with only beef cattle. This result corroborates previous work, where dairy herds were 3.5 times more likely to acquire neosporosis than beef animals (Fort *et al*., 2015; Ribeiro *et al*., 2019). The management practices of dairy farms, such as the intensive rearing system (higher animal density) and greater exposure to the agent due to longer stay on a farm (present in the feces of definitive hosts, carcasses, abortions, and fetal attachments of infected cattle) facilitate the distribution of the parasite in the herd. In addition, heifers from seropositive matrices can be used as breeders, thus maintaining vertical transmission of *N. caninum* (Fort *et al*., 2015).

Although the variable "slaughter of animals on the property" was not significant, the association of this risk factor with the "presence of dogs" that was reported in this study in most of the properties where bovines were diagnosed positive can result in a vertical transmission of the parasite. It is important to note that farms that

leave carcasses of dead animals, abortions, and fetal annexes on pastures, are twice as likely to have seropositive animals in the herd than rural properties where these biological materials are buried or incinerated (Cunha Filho *et al*., 2008; Bruhn *et al*., 2013). Even that, they can be ingested by canids, which are the definitive hosts of the parasite and responsible for the environmental dissemination of oocysts serving as a source of infection for cattle (Schmidt *et al*., 2018). All herds that presented seropositive animals belonged to the system "cow-calf, background-stocker and finishing operations" and thus confirms the vertical transmission of *N. caninum*. Infected females can abort, eliminate contaminated gestational content in the environment or even generate an infected calf, perpetuating the parasite cycle in the herd (Bruhn *et al*., 2013).

The risk factors evaluated in the present study suggested that the maintenance of the parasite in the herds studied is due to the vertical (transplacental) transmission of this agent, since the dog factor was not statistically relevant. However, the presence of dogs with access to cattle and the offering of remains from the clandestine slaughter in natura to these animals on farms must be considered during the elaboration of neosporosis control measures, since these animals are the definitive hosts of the parasite. Therefore, broader studies involving Brazilian herds are needed to determine endemic areas for *N. caninum*, in addition to the need to elucidate the role of definitive wild hosts, to better understand their epidemiology, which is not fully elucidated (Innes, 2007).

In conclusion, based on the data used, serological tests and analysis of risk factors, it is possible to affirm that the parasite is circulating in the region. This is reinforced by the presence of animals positive for anti-*N. caninum* antibodies in more than half of the microregions and in almost 31% of the sampled rural properties, indicating the exposure of these animals to the protozoan. The contact with the parasite was confirmed in 5.06% of the animals, with abortion being an important risk factor for the occurrence of infection in cattle in the state of Rondônia, Brazil.

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