Congenital *Neospora caninum* Infection in Dairy Cattle and Associated Calves' Mortality

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ABSTRACT

A prospective cohort study was undertaken on two central California dairies, A and B, to estimate prevalence of congenital infection with *Neospora caninum*, to characterize temporal variation in prevalence, to determine if occurrence of congenital infection was associated with specific dam and calf attributes, and to estimate the effect of congenital infection on calfhood mortality. Of the 405 calves enrolled over a period of 2½ y on dairy A and dairy B, 30.6% (85/278) and 53.5% (68/127), respectively, were seropositive precolostrally to *N. caninum*, as determined by an ELISA test. Adult cow seroprevalence at calving was 36.0% (82/228) for dairy A, and 57.9% (33/57) for dairy B. No evidence was found for a significant increasing or decreasing trend in adult and precolostral seroprevalence through the study period (*P* ≥0.26). For both herds combined, 81% of seropositive cows (93/115) and 5% of seronegative cows (8/170) had congenitally infected calves. Seroprevalence did not increase with cow age on either dairy (*P* ≥0.47). The probability of a calf being congenitally infected was not associated with dam age, dam lactation number, dam history of abortion, calf gender, or length of gestation (*P* ≥0.11). High dam ELISA values at calving were significantly associated (*P* < 0.001) with an increased probability of congenital infection in her calf. Results of survival analyses of female calves available for follow-up indicated a consistently greater survivorship to 90 d in congenitally infected calves than in noninfected calves on both dairies, which was significant for dairy A (0.07, *n* = 186) but not for dairy B (0.69, *n* = 72), thus indicating that congenital infection does not necessarily have a detrimental effect on calf health. The findings of a similar magnitude in congenital infection rate and adult cow prevalence, the lack of increasing seroprevalence with cow age, the lack of an effect of dam age on precolostral seropositivity, and the constant seroprevalences during the study period, suggest that, in the two dairies studied, congenital transmission constituted a substantial amount of infection and was likely the major mode of transmission of *N. caninum*.

RÉSUMÉ

Cette étude prospective, réalisée sur 2 fermes laitières de la Californie (A et B), avait pour objectifs : 1) d'estimer la prévalence d'infections congénitales à *Neospora caninum* et d'en caractériser les variations temporelles; 2) de déterminer les facteurs maternels et fetaux associés à l'infection congénitale; et 3) d'étudier l'effet de l'infection congénitale sur la mortalité pré-sevrage. Au cours des 2½ ans d'étude, des échantillons de sérum ont été prélevés chez 405 veaux et analysés par méthode ELISA afin de déterminer la présence d'anticorps contre *N. caninum*. Sur les fermes A et B, respectivement, 30.6 % (85/278) et 53.5 % (68/127) des veaux étaient séropositifs. Chez les vaches, la séroprévalence au veillage était 36.0 % (82/228) sur la ferme A, et 57.9 % (33/57) sur la ferme B. Au cours de l'étude, aucune augmentation ou diminution significative de la séroprévalence n'a été décelée chez les veaux ou chez les vaches (*P* ≥0.26). Au total, 81% des vaches séropositives (93/115) et 5% des vaches séronégatives (8/170) ont donné naissance à des veaux infectés congénitalement avec *N. caninum*. Les veaux plus âgés n'avaient pas une séroprévalence plus élevée que les plus jeunes (*P* ≥0.47). La probabilité d'un veau d'être infecté congénitalement n'était pas associée à l'âge, le nombre de lactations de la mère, ou un avortement antérieur chez la mère, ainsi qu'au sexe du veau, ou la durée de la gestation (*P* ≥0.11). Toutefois, la probabilité d'être infecté congénitalement avec *N. caninum* était significativement (*P* < 0.001) plus grande chez les veaux nés de vaches avec un haut taux d'anticorps contre *N. caninum* au moment du veillage. L'analyse de survie des génisses pour les 90 premiers jours de vie indique que les veaux infectés congénitalement avaient un taux de mortalité inférieur aux veaux non-infectés. Cette différence, significative pour la ferme A (0.07, *n* = 186), et non-significative pour la ferme B (0.69, *n* = 72), indique que l'infection congénitale avec *N. caninum* n'entraîne pas nécessairement de conséquences négatives sur la santé des veaux infectés. La similitude des taux de séroprévalence adultes et précolostraux sur chaque ferme, les

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séroprévalences adultes et pré-colostroales constantes au cours de l'étude, l'absence d'augmentation de la séroprévalence avec l'âge chez les vaches, ainsi que l'absence d'association entre l'âge de la vache et la probabilité d'infection congénitale suggèrent que, pour les 2 fermes étudiées, la transmission congénitale contribuait à une proportion importante des animaux infectés et était probablement le mode de transmission principal de *N. caninum*.

