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PARASITOLOGY

Anti-*Neospora caninum* antibodies in beef cattle from the northern region of Paraná state, Brazil

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ABSTRACT: *The presence of anti-Neospora caninum antibodies in beef cattle slaughtered in the northern region of the state of Paraná, Brazil, was evaluated. A total of 401 blood samples were collected; 281 samples from the municipality of Rolândia and 120 from the municipality of Borrazópolis, between April 2015 and November 2016. Of the total samples, 289 were from females and 112 from males, aged one and a half to eight years. Indirect fluorescent antibody test (IFAT) was performed, using a cut-off of 1:100. Variables were tabulated for statistical analyses (Fisher's exact test and chi-square tests, p≤0.05). The analysis showed that of the 401 samples, 37 were positive for N. caninum, indicating a prevalence of 9.2 %, and observed titers were 1:100 (16), 1:200 (14), and 1:400 (7). The variables sex, age, and location did not differ statistically (p>0.05). Our results showed a sero-occurrence of N. caninum in cattle slaughtered in the northern region of the state of Paraná.*
Key words: *Neosporosis, seroprevalence, IFAT, cattle, Paraná state.*

Anticorpos anti-*Neospora caninum* em bovinos de corte da região norte do estado do Paraná, Brasil

RESUMO: *A presença de anticorpos anti-Neospora caninum em bovinos de corte, abatidos na região norte do estado do Paraná, Brasil, foi avaliada. Foram coletadas 401 amostras de sangue, sendo 281 amostras no município de Rolândia e 120 no municipio de Borrazopolis, entre os meses de abril de 2015 e novembro de 2016. Do total de amostras, 289 eram de fêmeas e 112 amostras de machos, na faixa etária de um ano e meio até oito anos de idade. Foi realizada a reação da imunofluorescência indireta (RIFI) utilizando ponte de corte de 1:100. Em seguida, foram tabulados as variáveis para análise estatística (testes exato de Fisher e do qui-quadrado, p≤0,05). A análise mostrou que das 401 amostras, 37 foram positivas para N. caninum, indicando uma prevalência de 9,2 % e os títulos observados foram 1:100 (16), 1:200 (14) e 1:400 (7). As variáveis sexo, idade e local não diferiram estatisticamente (p>0,05). Nossos resultados demonstram uma soro-ocorrência de N. caninum em bovinos abatidos na região norte do Paraná.*
Palavras-chave: *Neospora caninum, prevalência, RIFI, bovino de corte, norte do estado do Paraná.*

INTRODUCTION

Neosporosis, caused by *Neospora caninum*, is one of the major reasons for reproductive failure in cattle and is responsible for fetal mummification, still births, and miscarriages (MARQUES et al., 2011; DUBEY & SCHARES, 2011; IBRAHIM et al., 2012). It has been estimated that the disease is responsible for worldwide economic losses of around 1 billion US dollars per year, and a loss of more than 100 million US dollars in the Brazilian beef industry (REICHEL et al., 2013).

Neospora caninum is prevalent in all continents (DUBEY & SCHARES, 2011), including Brazil, where different studies have been conducted to detect antibodies in beef cattle, with the prevalence ranging from 9.1 % to 97.2 % (HASEGAWA et al., 2004; VIANNA et al., 2008; ANDREOTTI et al., 2010; AMARAL et al., 2012; SILVA et al., 2017). In the state of Paraná, different regions have been studied; the north central and southwest have a prevalence of antibodies against the parasite in beef cattle of up to 30.3 % and 13.2 %, respectively (MARQUES et al., 2011; MOURA et al., 2012; NASCIMENTO et al., 2014); however, different diagnostic techniques and cut-offs were used in these studies, which could explain the difference in prevalence rates. Moreover, seroprevalence of neosporosis may vary according to the type of cattle (beef versus dairy), mainly due to the differences between the production systems (DUBEY & SCHARES, 2011).

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As there are few studies on neosporosis in beef cattle from the northern region of Paraná state, the aim of the present study was to evaluate the occurrence of anti-*N. caninum* antibodies in beef cattle from slaughterhouses in the northern region of Paraná state.

