

Neospora caninum: analysis of reproductive parameters in dairy herds in Brazil

Neospora caninum: análise dos parâmetros reprodutivos em rebanhos leiteiros no Brasil

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Abstract

Neosporosis is one of the most important causes of abortion in both dairy and beef cattle in many countries. The objective of this study was to quantify the effect of *Neospora caninum* seropositivity on reproductive parameters in three dairy herds and the dynamics of anti-*N. caninum* antibodies during gestation in naturally infected cows. Blood samples from all animals were collected nine times on each of the three farms over a two-year period. Serum was tested for antibodies against *N. caninum* using the indirect fluorescent antibody test (IFAT) with a cutoff value of 1:100. The relative risk of abortion between *N. caninum*-seropositive and seronegative cows varied between samplings at all farms, but there was only a statistically significant difference ($P < 0.05$) on Farm III in samplings 4, 7 and 8, with positive animals presenting higher risk of abortion. No significant differences ($P > 0.05$) were found regarding gestational age at abortion, repeated abortion, number of inseminations for conception and calving intervals, between seropositive and seronegative cows on all the farms.

Keywords: *Neospora caninum*. Abortion risk. Repeated abortion. Reproduction parameters.

Resumo

Neosporose é uma das causas mais importantes de abortamento em bovinos leiteiro e de corte em muitos países. O objetivo desse estudo foi quantificar o efeito da soropositividade para *Neospora caninum* nos parâmetros reprodutivos em três rebanhos leiteiros e a dinâmica dos anticorpos anti-*N. caninum* durante a gestação em vacas infectadas naturalmente. Amostras de sangue de todos os animais foram colhidas nove vezes em cada uma das três fazendas dentro do período de dois anos. O soro foi testado para anticorpos contra *N. caninum* usando o teste de reação imunofluorescência indireta (RIFI) com valor de corte de 1:100. O risco relativo de abortamento entre vacas *N. caninum*-soropositivas e soronegativas variou entre as amostragens em todas as fazendas, mas, houve somente diferença estatisticamente significativa ($P < 0,05$) na Fazenda III na amostragem 4, 7 e 8, com os animais soropositivos apresentando maiores risco de abortamento. Nenhuma diferença significativa ($P > 0,05$) foi encontrada em consideração a idade gestacional no abortamento, abortamentos repetidos, número de inseminações por concepção e intervalo entre partos entre vacas soropositivas e soronegativas em todas as fazendas.

Palavras-chave: *Neospora caninum*. Risco de abortamento. Abortamentos repetidos. Parâmetros reprodutivos.

Introduction

Neospora caninum is an apicomplexan parasite that infects a variety of wild and domestic animals¹. Currently, dogs, coyotes, foxes, dingoes and wolves are considered both definitive and intermediate hosts for *N. caninum*^{2,3,4,5,6}.

Neosporosis is one of the most important causes of abortion in both dairy and beef cattle in many countries⁷. Abortion due to *N. caninum* may occur at any time of gestation but the majority of abortions occur

from the fourth to the seventh month of gestation^{8,9,10}, and repeated abortions may occur^{11,12,13}.

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Serological studies have shown that *N. caninum*-seropositive cows are more likely to abort than seronegative cows^{11,14,15,16}, and no effects from *N. caninum*-serostatus on other reproductive performance parameters have been found^{17,18,19}.

The objective of this study was to quantify the effect of *N. caninum* on reproductive parameters in three dairy herds, through analyses on the abortion rate, gestational age at abortion, repeated abortion rates, number of inseminations for conception and calving interval.

Materials and Method

A prospective longitudinal study was carried out on three dairy herds, designated Farms I, II and III, which were located in the municipalities of Caçapava, Pindamonhangaba and Lagoinha, in the state of São Paulo, Brazil. These herds were selected and included in the study because they had at least one *N. caninum* seropositive animal at the first sampling and had a record system for individual zootechnical data. The percentage of *N. caninum*-positive samples at each sampling time ranged on Farm I from 3.32% to 11.71%, on Farm II from 3.90% to 22.06% and on Farm III from 28.57% to 37.10%, as shown by Cardoso et al.²⁰.

