Diagnostic Considerations for Bovine Respiratory Disease

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Introduction

Bovine respiratory disease remains a significant cause of economic loss to the cattle industry[1] and source of frustration to the cow/calf sector, the feedlot industry, veterinary practitioners and laboratory diagnosticians.

Numerous contributing factors are involved including weaning stress, shipping, commingling, anatomic and physiologic features of the bovine respiratory tract and multiple infectious agents. The role of many of these agents is currently not fully understood. This has led to the term bovine respiratory disease complex (BRD).[2] BRD can be a clinically useful term but to the laboratory diagnostician and the practitioner trying to design a control program or a treatment regimen a more specific etiologic diagnosis is generally necessary.

While numerous infectious agents have been reported to be involved in the BRD complex only a handful appear to be of major etiologic significance (Table 1). Identifying the specific risk factor(s) and agent(s) involved in a particular group of cattle best involves a team approach and this includes feedlot management and personnel, on-site and consulting veterinarians and diagnostic laboratory personnel. Good communication between field veterinarian and the diagnostic laboratory are particularly critical as is an understanding by field veterinarians of the limitations of the various laboratory tests.

Early identification and treatment of sick animals is considered to be the key to success in outbreaks of BRD and early, accurate diagnosis is prerequisite to successful treatment. The inherent time lag in many laboratory tests necessitates that the practitioner often has to act on their initial clinical impression but laboratory confirmation and monitoring can be essential to adjusting treatment and preventative protocols.

Sample Collection and Submission - Upper Respiratory Tract

Nasal swabs have been used to monitor potential pathogens in a group of cattle or as diagnostic procedures in early clinical respiratory disease.[3,4]

Collection technique is very important when using nasal swabs and different types of swabs are necessary for bacterial cultures versus virus isolation.

Dry cotton swabs are adequate for bacterial cultures.[a] Such swabs need to be inserted well into the nasal cavity taking care not to contaminate the swab with the many non-pathogens in the nostrils. The swab should then be placed in prepared transport medium or at least saline or lactated ringers solution for transport to a laboratory.

Pasteurella hemolytica and *Pasteurella multocida* are known to inhabit the nasal cavities and upper respiratory tract of normal cattle.[5] The rationale for culturing the upper respiratory tract of incoming or resident cattle, or cattle in early stages of respiratory disease, is to obtain antibiotic sensitivity data on suspected or potential pathogens. Unfortunately, strains that predominate in the upper respiratory tract may not be the same as those that under appropriate circumstances are capable of colonizing the lower respiratory tract and causing pneumonia.[3] Therefore, the antibiotic sensitivity patterns obtained may not be relevant and need be interpreted with care.

Lung lavage may provide a more accurate sample but is more difficult and generally impractical under feedlot conditions.[6]

Laryngotracheal cultures using guarded equine swabs is reported to be a compromise in that relevant organisms are more likely to be obtained than from nasal cultures and although still somewhat difficult is easier than lung lavage.

For identification of viral agents involved in the BRD complex, fluorescent antibody (FA) examination of smears from nasal mucosa is preferable to virus isolation. Fluorescent antibody examination is faster, cheaper and more reliable. Bovine herpesvirus 1 (IBR virus) and respiratory coronavirus are fairly reliably identified with this technique. Bovine respiratory syncytial virus (BRSV) and parainfluenza-3 virus (PI-3) can often be found but false negative results are more frequent while bovine virus diarrhea virus (BVD) does not lend itself to reliable identification in the nasal cavity.

[a]*Culturettes, Becton Dickinson, Cockeysville, Maryland 21030.*

If one chooses to attempt virus isolation (VI) on swabs of the upper or lower respiratory tract, the swabs need to be calcium alginate free cotton or Dacron™.[b] Regular cotton swabs used for bacterial cultures can be inhibitory to many viruses. The swabs should then be shipped, chilled but not frozen, in a viral transport media.[c] Some viruses such as BRSV cannot be recovered from mail-in samples. An ELISA test on swabs or lung tissue has been reported to be effective for BRSV diagnosis.[4]

It is always a good idea to contact the laboratory prior to obtaining samples to be sure just what specimens that laboratory prefers and how they would like it sent.

