Risk of abortion associated with *Neospora caninum* during different lactations and evidence of congenital transmission in dairy cows

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Objective—To examine the relationship between exposure to *Neospora caninum* and abortion in dairy cows during their first, second, third, and fourth or later lactations and to establish the main mode of transmission in female calves from birth until their first pregnancy was terminated by abortion or parturition.

Design—Prospective observational study.

Animals—460 Holstein cows and 79 female calves.

Procedure—Cows were classified as seropositive or seronegative to *N caninum* within 7 days after calving; incidence of abortion was compared between groups during different lactations. Blood samples were collected from female calves before ingestion of colostrum and every 6 months until their first pregnancy was terminated by abortion or parturition; number of seropositive calves was compared between seropositive and seronegative dams.

Results—During the first pregnancy of their second lactation, risk of abortion for seropositive cows was 2.8 times that of seronegative cows. Among 10 calves born to seropositive cows, 4 were classified as seropositive at birth and thereafter. Among 69 calves born to seronegative cows, all were classified as seronegative at birth; 67 calves remained seronegative thereafter.

Conclusions and Clinical Relevance—Exposure to *N caninum* alone was not significantly associated with abortion in cows during the first, third, and fourth or later lactations. Seropositive cows that have aborted previously may have subsequent abortions attributable to *N caninum*. Congenital infection was the main mode of *N caninum* transmission in a cohort of female calves. (*J Am Vet Med Assoc* 2002;221:1742–1746)

Neospora caninum is a protozoan parasite originally identified in dogs but now recognized as an important pathogen associated with abortions in cows[1-18] and occasionally with encephalomyelitis in congenitally infected calves.[19-24] Life cycles and routes of transmission of *N caninum* have not been completely elucidated. Because of taxonomic[25,26] and morphologic similarities with *Toxoplasma gondii*, *N caninum* is believed to have a life cycle similar to that of *T gondii*, for which infection can occur in utero or by ingestion of oocysts in the feces of a definitive host.[27] Despite the recent discovery that dogs can serve as a definitive host for *N caninum*,[28] congenital infection is generally accepted as the primary means of transmission and maintenance of *N caninum* in cattle.[12,13,29-34] During pregnancy, some fetal infections culminate in abortion, whereas most result in a new generation of chronically infected cattle.[12-14,30,31]

In commercial dairy herds, the economic importance of infection with *N caninum* is reportedly attributable to costs associated with abortion,[2,12,35] increased number of culled cows,[35] and decreased milk production.[36,37] In dairy herds in which congenital infection is recognized as the major confirmed method by which infection is maintained in herds, producers and veterinarians are interested in developing strategies of selective culling and replacement for control and eradication of the disease. Knowledge of the risk of abortion associated with *N caninum* is important for culling decisions and to reduce the frequency of new cases. In 1 study[12] conducted in a dairy herd in California, cows congenitally infected with *N caninum* had substantially more abortions, predominantly involving the initial pregnancy and the first pregnancy during their first lactation, compared with noninfected cows; in infected cows that had aborted previously, subsequent abortions were observed during the second lactation. Risk of abortion in cows during their third or later lactations was not assessed. No further studies have examined risk of abortion associated with *N caninum* among cows during their first or later lactations. The objectives of the study reported here were to examine the relationship between exposure to *N caninum* and abortion in dairy cows during their first, second, third, and fourth or later lactations and to establish the main mode of transmission of *N caninum* in a cohort of female calves evaluated from birth until their initial pregnancy was terminated by abortion or parturition.

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Materials and Methods

Study population—Holstein cows and calves from a 700-cow dairy in Florida were used. The dairy had a 23% seroprevalence to *N caninum*.[37] Cows were milked 3 times/d (rolling herd average milk production, approx 24,000 lb). Beginning 60 to 70 days after calving, bovine somatotropin (500 mg)[a] was administered every 2 weeks to all cows. Estrus detection involved visual observation of the cows and use of tail chalk markings. Cows were bred by means of artificial insemination beginning 60 days after parturition by trained farm personnel. Pregnancy diagnosis was performed by the attending veterinarian 42 to 49 days after insemination; additional reproductive examinations were performed transrectally to confirm pregnancy when a cow previ-

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ously confirmed pregnant had signs of estrus and at the time of dry-off. The vaccination program included vaccinations against *Brucella abortus*, 5 *Leptospira* serovars, bovine viral diarrhea virus, infectious bovine rhinotracheitis virus, and parainfluenza-3 virus at 4 to 12 months of age before heifers were bred by means of artificial insemination at 14 to 15 months of age. This dairy did not have a history of presence of dogs; however, many cats (> 20) were used for rodent control. It was selected for the study on the basis of a history of *N caninum* infection and willingness of the owner to participate.

