NEOSPORA CANINUM IN FLORIDA DAIRY CATTLE: EFFECT ON MILK PRODUCTION AND REPRODUCTIVE PERFORMANCE IN COWS, AND PREVALENCE OF CONGENITAL INFECTION IN NEWBORN CALVES

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Neospora caninum is a protozoan parasite originally identified in dogs but now recognized as an important pathogen associated with abortions in cows[1,2] and occasionally with encephalomyelitis in congenitally infected calves.[3-9] Life cycles and routes of transmission of *N caninum* have not been completely elucidated. Because of the taxonomic[10-11] and morphologic similarities with *Toxoplasma gondii*, *N caninum* is believed to have a similar life cycle to *T gondii*, where infection can occur in utero or by ingestion of oocyst in feces of a definite host.[12] So far, however, only congenital transmission has been demonstrated for *N caninum*[13-18].
Although the potential economic impact of *N caninum* infection is not known, costs associated with infection have been assumed to be related only to abortion, and possibly impaired reproductive performance, milk production[19], and premature culling.[20] Considerable histopathologic evidence exist, however, for infection to result in dysfunction of several organ systems, including brain, liver, heart, and kidney.[13,21-23] Impaired function of any of these organ systems conceivably could manifest in reduced milk production in cows, in turn resulting in premature culling. Knowledge of reproductive performance, milk production and cull rates among infected and noninfected cows would expand our understanding of the economic impact of the disease and of potential benefits expected if *N caninum* infection was eliminated from a herd. A prospective study is in progress in order to evaluate the effect of *N caninum* on milk production and reproductive performance in a dairy herd with a history of *N caninum* in Florida. In addition, in order to understand how *N caninum* 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 will depend on knowledge of infection risk experienced by cattle, beginning with exposure as a fetus and continuing through productive adulthood. A prospective study is also in progress in order to characterize and measure risk of infection in female calves between birth and first calving in a dairy herd with a history of *N caninum* in Florida.

Materials & Methods

Study population - Holstein cows and calves located on a 600 cow dairy located 35 miles south of Gainesville, Florida, will be used in the study. This herd was selected for the study on the basis of a history of *N caninum* infection and willingness of the owner to participate.
Milk production and reproductive performance – Data will be gathered prospectively until the end of the lactation for each cow that calved during 1999. Data gathered for each cow will include the eartag number, calving date, lactation number, days in milk, mean 10-day milk production, ME-305, calving related disorders (eg, dystocia, retained placenta, metritis, left displacement of the abomasum), conception date, number of services per conception. In

addition, from these data, average days open, proportion of cows pregnant at 90 and 120 days postpartum. Cows will be tested for detection of *N caninum* antibodies within one week after calving. Blood serum samples will be tested for *N caninum* antibodies by using a kinetic ELISA.[24]

Congenital infection in calves – Calves will be enrolled at birth when a sample of blood will be collected before colostrum intake. Additional blood samples will be collected from female calves at 6, 12, 18, and 24 months after birth. In order to confirm that initial samples were taken before consumption of colostrum, serum **gamma-glutamyl transferase (GGT)** concentration will be determined by using a commercially available test kit (Coultler, Hialeah, FL). Calves with serum GGT values equal or greater than 50 IU/L will be excluded from the study (serum activity of GGT is associated with consumption of colostrum and passive immune status). Blood serum samples will also be tested for *N caninum* antibodies by using a kinetic ELISA[24]. Data collected for each calf will include eartag number, gender, preweaning mortality, dam lactation number, dam history of abortion, dam length of gestation.

Analyses – The hypothesis that *N caninum* seropositive cows produce less milk than seronegative cows will be tested by using a repeated-measures ANOVA. Differences in milk weight attributable to age or season at calving will be accounted for by including season and lactation number in the model. The hypothesis that cows infected with *N caninum* are associated with increased number of days open will be evaluated using survival analysis. Kapplan-Mier function estimates will be used to determine the cumulative incidence of *N caninum* infected and noninfected cows becoming pregnant per time interval (eg, 90 & 120 days postpartum). The Cox proportional hazards model will be used to stratify by parity, calving season, breeding season, and calving related disorders. Prevalence of congenital infection at birth with *N caninum* will be estimated as the number of calves with a precalostrum ELISA value equal or greater than 0.45 divided by the number of calves sampled precolostrally.

Prevalence of *N caninum* in female calves at 0, 6, 12, 18, and 24 months after birth will be compared by a chi-square test. Preweaning mortality rates between precolostral seropositive and seronegative calves will be compared by a chi-square test. Finally, probability of a calf being congenitally infected and associated with dam lactation number, dam history of abortion, calf gender, or length of gestation will be assessed by using a logistic regression model.

