

Seroprevalence of an Endometriotropic Bovine Herpes Virus-4 in a Georgia Dairy

Mel Pence, DVM MS¹; Kendall S. Frazier, DVM, PhD¹; Charles A. Baldwin, DVM, PhD¹; Joe West, PhD²; John Bernard, PhD²; Alan Liggett, DVM, PhD¹; Debra Miller, DVM, PhD¹; Murray E. Hines III, DVM, PhD¹; Eloise Styer, PhD¹

¹University of Georgia, College of Veterinary Medicine, Tifton Veterinary Diagnostic and Investigational Laboratory, P.O. Box 1389, Tifton, GA 31973

²University of Georgia, Coastal Plain Experiment Station, Department of Animal and Dairy Science, Tifton, GA 31973

Introduction

The University of Georgia Veterinary Diagnostic Laboratory has now confirmed more than 60 cases of suppurative, ulcerative endometritis associated with Bovine Herpesvirus-4 (BHV-4) in postparturient dairy cows. These cases have a clinical pattern of metritis that occurs 3 to 28 days postpartum. Beginning at 3 to 7 days postpartum, the uterus is characterized by necrotic, ulcerated endometrial mucosa with only mild inflammation in the lamina propria and submucosa. Over the ensuing 21 days, the ulcers become confluent/diffuse and epithelium is replaced by fibrinonecrotic, suppurative mats resulting in severe bacterial pyometra by day 24, usually involving *Arcanobacterium pyogenes* or *Escherichia coli*. In addition to the gross pathology, histopathologic evidence demonstrates viral inclusion bodies and virus detection techniques yield positive results for BHV-4.³ Before 1999, BHV-4-associated endometritis had rarely been recognized in the United States,⁴ but a syndrome of epizootic metritis similar in timing, course and outcome attributed to BHV-4 was reported in Europe in the 1980s.^{1,2,6} Significant seroconversion to BHV-4 occurs in affected herds. The viral sequence of this endometriotropic strain of BHV-4 differs from the BHV-4 strain that is associated with mild respiratory disease (DN599).³ Herds affected by this endometriotropic strain of BHV-4 have experienced high morbidity and mortality rates.³ One endemic dairy herd had a period mortality rate of 70% due to BHV-4-associated metritis (7 of 10 dead in one two-week interval). Due to concurrent or subsequent bacterial metritis associated with this viral infection, large economic losses are incurred from death, delayed breeding and infertility, discarded milk from antibiotic therapy, and decreased milk production from systemic effects of the metritis. Similar reports from other diagnostic laboratories indicate BHV-4 appears to be an emerging cause

of endometritis in cattle throughout the southern and midwestern United States.

Materials and Methods

Serum neutralization assays were performed on all 296 cattle from a dairy that has had chronic, endemic BHV-4 metritis (16 clinical cases confirmed at necropsy) and on six barn cats from the same dairy. Eight cows from this high-incidence herd were selected for additional serological testing. Serum was obtained from the eight cows two weeks prepartum and at six, ten and 16 weeks post-partum. Samples were assayed using serum neutralization (SN) with polyclonal antibodies.^a Sera were tested for neutralizing antibodies using a standard microtiter neutralization assay. Briefly, twofold dilutions of the serum sample were made in minimal essential media in a row of wells of a 96-well microtiter plate. Fifty microliters of virus, containing 100-300 TCID₅₀ of BHV-4 virus, were then added to each well. The plates were incubated for at least one hour at 98.6°F (32°C) in a CO₂ incubator. After incubation, Madin Darby Bovine Kidney (MDBK) cells were added to each well. The plates were incubated as before for 5 to 7 days. The inverse of the last dilution that completely inhibited viral replication was recorded as the titer.

Results and Conclusions

The confirmation of more than 60 endometritis-associated diagnostic cases from 12 separate dairies using viral isolation polymerase chain reaction (PCR) and fluorescent antibody (FA) assays suggest that endometriotropic BHV-4 has wide distribution in Georgia. To investigate the level of infection and transmission within a herd, we serologically examined a high-incidence herd. This herd had lost 16 cows to a refractory postpartum metritis associated with BHV-4.

