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Anti-*Neospora caninum* antibodies among dairy cattle in a rural settlement, Paraná, Brazil

Prevalência de anticorpos anti-*Neospora caninum* em bovinos leiteiros de um assentamento rural, Paraná, Brazil

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Abstract

The aim of the present study was to evaluate the seroprevalence of antibodies against *Neospora caninum*, obtained two years apart, among dairy cattle in a rural settlement in southern Brazil. Blood samples from 734 dairy cattle on 41 farms were collected at two different times: in 2012, 406 animals on 30 farms were used; and in 2014, 329 animals on 31 farms. Serum samples were obtained and were used to detect antibodies against *N. caninum*, by means of the indirect fluorescence assay (IFA). Animals with titers ≥ 100 were considered positive. The total serum prevalence of anti-*N. caninum* antibodies was 19.7% (145/736) among all the dairy cattle, comprising 23.1% (94/406) in 2012 and 15.5% (51/329) in 2014. Serum from 91 animals was tested in both trials: 11(12.1%) showed positivity in 2012 and 10 (11%) in 2014. The variables of age, sex and breed did not show any associations with seropositivity. Thus, we showed that the cattle in this settlement presented high levels of antibodies against *N. caninum*, and that IFA showed good efficacy for epidemiological studies.

Key words: Neosporosis. Reproductive problems. Seropositive.

Resumo

O objetivo deste estudo foi avaliar a soroprevalência de anticorpos contra *Neospora caninum* em bovinos de leite de um assentamento rural, região sul do Brasil. Participaram do estudo um total de 734 bovinos de leite, 406 animais de 30 propriedades coletados em 2012, e 329 animais de 31 propriedades em 2014. Amostras de soro foram analisadas por meio da Reação de Imunofluorescência Indireta (RIFI), animais com títulos ≥ 100 foram considerados positivos. A soroprevalência total para anticorpos contra *N. caninum* foi 19,7% (145/736), 23,1 % (94/406) e 15,5% (51/329) em 2012 e 2014, respectivamente. Noventa e um animais tiveram amostras analisadas em ambos estudos, 11(12,1%) evidenciaram positividade em 2012 e 10 (11%) em 2014. Não houveram associações entre as variáveis estudadas (idade, sexo e raças) com a soropositividade para neosporose. Evidenciou-se com o presente estudo um alto nível de anticorpos contra *N. caninum* nos bovinos desse assentamento rural, bem como, a RIFI utilizada mostrou boa eficácia para estudos epidemiológicos.

Palavras-chave: Neosporose. Problemas reprodutivos. Soropositivo.

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Introduction

Neospora caninum is a protozoan parasite with worldwide distribution, and it is an important abortifacient in cattle (DUBEY, 2005). Brazil is a country with approximately 205.3 million cattle, including both dairy and beef cattle (IBGE, 2012), and this is the biggest commercial herd worldwide (ANUALPEC, 2011). The great majority of the herds are raised on pasture with a production of around 9.3 million tons of meat and milk production of around 30 billion liters per year (ANUALPEC, 2011). Thus, considering all these data taken together and assuming that neosporosis causes both direct and indirect losses relating to cattle-rearing (DUBEY et al., 1999) this parasite can be considered to be one of the main agents responsible for economic losses among cattle in Brazil.

The first study on the epidemiology of neosporosis in dairy cattle in Brazil was conducted by Gondim et al. (1999). These authors conducted a seroprevalence study among lactating dairy cows in northeastern Brazil and described an overall prevalence of 14.09% (0 – 23.53%). Additionally, histopathology and immunohistochemistry were used to characterize *N. caninum* as an important abortifacient agent in cattle. Corbellini et al. (2002) correlated bovine abortion with *N. caninum* in southern Brazil.

The aim of the present study was to evaluate the seroprevalence of *N. caninum* among dairy cattle in a rural settlement located in southern Brazil, at two different times: the first in 2012 and the second in 2014.