**INTRODUCTION**

*Neospora caninum* is a protozoan associated with abortion in cows (1,2) and occasionally with encephalomyelitis in congenitally infected calves (3–9). Because of the taxonomic (10,11) and morphologic similarities with *Toxoplasma gondii*, the parasite is believed to have a similar life cycle to *T. gondii*, where infection can occur in utero or by ingestion of oocysts in feces of a definitive host (12). So far, however, only congenital transmission has been demonstrated for *N. caninum* (13–18). Although some concern has been expressed for risk of encephalomyelitis in congenitally infected calves (19), the effect of congenital infection on calf health is unknown.

To understand how the disease is transmitted under typical cattle management environments, it is important to consider risk of infection associated with the various lifetime exposures experienced by cattle. Control and prevention strategies will depend heavily on knowledge of infection risk experienced by cattle, beginning with exposure as a fetus and continuing through productive adulthood.

In an effort to begin understanding the epidemiology of *N. caninum*, we undertook a long-term, prospective study aimed at characterizing and measuring risk of infection in dairy heifers between birth and first calving. Preliminary evidence of high precolostral seroprevalence, however, prompted us also to examine factors associated with congenital infection and to obtain a preliminary assessment of the importance of congenital infection compared to postnatal infection.

Objectives of the study were 1) to estimate the prevalence of congenital *N. caninum* infection in calves, and to characterize temporal variations of congenital infection prevalence on two dairies experiencing abortion associated with *N. caninum*; 2) to test the hypotheses that congenital infection was not associated with attributes of the dam, including serologic status, age, lactation number and history of abortion, or with attributes of the calf, including gender, or length of gestation; and 3) to test the hypothesis that congenitally infected calves had the same preweaning mortality as non-congenitally infected calves.

**MATERIALS AND METHODS**

**STUDY POPULATION**

Calves were enrolled on two dairies in the San Joaquin Valley of California between June 1992 and May 1994 (dairy A), and between September 1993 and December 1994 (dairy B). Herd size, vaccination programs, and calf management have been described elsewhere (20). Abortion rates were estimated to be 10.6% on dairy A, and 17.3% on dairy B, using life table methods previously described (21).

Of fetuses submitted for diagnostic workup, the percentage of abortions associated with *Neospora* was estimated previously to be 50% on dairy A (herd 22) and 60% on dairy B (herd 20)(22).

Both dairies were inhabited by potential definitive hosts, including dogs, cats, chickens, birds, rats, and coyotes. Feed management of both herds was typical of that for dry lot dairies. Corn silage, alfalfa hay, and almond hulls were stored outside, and grain and other commodities were stored in open barns on dairy A and in metal tanks on dairy B. All feed types on both dairies were accessible by wild and domestic animals. Cows on dairy A calved in a common corral, and cows on dairy B calved in an individual calving pen.

**DATA COLLECTION**

Calves were enrolled at birth when a sample of whole blood was collected before colostrum intake. Blood also was collected from the dam within a week of parturition. At birth, twinning and calf gender were recorded. Dam age at calving, length of gestation, lactation number, and abortion history (0 = no recorded abortion, 1 = at least one abortion recorded) were retrieved from computer databases available on the dairies (DairyComp305, Valley Agricultural Software, Tulare, California). Male calves and female calves with a twin male calf were sold within two days of age, and therefore were excluded from follow-up study. For those female calves that were retained, death dates were recorded during the preweaning period.

**SEROLOGY**

To confirm that samples were taken before consumption of colostrum, serum gamma-glutamyl transferase (GGT) concentration (23–25) was determined using a commercially available test kit (Coulter, Hialeah, Florida). Based on mean GGT activity of 13.1 and 28 IU/L (24,25) and a reported range of GGT activity from 10 to 31 IU/L in true precolostral calves (23), calves with serum GGT ≥50 IU/L were excluded from the study.

Sera were tested for antibodies against *N. caninum* using an ELISA as previously described (26). Sera with a sample-to-positive control Vmax ratio (S/P) <0.45 were considered negative and sera with an S/P ≥0.45 were considered positive. At a cutoff of 0.45, the sensitivity (Se) and specificity (Sp) have been estimated to be 88.6% and 96.5%, respectively (26). Samples were tested in duplicate and all results with a coefficient of variation greater than 15% were retested.

