Viruses in Bovine Respiratory Disease in North America
Knowledge Advances Using Genomic Testing

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INTRODUCTION

During the past several years, there have been conferences/symposiums for bovine respiratory disease (BRD) and research with several presentations on various aspects of BRD, including clinical disease, diagnosis, etiology, epidemiology, government and commercial diagnostic laboratories, treatment, prevention, economics of disease and prevention, and immunity. Participants included clinicians in private practice; university and government researchers; diagnostic laboratories; animal health firms; cattle owners including feedlots, beef cow calf operations, dairies for milk production, and dairy calf ranches for heifer replacements and dairy calves entering feedlots. A review published in 2009 summarized BRD research from 1983 (the initial BRD symposium) to

KEYWORDS

- Genetic sequencing
- Bioinformatics
- Cattle
- Influenza D virus
- Bovine coronaviruses
- Bovine viral diarrhea viruses
- Bovine herpesvirus 1

KEY POINTS

- In recent years, new bovine respiratory disease (BRD) viruses have been identified in bovine respiratory tract materials by viral sequencing and bioinformatics.
- Advances in viral identification have mirrored the use of viral sequencing, leading to identification of influenza D virus, bovine coronaviruses, and others.
- Diagnostic laboratories have moved to the use of viral sequencing and expanded the use of polymerase chain reaction for virus detection.
- New viruses sometimes are found in healthy cattle as well as in cattle with BRD, and more research is needed to confirm the importance of such viruses.
- Clinicians and diagnostic laboratories must work to confirm the role of new viruses in BRD and to determine whether vaccines have a place in their control.

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2009. This review covered infectious agents, including viruses, bacteria, and mycoplasmas. Coverage of viruses included the 4 most commonly discussed respiratory tract viruses in BRD: bovine herpes virus 1 (BoHV1), bovine parainfluenza type 3 virus (PI3V), bovine viral diarrhea viruses (BVDVs), and bovine respiratory syncytial virus (BRSV). There are commercial vaccines containing immunogens to these viruses. These 4 viruses were investigated extensively prior to 2009 in research studies and were described in published reports from state and federal diagnostic laboratories. These viruses were identified by the virologic methods in place at that time. These included isolation in cell culture based on cytopathology and confirmed by fluorescent antibody test using virus monospecific antisera. Other confirmatory tests included neutralization of infectivity using monospecific antisera. Numerous serosurveys permitted detection of viral exposure in selected populations. In the years prior to 2009, selected viruses such as BVDV were sequenced, resulting in the knowledge of genomic regions that could be used to identify these viruses. Eventually, technology produced many automated sequencing procedures and the field of bioinformatics facilitated alignment of newly identified sequences with reference sequences, permitting the identification of the entire or near full length of viral genomes. Terms, such as metagenomics, whole-genome sequencing, and next-generation sequencing, have become commonplace for both research and diagnostic laboratories. These new genomic tests permitted expansion of knowledge of the big 4 viruses: BoHV1, BVDV, PI3V, and BRSV.

EXAMPLES OF EXPANDED KNOWLEDGE OF BOVINE RESPIRATORY DISEASE VIRUSES: GENOMICS OF BOVINE HERPES VIRUS 1, BOVINE VIRAL DIARRHEA VIRUS, AND BOVINE PARAINFLUENZA TYPE 3 VIRUS