On all three farms, the cattle were of Holstein-Friesian crossbreed and were reared in a semi-intensive system, kept on pasture. The newborn calves were usually given the first colostrum, either milked from or by suckling from their dams, within a few hours after birth and they were separated from their dams 12 hours after birth. The calves were kept in pens until weaning at about two months of age, when they were transferred to the young stock area, composed of outdoor pens. Calves older than four months, heifers, milking cows and dry cows were kept on pasture.

Concentrate and mineral supplements were offered in accordance with animal stock type and milk production status. During the rainy season, forage

grass that was produced on the farm was harvested and offered to the cattle in troughs. During the dry season, the animals were fed with corn silage, which was also produced and stored on the farms. All the animals were bred by means of artificial insemination, and pregnancy diagnoses were made on day 40 post-insemination by means of palpation per rectum. The cows and heifers calved all year round and were milked twice per day. All animals were tuberculosis and brucellosis-free, and vaccination programs were followed for prevention of the main bovine diseases, such as brucellosis, leptospirosis, IBR/BVD, clostridiosis and rabies. Birds, chickens, rodents, feral and domestic cats and dogs were reported to have contact with the herds.

Blood samples were collected from the coccygeal or jugular vein of the animals. Each farm was visited over a two-year period, at three-month intervals. The samples were collected on Farm I from March 2004 to March 2006, on Farm II from September 2004 to September 2006 and on Farm III from October 2005 to October 2007. It was not possible to collect precolostral samples from the calves. After centrifugation at 1000 x g for 20 min, the serum samples were removed and stored at -20°C until analysis. Serum was tested for antibodies against *N. caninum* by means of the indirect fluorescent antibody test (IFAT), using whole culture-derived tachyzoites (NC-1 strain) as the antigen, with a cutoff value of 1:100²¹. Fluorescein isothiocyanate-conjugated rabbit anti-bovine IgG (Sigma®, St Louis, MO, USA) was used at a dilution of 1:3,000. Positive and negative control samples were added to each slide.

Because precolostral blood samples were not collected, and to minimize the impact of a false-positive or false-negative result, the animals were classified as seropositive if the first two consecutive blood samples were positive and were considered seronegative if the first two consecutive blood samples were negative²². Blood samplings from calves younger than four

months were not used, in order to avoid false positives resulting from the presence of colostral antibodies against *N. caninum*²³.

The following data were recorded for each animal: age, abortion, gestational age at abortion, number of inseminations per pregnant cow, calving interval and repeated abortion (defined as abortion subsequent to a history of previous abortion during the same lactation period). Abortion was defined as fetal death and expulsion at a gestational age of between 42 and 260 days, or presumed if the interval between two consecutive inseminations within the same parity was 55 days¹⁹. The risk of abortion was calculated as the percentage of seropositive/negative animals that aborted. The relative risk (RR) of abortion was calculated as the percentage of seropositive animals that aborted divided by the percentage of seronegative animals that aborted.

For descriptive purposes, univariate statistics were performed using Student's t-test, chi-square test or Fisher's exact test, depending on the type of variable and validation of test assumptions. The statistical analysis was performed using EPIDAT (Pan-American Health Organization, USA) and differences were considered statistically significant when $P < 0.05$. Values were expressed as the mean \pm standard deviation (SD).

Results

On Farm I, a total of 2,280 blood samples were collected from 308 pregnant cows; at Farm II, 985 samples from 128 pregnant cows; and on Farm III, 1,828 samples from 231 pregnant cows. The relative risk (RR) of abortion between *N. caninum*-seropositive and seronegative cows varied between the samplings on all the farms (Table 1). On Farms I and II, the differences were considered to be not statistically significant ($P > 0.05$). On Farm III, the RR was statistically significant ($P < 0.05$) only in relation to samplings 4,

7 and 8, with a higher abortion rate among the seropositive animals.