**STATISTICAL ANALYSIS**

Prevalence of congenital infection with *N. caninum* was estimated as the number of calves with a precolostral ELISA S/P ≥0.45 divided by the number of calves sampled precolostrally. Cow seroprevalence (Prev) was estimated as the number of cows seropositive divided by the total number of cows sampled. The expected number of false positive and false negative cows for each dairy were computed using positive (PVP) and negative (PVN) predictive values (27) as follows:

\[ \text{PVP} = \frac{\text{Prev}(\text{Se})}{\text{Prev}(\text{Se}) + (1 - \text{Prev})(1 - \text{Sp})} \]

\[ \text{False positive cows} = (1 - \text{PVP}) \times (\text{number of seropositive cows}) \]

\[ \text{PVN} = \frac{(1 - \text{Prev})(\text{Sp})}{(1 - \text{Prev})(\text{Sp}) + (\text{Prev})(1 - \text{Se})} \]
False negative cows = (1-PVN)* (number of seronegative cows) 

To test for evidence of a trend over time, the slopes of monthly precolostral and cow seroprevalences over the study period were estimated using a weighted-least-squares regression analysis (28). Seroprevalence was subjected to an arcsine square root transformation, and the square root of the number of calves or cows sampled during each month was used to weigh the regression. Similarly, weighted-least-squares regression analysis was used to test for any evidence of increase in dam seroprevalence with increasing dam age. 

For each dairy, univariate analysis of variance was used to test the hypotheses of no difference between precolostral seropositive and seronegative calves with respect to mean length of gestation, dam age, dam lactation number, and geometric mean of dam ELISA S/P. To fulfill the assumption of independence, for each dam with two calves enrolled in the study, a Bernoulli trial was used to randomly select one record to be used in the analyses. Pearson’s chi-square statistic was used to test the hypotheses of no association between congenital infection and calf gender, and the dam’s history of abortion. 

Multivariate logistic regression analysis was used to estimate the probability of a calf being precolostrally seropositive after considering effects of dam ELISA S/P, dam age, dam lactation number, dam history of abortion, length of gestation, and calf gender. Dam ELISA S/P was subjected to a logarithmic transformation before being included in the analysis. Season and year of birth were considered as potential confounding factors that might modulate the association between congenital infection and variables examined. Fall and the second year of study were used arbitrarily as reference season and year. Contribution of each variable was assessed in a forward stepwise procedure based on a likelihood ratio test (29). No interaction terms were tested. Odds ratios and 95% confidence intervals (CI) were computed using coefficients estimated for the final models. 

For each dairy, cumulative proportions of calves surviving to 30, 60 and 90 d were computed for precolostral seropositive and seronegative calves, using the product limit method (30). To test the hypothesis of no difference in preweaning mortality between precolostral seropositive and seronegative calves, rates of survivorship of the two groups were compared using the Breslow method (31). 

All statistical analyses were performed using a statistical computer program (BMDP Statistical Software, Version PC90, Los Angeles, California). Two sided tests and a level of significance of 0.10 were used for all analyses.

## RESULTS

### PREVALENCE

On dairy A and B respectively, 30.6% (85/278) and 53.5% (68/127) of calves sampled precolostrally were seropositive to *N. caninum*. Although a complete clinical neurologic examination was not performed, none of the calves sampled exhibited obvious neurologic signs.

Adult cow seroprevalence was 36.0% (82/228) for dairy A, and 57.9% (33/57) for dairy B. On dairy A, 18 seropositive cows gave birth to seronegative calves (Table I), compared to only 5 false positive cows expected from the calculated PVP of 93.4%. On dairy B, 4 seropositive cows gave birth to seronegative calves (Table I), compared to 1 expected false positive cow calculated from the PVP of 97.1%. The PVP was 93.8% on dairy A and 86.5% on dairy B, predicting 9 false negative cows on dairy A and 1 false negative cow on dairy B. The number of seronegative cows that gave birth to seropositive
TABLE II. Mean gestational length, mean dam age and lactation number, geometric mean of dam ELISA S/P, proportion of dams with a history of abortion and proportion of female calves for precolostrally N. caninum seropositive and seronegative calves on dairies A and B. Means were compared using an ANOVA and proportions were compared using Pearson’s chi-square test

<table>
<thead>
<tr>
<th>Dairy</th>
<th>Gestational length (d)</th>
<th>Dam age (mo)</th>
<th>Dam lactation number</th>
<th>Dam ELISA S/P</th>
<th>Dams previously aborted (%)</th>
<th>Female calves (%)</th>
<th>Seropositive calves (n)</th>
<th>Seronegative calves (n)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy A</td>
<td>277.7 (85)</td>
<td>43.4 (80)</td>
<td>2.41 (80)</td>
<td>0.87 (72)</td>
<td>11.3 (80)</td>
<td>63.5 (85)</td>
<td>279.3 (188)</td>
<td>279.3 (188)</td>
<td>0.68</td>
</tr>
<tr>
<td>Dairy B</td>
<td>278.9 (67)</td>
<td>56.0 (65)</td>
<td>3.15 (65)</td>
<td>0.94 (29)</td>
<td>15.4 (65)</td>
<td>60.3 (68)</td>
<td>278.4 (58)</td>
<td>278.4 (58)</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Calves was 8 on dairy A and 0 on dairy B (Table I).