Study design—Cows were used in a previous study[37] of exposure to *N caninum* and its effect on milk production. During January and December 1999, blood samples were obtained from cows within 1 week after calving and tested for *N caninum* antibodies by use of a kinetic ELISA.[38] To accomplish the first objective, cows were enrolled between 42 and 49 days after insemination when pregnancy was diagnosed. Seropositive and seronegative cows were evaluated from conception until pregnancy was terminated by abortion or parturition. The approach to the second objective was to compare the *N caninum* serologic status of calves born to seropositive or seronegative cows. Blood samples were collected from a cohort of 88 female calves at birth (before ingestion of colostrum) and thereafter every 6 months until their first pregnancy was terminated by abortion or parturition.

Detection of *N caninum* antibodies—Blood samples from cows and calves were tested for *N caninum* antibodies by use of a kinetic ELISA.[38] Blood samples were refrigerated until and during transportation to a laboratory at the University of Florida. In the laboratory, samples were centrifuged, and serum was harvested and dispensed into plastic vials with screw caps. Serum samples were properly identified and stored frozen at –20°C until shipped in dry ice via a commercial air carrier to the California Animal Health and Food Safety Laboratory for detection of *N caninum* antibodies. Serum samples with a sample-to-positive control ratio ≥ 0.45 were classified as seropositive, as described elsewhere.[38] The reported sensitivity (proportion of infected cattle with positive results) and specificity (proportion of noninfected cattle with negative results) of the kinetic ELISA are 89% (95% confidence interval, 78 to 95%) and 97% (89 to 99%), respectively.[38] In calves, **gamma-glutamyl transferase (GGT)** activity was determined from precolostral sera by use of a commercially available kit. Calves with GGT values ≥ 30 U/L (which were considered to represent postcolostral values) were excluded from the study. The GGT assay was conducted in a laboratory at the University of Florida Veterinary Medicine Teaching Hospital.

Abortion—In this herd, abortion was diagnosed in cows that had grossly appreciable signs of abortion or a return to service after being confirmed pregnant or in pregnant cows that were found to be nonpregnant during reconfirmation of pregnancy at the time of dry-off. Abortion and pregnancy diagnoses were made by the attending veterinarian, who was not aware of serologic status of cows.

Data collection—For each cow that was pregnant, data were collected, including ear tag number, calving date, serologic status, lactation number, conception date, days from calving to conception, breeding season (summer months: May through September; winter months: January through April and October through December), abortion (yes, no), abortion date, history of previous abortion (yes, no), culling (yes, no), and culling date. Data gathered for each female calf included ear tag number, birth date, serologic status, dam's ear tag number, and dam's serologic status.

Statistical analyses—The null hypothesis that risk of abortion during the first pregnancy associated with *N caninum* was the same among cows during their first, second, third, and fourth or later lactations was tested by use of Cox proportional hazards regression.[39] Records were right-censored when a cow died or was culled or at the time the study was discontinued. In the analysis, exposure status for *N caninum* was the main exposure variable of interest. Additional covariates (breeding season, number of days from calving to conception, and history of abortion in a previous lactation) were included in each model (ie, lactation 1, 2, 3, and 4+) to address possible modifying or confounding effects that these factors might have had on abortion. The continuous variable, days from calving to conception, was categorized into 2 groups on the basis of its frequency of distribution (median). The first pregnancy of each lactation was used to minimize effects of stage of lactation on abortion risk. In the model, adjusted **hazard ratios (HRs)** and 95% **confidence intervals (CIs)** were reported. In this study, the HR was used as an epidemiologic measure of association between a variable (eg, *N caninum* exposure) and the outcome of interest (eg, abortion). An assessed HR > 1.0 indicated that the probability of abortion in seropositive cows was increased, compared with seronegative cows. The upper and lower limits of a 95% CI indicated 95% confidence in the assertion that the true HR was within that interval. If the interval was broad, the precision was low. Fetal survival was characterized by the survivor function, which was the cumulative proportion of fetuses that survived from 42 to 260 days of gestation and was estimated by use of the product-limit method. The proportion that aborted between days 42 and 260 was estimated as 1 minus the survivor function. The attributable proportion was estimated as (HR – 1)/HR and interpreted to represent the proportion of seropositive cows that aborted because of exposure to *N caninum*.[40] For final comparisons, values of $P \leq 0.05$ were considered significant.

