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Prevalence of Bovine Viral Diarrhea Virus in Bovine Samples from the Intermountain West of the USA - Comparison between Age, Sex, Breed and Diagnostic Methods

David J Wilson*, Thomas J Baldwin, E Jane Kelly, Arnaud Van Wettere, Gordon Hullinger and Jennifer Bunnell

Utah Veterinary Diagnostic Laboratory, Department of Animal, Dairy and Veterinary Sciences, Utah State University, 950 East 1400 North, Logan, UT 84341, USA

***Corresponding author:** David J Wilson, Utah Veterinary Diagnostic Laboratory, Department of Animal, Dairy and Veterinary Sciences, Utah State University, 950 East 1400 North, Logan, UT 84341, USA, Tel: (435)760-3731; Fax: (435)797-2805; E-mail: David.Wilson@usu.edu

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Abstract

Prevalence of Bovine viral diarrhea virus (BVDV) ("detected" test results) among all bovine samples tested at the Utah Veterinary Diagnostic Laboratory from 2008 - 2013 was calculated, and results were compared by age, sex, or breed of the cattle and BVDV diagnostic test methods. Necropsies were tested for BVDV when lesions suggestive of infection were identified. Adults, juveniles and most calves were tested by antigen (Ag) capture ELISA, while fetuses and some calves were tested by real-time reverse transcriptase PCR. Cattle originated from Utah and surrounding states. Chi-square analyses were used to test for significant differences in BVDV prevalence between age, sex, breed and test methods. Bovine viral diarrhea virus was detected in 105/8,975 samples (1.2%), including 22/180 necropsies (12.2%). Detection of BVDV by each test method was: Ag Capture ELISA-skin 79/7,692 (1.0%); Ag Capture ELISA-serum 19/1,195 (1.6%); PCR 7/88 (8.0%). Detection of BVDV by age, sex, breed was: male 5/215 (2.3%); female 9/382 (2.4%); fetus 3/36 (8.3%); calf (1-200 days old) 29/579 (5.0%); juvenile (201-729 days old) 4/183 (2.2%); adult (≥ 730 days old) 4/75 (5.3%); dairy 25/750 (3.3%); beef 26/1,600 (1.6%). There were no significant differences in BVDV detection by age or sex. Necropsied animals (P<0.0001), those tested with PCR (P<0.0001) and dairy breeds (P=0.07), were more likely to be detected with BVDV. When prevalence of BVDV has been reported over the last 20 years, it has focused on the 0.1% prevalence of persistently infected (PI) cattle, but PI cattle are a source of infection for large numbers of herdmates. The 8% prevalence in aborted fetuses and overall prevalence of >1% demonstrates that despite the low reported prevalence of persistently infected cattle, BVDV remains an important bovine disease.

Keywords: BVDV; Bovine; Prevalence; ELISA; PCR; Abortion

Introduction

Bovine viral diarrhea virus (BVDV) infection is a costly disease of beef and dairy cattle [1-6]. Statistical modeling estimated the cost of BVDV in beef cow-calf herds in which infections were present at $90 per cow [3]. A 2003 review from Denmark estimated losses from BVDV at between $10 and $40 per dairy cow calving [6]. A Canadian study of direct BVDV costs including abortion and reproductive loss, milk loss, premature voluntary culling, reduced slaughter value, and mortality, estimated annual costs at more than $48 mean per cow [2] across all dairy cows, similar to cost estimates in a US study [5].

Most cattle infected with BVDV acquire transient infections, nearly all of which are non-fatal and result in seroconversion and immunity within 14 days [7,8]. The vast majority of these transient BVD infections are caused by viral shedding from herdmates that were persistently infected (PI) during gestation [7,8]. Fetuses infected with BVDV in utero between 30 and 120 days of gestation (while fetal immunity is being developed) develop immunotolerance and most become PI [7]. Viral replication persists in these PI animals throughout their lives. Many PI animals are eventually exposed to a strain of BVDV different from, or that is a mutation of the strain that caused in utero infection and subsequently develop severe clinical signs (mucosal disease) [7,8]. Effects of BVDV infection include gastrointestinal disease, respiratory disease, immunosuppression, infertility, abortions, congenital defects, stillbirths and lost meat and milk production [7,9].

