Pre-colostral Antibodies to *Neospora caninum* in Beef Calves Following an Abortion Outbreak and Associated Fall Weaning Weights

Cheryl L. Waldner, *DVM, PhD*
Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, 52 Campus Drive, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 0W0, cheryl.waldner@usask.ca

Abstract

Published reports have suggested that *Neospora caninum* infection may decrease milk production in dairy cows and affect rate of gain and feed efficiency in feedlot steers. This study examines whether there is an effect on the weaning weights of commercial beef calves. Calves that developed antibodies to *N. caninum* during gestation were compared to calves with no evidence of infection. Blood samples were collected from calves before colostrum ingestion and from all cows after calving. Sera were analyzed using a commercially available enzyme-linked immunosorbent assay for *N. caninum*. Detailed individual calving records, birth and weaning weights were available for all calves. Of the 180 samples collected from the cows and first-calf heifers after calving, 153 (85%) were positive for *N. caninum*. Seventy-five (67%) blood samples collected from 112 calves prior to colostrum ingestion also had positive antibody titers for *N. caninum*. Fall calf weights for calves with antibodies to *N. caninum* at birth were not significantly different than those of calves with no antibodies (difference, -9.2 lb [-4.18 kg]; 95% CI, -31.3 to 12.8 lb), after adjusting for calf sex, dam age, calf birth weight, calf age at weighing and sire group.

Résumé

Des publications semblent indiquer que l'infection causée par *Neospora caninum* pourrait réduire la production de lait chez les vaches laitières et influencer le gain de poids et le taux de conversion chez les bouvillons d'engraissement. Cette étude se penche sur un effet possible sur le poids au sevrage des veaux de boucherie. Les veaux qui avaient développé des anticorps contre *N. caninum* durant la gestation étaient comparés à des veaux sans preuve d'infection. Des échantillons de sang étaient recueillis chez les veaux avant la prise de colostrum et chez toutes les vaches après le vêlage. Les échantillons de sérum ont été analysés avec un test ELISA commercial pour *N. caninum*. Des fiches détaillant les évènements du vêlage et les poids à la naissance et au sevrage étaient disponibles pour tous les veaux. Sur l'ensemble des 180 échantillons provenant de vaches et de taures primipares après vêlage, 153 (85%) ont testé positif pour *N. caninum*. Un total de 75 (67%) des échantillons de sang provenant de 112 veaux avant la prise de colostrum montraient eux aussi des titres positifs d'anticorps contre *N. caninum*. Le poids au sevrage à l'automne des veaux avec des anticorps contre *N. caninum* n'était pas différent de celui des veaux sans anticorps (différence = -9.2 lbs [4.18 kg]; IC à 95%, -31.3 à 12.8), après ajustement pour le sexe du veau, l'âge de la mère, le poids du veau à la naissance, l'âge du veau à la pesée et l'origine paternelle.

Introduction

Infection with *Neospora caninum* is increasingly being recognized as an important cause of abortion and reproductive losses in beef cattle.[3,5,10-13] The estimated economic losses to the beef industry in Texas could exceed $7 million (USD) per year.[4]

In addition to the cost of increased abortion and premature culling, some authors have suggested that there may be a direct effect of chronic infection with the parasite on productivity parameters such as milk production and rate of gain. Milk production for a group of seropositive first-lactation cows was reported to be 2.5 lb (1.14 kg)/cow/d less than for seronegative cows.[8] Seropositive calves entering a Texas feedlot had significant reductions in average daily gain, live body weight

at slaughter, and increased cost of treatment for a projected economic loss of $15.62/calf.[1] To date no one has examined whether infection with *N. caninum* is associated with pre-weaning performance in cow-calf herds.

We have previously described an outbreak of neosporosis in a commercial 354-head beef herd in which the first abortions were observed in October 1997.[12] When the outbreak was over, only 57.9% (205/354) of the cows remained pregnant. *Neospora caninum* was identified in the four fetuses examined during the outbreak by using immunohistochemistry.

