PEER REVIEWED

A Survey of Southern Arizona Calves for Persistent Infection with Bovine Viral Diarrhea Virus

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Abstract

This study was conducted to determine the prevalence of persistent infection (PI) with bovine viral diarrhea virus (BVDV) in dairy and beef calves in southern Arizona. Formalin fixed skin biopsies of ear from the animals were used to perform immunohistochemistry (IHC). A total of 3,010 dairy bull calves, ranging in age from one day to approximately 14 days, were sampled and 15 PI calves were identified, giving an apparent prevalence of 0.49%. Range beef calves ranging from newborn to approximately 12 months were also sampled. One PI animal was identified out of 1,096 calves tested. Dairy calves testing positive for BVDV at the first sampling were retested using IHC and virus isolation at two-week intervals to confirm PI status.

Key words: bovine viral diarrhea virus, BVD, persistent infection, PI

Résumé

Cette étude a été menée afin de déterminer la prévalence de l'immunotolérance au virus de la diarrhée virale bovine (BVDV) chez des veaux laitiers et de boucherie dans le sud de l'Arizona. Des biopsies de la peau d'oreille, fixées dans la formaline, ont été analysées avec l'immunohistochimie. Un total de 3010 veaux laitiers mâles, dont l'âge variait entre 1 et 14 jours, ont été échantillonnés. L'immunotolérance a été détectée chez 15 de ces veaux pour une prévalence de 0.49%. Des veaux de boucherie au pâturage, incluant des nouveaunés et des individus jusqu'à 12 mois d'âge approximativement, ont aussi été échantillonnés. Seulement un individu immunotolérant a été détecté parmi les 1096 veaux de boucherie testés. Les veaux laitiers testant positifs pour le BVDV lors du premier échantillonnage ont été testés à nouveau avec l'immunohistochimie et l'isolement de virus à intervalle de deux semaines pour confirmer le statut d'immunotolérance.

Introduction

Avoiding infection with bovine viral diarrhea virus (BVDV) in cattle is important because the virus has been shown to increase production costs. Although BVDV was first recognized in the 1940s, it is only recently that comprehensive control of the virus is under discussion in the United States. Bovine viral diarrhea virus can infect and cause disease in cattle of any age. A major concern is the ability of BVDV to produce persistently infected (PI) calves that continually shed virus, thereby causing more infections.

Persistent infections occur as a result of infection with non-cytopathic BVDV *in utero* at 42-145 days, or prior to development of the fetal immune system at 90-125 days of gestation.[7,16,19] During immunological development the virus is not recognized as foreign, and will therefore establish persistent infection and not elicit an immune response. A PI calf may then shed large amounts of BVDV in body discharges, thereby spreading the virus. Dissemination of the virus can lead to more PI animals as well as acute, non-persistent infections that can cause reproductive failure, enteritis, and hemorrhagic disorders. Both acute and PI infections can suppress the immune system and allow opportunistic pathogens to infect the host.

In a prevalence study in cow-calf herds, Oklahoma researchers found 25 PI animals among 4,530 calves (0.55%) tested at 2-4 months of age.[9] Acute infections in seven Canadian cow-calf herds causing mortality and reproductive failures increased losses by $400 per cow.[3] While many PI animals die on the farm or ranch prior to weaning, others can survive calf rearing and feedlot settings, potentially causing financial loss. In a large feedlot study by Hessman *et al*,[11] high-risk calves

ranging in weight from 385 to 636 lb (175 to 289 kg) with direct exposure to BVDV PI cattle were compared to those without direct exposure. Calves without direct exposure had significant improvement in all performance outcomes, first relapse percentage, and mortality percentage. Economic analysis revealed that fatalities accounted for $5.26/head, and performance losses were $88.26/head. In contrast, others have reported no significant effect of exposure of feeder cattle to PI cattle or that testing was not cost-effective when a PI animal was present.[1,5,20,22,23] It may be important to identify the prevalence of the virus and use that information as a tool to formulate programs to interrupt transmission and/or eliminate PI calves.[10,13,15,16] By establishing such programs, ranches (seed stock and feeder calves) and dairies could potentially improve their marketability and productivity by testing and removal of PI animals.

Materials and Methods

Study animals

Two groups of calves were sampled in the study. One was comprised of 3,010 Holstein dairy bulls, and the other group had 1,096 range beef calves. Dairy calves tested were from 11 different dairies and ranged in age from one day to two weeks at sampling. All dairy calves were confined to individual crates upon arrival at one calf ranch. Sampling of newly arrived calves was performed weekly. Dairy calves that tested positive by immunohistochemistry (IHC) were retested two weeks later using IHC and virus isolation (VI). These animals were repeatedly retested using both tests at two-week intervals as long as the calves were available. Calves that died less than two weeks after initial testing were retested using IHC alone.