BVDV has been reported throughout the world [10]. It is estimated that 0.13% of cattle in the US are PI [11].

This descriptive case series reports the proportion of detection of BVDV in all samples tested for the disease at the Utah Veterinary Diagnostic Laboratory (UVDL) in the Intermountain West over a 4½-year period.

Materials and Methods

All samples/specimens included in this descriptive case series were of bovine origin and were tested for BVDV at the Utah Veterinary Diagnostic Laboratory (UVDL) from November 2008 through April 2013. Cattle originated from Utah and surrounding states. Blood, serum, and ear notch or other skin biopsy samples were submitted for BVDV testing in variably sized groups, nearly always without an accompanying history of whether or not there were clinical signs suggestive of infection with BVDV. Such testing was often required for interstate or international shipment of animals and/or for testing for the presence of PI animals within commercial herds, driven by the interest of the owners. These samples were representative of cattle from the study area.

Necropsied cattle (including non-mummified fetuses) or tissues submitted from field necropsies to the UVDL were tested for BVDV

when lesions suggestive of infection were identified. In fetuses, such lesions include microencephaly, cerebellar hypoplasia, cataracts, arthrogryposis, hydrocephalus, myelination defects, microphthalmia, thymic aplasia, hypotrichosis, brachygnathism, hydranencephaly, or pulmonary hypoplasia. In calves, juveniles and adults (see age groups below), BVDV-associated lesions include vasculitis, rumenitis, reticulitis, omasitis, abomasitis, crypt or gland centered enterocolitis, ulcers in any part of the digestive tract, or lymphoid depletion of Peyer's patches and/or lymph nodes.

Age groups

Age of tested animals was not provided for every submission. Where age was provided, animals were classified as: fetuses, calves (1-200 days old), juvenile (201-729 days old), and adult ≥ 730 days old. Similarly, sex or breed was not always provided, especially for samples submitted in large groups. When provided, sex and/or breed were recorded. Some animals were described by the submitter as simply "Beef" breeds. For animals necropsied at the laboratory, sex was recorded and breed was also if it was readily apparent, e.g., purebred Holstein, Charolais, etc.

Test methods for BVDV

Antigen capture ELISA-skin (Bovine Viral Diarrhea Antigen Test Kit, IDEXX, Inc., Westbrook, ME) was performed on ear notches or other skin biopsy samples according to the manufacturer's instructions. Ear notches or skin biopsy samples were soaked in phosphate-buffered saline (PBS) for a minimum of 10 min at room temperature, but where logistics allowed, they were soaked overnight in PBS at 4°C to facilitate viral antigen diffusion into PBS. When testing >20 samples, post-incubation PBS was placed into non-antigen coated, low protein binding microtiter plate wells and subsequently transferred to antigen-coated test wells using a multichannel pipetter. This resulted in rapid transfer of samples to antigen-coated test wells minimizing sample incubation time variability on the test plate.

Antigen Capture ELISA-serum was performed using the same commercial test kit on 100 µl of serum placed into antigen-coated test wells. When testing >20 samples, low protein binding microtiter wells and a multichannel pipetter were used to facilitate more rapid transfer of samples to the antigen-coated test plate at nearly the same time, so that samples did not incubate for variable times.

Real-time reverse transcriptase PCR for BVDV was performed as described previously [12]. Specimens tested included bovine blood collected into EDTA tubes and refrigerated, or fresh spleen, lymph node, tonsil or mucosa-associated lymphoid tissues, either refrigerated or frozen at -20°C. Primers and probes were obtained commercially (Applied Biosystems, Inc., Foster City, CA) and were: forward primer 5'GGG NAG TCG TCA RTG GTT CG3'; reverse primer 5'GTG CCA TGT ACA GCA GAG WTT TT3; probe 5'- 6 -FAM- CCA YGT GGA CGA GGG CAY GC [BHQ]3'. These primers amplify a 190 bp fragment in both BVDV type I and BVDV type II viruses. Following RNA extraction and PCR, interpretation of results was: if a sample exhibited amplification with Ct <38 cycles, and positive and negative controls performed correctly, the sample was classified as BVDV detected [12]. If no sample amplification curve was observed, or the amplification curve crossed the threshold with Ct >38, and positive and negative controls performed correctly, the sample was classified as BVDV not detected.

