

# Prevalence of respiratory viruses and *Mycoplasma bovis* in U.S. cattle and variability among herds of origin, production systems and season of year

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## Abstract

Bovine respiratory disease (BRD) is a significant cause of morbidity and mortality in beef and dairy calves in the United States. Although BRD is multifactorial in nature, viral infection often precedes bacterial infection; thus, diagnostic investigations often consider both bacterial and viral components of the disease. Prevalence estimates of respiratory viruses and *Mycoplasma bovis* would aid in the interpretation of diagnostic results. Therefore, the objective of this study was to determine the prevalence of bovine respiratory agents (bovine viral diarrhea virus, bovine respiratory syncytial virus, bovine respiratory coronavirus, bovine herpesvirus-1, influenza D virus, and *M. bovis*) in US cattle. Nasopharyngeal swabs (n=3205) were collected from cow-calf, dairy, feedlot, and stocker operations and tested for these respiratory agents using a multiplex PCR. Estimates of animal-level prevalence and associations with herd of origin, production system, and season of year were determined. Bovine coronavirus was the most prevalent respiratory agent with an overall animal-level prevalence of 36.05%, and was significantly associated with production class (highest in stocker) and season (fall). *Mycoplasma bovis* and influenza D virus were also detected frequently in this population of cattle, while bovine viral diarrhea, bovine herpesvirus-1, and bovine respiratory syncytial virus were detected in less than 5% of samples. The relatively high prevalence of bovine coronavirus and influenza D suggest that practitioners should consider testing for these agents as part of routine BRD diagnostic investigations.

**Key words:** bovine respiratory disease, bovine coronavirus, bovine influenza D, *Mycoplasma bovis*

## Résumé

Le complexe respiratoire bovin (CRB) est une cause importante de morbidité et de mortalité chez les veaux laitiers et de boucherie aux États-Unis. Bien que le CRB soit de nature multifactorielle, l'infection virale précède souvent l'infection bactérienne. Par conséquent, les tests diagnostiques prennent souvent en compte les composantes virales et bactériennes de la maladie. Des estimés de la prévalence des virus respiratoires et de *Mycoplasma bovis* seraient bien utiles pour l'interprétation des résultats de tests diagnostiques. L'objectif de cette étude était donc de déterminer la prévalence d'agents respiratoires bovins (le virus de la diarrhée virale bovine, le virus respiratoire syncytial bovin, le coronavirus respiratoire bovin, l'herpèsvirus bovin de type 1, le virus de l'influenza D et *M. bovis*). Des écouvillons nasopharyngés (n=3205) ont été recueillis dans des troupeaux allaitants et laitiers et dans des parcs d'engraissement et d'élevage. Ces échantillons ont été testés pour détecter la présence de ces agents respiratoires avec la PCR multiplex. On a examiné l'association entre le troupeau d'origine, le système de production et la saison de l'année et les estimés de prévalence au niveau individuel. Le coronavirus bovin était l'agent le plus souvent détecté avec une prévalence au niveau individuel de 36.05%. La prévalence de cet agent était significativement associée au type de production (plus élevée dans les parcs d'élevage) et à la saison (plus élevée l'automne). *M. bovis* et le virus de l'influenza D ont aussi été détecté souvent dans cette population de bovins. Moins de 5% des échantillons comportaient le virus de la diarrhée virale bovine, le virus respiratoire syncytial bovin et l'herpèsvirus bovin de type 1. La prévalence relativement élevée du coronavirus bovin et du virus de l'influenza D suggèrent que les praticiens devraient évaluer la présence de ces agents dans les tests diagnostiques routiniers du CRB.

## Introduction

Bovine respiratory disease (BRD) complex is 1 of the leading causes of morbidity and mortality in cattle, affecting approximately 12% of unweaned dairy heifers and 16% of feedlot calves in the United States.<sup>27,28</sup> Estimated losses due to BRD exceed \$1 billion annually in the US due to treatment costs, reduced performance, and mortality.<sup>11</sup> Both bacterial and viral agents have been implicated in the pathogenesis of BRD, and it is generally believed that stressors, such as transport, commingling, and dietary changes serve to compromise host immunity, which predisposes to viral infection.<sup>3</sup> Following viral infection, bacterial respiratory pathogens then colonize the lower respiratory tract, resulting in fatal bacterial pneumonia. Viral agents most commonly associated with the BRD complex include bovine respiratory syncytial virus (BRSV), bovine herpesvirus-1 (BHV-1), and bovine viral diarrhea virus (BVDV). Recent evidence suggests that bovine coronavirus (BCoV)<sup>25</sup> and bovine influenza D virus (IVD)<sup>5</sup> may also play a role in the BRD complex. Immunization of dams in late gestation (to provide passive colostral immunity) and calves early in life against these respiratory viruses is 1 management strategy that veterinarians and producers have chosen to help prevent BRD.