Extensive serological and pathological investigations were done to investigate other potential contributing infectious causes of the abortions. Two hundred and eighty-two of 347 cattle in the herd had antibodies to *N. caninum* in November 1997.[12] After adjusting for cow age and breeding group, the risk that seropositive cattle would be open or would abort was significantly higher than the risk for seronegative cattle (OR, 6.2; 95% CI, 2.9 to 13.1).[12]

One year after the outbreak (fall 1998), high percentages of both the cows (21.1%) and the 1997 heifers (13.5%) were open and remained positive for antibodies to *N. caninum* (38.7% of cows, 65.4% of heifers).[10] Very little information is available on the potential long-term implications of a *N. caninum* abortion outbreak in a cow-calf herd. Continued monitoring of this herd[12] provided an opportunity to compare the pre-weaning performance of calves with antibodies to *N. caninum* prior to colostrum ingestion to those without.

Materials and Methods

The outbreak involved a family-owned, 350-head, commercial cow-calf beef herd. Most of the seed stock for the herd were purchased during 1988 and 1989. The original herd consisted of Simmental, Charolais, Red Angus, Main Anjou, and Black Angus cattle. The herd had gradually increased in size, and a predominate Simmental influence was apparent. All cows and calves were individually identified, and a comprehensive computer-based herd record system was used.[a] The cows and replacement heifers were injected with a modified-live vaccine (IBRV, BVDV, parainfluenza-3 virus [PI$_3$V], and bovine respiratory syncytial virus [BRSV])[b] in the spring of each year prior to breeding and according to label recommendations.

In the spring of 1999, blood samples were collected and the body condition of all cows and heifers was scored prior to breeding. Blood samples had previously been collected from these cows and heifers in the fall of 1997, spring of 1998, and the fall of 1998 (results reported elsewhere).[10,15] Records of breeding groups and bull exposure were kept for all cows and heifers. Calving data, including the date of birth, calf identification, sex, calf birth weight, and condition at birth were recorded.

Blood samples were also collected prior to colostrum ingestion from 112 calves born in the spring of 1999 to identify calves that had been infected *in utero*. Samples were collected where calving was observed closely by the herd owner. Samples were not collected if the herd owner was unsure whether colostrum had been ingested. The serum samples were analyzed with a commercially available *N. caninum* antibody diagnostic kit[c]. Samples with a sample-to-positive (S/P) ratio greater than 0.5 were designated as positive.

All data were entered into a commercial statistical analysis program.[d] The association between serological status and fall calf weights were examined using general linear models with a normal distribution and identity link function. The effect of clustering by summer pasture group on the association between serological status and weaning weight were accounted for with generalized estimating equations (PROC GENMOD).[7] The initial model included fixed effects for cow age, prebreeding body condition score (1 to 9 scoring system), sire identification, calf birth weight, calf age at weighing, calf sex, twin (yes/no), and serological status for *N. caninum* before colostrum ingestion. Variables were considered significant when $p < 0.05$. First order interactions were assessed and reported where $p < 0.05$.

Results and Discussion

Of the 180 samples collected from the remaining cows and first-calf heifers prior to breeding in the spring of 1999, 153 (85%) were positive for *N. caninum*. The cow herd ranged in age from 2 to 13 years. Almost all cows had a body condition score of 5 or 6 out of 9 prior to summer pasture turnout. Only three cows scored 4 out of 9 and four cows scored 7 out of 9.

Because of the recent abortion outbreak and high prevalence of *N. caninum* infection, this herd provided an ideal opportunity to assess the impact of infection on pre-weaning performance. Previous reports had suggested no prolonged effects on stillbirth rates or calf viability in the herd, but the effect on pre-weaning gain has not been examined.[10,12] Seventy-five (67%) of the blood samples collected prior to colostrum ingestion from 112 of the calves born in spring 1999 had positive antibody titers for *N. caninum* (Figure 1). Crude fall calf weights did not appear to differ between the 64 calves with a positive *N. caninum* antibody result (mean, 660 lb [300 kg]; 95% CI, 640 to 678 lb) and the 36 calves with a negative result (mean, 666 lb [303 kg]; 95% CI, 638 to 694 lb) for which fall weights were available (Figure 2).

