SERO-PREVALENCE OF AND RISK FACTORS FOR NEOSPORA CANINUM IN BEEF CATTLE IN THE NORTHWESTERN UNITED STATES

Sanderson MW[1*], Gay JM[2], and Baszler TV[3]

[1] Department of Clinical Sciences, Kansas State University, Manhattan, KS 66506
[2] Field Disease Investigation Unit, Department of Veterinary Clinical Sciences, Washington State University, Pullman, WA 99164-6610
[3] Department of Veterinary Microbiology and Pathology, Washington Animal Disease Diagnostic Laboratory, Washington State University, Pullman, WA 99164-7040

Neospora caninum is now recognized as a major cause of abortion in California dairy cattle[1] and as a cause of abortion in beef cattle[12]. The life cycle of *N. caninum*, and the associated risk factors for bovine infection have not been completely defined, particularly for beef cattle. Vertical transmission[4] has been shown common and ingestion of oocysts from a definitive host[8] may be a route of horizontal transmission. Only vertical transmission has been established as a natural transmission route in the bovine[5]. The purposes of this study were to estimate beef cow-calf herd seroprevalence of *N. caninum* in the northwest USA and identify associated management and environmental risk factors.

Materials and methods

A management[9] and serologic survey for *N. caninum* in beef cattle was performed in a 5 state region of the Northwestern U.S. Private veterinary practitioners were recruited by telephone interview to enlist beef producer cooperators in Idaho, Montana, Oregon, Washington, and Wyoming. The number of herds enrolled in each state was proportional to the number of beef cows in each state, as reported by the USDA. Two producers were randomly selected for participation from willing producers in each cooperating practice. Serum was obtained from a systematic sample of cows during pregnancy examinations to enable estimation of a 90% confidence interval for within herd seroprevalence. Age category (2 years, 3-6 years, >6 years), origin (purchased or home raised), and pregnancy status was recorded for each cow sampled. The management questionnaire focused on animal purchasing feeding and grazing practices. Serum samples were analyzed for antibodies to *N. caninum* using a commercially available modification of a monoclonal antibody-based, competitive inhibition ELISA (CI-ELISA) previously described[2].

Data were analyzed[10] at the individual cow and the herd level. At the individual level, herd was included as a random effect to control for herd clustering. Models with dummy variables for state of residence as fixed effects were also examined. CI-ELISA inhibition percent for each cow was used as the outcome variable.
A linear model was fit to the herd level management data using herd prevalence as the

outcome variable. A full model was fit with all herd level variables that passed univariate screening ($p<0.25$). Final models were developed manually by backward elimination. State differences in prevalence were evaluated by one-way ANOVA with post-hoc comparison of means using Scheffe's pairwise comparisons. Square root transformation of seroprevalence was performed to normalize residuals and because of the presence of unequal variances between states.

Results and Discussion:
In total, 34 practices and 64 producers were enrolled as cooperators. 53 producers (83%) returned questionnaires, 55 returned blood samples and cow data (86%), and 49 returned blood samples, cow data, and management questionnaires (76.5%).

Based on this seroprevalence study, *Neospora* infection is common in northwestern U.S. beef cattle herds. Within herd individual seropositivity ranged from 2.5% to 67%. Mean individual seropositivity for all herds was 23% (607/2585). Linear regression of individual cow data showed that 2 year old cows had higher CI-ELISA inhibition percent values than did cows greater than 6 years of age ($p<0.05$). The observed association between age and seroprevalence has been inconsistent between studies, finding young cows more likely to be seropositive[11], no relationship[3], or increasing seroprevalence with age[7]. Increased prevalence in younger cows could be due to preferential culling of seropositive cows that had adverse reproductive outcomes previously. Alternately, if titers decrease over time[6], increasing cow age could result in an apparent decrease in seroprevalence.