All dairy calves received modified-live virus vaccine containing BVDV[a] on days 5 and 30. Some calves received an additional booster vaccination on day 15, however, the researchers were not aware of this change in the vaccination protocol while the study was being conducted.

Beef calves sampled ranged in age from neonatal to 12 months. Sampling was conducted when ranchers were branding, weaning, or performing herd work. Sample sizes for the study were determined by using numbers of calves greater or equal to the numbers needed to detect the disease at a prevalence of 0.5%.[2]

Sampling

Each animal was sampled using a commercial ear-notcher that removed a 2 cm x 2 cm triangular section of skin that was then placed in 10% buffered formalin for fixation. Tissues were fixed for a minimum of 24 hours and a maximum of seven days. Representative samples measuring approximately 8 mm x 10 mm were removed and embedded in paraffin blocks.

Immunohistochemistry

Blocks containing the ear notch samples were cut into two 5-micrometer tissue sections and mounted on positively charged glass slides. To detect BVDV antigen, the slides were tested using a DAKO automated IHC system. Five unknown samples per slide were incubated with a monoclonal 15C5 anti-BVDV antibody[b] and were run in tandem with a negative control slide incubated with an irrelevant antibody of the same IgG isotype. The samples were also run with slides containing known positive tissue as positive controls.

Virus isolation

Dairy calves that tested positive by IHC and survived for two weeks were retested using VI. Approximately 10 mL of whole blood was collected into EDTA tubes that were centrifuged to harvest the buffy coat. Buffy coats were inoculated into Leighton tubes with Madin-Darby bovine kidney (MDBK) cells and incubated at 98.6°F (37°C) for 30 minutes. The inoculum was then removed and maintenance medium containing 2% horse serum was added before returning to the 98.6°F incubator. Cells were examined daily for cytopathic effect. Cells were rinsed in phosphate buffered saline at the end of the third day and then fixed in 75% acetone: 25% ethanol for 20 minutes. After drying, staining of infected cells was performed using a protocol from a commercial direct fluorescent antibody conjugate directed against BVDV.[c]

Apparent prevalence calculations

When determining the apparent prevalence of a disease, the limitations of the individual test must be taken into consideration. These limitations include the sensitivity and specificity of the type of test being performed, and so must be incorporated into the equation to accurately determine prevalence.[12] Sensitivity and specificity values were both reported as 97% when using IHC procedures.[6]

Results

Immunohistochemistry testing

Fifteen of the 3,010 dairy calves were initially positive, giving an apparent prevalence of 0.49%. One of the 1,096 range beef calves was identified as PI, giving an apparent prevalence of 0.06%. Positive calves were identified if staining in two of three zones of the ear tissue occurred (epithelium, adnexa, and cartilage). In all positive specimens, a pronounced intracellular granular staining occurred throughout all layers of the epithelium, extending into the ostia of the hair follicles, and continuing into the dermal papillae and sebaceous glands. The adnexa had marked staining in the endothelial cells of vessels, apocrine glands, and fibroblasts. The chondrocytes stained in 10 of these animals. These

staining patterns were in accordance with the findings that PI animals have pronounced staining in keratinocytes, hair follicles, and dermal papilla.[18] Although most of the dairy calves died shortly after the secondary testing, one calf survived for several months and was tested at two-week intervals for two months with positive results using IHC and VI. Seven of the IHC-positive calves that died within two weeks after initial sampling were retested after death, and were positive by a repeat IHC assay. The beef calf positive with IHC testing died before secondary testing could be performed.

Virus isolation

Eight of the 15 IHC-positive dairy calves survived and were available for testing two weeks later. When retested, six of the eight calves tested positive and two showed negative results using VI. One of the negative calves died before subsequent VI testing could be performed; it was positive when tested postmortem with IHC. The other negative calf was tested every two weeks until day 50 using IHC and VI, and was positive on every test, leaving seven of the eight calves with positive VI results and the eighth calf positive with a second positive IHC test.

Necropsy

Necropsies were performed on three available animals. These calves had severe diffuse suppurative bronchopneumonia and marked lymphoid depletion. *Mannheimia haemolytica* was cultured from the lungs of two calves, and *Mycoplasma bovis* was cultured from the third one.

Discussion

In this study, approximately one out of every 200 dairy calves entering a south central Arizona calf ranch were identified as PI by IHC. Calves found positive were sampled again 14 days after the initial sample, or earlier if they died. All the calves were positive when retested, confirming their PI status and the apparent prevalence value of 0.49%. This value represents prevalence in calves entering the calf rearing facility, and does not necessarily reflect prevalence in southern Arizona dairies.