MATERIALS AND METHODS

Location and sampling

The sampling size was determined as 384, using the software EpiInfo, version 6, using a prevalence of 50 % and a 5 % confidence interval. Between April 2015 and November 2016, blood samples from 401 animals were collected, 221 samples from an abattoir in Rolândia and 180 samples from a slaughterhouse in Borrazópolis, both located at northern region of the state of Paraná, Brazil. The samples from beef cattle used in this study were from different municipalities in the northern region of Paraná State, including Borrazópolis, Cambé, Conselheiro Mairink, Floraí, Florestópolis, Guaraci, Ibiporã, Jaguapitã, Kaloré, Lobato, Londrina, Nova Londrina, Rolândia, Sabáudia, Sapopema, and Tamarana. Blood samples were collected in the slaughter line and serum samples stored at -20 °C until serological analysis.

Serology

Serum samples were analyzed using an indirect fluorescent antibody test to detect anti-*N. caninum* antibodies according to the methodology described by CONRAD et al. (1993). Tachyzoites of the Nc-1 strain previously cultivated in Vero cells in our lab were used as the antigen. Serum samples obtained from experimentally infected cattle with *N. caninum* and from healthy cattle were used as positive and negative controls, respectively, and included in all slides. The conjugate used was anti-bovine IgG FITC (whole molecule, Sigma-Aldrich®, St. Louis, Missouri, USA) and serum samples that showed fluorescence through all parasite contours and titers ≥ 100 were considered positive. Positive samples were titrated until the maximum dilution titer was reached.

Statistical analysis

Association between the variables was analyzed using a chi-square test (x^2) with a significance level of 5 % using the Epi Info software, version 6.04b (DEAN et al., 1994). P-values ≤0.05 were considered significant.

RESULTS AND DISCUSSION

Out of 401 analyzed samples, 37 (9.3%) were positive according to IFAT, with titers ranging from 100 to 400 (Table 1). Most of these samples were from the north central region of the state, with 8.8 % of animals positive for *N. caninum* (32/362, including the municipalities of Cambé, Floraí, Florestópolis, Guaraci, Ibipora, Jaguapitã, Lobato, Londrina, Rolândia, Sabáudia, Tamarana, and Kaloré); the Pioneer north, with 14% of animals positive (4/29, including the municipalities of Conselheiro Mairink, and Sapopema) and northwest of Paraná State, with 10% of animals positive (1/10, including the municipality of Nova Londrina).

Previous studies of *N. caninum* seroprevalence performed in Brazil have shown a prevalence ranging from 9.1% to 97.2% (CERQUEIRA-CÉZAR et al., 2017). Although, RAGOZO et al. (2003) in Minas Gerais State and AGUIAR et al. (2006) in Rondônia State observed a similar of prevalence using IFAT of 9.5% and 11.1%, respectively, these studies used a cut-off of 25, which is different to our study. Other studies, also using IFAT and beef cattle, have described higher prevalences, ranging from 29.6% to 62.5%; however, care should be taken when comparing the studies, since the animals were from different regions, and might have had different management, and the cut-off used in each study might have varied (MELO et al., 2006; ANDREOTTI et al., 2010; SILVA et al., 2017). Epidemiological studies have indicated considerable differences in the prevalence of neosporosis between different countries, regions, beef and dairy cattle, and types of production. However, it is difficult to compare these results, mainly due to the differences in serological techniques and cut-offs used (DUBEY & SCHARES, 2011).

Analyzing the results according to sex revealed that 9.7% (28/289) of females and 8.0% (9/112) of males were positive; however, there was no statistical difference (Table 1), which corroborates many seroprevalence studies previously performed (CERQUEIRA-CÉZAR et al., 2017).

The age of animals used in this study ranged from one and a half to eight years old, with most of them less than three years old (Table 1). The age of animals did not statistically influence the occurrence of *N. caninum* antibodies ($p>0.05$); although, the occurrence differed numerically (Table 1). These results do not allow us to identify whether horizontal or vertical transmission is the main route of infection and maintenance of the parasite in beef cattle herds in the region.