No relationship was found between origin or pregnancy status and CI-ELISA inhibition percent in the linear model. Abortions after the fall pregnancy check may have occurred, but at the time of these examinations, serostatus was not associated with pregnancy status. Other studies in beef herds[11,12] have shown a relationship between seropositivity and pregnancy

In the herd model, management of cows on summer range was negatively and density of cows during the winter was positively associated with herd prevalence ($p<0.05$). State of residence, particularly Montana and Wyoming, was strongly confounded with some categories of herd management variables including management of cows at range and percent of heifers in the herd. The association of management of cows on range and winter cow density with herd prevalence could have been due to the geographic distribution of herd management practices. However, the confounding of state with management of cows at range and winter density prevents proper assessment of the relationship between these variables and herd prevalence. The association of cow density with seroprevalence suggests horizontal transmission as a route of infection in these herds. Available data in dairy herds however, suggests horizontal transmission is a relatively rare event[4].

Seroprevalence and mean CI-ELISA inhibition percent were lower for Montana and Wyoming than Idaho, Oregon or Washington herds. Herds in Washington were

significantly less likely to manage herds on range. In a linear regression model of herd data, including variables for state, range, and winter cow density, Wyoming, and Montana were negatively, and Washington was positively associated with herd prevalence. The range and cow density variables were not significant in this model.

Exposure to *N. caninum* antigen is common in beef cow-calf herds in the northwest United States. Risk factors for horizontal transmission of *N. caninum* may be related to cow density. Factors associated with the geographical distribution of seroprevalence as well as the relative importance of vertical and horizontal transmission in the epidemiology of *N. caninum* in beef herds, are unknown and require additional investigation.

References:

1. Anderson, M.L., Blanchard, P.C., Barr, B.C., et al. *Neospora*-like protozoan infection as a major cause of abortion in California dairy herds. J. Am. Vet. Med. Assoc. 1991; 198,241-244.

2. Baszler, T.V., Knowles, D.P., Dubey, et al. Serological diagnosis of bovine neosporosis by *Neospora caninum* monoclonal antibody-based competitive inhibition enzyme-linked immunosorbant assay. J. Clin. Microbiol. 1996; 34,1423-1428.

3. Davison, H.C., French, N.P., Trees, A.J. Herd-specific and age-specific seroprevalence of *Neospora caninum* in 14 British dairy herds. Vet. Rec. 1999; 144,547-550.

4. Davison, H.C., Otter, O., Trees, A.J. Estimation of vertical and horizontal transmission parameters of *Neospora caninum* infections in dairy cattle. Int. J. Parasitol. 1999; 29,1683-1689.

5. Dubey, J.P., Lindsay, D.S., Anderson, M.L., et al. Induced transplacental transmission of *Neospora caninum* in cattle. J. Am. Vet. Med. Assoc. 1992; 201,709-713.

6. Dubey, J.P., Jenkins, M.C., Adams, D.S., et al. Antibody responses of cows during and outbreak of neosporosis evaluated by indirect fluorescent antibody test and different enzyme-linked immunosorbant assays. J. Parasitol. 1997; 83,1063-1069.

7. Jensen, A.M., Bjorkman, C., Kjeldsen, A.M., et al. Associations of *Neospora caninum* seropositivity with gestation number and pregnancy outcome in Danish dairy herds. Prev. Vet. Med. 1999; 40,151-163.

8. McAllister, M.M., Dubey, J.P., Lindsay, D.S., et al. Dogs are definitive hosts for *Neospora caninum*. Int. J. Parasitol. 1998; 28,1473-1478.

9. Sanderson, MW, Gay, JM. Veterinary involvement in management practices of beef cow-calf producers. J Am Vet Med Assoc. 1996; 208,488-491.

10. StataCorp. Stata Statistical Software: Release 6.0. 1999; College Station, TX: Stata Corporation.

11. Waldner, C.L., Janzen, E.D., Ribble, C.S. Determination of the association between *Neospora caninum* infection and reproductive performance in beef herds. J Am Vet Med. Assoc. 1998; 213,685-690.

12. Waldner, C.L., Janzen, E.D., Henderson, J., Haines, D.M. Outbreak of abortion associated with *Neospora caninum* infection in a beef herd. J Am Vet Med. Assoc. 1999; 215,1485-1490.