Electronic capture of data

Signalment and laboratory findings were entered into a laboratory information management system (Vetstar Animal Disease Diagnostic System [VADDS], Advanced Technology Corporation, Ramsey, NJ, USA) and transferred into data fields in a commercial database program (Excel, Microsoft Corporation, Redmond, WA). Data fields populated were accession number, date, animal ID, BVDV test method, test result, breed, age in days, age category and sex. Totals by category were calculated.

Statistical analysis

This is an observational study reporting diagnostic results; it was not a planned experiment, or even a natural experiment. The case could be made that no statistical analysis should be done for differences in BVD prevalence among the different types of samples or populations, such as aborted fetuses, necropsies, serum or ear notches. However, for the sake of interest, to supplement each reader's judgement of whether differences are biologically important, statistical analysis was performed. Comparison among animal or test method categories for proportion with BVDV "detected" or "not detected" was evaluated for statistical significance using Chi-square. Critical value of P for statistical significance was $\alpha=0.05$.

Results

There were 8,975 samples of bovine origin tested for BVDV during the study period. The virus was detected in 105/8,975 samples (1.2%). Test methods, the proportion of BVDV detected results for each type of test, and results by age, sex, or breed are shown in Tables 1 and 2. Specimens in two categories were significantly more likely to have BVDV detected: necropsied animals (22/180; 12.2%) (P<0.0001) and those tested with PCR (7/88; 8.0%) (P<0.0001), in comparison to specimens tested using Ag Capture ELISA-skin (79/7,692; 1.0%) or using Ag Capture ELISA-serum (19/1,195; 1.6%). A trend (P=0.07) was observed that dairy breeds (25/750; 3.3%) were more likely to be detected with BVDV than beef breeds (26/1600; 1.6%). There were no significant differences in BVDV detection by age or sex.

Table 1: Results by BVDV test method applied to bovine samples.

BVDV Test Method	Tested (n)	BVDV Detected (n) (%)
Ag Capture ELISA-skin	7,692	79 (1.0%)
Ag Capture ELISA-serum	1,195	19 (1.6%)
PCR	88	7 (8.0%)*
Necropsy (all methods)£	180	22 (12.2%)*

£: Subset of above 3 categories. *Higher prevalence of "BVDV detected" results in comparison with skin (usually ear notch) and serum samples, P<0.0001.

The breed of the tested animal was provided by submitters or determined at necropsy for 2,350 animals. Dairy breeds (n=750) consisted of: 713 Holsteins (95.1%), 29 Jerseys (3.9%), 7 Milking Shorthorns (0.9%), and 1 Ayrshire (0.1%). Beef breeds (n=1,600) consisted of: 959 Angus (59.9%), 365 "Beef" (22.8%), 99 Hereford (6.2%), 63 Red Angus (3.9%), 54 Charolais (3.4%), 42 Simmental (2.6%), 5 Corriente (0.3%), 4 Longhorn (0.3%), 4 Belted Galloway (0.3%), 1 Beefmaster (0.1%), 1 Maine Anjou (0.1%) and 1 Watusi

(0.1%). The sex of tested animals was provided by submitters or determined at necropsy for 597 animals, 382 females (64.0%) and 215 males (36.0%).

Table 2: BVDV test results by sex, age, and dairy vs. beef breeds.

