Prevalence of Bovine Viral Diarrhea virus in bovine samples from the Intermountain West of the U.S.A. - comparison between age, sex, breed and diagnostic methods

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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

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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 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 beef and dairy cattle [1-6]. Statistical modeling estimated the cost of BVDV 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 BVDV 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, hydropsychosis, bronchynathism, hydranencephaly, or pulmonary hypoplasia. In calves, juveniles and adults (see age groups below), BVDV-associated lesions include vasculitis, rumenitis, reticulitis, oesamitis, abomasitis, crypt or gland entered enterococci, 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 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 incubation PBS was placed into non-antigen coated, low protein binding microtiter plates. When testing >20 samples, post-incubation PBS was placed into non-antigen coated, low protein binding microtiter plates 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.

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 α=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.

<table>
<thead>
<tr>
<th>BVDV Test Method</th>
<th>Tested (n)</th>
<th>BVDV Detected (n) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag Capture ELISA-skin</td>
<td>7,692</td>
<td>79 (1.0%)</td>
</tr>
<tr>
<td>Ag Capture ELISA-serum</td>
<td>1,195</td>
<td>19 (1.6%)</td>
</tr>
<tr>
<td>PCR</td>
<td>88</td>
<td>7 (8.0%)*</td>
</tr>
<tr>
<td>Necropsy (all methods)²</td>
<td>180</td>
<td>22 (12.2%)*</td>
</tr>
</tbody>
</table>

£: 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.
Among necropsied bovines was not determined, but the virus was detected in a relatively high proportion (approximately 12%) of suspect laboratory, diagnostic samples in this study were of them. Necropsy specimens were tested for BVDV only if lesions.

In our study, 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 skin, including 16 of 114 necropsies (14.0%). By Ag Capture ELISA- serum, 1 by Ag Capture ELISA- skin, of 6 necropsies (16.7%). 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.

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.

**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].

**Acknowledgements**

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