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Results

Overall, 460 cows were enrolled in the study; 102 (22%) were classified as seropositive and 358 (78%) as seronegative to *N caninum* **(Table 1)**. Incidence of abortion during the current lactation was higher among seropositive cows (19%), compared with seronegative cows (14%). Proportion of cows that aborted in a previous lactation was higher among seropositive cows (21%), compared with seronegative cows (6%).

Risk of abortion during first pregnancy in first-lactation cows—Of 188 cows, 32 were classified as seropositive to *N caninum* and 156 as seronegative. Six of 32 (19%) seropositive cows aborted during the first pregnancy of the first lactation, and 1 (3%) had a history of abortion during the initial pregnancy. Twenty-

Table 1—Frequency distribution (No. [%]) of pregnant cows classified as seropositive or seronegative to *Neospora caninum*

Variable	Seropositive cows (n = 102)	Seronegative cows (n = 358)
Lactation		
1	32 (31)	156 (43)
2	31 (30)	104 (29)
3	13 (13)	57 (16)
4+	26 (25)	41 (11)
Breeding season		
Winter months	77 (75)	262 (73)
Summer months	25 (25)	96 (27)
Cows that aborted in current lactation	19 (19)	50 (14)
Cows that aborted in a previous lactation	21 (21)	20 (6)
Days from calving to conception*	164 ± 10	183 ± 5

*Data are reported as mean ± SEM.

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three of 156 (15%) seronegative cows aborted during the first pregnancy of the first lactation, and 1 (0.6%) had a history of abortion during the initial pregnancy. Among seropositive cows, 0 of 1 that aborted previously and 6 of 31 (19%) that had not aborted previously aborted during the first pregnancy of the first lactation. The attributable proportion of abortions in the first lactation associated with *N caninum* exposure was 0.23. Among seronegative cows, 0 of 1 that aborted previously and 23 of 155 (15%) that had not aborted previously aborted during the first pregnancy of the first lactation. In the Cox model analysis, interaction terms did not significantly contribute to the model for abortion and were removed from the model ($P > 0.50$). The risk of abortion for seropositive cows was 1.3 times greater than that of seronegative cows, although this difference was not significant ($P = 0.55$; **Table 2**).

Risk of abortion during first pregnancy in second-lactation cows—Of 135 cows, 31 were classified as seropositive to *N caninum* and 104 as seronegative. Nine of 31 (29%) seropositive cows aborted during the first pregnancy of the second lactation, and 7 (23%) had a history of previous abortion. Thirteen of 104 (13%) seronegative cows aborted during the first pregnancy of the second lactation, and 7 (7%) had a history of previous abortion. The attributable proportion of abortions in the second lactation associated with exposure to *N caninum* was 0.64. Among seropositive cows, 2 of 7 that aborted previously and 7 of 24 (29%) that had not aborted previously aborted during the first pregnancy of the second lactation. Among seronegative cows, 1 of 7 that aborted previously and 12 of 97 (12%) that had not aborted previously aborted during the first pregnancy of the second lactation. The risk of abortion for seropositive cows was 2.8 times greater than that of seronegative cows ($P = 0.02$; Table 2). Between 60 and 200 days of gestation, survival of fetuses of seropositive cows decreased relative to that of seronegative cows ($P = 0.02$).

Risk of abortion during first pregnancy in third-lactation cows—Of 70 cows, 13 were classified as seropositive to *N caninum* and 57 as seronegative. Two of 13 seropositive cows aborted during the first pregnancy of the third lactation, and 5 had a history of previous abortion. Nine of 57 (16%) seronegative cows aborted during the first pregnancy of the third lactation, and 2 (4%) had a history of previous abortion. Among seropositive cows, 1 of 5 that aborted previously and 1 of 8 that had not aborted previously aborted during the first pregnancy of the third lactation. Among seronegative cows, 0 of 2 that aborted previously and 9 of 55 (16%) that had not aborted previously aborted during the first pregnancy of the third lactation. The risk of abortion for seropositive cows was 0.7 times that of seronegative cows, although this difference was not significant ($P = 0.70$).