Age, Sex or Breed	Tested (n)	BVDV Detected (n) (%)
Male	215	5 (2.3%)[£]
Female	382	9 (2.4%)[£]
Fetus	36	3 (8.3%)[£≠]
Calf (1-200 days)	579	29 (5.0%)[£¥]
Juvenile (201-729 days)	183	4 (2.2%)[£γ]
Adult (≥ 730 days)	75	4 (5.3%)[£ λ]
Dairy[€]	750	25 (3.3%)[+]
Beef[±]	1,600	26 (1.6%)[+]

£: No significant difference among sex, age, or by test method within sex, age or breed. ≠All 3 by PCR at necropsy. ¥27 by Ag Capture ELISA-skin, including 16 of 114 necropsies (14.0%). γ3 by Ag Capture ELISA - serum, 1 by Ag Capture ELISA- skin, of 6 necropsies (16.7%). λ2 by Ag Capture ELISA-serum, 2 by Ag Capture ELISA- skin, of 24 necropsies (8.3%). €95.1% Holsteins, 3.9% Jerseys. ±59.9% Angus, 22.8% "Beef". +P = 0.07.

Discussion

As is true of samples submitted to any veterinary diagnostic laboratory, diagnostic samples in this study were of different types, such that the expected likelihood of detecting BVD differed between them. Necropsy specimens were tested for BVDV only if lesions suggested infection with the virus. Therefore, the prevalence of BVDV among all necropsied bovines was not determined, but the virus was detected in a relatively high proportion (approximately 12%) of suspect cases, as would be expected. A previous survey of US veterinary diagnostic laboratories reported that laboratories self-identified as predominantly testing samples from animals suspected to have BVDV had 6.6% BVDV-positive tests and those that tested mainly BVDV screening samples had 1.0% positive tests [9]. No detection of BVDV in the majority of animals tested based on suspicion of the disease at necropsy may be attributable to failure of the virus to survive after death of the host even when infection had been present. In addition, bovine coronavirus and bovine rotavirus in calves and malignant catarrhal fever in any age animal may mimic BVDV-induced lesions in cattle [1].

Because PCR tests were only performed on suspect necropsy specimens, it is not surprising that PCR resulted in relatively high proportion of BVDV detection. Samples collected at necropsy had BVDV detected at 80 to 100 times the 0.1% reported as overall prevalence of BVDV, but the latter focuses primarily on PI prevalence rather than that of transiently infected animals [11,13,14]. However, virus detection in the high-volume screening samples tested by ELISA that made up the majority of samples in this case series occurred at 10 times the 0.1% reported prevalence of PI animals [11]. Prevalence rates of cattle that may be transiently infected with BVDV at any given time are not well described. The relatively high proportion of detection of BVDV reported in the current study is similar to that in a previous survey of US diagnostic laboratories, which found that BVDV was detected in 4.3% of samples submitted for BVDV testing, ranging up to 26.1% for one laboratory [9]. Diagnostic laboratory samples, which include a subset of necropsies performed because BVD is suspected, might logically be expected to have more BVDV detected [9] than the proportion of the disease reported in investigations of BVDV PI prevalence that are not based on suspicion of BVD [11,13,14].

At the UVDL, the most commonly used BVDV test methods were Ag capture ELISA on ear notches, followed by the same ELISA on serum, while PCR constituted only about 1% of BVDV tests; this agrees with a previous report of test methods used at other veterinary diagnostic laboratories [9]. Sensitivity and specificity of diagnostic tests or procedures are important in order to correctly classify animals compared to their "true positive" or "true negative" disease status. In a previous validation study of a BVDV ELISA that used the same technology as the ELISA used in this study, 240 samples of serum or whole blood from herds certified BVD-free and 197 samples detected with BVDV using an NS3 (pestivirus protein) detecting ELISA were tested. For all 437 samples, the "gold standard" to determine "true" BVD-positive or BVD-negative status was an RT-PCR. Sensitivity of the BVDV ELISA was 99% and specificity was 99.5% (compared with PCR) [15]. The RT-PCR is often described as a highly accurate test for BVDV, with sensitivity and specificity nearly 100% [16], but there are no refereed scientific publications comparing it to "true" BVDV status, and calculating test sensitivity or specificity. This is largely because of the lack of an agreed upon standardized test or combination of tests to be used as a "gold standard" to classify animals or samples as "true" BVDV-positive or negative. However, the RT-PCR used in this study has published data [12] comparing BVD test results to a combination of other diagnostic tests including virus isolation and immunohistochemistry, that allow calculation of both statistics. The sensitivity was 60/61 (98.4%) and specificity was 2285/2290 (95.6%) [12]. In our opinion, the "false positives" were inflated because the other tests are not as sensitive as PCR; many of the 105 "false positive" cases likely had BVD correctly detected by PCR only.