Bovine herpesvirus-1 is widely distributed in the cattle population and is associated with a variety of syndromes such as infectious rhinotracheitis, pustular vulvovaginitis, abortion, infertility, conjunctivitis, and encephalitis.<sup>16</sup> Typically, BHV-1 causes mild upper respiratory disease in cattle with fever, depression, anorexia, coughing, excessive nasal discharge, dyspnea, and inflamed nares.<sup>12</sup> Acute cases without secondary bacterial infection generally result in low mortality; however, latent infection occurs with subsequent reactivation during periods of stress, resulting in virus shedding and transmission to herdmates.<sup>17</sup>

Bovine viral diarrhea virus infection can result in multiple clinical syndromes and pathology in cattle, including respiratory infections, thrombocytopenia, reproductive disease, mucosal disease, and persistently infected calves.<sup>23</sup> The contribution of BVDV to the development of BRD in an individual is ultimately dependent on a variety of factors, including virulence of the viral strain, whether the infection is acute or persistent, vaccination status, and the presence of secondary bacterial infection.<sup>24</sup>

Bovine respiratory syncytial virus has a predilection for causing disease in younger calves and cattle on high planes of nutrition or certain feedstuffs like corn silage.<sup>22</sup> As with other respiratory viruses, clinical signs are often non-specific and include depression, inappetence, fever (104 to 108°F; 40 to 42.2°C), and severe dyspnea as the disease progresses.<sup>1</sup>

Bovine coronavirus is a pneumoenteric virus, infecting epithelial cells of both the respiratory and intestinal tract. In cattle, this virus is associated with 3 distinct clinical syndromes: calf diarrhea, winter dysentery, and respiratory infections.<sup>9</sup> Bovine coronavirus-induced respiratory disease

primarily involves the upper respiratory tract which then predisposes the lower (pulmonary) respiratory tissues to infection by secondary (bacterial) pathogens. Although the role of BCoV in BRD has not been fully established, it has been repeatedly found in BRD cases, often in conjunction with other respiratory viruses and bacteria such as *Mannheimia haemolytica*.<sup>7</sup>

Bovine influenza D virus is a recently described virus that is phylogenetically and antigenically distinct from human influenza virus.<sup>10</sup> In a case-control study, IVD was significantly associated with BRD, as were bovine adenovirus 3 and bovine rhinitis A.<sup>18</sup> Clinically, IVD has been shown to cause mild respiratory disease and is easily transmitted between penmates.<sup>5</sup> Due to its recent discovery, the exact role of IVD in BRD is still under investigation; however, studies have shown that it has been circulating in cattle populations for over a decade.<sup>4</sup>

*Mycoplasma bovis* has been associated with a variety of clinical syndromes in cattle, including pneumonia, mastitis, otitis media, arthritis, keratoconjunctivitis, and reproductive system infections.<sup>19</sup> The clinical signs of *M. bovis* respiratory infection are generally non-specific in nature; however, pneumonia associated with arthritis or otitis is suggestive, as is chronicity and poor response to antibiotic therapy.<sup>2</sup> While *M. bovis* has been shown to produce primary respiratory disease experimentally, its role in natural infection is not as well defined as it is often found in chronic pneumonic lesions and can be isolated from the lungs of animals without respiratory disease.<sup>8,15</sup>

As these respiratory viruses and *M. bovis* are commonly associated with BRD, overall prevalence and associations with production type and season of year may be useful for practitioners considering diagnostic submissions or interpreting diagnostic test results. The objective of the current study was to determine the prevalence of bovine respiratory viruses (BVDV, BRSV, BCoV, BHV-1, IVD) and *M. bovis* in US cattle, and to evaluate associations with herd of origin, production type, and season of year.

