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Impact of spontaneous *Neospora caninum* infection on pregnancy loss and subsequent pregnancy in grazing lactating dairy cows

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ABSTRACT

The impact of spontaneous *Neospora caninum* infection on pregnancy loss and subsequent pregnancy in grazing lactating dairy cows was evaluated. Data from 1273 females (878 multiparous and 395 first-calving cows) from six preselected dairy herds were analyzed. Cows were classified as seropositive (SP) (prevalence, 24%; range, 11%–33%) or seronegative (SN) by indirect immunofluorescence detection of antibodies against *N caninum*. Seropostive cows (prevalence, 40.0%) presented higher ($P < 0.001$) incidence of abortion compared with SN cows (prevalence, 4.1%). *Neospora caninum* DNA was detected by real-time polymerase chain reaction in 44.4% of intact aborted fetuses from SP cows, whereas none was found in those aborted from SN cows. The average daily milk production adjusted to 305 days was lower ($P < 0.001$) in SP (22.5 ± 0.3 L/day) than in SN cows (24.8 ± 0.2 L/day). Furthermore, SP cows presented greater occurrence of retained placenta (17.1% vs. 6.0%; $P < 0.001$) and acute postpartum metritis (9.8% vs. 2.4%; $P < 0.001$). Despite similar pregnancy rates after first postpartum artificial insemination (27.6% vs. 31.8%; $P = 0.40$), cumulative pregnancy rates during 300 days in milk (94.7% vs. 98.5%; $P = 0.005$) were greater in SN cows. A reduced ($P = 0.0001$) Cox proportional hazard of pregnancy rate at 300 days in milk and a longer interval from parturition or abortion to conception (median, 111 vs. 101 days) were observed in SP compared with SN cows. Spontaneous *N caninum* infection is a significant contributing factor of pregnancy loss and occurrence of uterine disease (i.e., retained placenta and metritis), negatively affecting subsequent pregnancy in grazing lactating dairy cows.

1. Introduction

Neospora caninum is an obligate intracellular protozoon first isolated in Norwegian dogs [1]. Neosporosis has emerged as a serious disease causing abortions and neonatal mortality in cattle [2]. The dog was identified as the definitive host of *N caninum* when shown to be able to

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eliminate oocysts after experimental inoculation [3]. Infected cows demonstrate prominent transplacental transmission (~95%), and most calves born from infected mothers are clinically normal but are infected for life [2]. Serologic examination is an efficient diagnostic tool [4].

Cows infected with *N caninum* show greater likelihood of pregnancy loss than uninfected ones [5–8]. The gestational age of aborted Neospora-infected fetuses has been reported mostly from the fifth to seventh month of pregnancy [9]. Most studies evaluated the impact of *N caninum* infection in herds with high incidence of abortion, and generally, without a systematic control against other potential infectious agents causing abortion. The potential impact of *N caninum* infection on pregnancy after parturition or abortion in grazing dairy cows is not fully recognized.

The aim of this study was to evaluate the relationship between antibody titers against *N caninum* before pregnancy in grazing dairy cows from herds with a systematic control of other infectious diseases, such as brucellosis, infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), and leptospirosis. Cows were monitored from conception, throughout gestation, until the subsequent pregnancy or culling after parturition or abortion. The impact of spontaneous *N caninum* infection and its prevalence on postpartum alterations (i.e., retained placenta, metritis, and mastitis) and subsequent reproductive performance was analyzed.

2. Materials and methods

The Ethical and Animal Research Committee from the Federal University of Santa Maria (protocol number 45/2009) approved all procedures involving the cows in this study.

2.1. Herd and animals

This study was performed on six commercial dairy farms located in Rio Grande do Sul state, southern Brazil (27°30′–30° South; 51°30′–54°20′ West) from 2008 to 2012. All those were grazing dairy herds with similar genetics and management practices. Average milk production per cow of the entire herd was approximately 7000 kg (~24 L of milk adjusted for 305 days of lactation). All cows were milked twice a day, and daily milk yield was recorded once a week throughout the experiment in all herds.