Materials and Methods

Study location

The present study was conducted in a rural settlement located in the north of the state of Paraná. This settlement has an area of 756 hectares (ha), on which 93 families (369 people) had been settled on plots of 6.05 ha each, thus totaling 93 properties.

Cattle were reared on 56 of these farms. The cattle herd consisted of 1,009 head, with 277 dairy cows, 165 dry cows, 241 heifers, 170 calves up to one year of age, 70 steers, 61 calves and 25 bulls. The main management system for the cattle was semi-extensive. The animals had access to rotational grazing, supplemented with bulk feed at critical times of the year and concentrated diet all year round. The storage facilities and supply of these foods provided access to dogs, rodents and other animals. The population of dogs was 254 animals.

The climate according to Köppen's classification is CFA, i.e. subtropical, characterized by average temperatures in the coldest months < 18 °C (mesothermal) and in the warmer months > 22 °C; hot summers, winters with infrequent frosts and a tendency for rainfall to be concentrated in the summer months, but without any dry season (RITTA NETO, 2010). The average rainfall is about 1600-1800 mm per year and the average annual temperature is 21-22 °C (CAVIGLIONE et al., 2000).

Epidemiological questionnaire

Before the study began, an epidemiological questionnaire was applied to all the properties, to address health management, sex, age, breed, abortion, presence of other animals, and source of water. These questionnaires were filled out through individual interviews in the years 2012 and 2014. On this occasion, an authorization for the use and dissemination of data was obtained from all the producers who participated in this study. This study was approved by the Ethics Committee for Animal Use of the University of the North of Paraná (UNOPAR).

Samples

The number of samples was calculated by means of the Epi Info software, version 6, using a prevalence of 50% with 95% confidence interval.

Cattle blood samples were obtained in 2012 and 2014, two years apart, and 407 and 331 samples were collected, respectively. Blood samples were taken without anticoagulant and were properly identified. After removal of the clot, the serum was identified and stored at -20°C until the serological tests were performed.

Anti-Neospora caninum antibodies detection

To detect anti-*Neospora caninum* antibodies, the indirect fluorescence assay (IFA) was performed in accordance with the technique previously used by Conrad et al. (1993). Animals with titers ≥ 100 were considered positive (CARDOSO et al., 2012).

Statistical analysis

The association between seropositive animals and risk variables was investigated using the chi-square test (X^2) in the Epi Info 6 software, with the statistical significance level taken to be 5%. We calculated the Spearman correlation coefficient through the GraphPad Prism 5.0 software to investigate the relationships involved in the distribution of antibody titers over time.

Results and Discussion

In the present study, 145 (19.7%) out of the 735 animals sampled were considered positive for anti-*N. caninum* antibodies: 94/406 (23.1%) in 2012 and 51/329 (15.5%) in 2014 (Table 1), which showed statistical differences ($\text{OR}=1.49$, $1.01<\text{OR}<2.2$, $p=0.03$). When the prevalence of farms was studied 68.3 (28/41) and 61% (25/41) of farms had at least one animal considered as positive in 2012 and 2014, respectively. This variation of antibody levels was observed previously by Magalhães et al. (2014) in a longitudinal study with cross-breed dairy cattle from northeast Brazil using IFAT to detect IgG antibodies against *N. caninum*.

The seroprevalence observed in the present study (23.1%) are in agreement with the neosporosis seroprevalence from cross-sectional sampling studies on dairy cattle in Paraná state, which has been reported to range from 12 to 33% (LOCATELLI-DITTRICH et al., 2001; RAGOZO et al., 2003; GUIMARÃES JUNIOR et al., 2004; OGAWA et al., 2005;; CAMILLO et al., 2010) and 11.2 to 53.5% in Brazil (GONDIM et al., 1999; MELO et al., 2001; OGAWA et al., 2005; AGUIAR et al., 2006; MUNHOZ et al., 2006; MELO et al., 2006; MINERVINO et al., 2008; BENETTI et al., 2009; MUNHOZ et al., 2009; TEIXEIRA et al., 2010; BRUHN et al., 2013).