In a previous study performed in Pará State, it was not possible to observe a relationship between age or seropositivity and neosporosis (SILVA et al., 2015). In another study performed in Peru, the authors did not find a correlation between age or the origin of the animals and seropositivity

Table 1 – Results of indirect immunofluorescence antibody test (IFAT) for *Neospora caninum* in beef cattle from the north region of the state of Paraná, Brazil.

Variables		--------Animals-------		--------Positive-------		--------Negative------		P-value
		N	%	N	%	N	%	
Municipalities	A	95	23.69	6	6,32	89	93.68	0.6020
	B	10	2.49	1	10.00	9	90.00	
	D	26	6.48	3	11.54	23	88.46	
	E	4	1.00	0	0	4	100	
	F	51	12.72	7	13.73	44	86.27	
	G	3	0.75	0	0	3	100	
	H	37	9.23	6	16.22	31	83.78	
	I	85	21.20	4	4.71	81	95.29	
	F	7	1.75	1	14.29	6	85.71	
	G	14	3.49	0	0	14	100	
	I	18	4.49	2	11.11	16	88.89	
	J	11	2.74	2	18.18	9	81.82	
	L	1	0.25	0	0	1	100	
	C	22	5.49	4	18.18	18	81.82	
	K	7	1.75	0	0	7	100	
	H	10	2.49	1	10.00	9	90.00	
Sex	Male	112	27.93	9	8.04	103	91.96	0.7483
	Female	289	72.07	28	9.69	261	90.31	
Age (years)	<3	307	76.56	30	9.77	277	90.23	0.7056
	3 a 5	73	18.20	6	8.22	67	91.78	
	>5	21	5.24	1	4.76	20	95.24	
Total	-	401	100	37	9.23	364	90.77	-

A: Borrazópolis, B: Cambé, C: Mairink, D: Floraí, E: Florestópolis, F: Guaraci, G: Ibiporã, H: Jaguapitã, I: Kaloré, F: Lobato, G: Londrina, H: Nova Londrina, I: Rolândia, J: Sabáudia, K: Sapopema, L: Tamarana

(p>0.05). Risk factors associated with *N. caninum* infection in cattle were the presence of more than three dogs in the herd, the disposal of animal waste in the environment, and the herd being composed of more than one hundred animals (PORTOCARRERO et al., 2015). MARQUES et al. (2011) analyzed 159 samples obtained from the northern region of Paraná State and observed that the prevalence of *N. caninum* in dairy cattle did not proportionally increase with the age of the infected animals, corroborating the results of the present study.

Some of the municipalities had a prevalence below the average (9.2 %), including the cities Florestópolis, Ibiporã, Londrina, Sapopema, and Tamarana, where no sero-occurrence of the parasite was observed (Table 1). However, no statistical significance was observed. This could be due to the lower number of collected samples. In contrast, in the municipality Sabáudia and Conselheiro Mairink, a high percentage of positive animals was observed. Geographical differences in the distribution of animals infected by *N. caninum* has already been reported in other studies and may be related to the relative abundance of the definitive host (canids) and the type of production (NASIR et al., 2012).

In the present study we did not evaluate reproductive status of the animals. Previous studies have shown that *N. caninum* infection in beef cattle causes economic losses. In Brazil, ANDREOTTI et al (2010) observed that *N. caninum* seroprevalence in animals with disorders during pregnancy was higher than in cows with normal pregnancies, and pregnancy rate for seropositive heifers was lower than for uninfected animals.

CONCLUSION

Our results showed a sero-occurrence of *N. caninum* in beef cattle slaughtered in the northern region of the state of Paraná, showing that epidemiological studies performed in slaughterhouses could be used as a model for regional serum occurrence of *N. caninum* in beef cattle.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

BIOETHICS AND BIOSECURITY COMMITTEE APROVAL

The present study was approved by Animal Ethical Committee of the Universidade Norte do Paraná (n° 007/14).

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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