## Materials and Methods

### Sampling Methods and Collection

Veterinary practitioners were enrolled in study participation by members of the Merck Animal Health ruminant technical services team. Participation was strictly voluntary and samples were collected as part of routine practice procedures. Number of animals to be sampled per herd and classification of production type (cow-calf, stocker, feedlot, dairy) were at the discretion of the individual submitting veterinarian. The study population was a convenience sample (both herds and animals within herd) with the intent to sample clinically healthy animals; however, disease status and other relevant clinical data were not collected as part of this study.

For collection of nasopharyngeal swabs, veterinarians were instructed to restrain individual animals and remove dirt/debris from the external nares with single use towels. A

single-use double guarded swab<sup>a</sup> was passed into the nasopharynx through the ventral medial meatus (the approximate distance from the external nares to the medial canthus of the eye), passed through the first and second guards, rotated against the pharyngeal recess, and retracted into the guards. After removing the entire sampling apparatus from the nasal cavity, the swab was placed in commercial transport media<sup>b</sup> and shipped on ice for overnight delivery to the Kansas State Veterinary Diagnostic Laboratory. Specimens were processed by real time - polymerase chain reaction (RT-PCR) testing for BRSV, BHV-1, BVDV, IVD, BCoV, and *M. bovis*.

### Polymerase Chain Reaction (PCR) Methods

Nasopharyngeal swabs, collected and transported in liquid Amies media, were used for nucleic acid recovery. First, sample tubes containing swabs were gently vortexed for a few seconds followed by centrifugation for 15 seconds to collect the liquid at the bottom of the tube. Seventy microliters of supernatant was used for nucleic acid extraction. A magnetic beads-based nucleic acid extraction method was used to isolate the nucleic acids following the kit manufacturer's instructions.<sup>c</sup>

Following nucleic acid recovery, testing was conducted utilizing a real-time PCR-based bovine respiratory viral multiplex assay, offered at Kansas State Veterinary Diagnostic Laboratory (KSVDL). This RT-PCR targets 5 viral agents, BRSV, BHV-1, BVDV, BCoV, IVD, and 1 bacterial agent, *M. bovis*. Each sample of nucleic acid was assessed for these 6 targets in a 25 µl final reaction volume and the RT-PCR was performed in an Applied Biosystems 7500 Real-time PCR instrument. Prior to result analysis, the PCR run was validated by verifying the Cycle threshold (Ct) values of positive and negative internal controls included in each run. For this study, Ct values were interpreted as follows: sample is considered positive for a target if the Ct value is <37, and negative for a target if its Ct value is ≥37 or no Ct.

### Statistical Analysis

Data were analyzed in general linear mixed models with a binary outcome to represent the probability of a test positive. Individual animals were the unit of analysis. However, models included a random intercept term to account for the lack of independence among animals within herd of origin. Fixed effects of production system (cow-calf, dairy, feedlot, stocker) and season of year were evaluated for unconditional associations with the outcome variables. The type of production system was assigned by the submitting veterinarian and this information was collected as part of the diagnostic submission process. Season of year was defined by sample submission date. For purposes of this study, winter was defined as December – February; spring, March – May; summer, June – August; fall, September – November. Multivariable models were developed when more than 1 unconditional

association was statistically significant ( $P < 0.05$ ). Marginal means (model-adjusted means) and corresponding standard errors and confidence intervals were calculated from model outputs. Pairwise comparisons were made with adjustment for multiple comparisons using the Tukey method, when the overall treatment effect was significant. All models were fitted using maximum likelihood estimation and Kenward-Roger degrees of freedom method in Proc GLIMMIX SAS 9.3.