The IFA is the technique most commonly used for anti-*N. caninum* antibody detection in Brazil, but there is no consensus about which cutoff to use for determining positivity. Thus, a comparison between laboratory techniques could contribute toward evaluating test performance, and towards harmonizing and standardizing protocols (VAN MAANEN et al., 2004).

Serum from 91 animals was tested in both trials (Table 2): among these, 11(12.1%) showed positivity in 2012 and 10 (11%) in 2014; 73 (80.2%) and 3 (3.3%) remained negative and positive, respectively. A total of seven animals (incidence = 7.7%) that were negative at the first bleeding became positive at the second one, and eight animals (8.8%, all of them had more than 30 months) converted from positive to negative for antibodies. Fluctuating of *N. caninum* antibody levels in naturally infected cows have been described previously (CONRAD et al., 1993; MARQUES et al., 2011; MORE et al., 2009; PARE et al., 1996; STENLUND et al., 1999). Thus, Conrad et al. (1993) urged caution when interpreting the serologic results if they are to be used in strategies for the control of neosporosis in cattle.

Table 1. Outcome of seropositivities for *Neospora caninum* in dairy cattle from farms of a settlement in 2012 and 2014. Paraná, Brazil.

Farms	2012		2014	
	n	Positives- IFA(%)	n	Positives – IFA(%)
1	7	2 (28.57)	3	2 (66.7)
2	9	0	8	1 (12.5)
3	7	1 (14.28)	7	0
4	21	2 (9.52)	18	2 (11.1)
5	4	0	ND	
6	27	6 (22.22)	5	1 (20)
7	45	5 (11.11)	63	10 (16)
8	19	3 (15.78)	8	0
9	16	8 (50)	ND	
10	17	1 (5.8)	5	0
11	10	1 (10)	13	0
12	11	2 (18.18)		
13	2	1 (50)	ND	
14	25	6 (24)	14	2 (14.3)
15	14	9 (64.28)	4	1 (25)
16	12	2 (16.66)	14	1 (7.1)
17	13	2 (15.38)	10	1 (10)
18	22	10 (45.45)	15	2 (13.3)
19	9	1 (11.11)	ND	
20	10	4 (40)	ND	
21	14	1 (7.14)	12	4 (33.3)
22	6	2 (33.33)	ND	
23	5	1 (20)	ND	
24	6	1 (16.66)	12	1 (8.3)
25	12	5 (41.66)	7	0
26	7	6 (85.71)	11	4 (57.1)
27	9	0	6	1 (17)
28	7	5 (71.42)	7	1 (14.3)
29	16	3 (18.75)	ND	
30	24	4 (16.66)	ND	
31			8	0
32			9	3(33.3)
33			2	2 (100)
34			7	2 (28.6)
35			10	2 (20)
36	ND		7	2 (28.6)
37			16	3 (18.8)
38			9	2 (22.2)
39			3	3 (100)
40			7	1 (14.2)
41			9	1 (14)
Total	406	94(23.1)	329	51 (15.5)

ND= Not done.

Table 2. Seroprevalence of antibodies against *Neospora caninum* obtained from dairy cattle in 2012 and 2014. Paraná, Brazil.

Bleed		Positives (%)	Titer variation (IFA)	
1 st year-2012	406	94 (23.1)	100	25,600
2 nd year 2014				
Re-bleed	91*	11 (12.1)	100	800
New cattle	238	40 (16.8)	100	3,200
Total	329	51 (15.5)		
Serologic background*				
	First	Second		
	Neg.	Neg.		73 (80.2)
	Neg.	Pos.		7 (7.7)
	Pos.	Pos.		3 (3.3)
	Pos.	Neg.		8 (8.8)
Total				91 (100)*

* Animals that were re-bled a two years later of the first bleeding.