The BoHV1 represents one of the original viruses in BRD that was isolated and characterized in the 1950s with the advent of cell cultures. BoHV1 is a common component of bovine viral vaccines, including both modified live virus (MLV) vaccine and killed/inactivated viral vaccine. The MLV vaccine origin strains of BoHV1 have been identified in clinical cases postvaccination and in aborted fetuses. Thus, it became necessary to differentiate field strains from the MLV strains, but this posed significant challenges. Using whole-genome sequencing and analysis of the resulting nucleic segments, the viral genomes of BoHV1 reference strains, BoHV1.1 reference strains Cooper and Los Angeles, were sequenced. The resulting information on the BoHV1.1 genome was investigated further using the reference strain, Cooper, and multiple BoHV1.1 strains in the MLV vaccines available in North America. This genetic analysis found single-nucleotide polymorphisms (SNPs) among the viruses, which permitted the viruses to be classified into groups. The SNPs for various regions permitted the selection of multiple primers to be used and the polymerase chain reaction (PCR) products sequenced. These SNPs patterns then permitted the ability to separate the viruses into groups and each strain to have a specific identity. This information permitted isolates from clinic cases to be categorized as field/wild-type or MLV strain. Use of the SNPs and the sequencing of the PCR products of the primers were applied in multiple studies identifying wild-type strains of BoHV1.1 as vaccine or wild-type strains. In addition to the separation of vaccine from field strains, these genomic sequencing procedures identified a recombinant BoHV1.1 strain (including components of both a wild-type and a vaccine strain) from an aborted bovine fetus. Using this genetic sequencing, the BoHV1.2b reference strain K22 and multiple wild-type genital and respiratory BoHV1.2b strains were sequenced.
The BVDV strains are referred to as biotypes based on cytopathology in cell culture: cytopathic and noncytopathic, with 2 species, BVDV1 and BVDV2, based on genomics. Application of genetic testing of BVDV strains initially had focused on the sequencing of PCR products from multiple regions of the BVDV genome. Initial studies of the presence of BVDV subtypes in surveys of US and other North American cattle populations, diagnostic accession of bovine samples, or reports of respiratory disease outbreaks with viral identification led to detection of subgenotypes, BVDV1a, BVDV1b, and BVDV2a. Studies of the distribution of these 3 subtypes from diagnostic laboratory accessions indicated BVDV1b as the predominant BVDV subtype, and BVDV1b was the predominant subtype in multiple studies of beef calves with BRD, based on recovery of virus from acute cases of BRD and necropsy tissues. Investigation of the source of BVDV exposure identified the persistently infected calf as the most imported source of virus exposure, with persistently infected calves resulting from infection of susceptible heifers/cows during a critical stage of pregnancy.

A study to evaluate diagnostic tests used to detect persistently infected calves was performed, with the additional objective of determining prevalence of BVDV1a, BVDV1b, and BVDV2a subtypes in persistently infected calves entering a southwest Kansas feedlot. In a 2004 study, there were 86/21,743 (0.4%) persistently infected calves with the distribution of subtypes: BVDV1b (77.9%), BVDV1a (11.6%), and BVDV2a (10.5%). To determine if the distribution of the subtypes was consistent in succeeding years, samples from this same feedlot were tested over the following years and are summarized in Table 1. The distribution of the subtypes seems consistent for each collection from 2004 to 2008.

Another study, using complete genome sequences from samples from this same feedlot in August 2013 to April 2014, determined the distribution of subgenotypes among 119 samples. There were 82% BVDV1b, 9% BVDV1a, and 8% BVDV2. It was reported that the BVDV2 belong to at least 3 distinct genetic groups. This study indicated 2 points:

1. BVDV1b remained the predominant persistently infected subgenotype from 2004 to 2014.
2. BVDV2 may belong to at least 3 distinct genetic groups.

These studies demonstrated prevalence of the subgenotypes in beef cattle, yet information regarding the distribution in dairy cattle is limited. A study of samples from

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Table 1: Bovine viral diarrhea virus subtypes in persistently infected cattle entering a feedlot for beef cattle

| Study       | Number of Calves | Bovine Viral Diarrhea Virus 1a | Bovine Viral Diarrhea Virus 1b | Bovine Viral Diarrhea Virus 2a |
|-------------|------------------|-------------------------------|-------------------------------|-------------------------------|
| 2004–2005   | 86               | (10) 11.6%                    | (67) 77.9%                    | (9) 10.5%                     |
| 2005–2006   | 302              | (33) 10.9%                    | (229) 75.8%                   | (40) 13.3%                    |
| 2007 Spring | 201              | (28) 13.9%                    | (161) 80.1%                   | (12) 6.0%                     |
| 2007 Summer | 184              | (13) 7.1%                     | (152) 82.6%                   | (19) 10.3%                    |
| 2007 Fall   | 163              | (24) 14.7%                    | (124) 76.1%                   | (15) 9.2%                     |
| 2008 Spring | 180              | (28) 15.6%                    | (135) 75.0%                   | (17) 9.4%                     |
| 2008 Fall   | 147              | (14) 9.5%                     | (122) 83.0%                   | (11) 7.5%                     |
| Total: 2004–2008 | 1263          | (150) 11.9%                   | (990) 78.4%                   | (123) 9.7%                    |
39 persistently infected calves in a dairy calf ranch showed the distribution of BVDV subgenotypes: BVDV1a (2.6%), BVDV2a (12.8%), and BVDV1b (84.6%) (R. Fulton, unpublished data, 2020).