Because of the difficulty in getting blood samples before first colostrum ingestion in this large commercial herd, not all calves could be tested. The distribution of calf sex (Table 1), dam age (Figure 3), birth weights (Figure 4), and age at fall weighing (Figure 5) were compared across calves with antibodies to *N.*
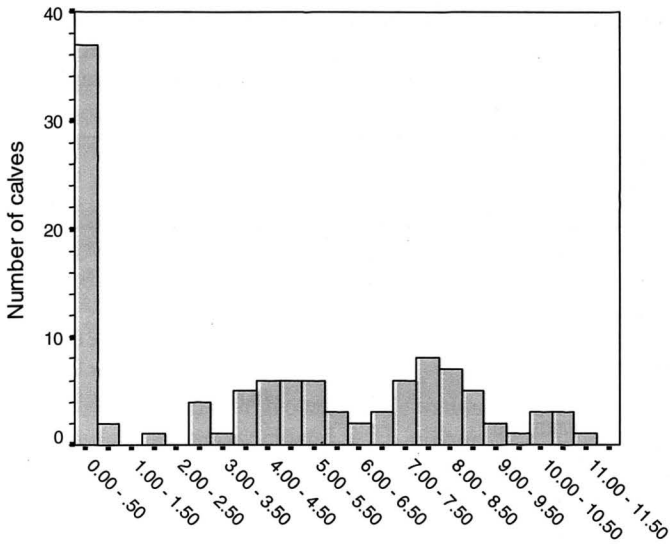
Figure 1. Distribution of serum ELISA results for antibodies to *N. caninum* for calf blood samples collected before first colostrum ingestion. Sample-to-positive ratios (S/P ratios) greater than 0.5 are considered positive.
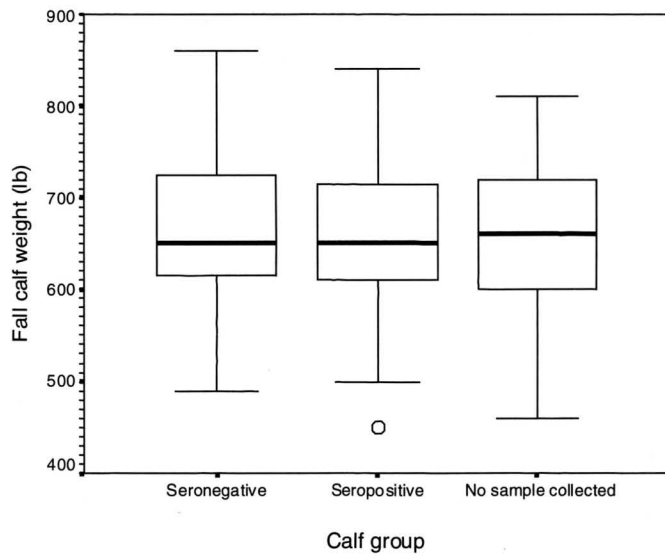

Figure 2. Box plots showing the distribution of crude fall calf weights (lb) by calf serological status. The center line reflects the median weight, the box the interquartile range, and the whiskers extend from the highest to lowest values excluding outliers. The circles indicate outliers between 1.5 and 3 box lengths from the upper or lower edge of the box. An asterisk represents an extreme outlier with a value greater than 3 box lengths from the upper or lower edge of the box.

caninum, calves without antibodies to *N. caninum*, and calves that were not sampled.

Fall calf weight was not associated with serological status after adjusting for calf sex, dam age, calf birth weight, calf age at weighing, and sire group (Table 2).

Table 1. Distribution of calf sex by serological status.

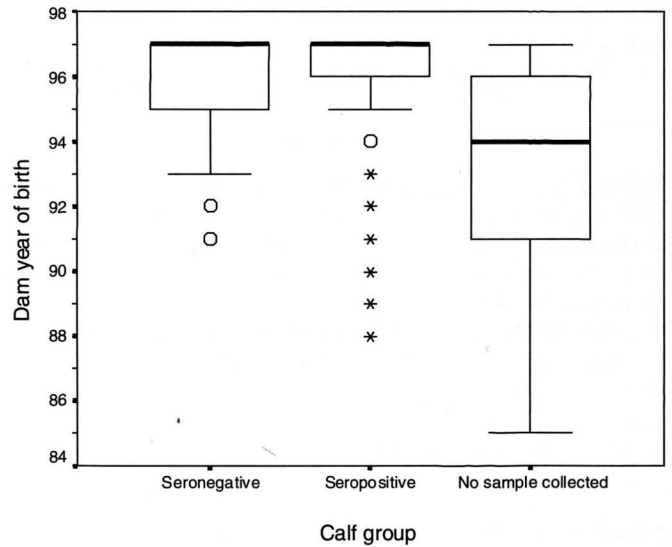| Calf group	Calf sex			
	Bulls	Heifers	Steers	Total
Seronegative	-	21	16	37
Seropositive	-	39	36	75
No sample collected	2	67	58	127
Total	2	127	110	239

