

Prevalence of Bovine Viral Diarrhea Virus in Young Purebred Beef Bulls in Kansas

David P. Gnad, DVM, MS, DABVP¹; Paul H. Walz, DVM, MS, PhD, DACVIM⁴;
Jan M. Sargeant, DVM, MS, PhD²; Peter J. Chenoweth, DVM, PhD, DACT³

^{1,3,4} Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506

² Department of Diagnostic Medicine / Pathology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506

Current addresses:

¹ Piper Heritage Veterinary Clinic, 3140 N. 99th, Kansas City, KS 66109, (913) 721-3126, dgnad@earthlink.net (corresponding author)

² Department of Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, Ontario, Canada L8N 3Z5

⁴ Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL 36849

Abstract

Bovine viral diarrhea virus (BVDV) causes severe economic losses in the cattle industry worldwide. To mitigate risk of these losses, BVDV should be considered when formulating beef cattle biosecurity programs. It is important to have accurate prevalence estimates for specific populations being tested when developing risk assessments as part of a biosecurity program. The objective of this study was to determine the prevalence of BVDV infection in young (10 months - two years of age), purebred beef bulls for sale in the state of Kansas.

Serum samples from young, purebred beef bulls were submitted to the United States Department of Agriculture (USDA) for brucellosis testing as part of the Kansas state requirements for the sale of breeding bulls. All serum samples (2,520) collected between January 1, 2001 and December 31, 2001 were tested for BVDV using an indirect immunoperoxidase monolayer assay. A total of 17 serum samples tested positive for BVDV, corresponding to a prevalence of 0.67% (95% CI; 0.35-0.99%) in this population. Therefore, although uncommon, young purebred beef bulls may be a source of infection for BVDV.

Résumé

Le virus de la diarrhée virale bovine (BVDV) entraîne des pertes économiques importantes au sein de l'industrie bovine partout dans le monde. Dans le but de réduire ces pertes, il serait important de prendre en ligne de compte le BVDV lorsqu'on élabore les plans

de biosécurité pour les bovins de boucherie. Il est important d'avoir des estimés précis de la prévalence dans les populations visées lors de l'évaluation du risque dans le cadre du programme de biosécurité. L'objectif de ce travail était de déterminer la prévalence de l'infection causée par le BVDV chez les jeunes (de 10 mois à 2 ans) taureaux de boucherie pur sang mis en vente dans l'état du Kansas.

Des échantillons de sérum provenant de jeunes taureaux de boucherie pur sang ont été recueillis par le *United States Department of Agriculture* (USDA) afin de tester pour la brucellose dans le cadre des exigences de l'état du Kansas pour la vente des taureaux de reproduction. Tous les échantillons de sérum (2520) recueillis entre le premier janvier 2001 et le 31 décembre 2001 ont été testés pour le BVDV à l'aide d'un dosage indirect à l'immunoperoxidase sur couche mince. Un total de 17 échantillons étaient positifs au BVDV correspondant à une prévalence de 0.67% (I.C. 95%; 0.35-0.99%) dans cette population. Les jeunes taureaux de boucherie pur sang pourraient donc être une source d'infection possible bien que peu commune du BVDV.

Introduction

Infection with bovine viral diarrhea virus (BVDV) occurs worldwide in cattle producing countries. BVDV is classified as a *Pestivirus* within the family *Flaviviridae*.²³ Once BVDV infects cattle, it can produce a variety of economically important clinical manifestations in the beef herd.^{1,3,10} The most important manifestation from a disease control standpoint is the

Materials and Methods

Serum Samples

The USDA received approximately 300,000 serum samples from the state of Kansas through the Federal Brucellosis Eradication Program (Don Evans, DVM, USDA, Personal Communication). Samples were derived from routine livestock market testing, slaughter facility testing and private testing. All breeding bulls sold in Kansas must be bled by an accredited veterinarian and tested for *Brucella abortus*.

