

3rd Annual TVMDL Amarillo
BOVINE RESPIRATORY DISEASE
Conference

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Clinical Utility and Interpretation of Diagnostic Tests for Bovine Respiratory Disease

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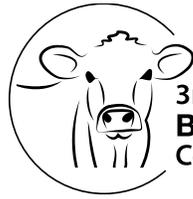
INTRODUCTION

Bovine respiratory disease (BRD) is the most common and costly disease of beef cattle in North America. In feedlots, BRD is responsible for 75% of all morbidity and 50-75% overall mortality. Approximately 16.2% of all cattle entering feedlots will be diagnosed with BRD and 2% will die. In stocker cattle, BRD morbidity occurs far more frequently than what is commonly seen in feedlot cattle and is estimated to be responsible for 90% of all morbidity and mortality in these operations. As a result, it is not unusual to see morbidity risk exceed 75% in certain cohorts of animals. Economically, BRD costs the beef industry \$2-4 billion annually with 20% of total losses due to medication costs and 80% of total losses due to reduced carcass weight and quality. While multiple factors play a role in the development of BRD, infection of the lower respiratory tract with viruses and bacteria is ultimately responsible for the clinical signs seen in affected cattle. As a result, monitoring and testing for pathogens associated with BRD might facilitate the development of medically appropriate preventive health programs. In addition, the stocker and feedlot segments have been encountering issues with antimicrobial resistance (AMR) in *Mannheimia haemolytica* and, based on recently published data, the isolation of strains of *Mannheimia haemolytica* that are resistant to multiple antimicrobials is becoming a more frequent occurrence. Thus, monitoring for AMR in common bacterial pathogens might help with the design of effective therapeutic regimens. The purpose of these proceedings is to review the basic characteristics of antemortem diagnostic tests available for BRD and provide recommendations for their use in field settings.

EPIDEMIOLOGY OF RESPIRATORY DISEASE IN CATTLE POPULATIONS

Bovine respiratory disease is encountered across all segments of the beef industry. Since various viruses and bacteria are commonly encountered in all animals with BRD, having an understanding of the overall prevalence within a specific population might help decisions on the submission of diagnostic samples and interpretation of diagnostic test results. In nursing beef calves, BRD is an emerging issue that is not well understood. Morbidity and mortality due to nursing calf pneumonia vary from farm to farm and year to year. Nevertheless, the disease can have a significant impact in some herds. While our understanding of nursing calf pneumonia is still in its infancy, common pathogens encountered in affected animals include *M. haemolytica*, *P. multocida*, BRSV, BVDV, and BHV-1.

In contrast to nursing beef calves, the epidemiology of BRD in stocker and feedlot cattle is relatively well understood. Generally, *M. haemolytica* is the most commonly isolated bacterial pathogen in these populations of animals. Nevertheless, recent studies have found an increase in the prevalence of *P. multocida* in some populations of animals. Interestingly, *Mycoplasma* spp and *M. bovis*, specifically, is found at a relatively high level in almost all populations. The prevalence of viral pathogens varies from study to study and cohort to cohort but, historically, BRSV, BVDV, and BHV-1 have been the pathogens of most concern. More recently, however, surveillance studies from feedlot cattle have shown a relatively high prevalence of both Influenza D and Coronavirus, making their inclusion in any diagnostic panel a necessity.



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CHARACTERISTICS OF DIAGNOSTIC TESTS – ABILITY TO DETECT HEALTH OR DISEASE

Generally, tests are used for either screening or diagnostic purposes. Screening tests are often applied to healthy animals or herds and further work up or scrutiny only given to those animals that are test positive. In contrast, diagnostic tests are used to confirm or classify disease, guide treatment, or aid in determining the prognosis of a diseased animal. Regardless of how they are used (screening vs diagnostic), tests should only be considered for use in a clinical decision-making context if the result of that test will change your management of a specific case.