Risk of abortion during first pregnancy in cows during their fourth or subsequent lactations—Of 67 cows, 26 were classified as seropositive to *N caninum* and 41 as seronegative. Two of 26 (8%) seropositive cows aborted during the first pregnancy of their fourth or later lactations, and 8 (31%) had a history of abortion in a previous lactation. Five of 41 (12%) seronegative cows aborted during the first pregnancy of the fourth or later lactations, and 10 (24%) had a history of abortion. Among seropositive cows, 1 of 8 that aborted previously and 1 of 18 that had not aborted previously aborted during the first pregnancy of the fourth or later lactations. Among seronegative cows, 2 of 10 that aborted previously and 3 of 31 (10%) that had not aborted previously aborted during the first pregnancy of the fourth or later lactations. The risk of abortion for seropositive cows was 0.5 times that of seronegative cows, although this difference was not significant ($P = 0.52$).

Evidence of congenital transmission of *N caninum*—For 88 female calves, blood was obtained and serum was initially tested for GGT concentrations. Nine calves had GGT ≥ 87 U/L (which was considered to represent a postcolostral value) and were excluded from the study. Thus, 10 calves born to seropositive cows and 69 calves born to seronegative cows were included **(Table 3)**. Among calves born to seropositive cows, 4 were classified as seropositive at birth (all 4 remained seropositive thereafter except 1 calf that tested negative once at 12 months of age). The ELISA ratio values of the 6 seropositive cows that gave birth to seronegative calves were 0.47, 0.49, 0.49, 0.55, 0.65, and 0.93. The calf born to the seropositive cow with an ELISA value of 0.93 was classified as seronegative at birth but seropositive thereafter. Among calves born to seronegative cows, 69 (100%) were classified as seronegative at birth. All calves remained seronegative thereafter, except 1 calf that tested positive once at 12 months of age (ELISA value, 0.49) and another calf that tested positive at 18 months of age (ELISA value, 0.57); 10 calves died within the first 5 months after birth. One of 79 female calves, born to a seronegative cow and classified as seronegative at birth and thereafter, aborted during the first trimester of the initial and second pregnancies before calving.

Table 2—Risk of abortion (proportion [%]) in cows classified as seropositive or seronegative to *N caninum* during various lactations

Lactation	Seropositive cows	Seronegative cows	Hazard ratio*	95% CI	*P* value	Attributable proportion
1	6/32 (19)	23/156 (15)	1.3	0.5, 3.2	0.55	0.23
2	9/31 (29)	13/104 (13)	2.8	1.1, 6.9	0.02	0.64
3	2/13 (15)	9/57 (16)	0.7	0.1, 4.1	0.70	ND
4+	2/26 (8)	5/41 (12)	0.5	0.1, 3.1	0.52	ND

*Estimated hazard ratios adjusted for breeding season, number of days from calving to conception, and history of abortion.
CI = Confidence interval. ND = Not determined.

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Table 3—Frequency distribution of seropositive female calves that were born to cows classified as seropositive or seronegative to *N caninum*

	Seropositive cows (n = 10)	Seronegative cows (n = 69)
Seropositive female calves		
At birth	4/10	0/69
At 6 months of age*	5/9	0/59
At 12 months of age	4/9	1/59
At 18 months of age	5/9	1/59
At 24 months of age	6/9	0/59

*One calf born to a seropositive dam and 10 calves born to seronegative dams died within the first 5 months after birth.

Discussion

Analysis of results of the study reported here indicated that cows classified as seropositive to *N caninum* were at 2.8 times greater risk of abortion during their second lactation, compared with seronegative cows. Attributable proportion analysis indicated that abortions in seropositive cows would be reduced for the first pregnancy during the second lactation by 64%, if those cows had been removed from the herd. Exposure to *N caninum* alone was not significantly associated with abortion during the first pregnancy of the first, third, and fourth or later lactations. We did not assess abortion risk for the initial pregnancy in heifers before their first lactation.