Figure 3. Distribution of dam year of birth by calf serological status. The center line reflects the median year of birth, the box the interquartile range, and the whiskers extend from the highest to lowest values excluding outliers. The circles indicate outliers between 1.5 and 3 box lengths from the upper or lower edge of the box. An asterisk represents an extreme outlier with a value greater than 3 box lengths from the upper or lower edge of the box.

Calves born with antibodies to *N. caninum* were approximately 9.2 lb (4.18 kg; 95% CI, -31.3 to 12.8 lb) lighter at weaning than calves without evidence of *in utero* infection. This difference is less than we might expect from measurement error alone. Most on-farm commercial scales would likely have an expected accuracy of not less than ± 10 lb (4.55 kg).

As vertical transmission is considered to be the most common source of new infections in the herd,[2] pre-colostral blood samples and cow status near the time of calving were examined rather than blood samples collected at weaning. A single blood sample at weaning may not accurately reflect calf infection status as substantial fluctuations in antibody levels have been previously documented.[10] Pre-colostral antibody measurements will not always reflect infection history
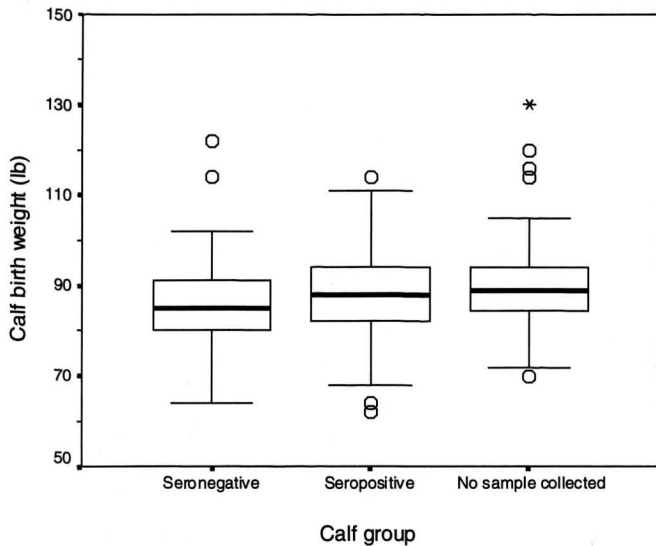
Figure 4. Distribution of calf birth weight (lb) by calf serological status. The center line reflects the median weight, the box the interquartile range, and the whiskers extend from the highest to lowest values excluding outliers. The circles indicate outliers between 1.5 and 3 box lengths from the upper or lower edge of the box. An asterisk represents an extreme outlier with a value greater than 3 box lengths from the upper or lower edge of the box.

Figure 5. Distribution of calf age at fall weighing (days) by calf serological status. The center line reflects the median age, the box the interquartile range, and the whiskers extend from the highest to lowest values excluding outliers. The circles indicate outliers between 1.5 and 3 box lengths from the upper or lower edge of the box. An asterisk represents an extreme outlier with a value greater than 3 box lengths from the upper or lower edge of the box.
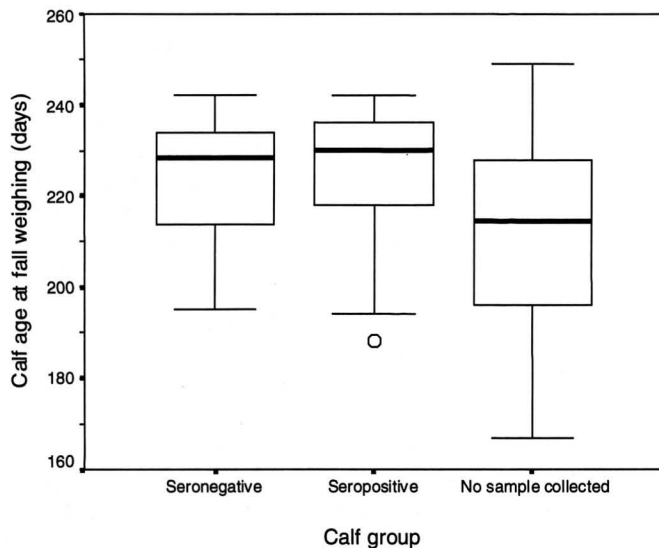
and some calves infected *in utero* could have been missed. Calves infected *in utero* prior to the time of immune competence might not always develop antibodies that could be detected at the time of birth.