To identify BVDV persistently infected calves in beef cow herds, 4530 calves from 30 ranches in south central Oklahoma and north central Texas were tested for BVDV PI status. There were 25/4530 (0.55%) persistently infected calves and 5/30 (16.7%) of the herds contained persistently infected calves. All of the PI BVDV strains were BVDV1b. In a summary of diagnostic laboratory samples over a 20-year period, the BVDV1b subtype predominated. In 2019 a study using clinical samples from diagnostic laboratories and the US Department of Agriculture National Animal Disease Center, BVDV Laboratory, BVDV2b and BVDV2c were identified. Thus, it appears with new genomics testing there may additional BVDV subtypes identified. A recent report on the global distribution of BVDV subgenotypes cited at least 21 subgenotypes for BVDV1 and 4 subgenotypes for BVDV2.

The application of genomics, PCR testing, and antigenic comparison has been applied to another of the BRD viruses, PI3V. There are 3 PI3V genotypes in the United States: PI3Va, PI3Vb, and PI3Vc. In addition to the genetic differences, there also are antigenic differences. These antigenic differences may have an impact on vaccine responses because the current PI3V vaccines contain PI3Va strains.

ADVANCES IN GENOMICS PERMITS IDENTIFICATION OF ADDITIONAL VIRUSES IN NORTH AMERICA: BOVINE CORONAVIRUS, INFLUENZA D VIRUS, AND OTHERS

**Bovine Coronavirus**

The bovine coronavirus (BoCV) has received considerable attention in recent years as another viral pathogen in respiratory disease. Although initially studied as a pathogen causing neonatal diarrhea/enteritis in young calves, growing evidence has suggested involvement in BRD, especially because of the recovery of BoCV from clinically ill cattle with BRD and necropsy cases of fatal pneumonias. The use of viral serology and more extensive use of PCR testing on respiratory tract swabs from BRD cases and necropsy cases by diagnostic laboratories have provided further evidence that BoCV plays a role in BRD. Interpretation of these diagnostic reports, however, often is difficult, especially because there is no US Department of Agriculture licensed BoCV vaccine for BRD prevention and control. A recent article by Ellis gives an excellent review of the history of BoCV in respiratory cases, both in field cases and in experimental studies. The purpose of the review was to seek evidence that BoCV is a biologically significant respiratory pathogen in cattle.

A study using nasal swabs from calves treated for BRD and BRD necropsy cases from multiple feedlots identified viruses with PCR testing and virus isolation. Of the 121 cases, the positives include: 14.9% (BoHV1), 15.7% (BVDV), 62.8% (BoCV), 9.1% (BRSV), and 8.3% (PI3V). In contrast to prior studies, the virus positives were tested by sequencing to differentiate vaccine strains from field strains. Often surveys collect samples from cattle recently vaccinated with MLV vaccines containing BoHV1, BVDV, PI3V, and BRSV. In a study of virus recovery from fatal cases in Ontario, Canada, feedlots, BoCV was recovered from 2/99 (2.0%) cases. A subsequent report in 2009 dealt with a year-long study of pathology and identification of infectious agents in fatal feedlot pneumonias. In that study, which used PCR testing of fresh lung samples, 21/194 (10.8%) were positive for BoCV. The BoCV has been isolated from both healthy calves and sick calves. Using a virus neutralization serotest, however, individual calf serums collected at feedlot entry in a retained ownership study (fresh from the ranch and no mixed-source auction calves) were tested for neutralizing antibodies,
and those calves with low antibody levels to BoCV (16 or less) were more likely to be treated for BRD during the feeding phase compared with calves with higher titers. In other research, BoCV from nasal swabs and bronchoalveolar washing fluids were evaluated for genetic and antigenic differences. A region of the viral spike protein in the envelope was the target of genomic sequencing, and virus neutralization tests in cell culture were used to compare antigenic relatedness. Testing demonstrated genetic differences that allowed classification of 2 clades, BoCV1 and BoCV2, which also demonstrated antigenic differences. The current reference BoCV is of enteric origin and is classified as BoCV1. This strain is included in most licensed BoCV vaccines for enteric disease in the United States. Another study of 15 isolates from 3 herds, using sequencing of the spike hypervariable gene region, indicated that there were 4 polymorphisms in the 15 isolates.