The entire herd had a sero prevalence of 36% (107 of 296). Five of the seropositive cows were subsequently euthanized with clinical and virological evidence of BHV-4 endometritis. Since the disease syndrome occurs as a post-partum metritis, we investigated seroconversion at parturition.

Approximately one year after the original herd BHV-4 testing, eight cows were randomly selected for further serological testing. Two of the cows (#1 and #2) had serum neutralization (SN) titers to BHV-4 of 1:32 and 1:8 in original samples taken the previous year, while the other six were all serologically negative. During this testing period, all eight cows were seronegative prepartum. Five of the eight cows seroconverted by two weeks postpartum. All eight cows were seronegative by 10 weeks postpartum (see Table 1) and remained negative at 16 weeks. It appears that BHV-4, like other herpes viruses, has the ability for latent infection. The recrudescence from latency may be associated with nutritional and/or physiological stresses at parturition. The vast majority (greater than 90%) of all cattle diagnosed at necropsy with endometriotropic BHV-4 to date have had moderate to severe hepatic lipidosis.³ In 15 of 17 where antemortem blood was available, hepatic enzymes (SGOT, alkaline phosphatase) were elevated.³ Lipidosis has recently been associated with predisposition to other viral diseases in cattle.⁵ Increased levels of nonesterified fatty acids and triacylglycerols in these lipidotic cows have been related to postparturient impaired immune function.⁵ Therefore, dairies with endemic BHV-4 that are also experiencing chronic nutritional or metabolic disease may be more likely to have a high incidence of BHV-4 associated metritis and pyometra. Preliminary anecdotal evidence from endemically infected dairies

indicates aggressive early intervention using antibiotics and uterine flushes in the first few days after parturition has decreased the incidence of refractory pyometra later. The role of the BHV-4 seroconversion in the cat population on transmission within this production unit is unknown. No clinical syndrome has been associated with the cat population.

Sources and Manufacturers

^aNational Veterinary Services Laboratory, Ames, IA.

Acknowledgments

We appreciate the technical assistance of Lisa Whittington, Dallas Ingram and Michele Farrar

References

1. Castrucci G, Frigeri F, Cilli V, *et al*: A study of a herpesvirus isolated from dairy cattle with a history of reproductive disorders. *Comp Immun Microbiol Infect Dis* 9:13-21, 1986.
2. Eged L, Ballagi-Pordany A, Bartha A, *et al*: Studies of in vivo distribution of bovine herpesvirus type 4 in the natural host. *J Clin Microbiol* 34:1091-1095, 1996.
3. Frazier K, Pence M, Mauel MJ, *et al*: Endometritis in postparturient cattle associated with bovine herpesvirus-4 infection: 15 cases. *J Vet Diagn Invest* 13:502-508, 2001.
4. Reed DE, Langpap TJ, Bergeland ME: Bovine abortion associated with mixed Movar 33/63 type herpesvirus and bovine viral diarrhea virus infection. *Cornell Vet* 69:54-66, 1979.
5. Rukkamsuk T, Kruip TA, Wensing T: Relationship between overfeeding and overconditioning in the dry period and the problems of high producing dairy cows during the postparturient period. *Vet Q* 21:71-77, 1999.
6. Van Opdenbosch E, Wellemans G, Ooms AA, *et al*: BHV-4 (Bovine herpes virus 4) related disorders in Belgian cattle: A study of two problem herds. *Vet Res Commun* 12:347-353, 1988.

Table 1. BHV-4 serum neutralization titers of cows from an endemically infected herd.

No	1 yr prior	2 wk pre	2 wk post	6 wk post	10 wk post	16 wk post
1	1:32	neg	1:16	1:4	neg	neg
2	1:8	neg	1:8	neg	neg	neg
3	neg	neg	1:4	neg	neg	neg
4	neg	neg	neg	neg	neg	neg
5	neg	neg	1:8	neg	neg	neg
6	neg	neg	neg	neg	neg	neg
7	neg	neg	1:4	neg	neg	neg
8	neg	neg	neg	neg	neg	neg