### Results

Overall, 3,215 samples were submitted for PCR testing between May 2015 and July 2016. Ten samples were excluded from statistical analyses because the submission did not include information on premise. The remaining 3,205 samples originated from 80 different premises and samples per location ranged from 1 to 562 (mean - 39.2; median - 13.5). The overall percent positive, animal-level adjusted prevalence, prevalence (adjusted) by production class, and prevalence (adjusted) by season can be found in Table 1. Briefly, bovine coronavirus was the most prevalent virus in US cattle, with an overall animal-level prevalence (adjusted for clustering within premise/source farm) of 36.05%. Production class was statistically significant for BCoV, with highest prevalence of this respiratory agent in stocker class cattle. Season of year was also significant for BCoV, with the highest prevalence in the fall months (Sep – Nov); however, the prevalence was greater than 20% for all seasons in the present study. The interaction between production class and season was not significant for BCoV. For BVDV, IVD, and *M. bovis*, only season of year was significant, with the highest prevalence of these agents in winter months (Dec – Feb). Production class and season were not significantly associated with the prevalence of BRSV and BHV-1. The animal-level adjusted prevalence for multiple agent positive (in any combination) was 15.39%. Season was significantly associated with multiple positive results, with winter having the highest prevalence. The respiratory agents most commonly found in combination were BCoV/*M. bovis* and IVD/*M. bovis* (Figure 1). Any combination of BVDV, BHV-1, and BRSV (as might be present in commercial vaccines) was detected in only 24 samples (<1%) with no specific combination occurring in more than 5 samples.

### Discussion

The overall objective of our study was to determine the prevalence of bovine respiratory viruses (BRSV, BHV-1, BVDV, IVD, BCoV) and *M. bovis* in US cattle from a convenience sample of herds and to evaluate associations with herd of origin, production type, and season of year. Our survey results show a high animal-level adjusted prevalence of BCoV (36.05%), moderate prevalence of *M. bovis* (16.73%) and IVD (5.55%) with nominal levels of BRSV, BHV-1, and BVDV (all <5%). In addition, there were a moderate number of samples positive for multiple agents (15.39%).

Overall, the results reported here generally agree with previous reports of prevalence for bovine respiratory viruses and *M. bovis* with considerations for differences in study design and sample populations. In a previous retrospective analysis conducted by O'Neill et al, 1363 nasal swabs collected

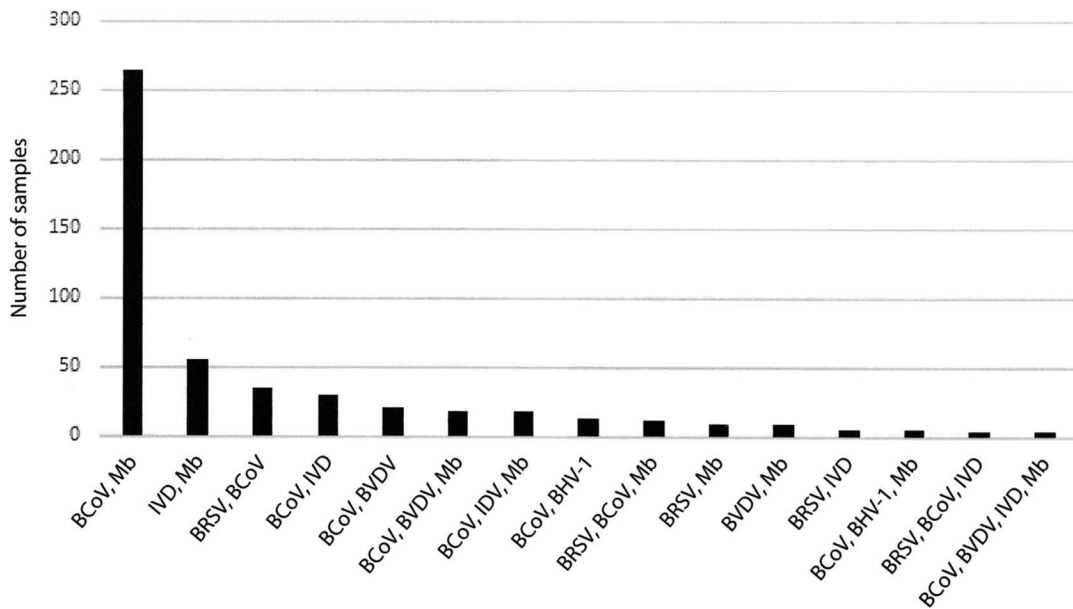
from cattle during outbreaks of BRD were used to determine the prevalence of respiratory agents in Ireland.<sup>21</sup> In that study, 1 or more respiratory viral pathogens were detected in 34.6% of the samples, with BCoV detected most frequently (22.9%) compared to 11.6%, 6.1%, and 5.0% for BRSV, BHV-1,

**Table 1.** Overall percent positive, animal-level prevalence\*, prevalence by production class, and prevalence by season for viruses detected in nasopharyngeal swabs of US cattle.