For this study, all serum samples received by the USDA from young (<2 years of age) beef bulls for sale in Kansas during 2001 were segregated after brucellosis testing. The samples were frozen at -4°F (-20°C) and sent to Kansas State University for storage at -112°F (-80°C) within three months of submission. Samples were individually identified with a sample number. Data were available on breed, age, county of origin and group number, which was recorded by the submitting veterinarian. Group number was identified as a group of samples received by the USDA from a single veterinarian on a single day. A total of 2520 samples were collected throughout 2001.

Laboratory Methods

Samples were stored at -112°F (-80°C) until all samples (2,520) from 2001 were received. Serum samples were thawed using a water bath at 98.6°F (37°C). Twenty-five microliters of each sample was inoculated into wells on 96-well microtiter plates. Each well contained monolayers of embryonic bovine kidney cells in Eagle's minimum essential medium with 7% fetal bovine serum, L-glutamine, penicillin (100 units/ml) and streptomycin (100 µl/ml). After three days of incubation at 98.6°F in humidified air containing 5% CO₂, the embryonic bovine kidney cells were stained for BVDV antigen by an immunoperoxidase monolayer assay (IMPA).¹² Prior to use, all cells and media were determined to be free of adventitious BVDV by virus isolation procedures. In addition, positive and negative controls were included in each 96-well microtiter plate used to assay for BVDV.

Geographical Stratification

The state of Kansas was divided into north (NH) and south (SH) halves using county lines as the division between halves. Similarly, the state was divided into west (WH) and east (EH) halves using county lines as the division between halves. An effort was made, *a priori*, to create halves that were approximately equal in size geographically. The state was also divided into four quadrants (Q1, Q2, Q3, Q4), using the two division lines that were utilized for dividing the state into halves.

development of an immunotolerant, persistently infected (PI) calf.² PI calves can be created if the calf is infected, *in utero*, before fetal immunocompetence occurs. Immunocompetence generally occurs between 1 1/2 and four months of gestation.^{13,14,18} Transplacental infection with BVDV occurs efficiently if the dam is PI or if she is acutely infected during gestation. In addition to creating a PI calf, transplacental infection can result in fetal death or malformations.⁴ The mortality rate in PI calves is high and growth rates are generally poor,^{11,19} although some calves will grow normally and survive to maturity.¹ PI cattle shed large levels of virus into the environment, creating an opportunity to maintain virus within the herd or infect other herds if the PI animal is introduced.^{1,2,9,19} This can be economically significant to a breeding herd since BVDV can cause an increased incidence of embryo-fetal loss^{6,7} and a decrease in fertilization/conception rate.^{8,22}

The risk of introduction of BVDV into a beef breeding herd needs to be addressed, considering the common practice by United States (US) cow/calf producers of importing cattle into their herds.²⁰ In 1996, the USDA conducted a cross-sectional survey of 1,190 cow/calf operations to identify management practices.²⁰ Within this survey, it was determined that the class of cattle most commonly purchased, leased, or borrowed was weaned bulls. In 1996, 26.8% of cow/calf operations brought a new weaned bull onto their farm or ranch operation.²⁰

Vaccinating against BVDV infection is a widely accepted management tool, however estimates of vaccination rates in US beef herds vary considerably.^{21,24} Vaccinating female cattle before breeding provides partial, but not complete, fetal protection against BVDV infection.⁵ PI calves have been identified within vaccinated herds; therefore vaccination should be considered an inadequate means of controlling or preventing BVDV infection if used alone, without identification and removal of PI cattle.