Results

During December 1998 and October 1999, a total 464 cows have been blood sampled within one-week after calving and tested for detection of *N caninum* antibodies by using an ELISA. One-hundred and eleven cows (24%) have been classified as seropositive. Data collection associated with milk production and reproductive performance is in progress. A preliminary analysis of the effect of *N caninum* on milk production during the first 100 **days in milk (DIM)** in 282 cows has been completed. Ten-day mean daily milk production of 20 (22%) seropositive heifers was consistently less than that of 71 (78%) seronegative heifers. Differences in milk weights between groups of seronegative and seropositive heifers ranged from 0.9 (60 DIM) to 3.6 lbs/heifer/day (90 DIM). Analysis of results for the first 100 DIM revealed that after adjusting for effects of clinical mastitis, season and age at calving, milk production of seropositive heifers was less 2.3 lbs/heifer/day than that of seronegative heifers, but the difference was not statistically significant ($P = 0.56$). A decrease of 2.3 lb of milk/heifer/day would amount to 701 lb of milk for a typical 305-day lactation. In addition, milk production of

73 (26%) seropositive cows (all lactations) was also consistently less 1.9 lbs/cow/day than that of 209 (74%) seronegative cows ($P = 0.73$).
Among 73 "paired" dams and offspring tested for *N caninum* antibodies, 16 dams and 9 offspring were classified as positive to *N caninum* antibodies. Eight of 9 seropositive calves were born to seropositive cows. Eight of 16 seropositive cows gave birth to seronegative calves.

References

1. Dubey JP, et al. Newly recognized fatal protozoan disease of dogs. J Am Vet Med Assoc 1988;192:1269-1285.
2. Pare J, et al. Seroepidemiologic study of *Neospora caninum* in dairy herds. J Am Vet Med Assoc 1998;213:1595-1598.
3. Barr BC, et al. Neospora-like encephalomyelitis in a calf: pathology, ultrastructure, and immunoreactivity. J Vet Diagn Invest 1991;3:39-46
4. Bryan LA, et al. Bovine neonatal encephalomyelitis associated with a *Neospora caninum* protozoan. Can Vet J 1994;35:111-113.
5. O'Tolle D, Jeffrey M. Congenital sporozoan encephalomyelitis in a calf. Vet Rec 1987;121:563-566.
6. Dubey JP, et al. Congenital *Neospora caninum* infection in a calf with spinal cord anomaly. J Am Vet Med Assoc 1990;197:1043-1044.
7. Dubey JP, et al. Clinical neosporosis in a 4-week old Herford calf. Vet Parasitol 1992;43:137-141.
8. Gunning RF, et al. Neospora infection and congenital ataxia in calves. Vet Rec 1994;134:558.
9. Parish SM, et al. Myelitis associated with protozoal infection in newborn calves. J Am Vet Med Assoc 1987;191:1599-1600.
10. Holmdahl OJM, et al. The phylogeny of *Neospora caninum* and *Toxoplasma gondii* based on ribosomal RNA sequences. FEMS Microbiol Letters 1994;119:187-192.
11. Ellis J, et al. The phylogeny of *Neospora caninum*. Mol Biochem Parasitol 1994;64:303-311.
12. Dubey JP. Toxoplasma, Neospora, Sarcocystis, and other tissue cyst-forming coccidia of humans and animals. In: Kreier JP, ed. Parasitic Protozoa. 2nd ed. Vol. 6. San Diego: Academic Press, 1993:31-36.
13. Barr BC, et al. Congenital Neospora infection in calves born from cows that had previously aborted Neospora-infected fetuses: four cases (1990-1992). J Am Vet Med Assoc 1993;202:113-117.
14. Dubey JP, Lindsay DS. Transplacental *Neospora caninum* infection in cats. J Parasitol 1989;75:765-771.
15. Dubey JO, Lindsay DS. Transplacental *Neospora caninum* infection in dogs. Am J Vet Res 1989;50:1578-1579.
16. Dubey JP, Lindsay DS. *Neospora caninum* induced abortion in sheep. J Vet Diagn Invest 1990;2:230-233.
17. Dubey JP, et al. Induced transplacental transmission of *Neospora caninum* in cattle. J Am Vet Med Assoc 1992;201:709-713.
18. Pare J, et al. Interpretation of an indirect fluorescent antibody test for diagnosis of *Neospora caninum* infection in cattle. J Vet Diag Invest 1995;7:273-275.
19. Thurmond MC, Hietala SK. Effect of *Neospora caninum* infection on milk production in first-lactation dairy cows. J Am Vet Med Assoc 1997;210:672-674.
20. Thurmond MC, Hietala SK. Culling associated with *Neospora caninum* in dairy cows. J Am Vet Med Assoc 1996;57:1559-1562.
21. Barr BC, et al. Bovine fetal encephalitis and myocarditis associated with protozoal infections. Vet Pathol 1990;27:354-361.
22. Barr BC, et al. Experimental reproduction of bovine fetal Neospora infection and death with a bovine Neospora isolate. J Vet Diagn Invest 1994;6:207-215.
23. Dubey JP, et al. *Neospora caninum* associated myocarditis and encephalitis in an aborted calf. J Vet Diagn Invest 1990;2:66-69.
24. Pare J, et al. An enzyme-linked immunosorbent assay (ELISA) for serological diagnosis of *Neospora caninum* infection in cattle. J Vet Diagn Invest 1995;7:352-359.