A critical question posed for BoCV as a respiratory pathogen has been whether BoCV infection with isolates of respiratory tract origin (as opposed to the reference enteric strain or other enteric strains) in susceptible cattle results in measurable gross and microscopic lesions on the respiratory tract. Such challenge studies are required in order to have a model to measure efficacy of BoCV vaccines in the respiratory tract protection. A series of studies was performed with multiple isolates from the respiratory tract of calves and the reference enteric strain to study dynamics of the BoCV infection. BVDV exposure was used in dual infections (BVDV and BoCV) as well as BVDV alone, BoCV alone, and controls. Respiratory disease was observed in calves inoculated with BoCV 6 days or 9 days after BVDV. Lung lesions were present in calves in dual infection groups; however, lesions were more pronounced in calves inoculated with BVDV followed by BoCV inoculation 6 days later. Immunohistochemistry (IHC) confirmed the presence of BoCV antigen in the respiratory tract. Gross lung lesions of the dual infected calves were multifocal and randomly distributed throughout the lungs in most cases. Histologically, lung lesions consisted of interstitial to bronchointerstitial pneumonia (BIP), with inflammatory changes ranging from mononuclear infiltrates to fibrin and neutrophils in more severely affected lungs. Similarly, less severe changes could be seen in several of the BVDV or BoCV inoculated calves. In this study, BoCV antigen was found via IHC in bronchial and tracheal epithelium, alveolar interstitium, and macrophages, whereas BVDV antigen was not detected by IHC. This study confirms the potential for BoCV isolates from the respiratory tract to cause clinical disease detected by gross and microscopic lesions, in particular, with a sequential dual infection with BVDV. In addition, IHC detected the present of BoCV antigen in multiple respiratory tract sites. This study indicates that sequential dual infections may have potential as models for vaccine and therapy development and efficacy studies.

Additional information on BoCV will follow in the section on various surveys using metagenomics and PCR testing as well as serotesting in samples from North America.

**Influenza D Virus**

The influenza D virus (IDV) has gained considerable attention in the etiology of BRD. Use of genomic testing, including sequencing of the viral genome and use of PCR testing, along with antibody testing has resulted in numerous studies indicating the widespread presence of IDV in North America. The virus ultimately identified as IDV initially was isolated from swine, and designated C/swine/Oklahoma/1223/2011 (C/OK); this virus was found to have homology to human influenza C viruses. Respiratory tract samples from cattle were submitted to a commercial laboratory for testing for BRD diagnosis using PCR testing, which included testing with primers derived
from this swine influenza C virus. Viruses from these PCR positives were isolated from cell cultures and confirmed to be influenza virus by hemagglutination and PCR. The viral genomes were sequenced and found different from the influenza C viruses. Using serotesting, these bovine isolates also were found antigenically different from the influenza C viruses. The swine strain C/OK, and these new bovine influenza viruses are now classified as D influenza viruses referred to as IDVs (influenza D viruses).

With this new information, multiple studies have reported the presence of IDV in several regions of North America using respiratory tract samples for detection of the viral genome, PCR positives, and/or serology.32–41 These reports are summarized later, not only for presence of IDV but also for other viruses. As with other viruses detected in BRD cases, the question of whether the virus causes disease (acts as a pathogen) or is a resident in healthy cattle without disease potential has been raised. Another question posed for IDV in cattle is whether a vaccine might provide protection in vaccinated and challenged cattle. A subsequent report found that an inactivated IDV vaccine using an isolate from cattle provided partial protection in vaccinated calves compared with controls, and the challenge virus caused inflammation in the nasal turbinates and trachea but not appreciably in the lungs.42 These results give evidence for the role of IDV of cattle in BRD and that partial protection may result from an inactivated vaccine.