There is a strong association between seropositivity and abortion among cattle, and high prevalence of anti-*N. caninum* antibodies in the herd increases the risk of abortion at herd level (DUBEY et al., 2007). Corbellini et al. (2002) in Rio Grande do Sul observed that seropositive cows were 3.3 times more likely to abort than seronegative cows. Landmann et al. (2011) in Australia estimated that the risk of miscarriage among seropositive cows was 2.9 to 3.9 times higher than among seronegative cows, and that the likelihood that calves from seropositive mothers might become positive was 3.5 times higher than that of calves from seronegative cows.

In the present study, the variables of age, sex and breed did not show any statistically significant differences in relation to seropositivity (Table 3). Melo et al. (2001), in the state of Minas Gerais,

did not observe any significant association between the distribution of seropositivity and the ages of the groups, which could be explained by vertical transmission. However, Guimarães Junior et al. (2004) found that age was a risk factor for occurrence of anti-*N. caninum* antibodies. Munhoz et al. (2009) examined 563 cows on 57 farms in the state of Rio de Janeiro and observed that the Holstein breed was 2.65 times more likely to be seropositive than zebu breeds.

We were unable to correlate the presence of dogs with seropositive cattle, because there was a huge population of dogs in this settlement (approximately six dogs/farm). Among farms in northern Paraná that produced “type B” milk, Guimarães Junior et al. (2004) showed that the presence of seropositive dogs had a correlation with occurrence of anti-*N. caninum* antibodies.

Table 3. Association between variables and presence of antibodies against *Neospora caninum* from dairy cattle in 2012 and 2014. Paraná, Brazil.

Variables	2012				2014				Total			
	n	+(%)	X ²	p	n	+(%)	X ²	p	n	+(%)	X ²	p
Age ¹												
≤ 12	150	38 (25.3)			10	2 (20.0)			160	40 (25.0)		
13 a 24	80	20 (25.0)	1.27	0.53	21	3 (14.3)	0.18	0.91	101	23 (22.8)	5.1	0.08
≥ 25	176	36 (20.4)			298	46 (15.5)			473	82 (17.3)		
Sex												
Male	49	13 (26.5)	0.17	0.67	34	2 (5.9)	1.92	0.16	83	15 (18.1)	0.07	0.8
Female	357	81 (22.7)			295	49 (16.6)			652	130 (19.9)		
Breed												
Jersey	164	33 (20.1)			120	17 (14.1)			284	50 (17.6)		
Girolando	90	16 (17.8)			74	14 (18.9)			164	30 (18.3)		
Holstein	25	7 (28.0)	8.53	0.07	57	10 (17.5)	1.82	0.77	82	17 (20.7)	5.71	0.22
Cross-breeding	122	38 (31.1)			75	10 (13.3)			197	48 (24.4)		
Others	5	0 (0)			3	0 (0)			8	0 (0)		

Age is in months.

The highest antibody titer observed in the present study was 25,600, in the first trial. However, the majority of the animals showed 100 (45/145, 31%, Table 4). Eighteen animals (19.1%, 18/94) and just four animals (7.8%, 4/51) showed titers ≥

1,600 in 2012 and 2014, respectively. This elevated titers may have been associated with the acute form of infection, but further studies would need to be conducted in order to confirm this hypothesis.

Table 4. Titers of antibodies against *Neospora caninum* obtained by Indirect fluorescence Assay (IFA) from dairy cattle in 2012 and 2014. Paraná, Brazil.

Titers	2012		2014		Total	
	n	%	n	%	n	%
100	33	35.1	15	29.4	45	31.0
200	22	23.4	14	27.4	36	24.8
400	5	5.3	10	19.6	15	10.3
800	16	17.0	8	15.7	24	16.5
1,600	3	3.2	3	5.9	6	4.1
3,200	10	10.6	1	1.9	11	7.6
6,400	2	2.13	0	0	2	1.4
12,800	2	2.13	0	0	2	1.4
25,600	1	1.0	0	0	1	0.7
Total	94	100	51	100	145	100

In summary, we observed a high level of antibodies against *N. caninum* among cattle in this settlement in both years (2012 and 2014). The IFA used here showed good efficacy for epidemiological studies, given that the 91 animals that were retested showed similar prevalences.

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