Substantial variability was noticed in monthly prevalence of N. caninum congenital infection over the study period, with estimates of prevalence ranging from 0% (0/9) to 67% (2/3) for dairy A, and 0% (0/3) to 2% (2/2) on dairy B (Fig. 1). No increasing or decreasing trend in monthly precolostral seroprevalence was detected, however, over the study period for either dairy, as indicated by the lack of linear association between seroprevalence and study month (P=0.26, dairy A; P=0.36, dairy B). Similarly, results of weighted least squares regression indicated no increasing or decreasing trend for monthly cow prevalence during the course of the study (P=0.62, dairy A; P=0.52, dairy B).

Slopes of weighted-least-squares regressions of cow seroprevalence by age were not significantly different from zero (P=0.47, dairy A; P=0.64, dairy B), thus indicating that older cows did not have a greater seroprevalence than younger cows.

FACTORS ASSOCIATED WITH CONGENITAL INFECTION

No difference was detected between precolostrally seropositive and seronegative calves with respect to mean gestational length, dam age, or dam lactation number (Table II). Precolostral seroprevalence was similar for male and female calves and congenital infection was not associated with the dam’s history of abortion (Table II). Geometric mean of dam ELISA S/P was significantly greater for dams of precolostral seropositive calves than for dams of precolostral seronegative calves (Table II).

Results of multivariate analysis, adjusted for season and year of birth, indicated high dam ELISA S/P at calving was significantly associated (P<0.0001) with a high probability of her calf being born seropositive. Coefficients for dam log(ELISA S/P) were 6.12 for dairy A and 30.88 for dairy B. The odds ratio (OR) of being congenitally infected for a calf born to a cow with an ELISA S/P of 0.60, for example, compared to a calf born to a cow with a negative ELISA S/P of 0.30, was 6.3 (95% CI 4.1 to 9.6) on dairy A (n=228) and 10.6×10³ (95% CI 1.7 to 6.7×10⁷) on dairy B (n=57). No other variable was associated with congenital infection for either dairy.

MORTALITY

Of the 186 female calves retained for follow-up on dairy A, 51 (27.4%) were seropositive precolostrally. On dairy B, 39 of the 72 (54.2%) heifers available for follow-up were seropositive precolostrally. For calves on dairy A, survivorship was consistently greater in precolostrally seropositive calves than in precolostrally seronegative calves (P=0.07) through 90 days of age (Table III and Fig. 2). For dairy B, no significant difference was detected in survivorship of precolostrally seropositive and seronegative calves (P=0.69) through 90 d of age, although seropositive calves tended to survive better than seronegative calves (Table III and Fig. 2).

DISCUSSION

Currently, it is assumed that N. caninum is transmitted mainly via feces of a hypothetical definitive host (12,32), even though congenital infection is the only demonstrated mode of transmission (13–18). Results of the present study challenge that dogma and indicate that congenital infection can constitute a substantial amount of infection and may be the major mode of transmission of N. caninum in cattle.

Several findings of the present study support congenital infection as the main means by which infection was maintained in the herds studied. The surprisingly high rates of congenital infection of calves from seropositive cows (93/115, 81%) and the strong association between presence of antibodies in the dam and congenital infection demonstrate that infected cows can readily transmit infection to their fetus, thereby perpetuating infection in a population without a definitive host. Similar findings were reported by Webster (33), who demonstrated that T. gondii infection passed from generation to generation of wild rats in the absence of Felidae. Moreover, the failure to demonstrate specific associations expected under the hypothesis of postnatal transmission provides strong evidence favoring congenital transmission. Specifically, the lack of an association between congenital infection in a calf and dam age or lactation, the absence of change in cow seroprevalence with increasing age, the similar magnitude of precolostral and adult cow seroprevalences, and the absence of a trend in precolostral and cow seroprevalences during the study period support the hypothesis that congenital transmission was the main means, if not the only means, by which cattle became infected in the herds studied.