Respiratory agent	Overall percent (proportion) positive	Animal-level prevalence (95% Confidence Intervals)	Prevalence by production class (95% Confidence Intervals)				Prevalence by season (95% Confidence Intervals)			
			Cow-calf	Dairy	Feedlot	Stocker	Winter (Dec – Feb)	Spring (Mar – May)	Summer (Jun – Aug)	Fall (Sep – Nov)
BRSV	3.81% (122/3205)	2.88% (1.9-4.4)	0.69% (0.1-3.5)	3.89% (2.2-6.8)	1.92% (0.6-5.8)	3.22% (1.3-7.6)	5.26% (2.7-10.1)	3.64% (1.8-7.2)	2.90% (1.7-5.1)	2.14% (1.2-3.8)
BHV-1	1.59% (51/3205)	1.59% (1.2-2.1)	1.49% (0.3-7.6)	1.45% (0.6-3.3)	0.53% (0.1-3.1)	0.47% (0.1-2.4)	2.92% (1.7-5.1)	0.88% (0.3-2.7)	1.88% (1.2-2.9)	1.15% (0.7-1.9)
BVDV	3.56% (114/3205)	3.92% (2.8-5.5)	1.21% (3.4-4.1)	3.94% (2.4-6.3)	4.98% (2.4-10.2)	5.06% (2.6-9.6)	9.90% (5.1-18.5)	5.18% (2.6-10.2)	4.34% (2.5-7.4)	2.23% (1.3-3.8)
IVD	8.30% (266/3205)	5.55% (3.4-9.0)	2.20% (0.5-9.5)	4.90% (2.4-9.6)	9.91% (3.2-26.9)	6.21% (2.1-17.0)	17.24% (9.9-28.4)	12.44% (6.4-22.7)	4.93% (2.7-8.9)	2.66% (1.4-4.9)
BCoV	43.81% (1404/3205)	36.05% (26.3-47.2)	15.98% (5.0-40.6)	32.75% (21.4-46.5)	24.96% (10.1-49.8)	69.51% (46.0-85.9)	25.07% (15.3-38.2)	21.73% (13.9-32.4)	32.09% (22.7-43.2)	50.46% (38.7-62.1)
<i>M. bovis</i>	20.12% (645/3205)	16.73% (11.4-24.0)	11.09% (3.1-33.1)	16.51% (9.7-26.7)	23.69% (9.4-48.1)	15.03% (5.9-33.2)	31.05% (19.6-45.4)	17.05% (10.3-26.9)	19.41% (12.6-28.8)	12.37% (7.7-19.4)
Multiple positive	17.32% (555/3205)	15.39% (11.3-20.7)	9.65% (3.4-24.5)	15.41% (10.0-23.0)	15.64% (7.1-31.2)	18.27% (9.2-33.0)	35.64% (24.3-48.8)	11.91% (7.3-18.8)	17.01% (11.5-24.4)	12.45% (8.3-18.4)

BRSV – bovine respiratory syncytial virus; BHV-1 – bovine herpesvirus-1; BVDV – bovine viral diarrhea virus; IVD – bovine influenza type D virus; BCoV – bovine coronavirus; *M. bovis* – *Mycoplasma bovis*; multiple positive – samples positive for 2 or more agents in combination

\* Prevalence estimates were from mixed models adjusting for the lack of independence within herds/premises



**Figure 1.** Number of nasopharyngeal samples positive for combinations of respiratory agents (3,205 total samples\*).

BRSV – bovine respiratory syncytial virus; BHV-1 – bovine herpesvirus-1; BVDV – bovine viral diarrhea virus; IVD – bovine influenza type D virus; BCoV – bovine coronavirus; Mb – *Mycoplasma bovis*

\* Only combinations detected in 5 or more samples shown

and BVDV, respectively. The numerical differences between the O'Neill study and the present study could be due to differences in sample populations as the O'Neill study targeted clinically ill cattle; or differences in virus prevalence over time as the O'Neill study was conducted from 2008 through 2012, whereas the current study was conducted in 2015 and 2016. However, the observed numerical differences may not be significant, but rather a reflection of the significant variability in viral prevalence in both studies. Although a statistical analysis was not performed in the O'Neill study, the authors noted a seasonal pattern of viral detection, similar to the results the present study.