There is a paucity of data identifying the prevalence of BVDV in specific segments of the beef industry within the US. There is one study that addressed the prevalence of randomly selected US beef herds with PI BVDV calves (3.0%).²⁴ This study tested calves, less than four months of age, in 52 herds within five states for BVDV. Unfortunately, this study did not identify prevalence of PI BVDV at the individual animal level. Identifying prevalence of BVDV within specific segments of the beef industry, at the individual animal level, is important if veterinarians are to customize biosecurity programs for individual producers. The present study was designed to estimate the prevalence of BVDV in young, purebred beef bulls in Kansas. This population is critical to evaluate, since weaned bulls are reportedly the most common category of cattle into be imported into a cow/calf herd.²⁰

Statistical Methods

Prevalence was determined by calculating the proportion of positive samples in the population tested. Data were analyzed to determine evidence of differences in prevalence among the four quadrants or among the two sets of halves, utilizing the test for homogeneity of proportions. The statistical software Statistix®^a was used for all statistical analysis.

Results and Discussion

Seventeen samples of the 2,520 tested were positive for BVDV (0.67%; 95% CI; 0.35-0.99%). There was no statistical evidence of clustering within quadrants or halves of the state, suggesting there was no difference in geographic distribution of BVDV infected herds within the state. However, the inability to identify significant clustering may have been due to the small number of positive samples, minimizing the power to detect a significant difference.¹⁷

Our testing method identified the presence of BVDV in serum at the time of initial serum collection. It was not possible to retest any of the positive bulls to determine if the bulls were PI or acutely infected with BVDV. Ideally, cattle should be retested to confirm a persistent viremia, differentiating a persistent infection from an acute infection.¹⁶ The testing method used in this study was a serum-based test. Serum-based tests are ideal for identifying the PI animal due to their high circulating viral level.¹⁵ Acutely infected animals generally have lower circulating viral levels; therefore, serum-based testing for the presence of acute infections is less desirable than whole blood testing, considering BVDV's affinity to leukocytes.¹⁶ This would support the opinion that our positive samples were likely PI, although not confirm PI status. Since mortality and growth rates are poor in PI calves, it is likely that not all PI bulls survive or perform well enough to be offered for sale. Therefore, the prevalence of all young, purebred, beef bulls in Kansas could be higher than the reported prevalence in bulls being offered for sale.

Bovine viral diarrhoea virus can lose infectivity after freezing at -4°F (-20°C). Samples in this study were frozen at -4°F (-20°C) before being transported to Kansas State University and freezing at -112°F (-80°C). If the BVDV lost infectivity in these samples then it would decrease the sensitivity of the IMPA to detect positive samples. This would cause an underestimation of prevalence in the study population.

Conclusion

Considering the devastating impact BVDV can have on a breeding herd, BVDV must be considered in a biosecurity program for beef breeding herds. PI ani-

mals shedding large amounts of virus can be a source of pathogen entrance into a herd. This study identified the prevalence of BVDV in young, purebred beef bulls for sale in Kansas, enabling veterinarians to make accurate risk assessments regarding importation of bulls similar to those described in this paper. This information is critical since it is a common practice for cow/calf producers to import young bulls into their herds.

Footnote

^a Statistix 7®, Analytical Software 850-893-9371.

Acknowledgement

The authors would like to thank Fort Dodge Animal Health for partial funding of this project.