**STUDIES OF ADDITIONAL VIRUSES BEYOND BOVINE HERPES VIRUS 1, BOVINE VIRAL DIARRHEA VIRUS, BOVINE PARAINFLUENZA TYPE 3 VIRUS, AND BOVINE Respiratory Syncytial Virus**

Use of testing for influenza viruses in cattle in the United States first was published in 2014.32 Nasal swabs or lung samples were submitted for testing for BRD diagnosis and consisted of 45 samples. These samples were from 6 different states and were tested using a real-time/reverse transcriptase (RT)-PCR assay, which included primers for the influenza C viruses. There were 8 samples (18%) positive for the influenza C virus, representing samples from Minnesota and Oklahoma. Five of the positives were isolated in cell culture and were tested further by PCR and hemagglutination assays. Four positives were from 1 herd in Minnesota and 2 were chosen for further study, C/bovine/Minnesota/628/2013 and C/bovine/Minnesota/729/2013, and 1 remaining isolate was from a case in Oklahoma, C/bovine/Oklahoma/660/2013. Eventually these viruses were placed into a new group based on genomic and antigenic differences from influenza C virus group, leading to designation of a new genus (D) in the viral family *Orthomyxoviridae*. Seroprevalence of IDV in bovine populations was examined with hemagglutination inhibition (HI) with the C/swine/Oklahoma/1334/2011 (C/OK) virus and C/bovine/660/2013 (C/660) as antigen and bovine sera from 8 herds in 5 different states tested individually. With the exception of 1 herd, all herds had high geometric mean titers of greater than 40, and antibodies against the bovine C/OK virus and C/660 virus were cross-reactive in the HI assay. These results indicated that cattle are a reservoir for these viruses.

The epidemiology of IDV was reported further in 2015 using samples from cattle in Mississippi.33 Sera, nasal swabs, and nasopharyngeal swabs were collected from calves at a cattle buying facility in Mississippi. Respiratory swabs testing positive by RT-PCR revealed that 16/55 (29.1%) of the sick calves and 2/84 (2.4%) of the healthy calves were positive for IDV, and the virus was isolated in cell culture from 15 of these 18 RT-PCR positive samples. The genome of D/bovine/Mississippi/C00046/2014 was fully sequenced. Phylogenetic analysis of the HE gene aligned the IDV isolates from Mississippi into 2 clades. Using serology with the HI test on serum samples from
neonatal samples indicated transfer of IDV antibodies to the calf from the dam. Also, testing of sera archived from 2004, 2005, and 2006 found the seroprevalence by year of 18.3% (n = 241), 14.8% (n = 223), and 13.5% (n = 141), respectively, indicating presence of IDV in Mississippi since at least 2004. Distinct genetic and antigenic lineages of IDV in cattle were reported. Samples from BRD cases submitted to the Kansas State University Veterinary Diagnostic Laboratory were screened by BRD PCR panel, which detects BoHV1, BVDV, PI3V, BRSV, and BoCV. The samples were later tested for IDV by an RT-PCR assay. These 208 samples represented nasal and pharyngeal swabs and lung tissues from 12 Midwestern states. There were 10 (4.8%) positive for IDV along with other positives: 36% (BoCV), 7% (BoHV1), 5% (BVDV), 3% (BRSV), and less than 1% (PI3V). The 10 IDV positives were from Kansas, Texas, and Nebraska. Of the 10 PCR IDV positives, 6 were positive via cell culture inoculation. Full-genome sequencing was performed on all 6 cell culture IDV positives. The phylogenetic analysis showed 2 distinct lineages of the IDV from cattle. Using polyclonal antiserum against 2 IDV, D/OK (D/swine/Oklahoma/1334/2011 and the D/660 (D/bovine/Oklahoma/660/2013 in the HI serotest, antigenic differences were noted based on varied HI results.

Metagenomics and PCR testing were used to detect viruses in a study of BRD in California dairy calves. Dairy calves between the ages of 27 days and 60 days were enrolled as either BRD cases or controls. Nasopharyngeal and pharyngeal recess swabs were collected. Using metagenomics and subsequent PCR testing, numerous viruses were identified. Viruses were detected in 68% of the BRD cases and 16% of the healthy controls. Multiple viruses were found in 38% of the sick animals versus 8% of the controls. Based on the viral hits of the genome sequences, the following viruses were detected in descending order: bovine rhinitis A virus, which was greater than bovine adenovirus 3, which was greater than bovine adenovirus-associated virus, which was greater than bovine rhinitis B virus, which was greater than astrovirus, which was greater than bovine IDV, which was greater than picobirnavirus, which was greater than bovine parvovirus 2, which was greater than bovine herpesvirus 6. Those viruses significantly associated with BRD compared with matched controls included bovine adenovirus 3 (P < .0001), bovine rhinitis A virus (P = .009), and bovine IDV (P = .012).