Three explanations may be considered for the observed association between exposure to *N caninum* and high risk of abortion in cows during their second lactation but decreased risk of abortion during the first, third, and fourth or later lactations: maternal immunity, dairy management factors, and preferential culling. In 2 studies,[13,41] an increase in *N caninum* antibody concentrations developed in naturally infected cows during pregnancy, suggesting that reactivation of the parasite and maternal immune response may influence abortion. In 1 study,[41] an increase in *N caninum* antibody concentrations at 5 to 6 months of gestation (and a later decrease) during the initial pregnancy and the first pregnancy during the first lactation suggested a midpregnancy reactivation of the parasite in infected cows, rather than a reinfection. In another study,[13] seropositive cows with high antibody concentrations at 60, 90, 120, and 150 days of gestation were more likely to abort than were seronegative cows with low antibody concentrations at those times. In a previous study,[12] the decreasing risk of abortion associated with congenital *N caninum* infection as parity decreased was probably related to dairy management factors and preferential culling; risk of abortion was 7.4 and 1.7 times higher in seropositive cows during the initial pregnancy and the first pregnancy during the first lactation, respectively, compared with seronegative cows. In that study,[12] heifers were moved at 6 months of age from the dairy to a facility containing only replacement heifers; pregnant heifers returned to the dairy approximately 1 month before calving (approx age, 25 months). Heifers were exposed for the first time to the cow herd and associated pathogens in the late stages of the initial pregnancy or at the time of calving.[12] In subsequent pregnancies, cows had increased opportunities for exposure to a range of abortifacients that may have masked or overridden the role of *N caninum* as an abortifacient.[12] Competing risk for fetal death would be expected to diminish the role of *N caninum* as an abortifacient if fetuses so predisposed were selectively removed early in pregnancy because of other causes.[12] In our study, cows were exposed to the cow herd and associated pathogens from birth until their first calving and thereafter. Thus, it is possible that failure to detect an association between exposure to *N caninum* and abortion among cows during their first lactation was indeed the result of competing risk by other agents for fetal death. In our study, the possibility that preferential culling was related with lower risk of *N caninum*-associated abortion in cows during their fourth or later lactations was not likely. If there was a preferential culling of aborted cows, and if more *N caninum*-infected cows had a history of abortion in early lactations, then one might expect a diminished proportion of previous abortions in cows in the third and fourth lactations to affect the relationship between infection with *N caninum* and abortion during those lactations. However, the proportions of cows that had aborted previously were 1, 10, 10, and 27% in cows during the first, second, third, and fourth or later lactations, respectively. Furthermore, the proportion of cows in their fourth or later lactations was higher for seropositive cows (26/102 [25%]), compared with seronegative cows (41/358 [11%]). These findings raise questions about the pathogenesis of *N caninum* infection during the first and subsequent pregnancies and means by which maternal immunity may protect against *N caninum*-associated abortion in later lactations.

A significant interaction between exposure to *N caninum* and previous abortion and risk of abortion during the current lactation was not identified, in contrast with a previous study.[12] However, our study results suggest that seropositive cows that aborted were not protected from subsequent abortion caused by *N caninum*, because the proportions of seropositive cows with a history of abortion that aborted in the current lactation were similar to that of seronegative cows during the second lactation (29 vs 29%, respectively) and higher during the third (20 vs 13%, respectively) and fourth or later lactations (13 vs 6%, respectively).

The results of the study reported here suggest that congenital transmission was the main method by which *N caninum* was maintained in the study herd. Female calves born to seropositive or seronegative cows were tested at birth and thereafter every 6 months until their first pregnancy was terminated by abortion or parturition. It was assumed that congenital transmission would be associated with a higher proportion of seropositive calves born to seropositive cows, compared with seronegative cows. Further, it was assumed that postnatal transmission (eg, through ingestion of contaminated colostrum, direct contact with tissues or fluids from aborting seropositive cows, or ingestion of oocysts shed from a definitive-host carnivore) would be associated with seroconversion of seronegative calves born to seronegative cows during the study period. Evidence of congenital transmission was confirmed in 4 of 10 calves born to seropositive cows. It is known that infected cows with high *N caninum* antibody concentrations during pregnancy are more likely to give birth to seropositive calves than are cows with low antibody concentrations.[13] In our study, among the 10 seropositive cows, antibody concentrations were higher in 4

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cows that gave birth to seropositive calves (mean ELISA value, 1.18), compared with 6 cows that gave birth to seronegative calves (0.59). Finally, of the 69 calves classified as seronegative at birth, 1 calf was classified as seropositive at 12 months of age and another calf at 18 months of age. Because both calves were born to seronegative cows, were classified as seronegative at birth, had ELISA values at 12 (0.49) or 18 (0.57) months of age that were not high, and did not remain seropositive after seroconversion, the possibility that these 2 calves had postnatal exposure is low.

In a previous study,[12] risk of abortion in cows during their third or later lactations was not examined. Our study results suggest that risk of abortion associated with exposure to *N caninum* is different in cows during different lactations in a dairy herd in which *N caninum* is transmitted mainly by congenital infection. Results of our study raise questions about the pathogenesis of *N caninum* infection during different pregnancies and means by which maternal immunity may protect against *N caninum*-associated abortion in later lactations.

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[a]Posilac, Monsanto Co, St Louis, Mo.

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