The productive system could be generally classified as semi-intensive pasture grazing. Stocking rate of the grazing area was approximately 5.5 cows/ha. Pasture was composed of perennial Bermuda grass Tifton 85 (*Cynodon* spp) during late spring and summer (warm season) and *Raygras/Trifolium* spp during fall and winter (cold season). Lactating cows were maintained exclusively on pasture, receiving corn silage and concentrate after each milking to provide nutrition requirements; nutrient requirements of dairy cattle [10]. Concentrate was made of soybean meal (44%–48% crude protein), citrus pulp, rice bran, milled sorghum grain, wheat bran, and a mineral–vitamin premix, containing approximately 20.0% to 22.0% crude protein, 3.5% ether extract, 85.0% dry matter, and approximately 70% total digestible nutrients. The amount of concentrate offered changed according to milk production and stage of lactation. An anionic prepartum supplementation was provided for at least 21 days and a maximum of 30 days, based on the nutrient requirements of dairy cattle (2001) recommendations and urine pH monitored (pH 6.0–6.5 indicating anionic supplementation effectiveness).

A total of 1273 females (878 multiparous and 395 primiparous heifers) were enrolled in the study. A detailed description of the characteristics of each herd is presented in Table 1. Herds had all individual records registered on a computer program (ALPRO herd management system, Tetra Laval Group, Sweden).

Herds were previously selected (42.9%; 6/14) according to the selection process shown in Figure 1. These selection criteria were applied aiming to use only dairy herds without occurrence of infectious diseases, such as brucellosis, tuberculosis, and having a systemic

Table 1
Descriptive analysis of the dairy herds.

End points	Herd						Overall	P value[a]
	A	B	C	D	E	F		
No. of cows	229	228	246	110	203	257	1273	—
Heifers/primiparous, % (n)	37.6 (86)	37.7 (86)	36.2 (89)	28.1 (31)	19.7 (40)	24.5 (63)	31.0 (395)	<0.0001
Seropositives, % (n)	11.4 (26)	22.8 (52)	27.2 (67)	36.4 (40)	24.6 (50)	27.2 (70)	24.0 (305)	<0.0001
Pregnancy loss, % (n)	5.7 (13)	8.8 (20)	12.6 (31)	14.6 (16)	12.1 (26)	21.8 (56)	12.7 (162)	<0.0001
Time of pregnancy loss, days of pregnancy	157.2 ± 11.4	172.5 ± 6.3	167.3 ± 8.0	178.1 ± 7.3	178.9 ± 5.5	170.8 ± 4.2	171.5 ± 2.6	0.44
Retained placenta, % (n)	3.5 (8)	8.3 (19)	8.9 (22)	11.8 (13)	6.4 (13)	13.6 (35)	8.7 (110)	0.002
Metritis, % (n)	0.8 (2)	2.2 (5)	3.7 (9)	3.6 (4)	4.0 (8)	9.7 (25)	4.2 (53)	<0.0001
Culling, % (n)	2.6 (6)	0.9 (2)	3.7 (9)	6.4 (7)	3.9 (11)	4.7 (12)	3.5 (44)	0.10
Daily milk 305 days of production, L/day	23.7 ± 0.3	24.0 ± 0.3	24.5 ± 0.2	24.4 ± 0.5	24.6 ± 0.4	24.7 ± 0.3	24.0 ± 0.1	0.28
First postpartum AI, days	74.5 ± 1.4	75.4 ± 1.5	77.4 ± 1.7	73.4 ± 2.0	73.9 ± 1.6	77.7 ± 1.4	75.7 ± 0.6	0.27
Pregnancy at first postpartum AI, % (n/n)	32.6 (73/224)	29.4 (67/228)	29.5 (71/241)	31.8 (34/107)	28.9 (57/197)	32.9 (83/252)	30.8 (385/1249)	0.89
Days open, days	110.8 ± 3.2	115.1 ± 3.5	117.8 ± 3.8	118.5 ± 6.0	117.4 ± 4.0	125.1 ± 4.0	117.5 ± 1.6	0.15
Pregnancy rate at 300 DIM, % (n)	99.1 (222/224)	98.7 (225/228)	97.9 (236/241)	96.3 (103/107)	97.5 (193/198)	95.6 (241/252)	97.6 (1220/1250)	0.14

Abbreviations: AI, artificial insemination; DIM, days in milk.
[a] Univariable analysis.