There was no significant difference ($P > 0.05$) in gestational age at abortion between the *N. caninum*-seropositive and seronegative cows, on all the farms. On Farm I, three seropositive and 45 seronegative pregnant cows suffered abortion. The mean gestational ages at abortion for the seropositive and seronegative cows were 211.75 ± 83.36 days (ranging from 104 to 281 days) and 192.52 ± 83.21 days (ranging from 55 to 294 days), respectively. On Farm II, six seropositive and 14 seronegative pregnant cows aborted and the mean gestational ages at abortion for the seropositive and seronegative cows were 167.71 ± 53.44 days (ranging from 97 to 242 days) and 204.88 ± 74.04 days (ranging from 69 to 288 days), respectively. On Farm III, 31 seropositive and 20 seronegative pregnant cows suffered abortion. The mean gestational ages at abortion for the seropositive and seronegative cows were 162.23 ± 48.61 days (ranging from 79 to 288 days) and 140.59 ± 77.08 days (ranging from 60 to 306 days), respectively.

On Farm I, repeated abortion occurred in 11% (5/52) of the seronegative animals, while no seropositive cows (0/4) presented repeated abortions. On Farm II, repeated abortion occurred in 35.29% (6/17) of the seronegative and 28.57% (2/7) of the seropositive cows (RR 0.89; 95% CI = 0.21-3.08). On Farm III, repeated abortion occurred in 18.18% (4/22) of the seronegative and 32.50% (13/40) of the seropositive cows (RR 1.79; 95% CI = 0.66-4.82). Repeated abortion was not associated with the presence of *N. caninum* antibodies on any of the farms.

No differences ($P > 0.05$) in the number of inseminations for conception were found in relation to the presence of antibodies to anti-*N. caninum* in the cows, on any of the farms. On Farm I, 25 seropositive and 439 seronegative cows were inseminated and the mean numbers of inseminations for conception were, respectively, 1.96 times (SD = 1.02) and 1.87 times (SD

Table 1 – Relative risk of abortion between *N. caninum*-seropositive and seronegative cows at the different blood samplings on Farms I, II and III

Sampling	Status	Farm I				Farm II				Farm III			
		n	Abortion	Risk (%)	Relative Risk (95% CI)	n	Abortion	Risk (%)	Relative Risk (95% CI)	n	Abortion	Risk (%)	Relative Risk (95% CI)
1	Positive	10	1	10.00	2.32 (0.31-17.42)	2	0	0.00	nd	44	5	11.36	1.89 (0.58-6.17)
	Negative	139	6	4.32		57	3	5.26		83	5	6.02	
2	Positive	11	0	0.00	nd	8	1	12.50	3.00 (0.31-29.35)	44	5	11.36	3.33 (0.83-13.31)
	Negative	148	8	5.41		48	2	4.17		88	3	3.41	
3	Positive	11	0	0.00	nd	8	1	12.50	2.13 (0.25-18.01)	38	4	10.53	2.34 (0.62-8.88)
	Negative	146	4	2.74		51	3	5.88		89	4	4.49	
4	Positive	0	0	nd	nd	10	2	20.00	8.60 (0.86-85.75)	39	6	15.38	3.73 (1.11-12.50)*
	Negative	128	7	5.47		43	1	2.33		97	4	4.12	
5	Positive	7	1	14.29	2.84 (0.40-20.00)	8	1	12.50	2.94 (0.30-28.73)	33	2	6.06	1.84 (0.32-10.52)
	Negative	139	7	5.04		47	2	4.26		91	3	3.30	
6	Positive	7	2	28.57	4.29 (1.15-15.97)	10	1	10.00	1.60 (0.18-13.85)	38	3	7.89	nd
	Negative	150	10	6.67		48	3	6.25		76	0	0.00	
7	Positive	5	0	0.00	nd	12	0	0.00	nd	42	7	16.67	6.67 (1.45-30.68)*
	Negative	143	3	2.10		48	2	4.17		80	2	2.50	
8	Positive	4	0	0.00	nd	11	1	9.09	3.82 (0.26-56.31)	48	8	16.67	14.50 (1.87-112.50)*
	Negative	137	7	5.11		42	1	2.38		87	1	1.15	

nd = non-determinate; n = number of animals

* $P < 0.05$

= 1.33). On Farm II, 21 seropositive and 116 seronegative cows were inseminated and the mean numbers of inseminations for conception were, respectively, 2.10 times (SD = 1.18) and 2.42 times (SD = 1.67). On Farm III, 110 seropositive and 203 seronegative cows were inseminated and the mean numbers of inseminations for conception were, respectively, 2.49 times (SD = 2.22) and 2.24 times (SD = 1.99).