There have been previous surveys conducted to determine the prevalence of persistent infection (PI) with BVDV in cattle. Generally, these surveys have reported a very low sample-level prevalence (<1%) in the US cattle population.<sup>14,20</sup> It is not unexpected that the prevalence of BVDV reported in the present study is much higher as the testing platform (PCR) used in our study would potentially detect not only virus shed by BVDV – PI animals, but also transiently infected calves. Additionally, the PCR test will detect, but not differentiate, both BVDV type 1 and BVDV type 2, so these estimates represent the summation of both genotypes.

As IVD has only recently been described in cattle, there are only a limited number of prevalence studies available for comparison. In a survey of Mississippi cattle, IVD was found in 29% of sick calves, but only 2.4% of healthy calves were positive by PCR.<sup>5</sup> Our adjusted prevalence of 5.5% is closer to the lower estimate for healthy Mississippi calves. As our intention was to sample clinically normal calves, with likely inclusion of some diseased cattle in our study population, the prevalence estimates for IVD between the previous and current studies are quite similar.

One of the limitations of this study is that the enrollment of herds and method for determining which cattle to sample within a herd (e.g. convenience sampling) could have introduced bias into these prevalence estimates. For example, if well-managed herds with good biosecurity and low BRD morbidity were more likely to be selected for study participation, the prevalence estimates reported here may underestimate “true” prevalence of these pathogens in the cattle population. In addition, if study participants in 1 production class preferentially selected clinically diseased animals compared to participants in other production systems, the association with production class could be biased.

Another limitation of this study is that disease causality cannot be inferred from the sample-level prevalence estimate. For viruses that are well-established BRD pathogens, this is less of an issue, because detection of the pathogen is known to be associated with an increased risk for BRD. However, for new viruses or viruses where the role in disease pathogenesis is not as clear, this study design can only provide a “benchmark” expectation of how frequently a particular organism will be encountered. When conducting disease outbreak investigations, this information becomes useful as clinicians

attempt to interpret the prevalence of a pathogen as normal or abnormal (below or above the expectations). The results presented here also showed that there was an association between production system and season for BCoV, and season for IVD. Practitioners could adjust their expectations based on these factors.

Another limitation of this study is that PCR was utilized as the testing platform. The primary disadvantage of this methodology is that as the test detects viral nucleic acid, it cannot be assumed that test positive animals are truly infected. Therefore, our prevalence estimates would include animals that represent “true” viral infections, as well as animals in which the virus is only transiently present.

The impact of recent vaccination on PCR test results is equivocal in the published literature and may be influenced by the respective vaccine, viral agent, and time since vaccination. Fulton *et al* demonstrated that persistently-infected calves shed BVDV vaccine virus into the nasal cavity following vaccination with modified-live vaccine,<sup>6</sup> while Klieboeker *et al* did not detect either BVDV or BHV-1 in the nasal cavity of beef calves following vaccination.<sup>13</sup> Socha *et al* reported positive PCR results for BRSV (and PI3) for up to 8 days following the use of an intranasal modified-live vaccine.<sup>26</sup> The prevalence estimates reported here for BVDV, BHV-1, and BRSV were likely impacted minimally by the use of commercial combination vaccines as the prevalence of all 3 agents was relatively low, and these viruses were infrequently detected together in any combination (Figure 1). However, as we have no information on vaccination status at the time of sampling, prevalence of these agents should be interpreted accordingly.

Detection of vaccine virus would not affect the prevalence of IVD in the current study as this virus is not available in any commercially available vaccines. Although BCoV was available in a commercial vaccine at the time of this study, it is not as widely used as other BRD vaccines and it likely contributes minimally to the prevalence estimate in our survey. The RT-PCR used here does not distinguish between the enteric and respiratory forms of this virus, so the sample-level prevalence here may overestimate the respiratory form; however, a previous study reported antibody neutralization and hemagglutination activity to be similar between respiratory and enteric bovine coronaviruses.<sup>9</sup> Any prevalence estimate of BCoV as a respiratory pathogen should be interpreted in light of the testing limitations.