Two important characteristics of a test's ability to determine health status of a clinical patient are sensitivity (Se) and specificity (Sp). Sensitivity is the probability of an animal testing positive given that it is truly diseased. Specificity, in contrast, is the probability of an animal testing negative given that it is truly healthy. In order to determine sensitivity and specificity of a diagnostic test, the test in question must be compared to a gold standard. A gold standard test is a test that is absolutely accurate and diagnoses all of a specific disease that is present while misdiagnosing none. Unfortunately, there are very few true gold standards in existence, a factor that makes even the earliest stages of test evaluation fraught with imperfections.

While much attention is given to sensitivity and specificity when considering the interpretation of a specific test, these parameters only describe the test being used and give diagnosticians some sense of how one test might compare to another. In other words, sensitivity and specificity given absolutely no indication of how useful a test will be when it is applied to animals of unknown disease status. To put it more simply, we are more interested in the probability of an animal truly being disease positive or negative given a positive or negative test result. These probabilities are called predictive values and, unlike sensitivity and specificity, change with different populations of animals tested because they are dependent on both the true prevalence of disease and the test characteristics. There are two predictive values commonly used in clinical test interpretation: positive predictive value (PPV) and negative predictive value (NPV). Positive predictive value is the probability that an animal testing positive for a disease truly has the disease. In contrast, negative predictive value is the probability that an animal testing negative for a disease is truly free of disease. As stated previously, predictive values are dependent on the true prevalence of disease within a population and, as prevalence increases, so too does positive predictive value. The relationship between Se, Sp, PPV, and NPV is shown below in Table 1.

Table 1. Calculating Sensitivity, Specificity, and Predictive Values

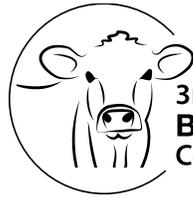
	Test +	Test -
Disease +	TP	FN
Disease -	FP	TN

$$\text{Se} = \text{TP}/\text{TP}+\text{FN}$$

$$\text{Sp} = \text{TN}/\text{TN}+\text{FP}$$

$$\text{PPV} = \text{TP}/\text{TP}+\text{FP}$$

$$\text{NPV} = \text{TN}/\text{TN}+\text{FN}$$



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The clinical importance of making a distinction between these different parameters is illustrated in the example below evaluating the diagnostic utility of an ear notch for detecting BVD persistent infection in beef cattle:

Population = 1000 auction market derived beef calves

True prevalence of BVD PI = 0.4%

Sensitivity of test = 99%

Specificity of test = 99%

	Test +	Test -
Disease +	4	0
Disease -	11	985

PPV = $TP/TP+FP = 4/4+11 = 27\%$

NPV = $TN/TN+FN = 985/0+985 = 100\%$

As you can see, both the Se and Sp are quite high and make the test in question seem perfect on paper when not being applied to a relevant population. However, because prevalence is so low (0.4%), PPV is only 27%. This means that, in this population, approximately 3 out of every 4 animals that test positive for BVD will be false positives. If we now apply the same test to another population with different disease prevalence, the performance of the test can change dramatically:

Population = 1000 auction market derived beef calves

True prevalence of BVD PI = 2%

Sensitivity of test = 99%

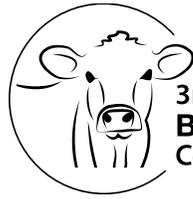
Specificity of test = 99%

	Test +	Test -
Disease +	20	0
Disease -	11	969

PPV = $TP/TP+FP = 20/20+11 = 65\%$

NPV = $TN/TN+FN = 969/0+969 = 100\%$

Another concept with which it is important to be familiar is agreement. Often, another diagnostic test becomes available that is cheaper, more convenient, or technically less challenging. Generally, these tests are measuring the same parameter or quantity and, when this situation arises, we want to know how well the new test agrees with the old test to ensure that the results of the new test can be trusted. For example, a study recently evaluated the agreement between 4 different antemortem diagnostic tests to identify respiratory pathogens in dairy calves with BRD. In this study, the transtracheal wash (TTW)



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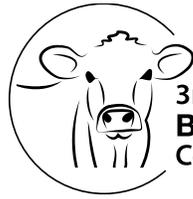
was considered the reference standard since it bypasses the contaminants commonly found in the upper airway. However, the TTW is considered to be invasive, somewhat expensive, and technically challenging. The goal was to determine how well the results of culture and PCR for bacterial and viral respiratory pathogens from a nasopharyngeal swab (NPS), nasal swab (NS), and bronchoalveolar lavage (BAL) agreed with TTW so that results derived from samples collected with these techniques could be interpreted with confidence. Ultimately, this study found that, by and large, agreement between TTW and the other diagnostic tests was very good for the majority of pathogens. This means that a veterinarian can collect samples using less invasive and time consuming techniques and be provided with results that are in line with a test that is considered to be accurate. There are multiple methods commonly used to measure agreement and each method has advantages, disadvantages, and datasets to which they are most appropriately applied.