References

1. Bolin SR, McClurkin AW, Coria MF: Frequency of persistent bovine viral diarrhoea virus infection in selected cattle herds. *Am J Vet Res* 46:2385-2387, 1985.
2. Brock KV: Strategies for the control and prevention of bovine viral diarrhoea virus. *Vet Clin North Am Food Anim Pract* 20(1):171-180, 2004.
3. Chi J, VanLeeuwen JA, Weersink A, Keefe GP: Direct production losses and treatment costs from bovine viral diarrhoea virus, bovine leukosis virus, *Mycobacterium avium* subspecies *paratuberculosis*, and *Neospora caninum*. *Prev Vet Med* 55:137-153, 2002.
4. Cortese SV: Bovine viral diarrhoea virus and mucosal disease, in Howard JL, Smith RA (eds): *Current Veterinary Therapy, Food Animal Practice*, ed 4. Philadelphia, WB Saunders, 1999, pp 286-290.
5. Cortese SV, Grooms D, Ellis J, Bolin SR, Ridpath JF, Brock KV: Protection of pregnant cattle and their fetuses against infection with bovine viral diarrhoea virus type 1 by use of a modified-live virus vaccine. *Am J Vet Res* 59:1409-1413, 1998.
6. Done JT, Terlecki S, Richardson C, Harkness JW, Sands JJ, Patterson DSP, Sweaney D, Shaw IG, Winkler CE, Duffell SJ: Bovine virus diarrhoea-mucosal disease virus: pathogenicity for the fetal calf following maternal infection. *Vet Rec* 106:473, 1980.
7. Duffell SJ, Harkness JW: Bovine viral diarrhoea-mucosal disease infection in cattle. *Vet Rec* 117:240-245, 1985.
8. Graham TC, Fahning ML, Zemjanis R: Nature of early reproductive failure caused by bovine viral diarrhoea virus. *J Am Vet Med Assoc* 185:429, 1984.
9. Holland RE, Bezek DM, Sprecher DJ, Patterson JS, Steficek BA, Trapp AL: Investigation of an epizootic of bovine viral diarrhoea virus infection in calves. *J Am Vet Med Assoc* 202: 1849-1854, 1993.
10. Houe H: Epidemiological features and economic importance of bovine viral diarrhoea virus (BVDV) infections. *Vet Micro* 64:89-107, 1999.
11. Kelling CL, Stine LC, Rump KK, Parker RE, Kennedy JE, Stone RT, Ross GS: Investigation of bovine viral diarrhoea virus. *Can J Comp Med* 48:156-161, 1990.
12. Meyling A: Detection of BVD virus in viremic cattle by an indirect immunoperoxidase technique, in McNulty M, MacFerran J (eds): *Recent Advances in Virus Diagnosis*. Boston, Martinus Nijhoff Publishing, 1988, pp 37-46.
13. McClurkin A, Littledike E, Cutlip R, Frank G, Coria M, Bolin S: Production of cattle immunotolerant of bovine viral diarrhoea virus. *Can J Comp Med* 48:156-161, 1984.
14. Perdizet J, Rebhun W, Dubovi E, Donis R: Bovine viral diarrhoea - clinical syndromes in dairy herds. *Cornell Vet* 77:46-74, 1987.

15. Saliki JT, Fulton RW, Hull SR, Dubovi EJ: Microtiter virus isolation and enzyme immunoassays for detection of bovine viral diarrhea virus in cattle serum. *J Clin Micro* 35(4): 803-807, 1997.
16. Saliki JT, Dubovi EJ: Laboratory diagnosis of bovine viral diarrhea virus infections. *Vet Clin North Am Food Anim Pract* 20(1):69-84, 2004.
17. Smith RD: Statistical Significance, in Smith RD (ed): *Veterinary Clinical Epidemiology*. Boca Raton, CRC Press, 1995, pp 91-159.
18. Steck F, Lazary S, Gey H, Wandeler A, Huggler C, Oppliger G, Baumberger H, Kaderli R, Martig J: Immune responsiveness in cattle fatally affected by bovine viral diarrhea-mucosal disease. *Zentralbl Veterinarmed Med* 27:429-445, 1980.
19. Taylor L, Janzen E, Ellis J, Van den Jurk J, Ward P: Performance, survival, necropsy, and virological findings from calves persistently infected with the bovine viral diarrhea virus originating from a single Saskatchewan beef herd. *Can Vet J* 38:29-37, 1997.
20. USDA: APHIS: VS. *Part II: reference of 1997 beef cow-calf production management practices*. No. N247.198. Fort Collins, CO: Centers for Epidemiology and Animal Health, 1997.
21. USDA: APHIS: VS. *Part III: reference of 1997 beef cow-calf production management practices*. No. N247.198. Fort Collins, CO: Centers for Epidemiology and Animal Health, 1997.
22. Virakul P, Fahning ML, Joo HS, Zemjanis R: Fertility of cows challenged with a cytopathic strain of bovine viral diarrhea virus during an outbreak of spontaneous infection with a noncytopathic strain. *Therio* 29:441, 1988.
23. Wenger DG, Bradley DW, Collett MS, Heinz FX, Schlesinger RW, Strauss JH: Family flaviviridae, in FA Murphy, CM Fauquet, DM Bishop, et al (eds): *Viral taxonomy*. New York, Springer-Verlag, 1995, pp 414-427.
24. Wittum TE, Grotelueschen DM, Brock KV, Kvasnicka WG, Floyd JG, Kelling CL, Odde KG: Persistent bovine viral diarrhea virus infection in US beef herds. *Preventive Vet Med* 49:83-94, 2001.