When the sex was determined or provided, approximately two-thirds of the specimens were from females. This likely reflects that bovine females outnumber males, and especially in the case of dairy cattle, are often of greater financial value. When the breed was determined or provided, the breeds of cattle tested were representative of the proportions of dairy and beef breeds in the Intermountain West.

When prevalence of BVDV has been reported over the last 20 years, it has focused on the 0.1% prevalence of PI cattle, but PI cattle are a source of infection for large numbers of herdmates. The 8% BVDV prevalence in aborted fetuses, 12% prevalence in necropsied cattle and overall prevalence of >1% found in this study all indicate that BVDV remains an important bovine disease in the Intermountain West of the USA.

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References

1. Center for Food Security and Public Health (2012) Malignant Catarrhal Fever. Iowa State University, pp: 1-10.
2. Chi J, VanLeeuwen JA, Weersink A, Keefe GP (2002) 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.
3. Gunn GJ, Stott AW, Humphry RW (2004) Modelling and costing BVD outbreaks in beef herds. Vet J 167: 143-149.
4. Hessman BE, Fulton RW, Sjeklocha DB, Murphy TA, Ridpath JF, et al. (2009) Evaluation of economic effects and the health and performance of the general cattle population after exposure to cattle persistently infected with bovine viral diarrhea virus in a starter feedlot. Am J Vet Res 70: 73-85.
5. Houe H (1999) Epidemiological features and economical importance of bovine virus diarrhoea virus (BVDV) infections. Vet Microbiol 64: 89-107.
6. Houe H (2003) Economic impact of BVDV infection in dairies. Biologicals 31: 137-143.
7. Lanyon SR, Hill FI, Reichel MP, Brownlie J (2014) Bovine viral diarrhoea: pathogenesis and diagnosis. Vet J 199: 201-209.
8. Lindberg A, Houe H (2005) Characteristics in the epidemiology of bovine viral diarrhea virus (BVDV) of relevance to control. Prev Vet Med 72: 55-73.
9. Driskell EA, Ridpath JF (2006) A survey of bovine viral diarrhea virus testing in diagnostic laboratories in the United States from 2004 to 2005. J Vet Diagn Invest 18: 600-605.
10. Houe H (1995) Epidemiology of bovine viral diarrhea virus. Vet Clin North Am Food Anim Pract 11: 521-547.
11. Houe H, Baker JC, Maes RK, Wuryastuti H, Wasito R, et al. (1995) Prevalence of cattle persistently infected with bovine viral diarrhea virus in 20 dairy herds in two counties in central Michigan and comparison of prevalence of antibody-positive cattle among herds with different infection and vaccination status. J Vet Diagn Invest 7: 321-326.
12. Mahlum CE, Haugerud S, Shivers JL, Rossow KD, Goyal SM, et al. (2002) Detection of bovine viral diarrhea virus by TaqMan reverse transcription polymerase chain reaction. J Vet Diagn Invest 14: 120-125.
13. Ridpath J (2012) Preventive strategy for BVDV infection in North America. Jpn J Vet Res 60 Suppl: S41-49.
14. Van Campen H (2010) Epidemiology and control of BVD in the US. Vet Microbiol 142: 94-98.
15. Mars MH, Van Maanen C (2005) Diagnostic assays applied in BVDV control in The Netherlands. Prev Vet Med 72: 43-48.
16. Sandvik T (2005) Selection and use of laboratory diagnostic assays in BVD control programmes. Prev Vet Med 72: 3-16.