Additionally, the intent of our study was to sample clinically healthy calves; however, based on submission form histories, not all samples met this intent. No samples were excluded from testing or final data analysis based on disease status for 2 reasons: 1) it is likely that the sample set would still include clinically ill calves although it was not indicated on the submission form and 2) the relatively poor diagnostic sensitivity of visual exam to detect clinical illness. In reality, the submissions described here likely represent samples from calves at all stages of disease: clinically normal, subclinical, acutely infected, and chronically ill.

## Conclusions

We describe here a high animal-level prevalence of BCoV in nasopharyngeal samples from US cattle and its significant associations with production system (highest in stocker cattle) and season of year (highest in fall). In addition, the prevalence (adjusted for premise) of IVD, a newly described virus associated with clinical BRD, was 5.5% and was significantly associated with season (highest prevalence in winter, followed by spring). Results of this study suggest that BCoV and IVD should be investigated as part of an overall BRD diagnostic work-up. Additionally, as the role of these agents in the BRD complex is further elucidated, practitioners should interpret results with regard to both season and production class.

## Endnotes

<sup>a</sup>Guarded Culture Swab, Continental Plastic Corp., Delavan, WI  
<sup>b</sup>ESwab™ Liquid Amies Collection and Transport system, Copan Diagnostics, Murrieta, CA  
<sup>c</sup>MagMAX™-96 Viral RNA Isolation Kit, Life Technologies, Carlsbad, CA

## Acknowledgements

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: B.V. Lubbers and D.G. Renter have active consulting agreements with Merck Animal Health. B.V. Lubbers, R.A. Hesse, and L. Peddireddi are employed by the Kansas State Veterinary Diagnostic Laboratory. M.T. Zerse, B.D. Meyer, and E.A. Cox are employees of Merck Animal Health.

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: funding (diagnostic testing fees) was provided by Merck Animal Health.

## References

1. Baker JC, Ellis JA, Clark EG. Bovine respiratory syncytial virus. *Vet Clin North Am Food Anim Pract* 1997; 13:425-454.
2. Caswell JL, Archambault M. *Mycoplasma bovis* pneumonia in cattle. *Anim Health Res Rev* 2007; 8:161-186.
3. Cusack PM, McMeniman N, Lean IJ. The medicine and epidemiology of bovine respiratory disease in feedlots. *Aust Vet J* 2003; 81:480-487.
4. Ducatez MF, Pelletier C, Meyer G. Influenza D virus in cattle, France, 2011-2014. *Emerg Infect Dis* 2015; 21:368-371.
5. Ferguson L, Eckard L, Epperson WB, Long LP, Smith D, Huston C, Genova S, Webby R, Wan XF. Influenza D virus infection in Mississippi beef cattle. *Virology* 2015; 486:28-34.
6. Fulton RW, Step DL, Ridpath JF, Saliki JT, Confer AW, Johnson BJ, Briggs RE, Hawley RV, Burge LJ, Payton ME. Response of calves persistently infected with noncytopathic bovine viral diarrhoea virus (BVDV) subtype 1b after vaccination with heterologous BVDV strains in modified live virus vaccines and *Mannheimia haemolytica* bacterin-toxoid. *Vaccine* 2003; 21: 2980-2985.
7. Fulton RW. Bovine respiratory disease research (1983-2009). *Anim Health Res Rev* 2009; 10: 131-139.