A metagenomics study investigated viral genomes in nasal swabs from 103 cattle from Mexico (63) and the United States (40), representing 6 Mexican feedlots and 4 Kansas feedlots in 2015. Cattle with acute BRD and asymptomatic pen mates were included. There were 21 viruses detected, with bovine rhinitis A (52.7%), bovine rhinitis B (23.7%), and BoCV (24.7%) the most commonly reported. Comparing the recovery of viruses from cattle with BRD versus asymptomatic controls, bovine IDV tended to be significantly associated with BRD (P = .134; odds ratio 2.94). The other viruses historically associated with BRD, including BoHV1, BVDV, PI3V, and BRSV, were detected less frequently.

A Canadian survey of beef cattle utilized metagenomics to detect viruses in western Canadian feedlot cattle with or without BRD. There were 116 cattle sampled with deep nasal swabs and transtracheal washes collected and included samples from animals with or without BRD. The cattle on arrival received an MLV vaccine containing BoHV1, BVDV1, BVDV2, PI3V, and BRSV. There were 21 viruses identified via metagenomics. Viruses associated with BRD based on statistical comparison included bovine IDV (P < .015), bovine rhinitis B virus (P < .02), BRSV (P < .022), and BoCV (P < .021). This report represents the first report of bovine IDV in western Canada. The BoHV1 was not identified in any sample, and BVDV1 and PI3V were found only in 1 sample and 2 samples, respectively. Perhaps the efficacy of the MLV resulted
in reduced or absence of recovery of BVDV, PI3V, and BoHV1. The BRSV was found in 17% of BRD cases and 2% of the controls. There was weak agreement in the identification of viruses in the nasal swabs and transtracheal swabs, suggesting that sample location affects the recovery of viruses.

A study was performed to determine prevalence of BRD viruses and Mycoplasma bovis in US cattle. Samples were from different production classes, including cow calf, stocker, feedlot, and dairy, and from varied seasons of the year. There were 3205 samples collected between May 2015 and July 2016 and from 80 different premises. The intent was to test healthy animals; however, disease status and other clinical data were not collected. These nasopharyngeal swabs were assayed using RT-PCR assay using primers for BoHV1, BVDV, BoCV, IDV, BRSV, and Mycoplasma bovis. The overall percent positive rates for each agent were 3.81% for BRSV, 1.59% for BoHV1, 3.56% for BVDV, 8.3% for IDV, 43.81% for BoCV, 20.12% for M bovis, and 17.32% for multiple-agent positives. The high percentage of IDV and BoCV positives suggested that more emphasis should be placed on these viruses in BRD. The BoCV was significantly more associated with stocker production class and the fall season. This study did not differentiate vaccine viruses from field strains.