Abstract

Effects of Diets Fed to Dairy Cows Before and After Calving on Their Plasma Progesterone Profiles After Calving

Fahey J., McNamara S., Murphy J.J., O'Callaghan D., Mee J.F.
Veterinary Record 156:505-509, 2005

Four weeks before their predicted mean second lactation calving date, 60 spring-calving Holstein-Friesian cows were blocked into groups of six on the basis of their predicted calving date and body condition score, and allocated at random to one of six dietary treatments in a factorial design: ad libitum grass silage, ad libitum grass silage plus barley straw or ad libitum grass silage plus 3 kg of concentrates, was offered for four weeks before the expected calving date, and after calving they were offered either 4 kg or 8 kg of concentrates plus ad libitum grass silage for eight weeks. On average, the first luteal activity occurred in all the groups at 29 days after calving. Seventeen of

the cows had an atypical first plasma progesterone profile; 12 were anovulatory, three had prolonged luteal phases and two became anovulatory after having been cyclic. The cows offered grass silage only before calving had a significantly shorter mean (se) interval between calving and second luteal activity (44.9 [2.1] days), than the cows offered grass silage and straw (53.5 [1.9] days) or grass silage and concentrates (51.5 [3.2] days) ($P < 0.05$). After calving none of the 28 cows offered grass silage and 4 kg of concentrates started cycling before day 21, whereas five of the 30 cows offered grass silage and 8 kg of concentrates cycled before day 21 ($P < 0.05$).

HARDJO-BOVIS, THE BAD GUY, IS OUT THERE.



© Copyright American Association of Bovine Practitioners; open access distribution.

But, Spirovac[®], the original hardjo-bovis protection, is now even better.

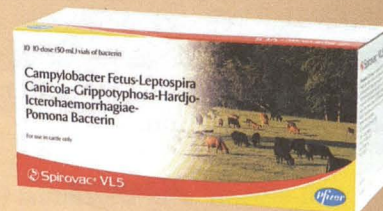


Leptospira

Hardjo-bovis—a different kind of leptospirosis—causes infertility and early embryonic death. The clinical signs of hardjo-bovis are not easy to see; you may not even know when it's in your herd.

Spirovac is today's most complete lepto vaccine line.

- Spirovac offers convenient L5 and VL5* combinations for a unique way to fight hardjo-bovis plus other common leptos. No other L5 or VL5 is labeled to protect against hardjo-bovis.
- Spirovac prevents hardjo-bovis shedding¹; is safe for pregnant cows and has demonstrated 12-month hardjo-bovis protection.
- Spirovac delivers strong placental and fetal protection to help prevent long-term hardjo-bovis maintenance host infections.²



Spirovac[®]
One Vaccine.
Best Protection.



Pfizer Animal Health

For more details, contact your Pfizer representative.
Or visit www.spirovac.com.

*VL5 not recommended for dairy cattle.

¹Bolin CA, Alt DP. Use of a monovalent leptospiral vaccine to prevent renal colonization and urinary shedding in cattle exposed to *Leptospira borgpetersenii* serovar hardjo. *Am J Vet Res* 2001;62:995-1000.
²Data on file, USDA.

Spirovac[®] is a registered trademark of Pfizer Inc. ©2005 Pfizer Inc. All rights reserved. SPV05013