Table 2. Association between serological status and fall calf weight (lb) adjusted for cow age, calf age at weighing, calf birth weight, calf sex, and sire group.

Factors affecting fall calf weight	β	95% CI	p
Antibody status (positive compared to negative)	-9.2	-31.3 to 12.8	0.41
Age at fall weighing	2.8	2.1 to 3.5	< 0.0001
Birth weight	2.7	1.6 to 3.8	< 0.0001
Calf sex (heifers compared to steers)	-32.7	-46.5 to -18.9	< 0.0001
Cow age (all age groups compared to 3-year-old cows)			
• 2-year-olds	-57.7	-78.5 to -36.5	< 0.0001
• 4-year-olds	-15.7	-46.2 to 14.7	0.31
• 5 to 7-year-olds	11.9	-22.6 to 46.5	0.49
• 8 years and older	-86.7	-146.9 to -26.5	0.005

The pre-colostral blood samples should be a very specific indicator of *in utero* infection if not a completely sensitive one. No seropositive calves were born to either cows or heifers that had been negative for *N. caninum* on all previous antibody tests.[10,12] The sensitivity and specificity of the ELISA is reported to be 98.6 and 98.9%, respectively, for adult animals.[12] The performance of the test has not been reported for pre-colostral blood samples, but the agreement between the ELISA and the IFAT has been previously reported to be excellent (k 0.83; 95% CI 0.65 to 1.00).[10] The distribution of serological titers from the pre-colostral samples also suggests that there does seem to be a very clear distinction between positive and negative samples with very few values falling on or near the cut-point. Using an apparent prevalence of 67% and the sensitivity and specificity of approximately 98%, the positive predictive value for the pre-colostral samples is 99% and the negative predictive value is 97%. The effects of misclassification on the assessment of association between infection and pre-weaning performance should be minimal.

The serological status of the cows was reasonably stable over a prolonged period and there was a very high rate of vertical transmission to the calves.[10] Because of these factors, we might expect to see an association between antibody status of the cows near calving and weaning weights if infection with *N. caninum* affects calf performance. The calves from cows with antibodies near the time of calving had similar weaning weights to calves from cows without antibodies (-9.7 lb [-4.4 kg]; 95% CI, -32.1 to 12.6 lb). This estimate is very similar to that based on the pre-colostral titer assessment.

Previous reports have suggested that the prevalence of *N. caninum* infection in beef cattle could range between 4.8 and 24%.[6,9] One recent report estimated the prevalence of antibodies to *N. caninum* in cows from the northern Alberta region where this beef herd originated to be 9.0%.[11] If *N. caninum* infection is relatively common in commercial beef herds, any effect on either pre-weaning performance or feedlot performance could have a substantial economic impact on the beef industry.

Conclusions

An effect on calf growth prior to weaning, if present, could have been associated with either decreased milk production from the cow or decreased feed efficiency in the calf. Other studies have shown economically significant associations between *N. caninum* infection and milk production in dairy herds or feedlot performance in beef calves.[1,8] There was, however, no statistical or economically substantial effect of the presence of pre-colostral antibodies to *N. caninum* on pre-weaning performance detected in this herd either before or after adjusting calf weights for known biologically important confounders. Additional studies found that stillbirth rates, pre-weaning treatment and mortality for this herd were all within expected limits during the three calving seasons following the abortion outbreak.[10,12]

Sources and Manufacturers

[a]Microsoft Works
[b]BRSV VAC® 4, Intervet, Whitby, Ontario, Canada
[c]IDEXX, Westbrook, Maine, USA
[d]SAS Institute, Cary, North Carolina, USA

Acknowledgements

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