8. Gagea MI, Bateman KG, Shanahan RA, van Dreumel T, McEwen BJ, Carman S, Archambault M, Caswell JL. Naturally occurring *Mycoplasma bovis*-associated pneumonia and polyarthritis in feedlot beef calves. *J Vet Diag Invest* 2006; 18:29-40.
9. Hasoksuz M, Lathrop SL, Gadfield KL, Saif LJ. Isolation of bovine respiratory coronaviruses from feedlot cattle and comparison of their biological and antigenic properties with bovine enteric coronaviruses. *Am J Vet Res* 1999; 60:1227-1233.
10. Hause BM, Collin EA, Liu R, Huang B, Sheng Z, Wuxun L, Wang D, Nelson EA, Li F. Characterization of a novel influenza virus in cattle and swine: proposal for a new genus in the *Orthomyxoviridae* family. *mBio* 2014; 5:e00031-14.
11. Irsik M, Langemeier M, Schroeder T, Spire M, Roder JD. Estimating the effects of animal health on performance of feedlot cattle. *Bov Pract* 2006; 40:65-74.
12. Jones C, Chowdhury S. A review of the biology of bovine herpesvirus type 1 (BHV-1), its role as a cofactor in the bovine respiratory disease complex and development of improved vaccines. *Anim Health Res Rev* 2007; 8:187-205.
13. Kleiboeker SB, Lee SM, Jones CA, Estes DM. Evaluation of shedding of bovine herpesvirus 1, bovine viral diarrhoea virus 1, and bovine viral diarrhoea virus 2 after vaccination of calves with a multivalent modified-live virus vaccine. *J Am Vet Med Assoc* 2003; 222: 1399-1403.
14. Loneragan GH, Thomson DU, Montgomery DL, Mason GL, Larson RL. Prevalence, outcome, and health consequences associated with persistent infection with bovine viral diarrhoea virus in feedlot cattle. *J Am Vet Med Assoc* 2005; 226:595-601.
15. Maunsell FP, Woolums AR, Francoz D, Rosenbusch RF, Step DL, Wilson DJ, Janzen ED. *Mycoplasma bovis* infections in cattle. *J Vet Intern Med* 2011; 25: 772-783.
16. Muylkens B, Thiry J, Kirten P, Schynts F, Thiry E. Bovine herpesvirus 1 infection and infectious bovine rhinotracheitis. *Vet Res* 2007; 38:181-209.
17. Nandi S, Kumar M, Manohar M, Chauhan RS. Bovine herpesvirus infections in cattle. *Anim Health Res Rev* 2009; 10:85-98.
18. Ng TFN, Kondov NO, Deng X, Van Eenennaam A, Neilbergs HL, Delwart E. A metagenomics and case control study to identify viruses associated with bovine respiratory disease. *J Virol* 2015; 89:5340-5349.
19. Nicholas RAJ, Ayling RD. *Mycoplasma bovis*: disease, diagnosis, and control. *Res Vet Sci* 2003; 74:105-112.
20. O'Connor AM, Reed MC, Denagamage TN, Yoon KJ, Sorden SD, Cooper VL. Prevalence of calves persistently infected with bovine viral diarrhoea virus in beef cow-calf herds enrolled in a voluntary screening project. *J Am Vet Med Assoc* 2007; 230:1691-1696.
21. O'Neill R, Mooney J, Connaghan E, Furphy C, Graham DA. Patterns of detection of respiratory viruses in nasal swabs from calves in Ireland: A retrospective study. *Vet Rec* 2014; 175:351.
22. Prieksat P, Thompson J. Bovine respiratory syncytial virus infection. *Iowa State University Veterinarian* 1988; 50:57-60.
23. Ridpath JF. Bovine viral diarrhoea virus: Global status. *Vet Clin North Am Food Anim Pract* 2010; 26:105-121.
24. Ridpath JF. The contribution of infections with bovine viral diarrhoea viruses to bovine respiratory disease. *Vet Clin North Am Food Anim Pract* 2010; 26:335-348.
25. Saif LJ. Bovine respiratory coronavirus. *Vet Clin North Am Food Anim Pract* 2010; 26:349-364.
26. Socha W, Rola J, Bednarek D, Urban-Chmiel R, Zmudzinski JF. Shedding course of bovine respiratory syncytial virus and bovine parainfluenza 3 virus in calves vaccinated intranasally. *Bull Vet Inst Pulawy* 2013; 57:479-483.
27. U.S. Department of Agriculture – Animal and Plant Health Inspection Service. 2007. Dairy 2007 Part III: Reference of dairy cattle health and management practices in the United States, 2007. Available at: [https://www.aphis.usda.gov/animal\\_health/nahms/dairy/downloads/dairy07/Dairy07\\_dr\\_PartIII\\_rev.pdf](https://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy07/Dairy07_dr_PartIII_rev.pdf) Accessed: 2 December 2016.
28. U.S. Department of Agriculture – Animal and Plant Health Inspection Service. 2011. Feedlot 2011 Part IV: Health and health management on U.S. feedlots with a capacity of 1,000 or more head. Available at: [https://www.aphis.usda.gov/animal\\_health/nahms/feedlot/downloads/feedlot2011/Feed11\\_dr\\_PartIV.pdf](https://www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot2011/Feed11_dr_PartIV.pdf) Accessed: 2 December 2016.