If infection were transmitted postnatally, as by exposure to feces of a definitive host, prevalence would be expected to be higher among older cows than younger cows in the herd, based on a greater lifetime opportunity for exposure. Also, if postnatal transmission had occurred through feed contamination, incidence of
infection in cows would be expected to have increased, resulting in an increasing prevalence of congenital infection over the course of the study. Although the presence of domestic and wild animals and the accessibility to feed storage facilities on the dairies studied indicated the potential for postnatal transmission clearly existed, no evidence was found that postnatal transmission contributed to overall herd infection.

Results of the present study also challenge the view that congenitally infected calves can be expected to experience higher mortality rates than noninfected calves (19), presumably due to neurologic disorders (3–9). In the present study, congenitally infected calves on dairy A had consistently higher survivorship than noninfected calves. In addition, calves congenitally infected with *N. caninum* did not experience a shorter gestation than noninfected calves, as would be expected if *N. caninum* fetal infection were sufficiently harmful to initiate early parturition (3–5).

Reasons for the higher survivorship in congenitally infected calves compared to noninfected calves on dairy A are unknown. Immune stimulation following in utero exposure to *N. caninum* may have contributed to an earlier or more generalized immunocompetence in infected calves. In addition, shared antigens between *N. caninum* and common calfhood apicomplexa parasites, such as *Cryptosporidium* sp. and *Eimeria* sp. (18,34), may have provided cross-protective antibodies, which may have contributed to a reduced morbidity and subsequently reduced mortality in congenitally infected calves.

An interesting finding was that dam and precolostral calf serological status to *N. caninum* differed only for a few dam-calf pairs (30/285, 10.5%). For dairy A, the expected number of false negative dams (*n* = 9) indicated that sensitivity of the test could explain all the seronegative cows that gave birth to seropositive calves (*n* = 8). ELISA specificity, however, is too high to explain all 22 of the seropositive cows that gave birth to seronegative calves. One explanation may be that immunity of some cows was sufficient to prevent exposure of the fetus to an infective dose of tachyzoites. It appears, however, that protection from fetal infection would be expected to be rare, as indicated by a very high risk (eg, OR > 6) of a calf being congenitally infected if it is born to a cow with a high ELISA S/P, compared to a calf born to a dam with a low ELISA S/P.

Other explanations for infected cows giving birth to precolostrally negative calves relate to the system for which the ELISA was optimized and the fetal immune response. The ELISA was optimized using adult sera, which would be expected to have higher antibody concentrations than newborn calf sera. Consequently, sensitivity of the test used on newborns would not be expected to be as high as it is in adults. In addition, some fetuses could have been infected late in gestation or with a low dose of tachyzoites, resulting in insufficient immune stimulation for detectable antibodies to be present at birth.

A fourth explanation relates to the possibility of dam and calf mismatching on dairy A. The potential existed for misidentifying dam and calf pairs when several cows calved at the same time in the same corral. Because cows calved in individual calving pens on dairy B, the likelihood of misidentification was considerably less than on dairy A.

Interpretation of the results of the present study assumes that congenital infection results in a persistent, lifelong infection with *N. caninum*. Justification for this assumption is based on evidence for persistent infection in
animals congenitally infected with *T. gondii* and on the close relationship between *N. caninum* and *T. gondii*. Tissue cysts of *T. gondii* are known to persist at least several years after infection, if not for life of the animal (12). Support for persistence of *N. caninum* has been provided by two phylogenetic studies that concluded that *N. caninum* and *T. gondii* are very closely related and both should be classified in the *Toxoplasma* genus (10,11). Moreover, cysts of *N. caninum* have been identified up to 13 mo post-infection in mice (35,36), suggesting the parasite can be harbored in a latent form for long periods of time.

The assumption of persistent infection in congenitally infected calves is supported further by the finding of cysts and tachyzoites of *N. caninum* in a precolostrally seropositive, clinically normal calf (13).

Results of the present study provide new insight into the epidemiology of *N. caninum* infection in cattle. The high rate of congenital infection and the high survivorship of congenitally infected calves compared to noninfected calves suggest that retention of persistently infected calves contributed to maintenance of *N. caninum* infection in the herd. Congenital infection, therefore, should be considered an important means of transmission of *N. caninum*, and development of prevention and control strategies for infection will need to consider and possibly focus on congenital infection. Although congenital transmission appeared to be the main mode of transmission on the dairies studied here, further studies are necessary to characterize and quantify risk of postnatal infection. In addition, information is needed on the extent to which congenitally infected cows abort, compared to cows infected postnata- tally. Without such knowledge, the impact of congenital infection on abortion risk cannot be determined.

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REFERENCES