DIAGNOSTIC TESTS FOR BOVINE RESPIRATORY DISEASE

The nasal swab (NS), guarded nasopharyngeal swab (NPS), transtracheal wash (TTW), and bronchoalveolar lavage (BAL) are all used to collect samples from animals suspected of having BRD. However, each of these methods has advantages and limitations. The TTW allows collection of a sample from the lower airways and bypasses the normal flora of the nasopharynx but the procedure is invasive, expensive, and time consuming. Nasal swabs, however, are simple to collect. Unfortunately, the results of bacterial culture of nasal swabs may be difficult to interpret due to the potential for contamination by commensal organisms. The guarded NPS has been proposed to provide a more reliable sample of bacteria causing pneumonia but these can be unwieldy to collect and they are also relatively expensive. The BAL has been proposed to provide a representative sample of the lower respiratory tract but the method of collection provides the possibility of upper airway commensal contamination of the sample. Moreover, it is possible to miss pathogens not evenly distributed throughout the lung.

Anecdotal comments have indicated that NS are unreliable for diagnosis of bacterial BRD agents, because NS cultures are often overgrown by contaminants. Nevertheless, overgrowth of contaminants does not make it impossible to identify the bacterial agents of interest and studies have shown good agreement between NS and other diagnostic testing modalities. Generally, it is recommended to wipe the nostrils clean with a single use paper towel to remove excess respiratory secretions to reduce the risk of sample contamination.

Like the NS, the ability of a NPS to provide a representative sample of the respiratory tract flora is a matter of debate. One study found that *Mh* could be isolated from the tonsils when it could not be isolated from the nasopharynx. Nevertheless, other studies have shown better agreement between NPS and more invasive collection techniques (trans-tracheal swab, trans-tracheal aspirate or bronchoalveolar lavage) used for sampling the lower respiratory tract. Indeed, work has shown that paired isolates from nasal swabs and trans-tracheal swabs were the same species 96% of the time, and genetically identical 70% of the time, while other groups have found that paired samples from NPS and lung samples collected at necropsy were the same 77% of the time. Another study also found that the observed agreement between NPS and trans-tracheal aspirates was nearly perfect at 77%. Research has shown that the positive and negative predictive value of NPS relative to lung lavage were 100 % and 67%, respectively. More recent work comparing the agreement between TTW, BAL, NS, and NPS to identify respiratory pathogens in dairy calves found that agreement between TTW, BAL, NS, and NPS was very good for *P. multocida*, *M. haemolytica*, and *M. bovis*. In contrast, relative agreement between NS, NPS, or BAL and TTW were not the same for the two viral pathogens identified, BRSV and Bovine Coronavirus (BCV). It was more common



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to find BCV with the NS or NPS than with the TTW, and it was more common to find BRSV with the TTW than the NS. Finally, work from Kansas State University comparing BAL to NPS found that the PPV and NPV of NPS relative to BAL were 67% and 100% for *M. haemolytica*, 75% and 100% for *P. multocida*, and 100% and 96% for *H. somni*. Agreement between results of the samples was substantial for *M. haemolytica* and *H. somni* and almost perfect for *P. multocida*. As it relates to antimicrobial susceptibility, there are times where disagreement can occur and this is a finding that suggests that more than 1 susceptibility phenotype can exist in the airway of the same animal.

CONCLUSIONS

Multiple diagnostic tests are available to help the practitioner better characterize the pathogens associated with BRD in cattle. Many of the diagnostic tests agree relatively well with one another and give flexibility to the collection and submission of diagnostic samples.



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