Dairy calves that died within two weeks after initial testing had a second positive IHC test result after they died. Blood could not be collected from all of the IHC-positive calves, therefore only seven of the 15 dairy calves were found positive by secondary VI testing. Using the same vaccine as the calf ranch, a study in 2003 found that vaccine-induced viremia in calves was present up to 10 days after primary vaccination.[14] Of the seven animals testing positive by VI, four were outside that time range and three were bled when vaccine-induced viremia was possible. Although the positive results for three of the calves could have been produced by the first booster vaccination, the viremia (if produced) was likely limited in duration due to the initial vaccination establishing immunity. Also, vaccine exposure has not been shown to produce positive staining by IHC.[4] Further, all the calves that tested positive died, further supporting the laboratory results.

While this dairy calf study focused solely on bull calves, it could be hypothesized that the prevalence of PI heifers could be different. In a 2009 report, Shelton and Hoffman[21] reported the prevalence of PI dairy calves entering a calf ranch was 0.27% (149/54,260); most calves sampled in that study were heifers. Increased husbandry efforts are often directed at heifer calves, which could increase their chance of survival and subsequent spread of the virus. Calves that arrived at the calf ranch in this study may or may not have received colostrum, and were housed in hutches that held three animals side-by-side. These hutches were then placed beside one another in rows that contained approximately 500 mixed-origin animals. This close proximity of calves leads to nose-to-nose contact on a daily basis that could spread the virus and other pathogens in a horizontal manner, leading to increased acute infections.[8]

Loneragan *et al*[17] reported the prevalence of beef cattle PI with BVDV arriving into a commercial feedyard was 0.3%. In that study, PI animals comprised 2.6% and 2.5% of the chronically ill and dead cattle, respectively. Animals in that study were at 43% greater risk of developing respiratory disease when exposed to a PI than those that were not. Although the animals in this study were of different age and origin, they were also destined for a feedlot, however, all of the PI animals died. A possible reason for the high death loss was that most of the PI calves in the study arrived in the first few months of the year, and were exposed to considerable cool and wet weather. One PI calf identified near the end of the study lived for over four months. It is possible, under more ideal conditions, that these calves could have survived long enough to enter a commercial feedlot and spread BVDV to other cattle.

The one range beef calf found PI positive in the present study died before secondary testing could be performed. Sample collection from the beef calves was limited because of the low stocking rates in Arizona and difficulty in confining animals for testing. Cattle numbers in Arizona also were markedly reduced because of drought. Because of this, the maximum age of beef calves tested was increased to 12 months, and whole-herd calf testing was initiated. This may have limited our ability to identify some young PI calves before they died. Alternatively, the wide distribution and reduced numbers of calves in the sample population may have restricted the spread of the virus, thereby limiting the number of PI animals altogether. Because of insufficient

sample size, the prevalence reported in this study may not adequately represent the general beef cattle population in Arizona.

Determining prevalence of PI calves is fundamental for mapping the epidemiology of the virus. This knowledge can possibly lead to methods of eradication or control that might be attainable at reasonable cost, especially in range cattle with very low prevalence.

Alternatives for determining PI status of animals now include IHC, polymerase chain reaction with or without pooling of samples for efficiency, and antigen capture ELISA.[10,15] One or a combination of these tests may be used, depending on the type of livestock operation and specific objectives. Veterinarians can work with producers to identify PI animals and eliminate them as sources of infections in individuals, groups or entire areas.

Eradication of PI cattle in dairy herds is possible using described tests and protocols because dairy cattle are closely confined, making sampling more convenient than on beef ranches. Veterinarians are well equipped to develop protocols for eradication of BVDV-PI animals for herd owners/managers that choose to make that management commitment.

Conclusions

This study demonstrated that BVDV is present in southern Arizona cattle, and that testing calves at a very early age may be necessary to establish prevalence because the high mortality rate within two weeks of initial testing in this study demonstrates that the mortality rate can be high in young, BVDV PI calves.

Acknowledgements

The authors thank Boehringer-Ingelheim Vetmedica Inc. for providing financial support for laboratory costs. We thank Dr. J. D. Biwer of Paradox Veterinary Services and Mr. Richie Kennedy of Kennedy Achers Inc. in Casa Grande, AZ, as well as many others in the Arizona cattle industry for extensive assistance in conducting the study. We also thank Dr. S. M. Dial of the University of Arizona Veterinary Diagnostic Laboratory for her technical support.

Endnotes

[a]Express®5, Boehringer-Ingelheim Vetmedica Inc., St. Joseph, MO

[b]Antibody kindly provided by Dr. E. J. Dubovi, Animal Health Diagnostic Center, Cornell University, Ithaca, NY

[c]BVDV FITC conjugate, 210-61-BVD, VMRD, Inc., Pullman, WA

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