Serology

Serologic examination of multiple sera can be used to evaluate new arrivals and for monitoring certain infectious diseases in resident animals. For best results, blood should be collected in clean tubes, not Bangs tubes or other washed tubes as the detergent used to clean the tubes may be toxic to the cells used for serum neutralization (SN) and invalidate that test. Paired samples (acute and convalescent) are necessary for accurate interpretation. At least a four-fold rise in titer is needed to indicate recent exposure to a particular agent. Negative titers can help rule out a particular agent. A pitfall is that even demonstrating seroconversion doesn't necessarily prove that agent was responsible for the disease. Whenever possible serum should be separated from the cells prior to shipping. When submitting paired serum samples most laboratories prefer the practitioner or feedlot to hold the acute samples until convalescent samples are obtained and send both sets together. This eliminates the possibility of variation in titers between test runs.

Necropsy Examination and Interpretation

Necropsy evaluation is a valuable tool for disease diagnosis and monitoring. Ideally a veterinarian will glean more out of a necropsy than a non-veterinarian but trained lay personnel are often utilized and can do an adequate job of recognizing important lesions and collecting specimens. Further, while in a feedlot setting, necropsy procedure can often be tailored to the situation but an orderly and systematic approach is still necessary for maximum value and at least a cursory look at all tissues and organ systems will pay dividends in the long run.

Equipment necessary for necropsy examination of cattle need only include one or more knives, a sharpening stone or steel, axe and/or rib cutters, scissors and forceps and protective gloves. Having sharp knives and axe takes much of the labor out of performing a necropsy.

For collection of specimens a supply of plastic bags, Whirl-pak[d] or Zip-Lock bags, wide-mouth plastic or glass containers with 10% neutral buffered formalin, sterile cotton swabs and containers for bacterial culture and calcium alginate-free cotton or Dacron™ swabs for virus isolation, glass slides for smears and blood tubes for serology are all that should be necessary.[7] Specimens of lung or trachea for culture should be "fist sized" and from representative lesions. The specimen needs to be large enough that the laboratory can sear the surface and obtain a non-contaminated culture from the interior. Preferably specimens should be submitted chilled and not frozen.

Specimens for histologic examination should be thin slices, not more than 1 cm thick so that formalin can penetrate, and large enough, at least several cm square, for the pathologist to observe the overall architecture. It is important that tissues be fixed in an adequate amount of formalin. A formalin to tissue ratio of roughly 10:1 should be used for at least overnight, after which a smaller volume of formalin, or gauze sponges heavily soaked in formalin, can be used to keep the specimens moist while in transit. Inadequate fixation can seriously compromise the diagnostic value of a specimen and negate a lot of work and expense on the part of the veterinarian and feedlot owner or manager. Several specimens from representative lesions in various stages of development are helpful to the pathologist to get the overall picture.

Specimens for virus isolation and fluorescent antibody examination should be unfixed and selected from representative lesions. Most laboratories prefer FA and VI specimens to be submitted chilled and not frozen. If the specimens for virus isolation will be more than 24 hrs in transit, freezing on dry ice will result in greater isolation success. If specimens are submitted on dry ice, the ice should be tightly sealed since the fumes can lower pH in the container and thereby lower viability of enveloped viral agents such as IBR.

A written record of the clinical and necropsy observations is important to identify trends and compare cases over time, as an aid to the laboratory pathologist and also to aid one's memory if legal questions later arise.

A good way of shipping specimens is in a large insulated, styrofoam box with adequate padding and refrigerant to hold specimens in place and keep unfixed tissues chilled until arrival at the laboratory. Specimens with unfixed samples should be shipped so that they arrive within 24 hours.