A metagenomics study was performed using cases submitted to a western Canadian diagnostic laboratory BRD diagnosis. The samples from pneumonia cases (130) were submitted between September 2017 and December 2018. There were 90.8% of the samples from beef cattle and 9.2% from dairy cattle. Formalin-fixed tissues were processed for histologic examination and fresh tissues frozen until further testing. Cases were classified as suppurative bronchopneumonia (SBP), fibrinous bronchopneumonia (BP), interstitial pneumonia, BP + BIP, and bronchiolitis. The metagenomics identification was performed on fresh lung tissues. From 34 samples with metagenomics sequencing results, an RT-PCR test with primers for BVDV, PI3V, BRSV, BoHV1, and BoCV was used in all cases of these viruses detected. In 4 cases, however, a virus was detected by RT-PCR that was not detected by metagenomics sequencing. The recovery of viruses was low, with only 36.9% (48/130) positive. There were 16 viruses identified. The bovine parvovirus 2 was the most prevalent virus, 11.5%, followed by unulate tetraparvovirus 1 and BRSV, both 8.3%. The BRD viruses—BRSV, 8.5%, and BVDV1 and BVDV2, 2.3%, and 3.8%, respectively—and PI3V, 2.3%, were found infrequently. None of these viruses was associated with a particular pneumonia. Animal viruses were identified in only 1 animal each: bovine rhinitis B, IDV, fowl aviadenovirus, avian adenovirus–associated virus, and bovine polyomavirus. The most prevalent virus in each type of pneumonia was bovine parvovirus 2, at 5.9% in FDP; bovine astrovirus, at 3.1% in SBP; BRSV, at 1.5% in interstitial pneumonia; and unulate tetraparvovirus 1 in BP and BIP, at 1.5%. However, for every type of pneumonia, samples in which no virus was detected, this was the most common result compared to the percentage virus recovery in each pneumonia category. None of these viruses detected were significantly associated with any type of pulmonary pathology. This virus detection in lung tissue provides low analytic sensitivity relative to ante mortem sampling of the upper respiratory tract for virus surveillance. In this study, however, the bacterial agents Histophilus somni, Mannheimia haemolytica, and Pasteurella multocida were found to have strong associations with SBP, fibrinous BP, and BP and BIP, respectively. These results were in contrast to a prior western Canadian study using swabs from the upper respiratory tract (nasal and tracheal) of beef cattle where IDV, bovine rhinitis B, BRSV, and BoCV were significantly associated with BRD. A potential explanation for these divergent findings is that the lungs of the fatal cases may have cleared the viruses and the bacterial pathogens remained predominant.
Serologic surveys often are used to determine presence and prevalence of viruses in various populations of cattle, based on production class and/or geographic regions. Such surveys preferably should rely on samples from animals that have lost their maternal antibodies; thus, antibodies identified result from active infections. Using HI testing, the seropositive rate for IDV ranged from 13.5% to 80.2% in 2 studies.\textsuperscript{33,40} In the latter study, sera from animals 2 years of age or older from beef cattle herds in Nebraska were tested for IDV antibodies via the HI assay. These were from samples collected from September 2003 to May 2004. The HI assay used 2 IDV from Mississippi, representing 2 reported IDV clusters that were antigenically distinct. There were 240 (81.9%) samples seropositive to 1 or both of the 2 IDVs. There were log\textsubscript{2} differences in titers in some samples, suggesting there were 2 antigenic clusters circulating in these Nebraska herds. The cattle from all the 40 farms had evidence of exposure and were from farms across Nebraska.

A subsequent serosurvey was performed using samples from throughout the United States as part of the US brucellosis surveillance program.\textsuperscript{41} Both male and female cattle 2 years of age or older representing 42 US states were randomly sampled in 5 slaughter plants. The cattle represented 6 US regions: Pacific West, Mountain West, Upper Midwest, South Central, Northeast, and Southeast. The antigen in the HI test was selected as D/bovine/Kansas/14-22/12. Of the 1992 samples, 1545 (77.5%) were positive for IDV antibodies. Positives were found in samples from 41 of 42 states, with a seropositive rate by state ranging from 25% to 93.8%. Sample size by state or titer level may have caused bias. The range among geographic regions for seropositivity was 47.7% to 84.6%. The Mountain West region had the highest, 84.6%, and the Northeast the lowest, 47.7%.

SUMMARY

Advances in viral detection in BRD mirrors advances in viral sequencing using respiratory tract samples. Additional viruses beyond BoHV1, BVDV, PI3V, and BRSV include, as examples, IDV, BoCV, bovine rhinitis A, bovine rhinitis B, adenoviruses, astrovirus, bovine parvovirus, and others. Diagnostic laboratories are now using PCR testing based on primers learned from sequencing. In selected instances, such as IDV and BoCV, serosurveys have demonstrated the widespread presence of these viruses in North American cattle. In limited studies, these viruses, such as IDV and BoCV, have caused disease in animal studies. In various studies, some of these viruses, but not all, have been found in BRD cases more frequently than in healthy cattle. It is important that reagents be developed by diagnostic laboratories to use in diagnostic testing for the new viruses. The pathogenicity of these new viruses should be determined in controlled challenge studies. Vaccine development and evaluation in controlled studies for these viruses should be considered to determine if vaccinations have a role in their control.

DISCLOSURE

The author has nothing to disclose.

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