No significant differences ($P > 0.05$) in calving interval were found in relation to the presence of antibodies to anti-*N. caninum*. On Farm I, 11 seropositive and 188 seronegative cows calved during the study, and the calving intervals for the seropositive and seronegative cows were, respectively, 393.27 days (SD = 47.68) and

420.77 days (SD = 98.46). On Farm II, 11 seropositive and 76 seronegative cows calved during the period, and the calving intervals for the seropositive and seronegative cows were, respectively, 529.36 days (SD = 129.08) and 464.13 days (SD = 99.94). On Farm III, 52 seropositive and 136 seronegative cows calved during the study, and the calving intervals were, respectively, 407.25 days (SD = 113.69) and 412.29 days (SD = 142.21).

Discussion

A more definitive diagnosis can be achieved when the reproductive problem (abortion, stillbirth or neonatal mortality) is examined in such a way that all the

dams at risk are included or the examination is made at herd level. To clarify the reason for an abortion problem in a herd, a seroepidemiological approach has been proposed by Thurmond and Hietala²⁴. The idea is to use statistical methods to determine whether the proportion of seropositivity among aborting cows is higher than among non-aborting cows (i.e. to determine whether abortion is statistically associated with seropositivity for *N. caninum*). It must be stressed that only serological results that have been obtained from dams at risk (i.e. the dams that were pregnant during the period of time when the abortion problem occurred) should be included in the analysis.

The present study analyzed the risk in which pregnant cows might suffer an abortion, with data obtained on eight occasions, every three months over a two-year period. Thus, this study differed from previous studies that sampled only on one occasion^{11,14,15}. *N. caninum*-seropositive cows only presented greater risk of suffering abortion than the seronegative cows at three of the measurement times, on one of the three farms studied. Similar results were shown by Pfeiffer et al.¹⁶, who correlated the risk of abortion due to neosporosis at only one of the nine times analyzed. Differences in the risk of abortion in relation to the times analyzed may result from environmental factors that cause stress and suppression of immunity²⁵, thereby giving rise to recurrence of persistent infection, parasitemia and abortion^{11,15,16}.

The present study did not find any significant differences in gestational age at abortion between the animals that were seropositive and seronegative for *N. caninum*. Abortions among cows that were seropositive occurred throughout the pregnancy, at ges-

tational ages ranging from 162.23 to 211.75 days, as also observed in other studies conducted in the USA and Europe^{8,9,26,27}.

Some studies have shown that *N. caninum*-seropositive cows were at higher risk of repeated abortion^{11,12,13}. These authors suggested that *N. caninum*-infected cows that aborted were unable to develop adequate protective immunity and that the immunity provided by an initial infection was insufficient to prevent repeated abortion in mature cows. However, differing from these studies, no association was found in the present study between seropositive and repeated abortion as also observed by Del Fava et al.²⁸.

In this study, there was no difference in the number of inseminations for conception or calving interval in relation to seropositivity to *N. caninum*, as previously demonstrated by López-Gatius, Santolaria and Alméria¹⁷, Romero et al.¹⁸ and Bartels et al.¹⁹.

Conclusions

Serological results from this study showed that abortions were not associated with neosporosis at most of the times analyzed on each farm, thus showing that knowledge of the animals' serological status, together with statistical analysis on the risk of abortion, is necessary. However, abortions associated with *N. caninum* occurred at three times on one farm, thus confirming the importance of this parasite. No differences in gestational age at abortion, repeated abortion, number of inseminations for conception and calving interval were found in relation to seropositivity.

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