When performing a necropsy an often neglected part is the external examination. The animal's body condition and hair coat, state of hydration, wounds, ex-

[b]Available from Baxter Diagnostics, Inc., Scientific Products Division, McGraw Park, Il.
[c]Hanks Balanced Salt Solution, Gibco Laboratories, Grand Island, NY 14072
[d]Whirl-Pak, Nasco, available from Fisher Scientific, St. Louis, MO.

ternal parasites, discharges from body cavities and as one proceeds with the dissection noting whether the tissues are icteric or pale, bruised or hemorrhagic can all be important clues. In food animals, looking for injection site lesions is also important.

When respiratory disease is suspected emphasis is, of course, on that system and should start with examination of the nasal cavity, larynx, trachea, lungs and thoracic cavity. The upper respiratory system should be observed for rhinitis, laryngitis and tracheitis.[8] These tissues may display important lesions with diseases such as IBR and necrotic laryngitis. Occasionally a traumatic laryngitis will be found indicating poor medication technique. At the same time the oral cavity and esophagus should always be examined for the linear erosions and ulcers suggestive of BVD.

Intense congestion of the trachea often with whitish or pinkish froth can be a terminal feature of many diseases including bloat and are not necessarily indicative of respiratory disease.

Many cases of bronchopneumonia will have some mucus and purulent exudate in the trachea and this is not necessarily indicative of a primary tracheitis such as IBR. Cattle dying with IBR will have a necrotizing rhinitis and tracheitis with fibrinopurulent pseudomembranes lining much of the trachea. Careful examination should distinguish between the necrotizing tracheitis and bronchitis of IBR and just congestion or coughed up exudate from a bronchopneumonia. The necrotizing laryngitis of calf diphtheria can look similar but should be limited to the larynx and proximal trachea. Cattle dying with the so-called honker syndrome will have frank hemorrhage in the tracheal submucosal tissue and without necrosis or inflammatory pseudomembrane formation.

Infectious bovine rhinotracheitis can usually be readily confirmed by fluorescent antibody examination. The preferred specimens are a couple of short segments of unfixed trachea and bronchi. Histopathologic examination can be suggestive but may not be confirmatory.

As noted above, BRD is a multifactorial syndrome. Uncomplicated viral pneumonias tend to be mild and seldom come to necropsy. Current concepts are that the various viral, mycoplasma and chlamydial agents compromise pulmonary defenses resulting in secondary bacterial pneumonia which then can result in severe clinical disease. Bovine virus diarrhea is an example. While BVD is not considered to be a primary respiratory pathogen it is thought to initiate bacterial bronchopneumonia by depressing the immune response. If a practitioner suspects BVD involvement in a respiratory disease outbreak the preferred samples are unfixed lung tissue for FA examination.

While numerous bacteria have been isolated from pneumonic bovine lungs, only three appear to be of major importance, i.e. *Pasteurella hemolytica, Pasteurella multocida* and *Hemophilus somnus*. Prior viral infections may not always be necessary for these bacteria to cause clinical pneumonia but some form of stress to the respiratory defense mechanisms usually has occurred.

Grossly, the pneumonia in feedlot cattle has been classified into three main categories; fibrinous pneumonia, bronchopneumonia, and interstitial pneumonia.[9] Differentiating fibrinous from bronchopneumonia can be difficult both grossly and histologically. All three of the main bacterial species involved in feedlot pneumonias, i.e. *P. hemolytica, P. multocida*, and *H. somnus* can cause overlapping morphologic lesions making an etiologic diagnosis based on gross or histologic criteria unreliable.[10] Complicating matters even more is the fact that these three pathogens may coexist in the same set of pneumonic lungs and the isolate recovered on culture may not represent the primary agent responsible for causing the pneumonia.

Typically, the "shipping fever" pneumonia of weanling and feedlot calves that come to necropsy will be cranial-ventral in distribution.[11] There is generally a rather sharp demarcation between affected and non-affected lung with varying degrees of fibrin on the pleural surface and within interlobular septa and with time a progression of lesions caudally and dorsally. A secondary interstitial emphysema is often present in the more caudal-dorsal regions. On cut surface, varying amounts of mucus and purulent exudate will be present in airways. It is important not to base a diagnosis of pneumonia on the color of the lung tissue but on the palpable texture. A reddened or purple lung may be nothing more than congestion or postmortem change if not accompanied by palpably firm lungs.

The lesions of bacterial pneumonia tend to develop through the stages of red and gray hepatization and will have a distinct "hepatized" or liver-like consistency. The lobular structure of the bovine lung often gives pneumonic lesions a checkerboard pattern with adjacent lobules in various stages of red hepatization, gray hepatization, or necrosis. This feature of the various lobules being in different stages of lesion development at the same time can make aging of lesions difficult.

Fatal lesions can develop surprisingly fast, within 2-3 days.

With increasing chronicity, pale areas of necrosis develop into foci of abscessation. Progressive fibroplasia develops around these abscesses, within intralobular septa and the early fibrinous pleural adhesions become organized into firm fibrous adhesions. Irregularly dilated bronchi and bronchioles (bronchiectasis) are also evidence of chronicity.

Microscopically, fibrosis can be recognized within

5-7 days but grossly fibrosis may be difficult to recognize in less than about 3 weeks.[12]

Actinomyces (Corynebacterium) *pyogenes* is frequently recovered from these "abscesses" and generally considered to be a secondary invader. Antibiotic sensitivities on these isolates will be of little value.

In general, histologic examination is of limited value in differentiating the common "shipping fever-type" feedlot pneumonias but it can be helpful in giving a rough estimate of the age of lesions and in differentiating these bronchopneumonias from interstitial pneumonia and other respiratory conditions such as lungworms and metastatic pneumonia.

Interstitial pneumonia is less common in feedlots than bronchopneumonia and fibrinous pneumonia and usually not considered part of the BRD complex. There is some confusion in terminology of interstitial pneumonia. Some of the synonyms used include: atypical interstitial pneumonia (AIP), pulmonary adenomatosis and acute respiratory distress syndrome (ARDS).[13]

Typically, upon opening the thorax these lungs nearly fill the thoracic cavity and do not collapse, are pale and with diffuse emphysema consisting of distended, air and/or edema filled interlobular septa and frequently air-filled bullae. There may be some cranial-ventral consolidation in which case differentiating this condition from bronchopneumonia with secondary dorsal-caudal agonal emphysema can be difficult. Interstitial pneumonia should have a diffuse distribution, a subtle "meaty" texture on palpation and often a dull grayish overall appearance. Histologic examination may be necessary to distinguish between the two. The best histologic sample in these cases is a slice of tissue from the junction of the consolidated or "hepatized" tissue and the emphysematous tissue.

The etiology of interstitial pneumonia in feedlot cattle is still unclear and may be multifactorial. In adult pastured cattle, the pathogenesis is thought to involve rumen production of 3-methyindole from ingestion of high levels of L-tryptophan in cattle recently moved from low to high L-tryptophan forage. Lungworms, hypersensitivity reactions to moldy feed and plant toxins have also been implicated but do not explain most feedlot cases. Bovine respiratory syncytial virus has been incriminated as a cause of interstitial pneumonia in weanling and feedlot cattle but is not proven.

Another condition that may be encountered at necropsy of cattle dead with a suspected respiratory condition is so-called metastatic pneumonia.[14] The finding of multiple abscesses, perhaps with hemorrhage in the airways, and fairly normal lung in between lesions, suggests caudal vena-cava syndrome resulting from a liver abscess that has ruptured into the posterior vena-cava.

Pneumonia due to lungworms (*Dictyocaulus viviparus*) may also be encountered and can, at least during certain stages, be diagnosed by careful dissection and observation of the adult worms in bronchi and bronchioles. At gross necropsy these lungs will have a little "different" look than the typical shipping fever bronchopneumonia with more involvement of the diaphragmatic lobes and copious mucus and exudate in the airways. Typically, there are dark red or grayish "wedge-shaped" areas along the periphery of the diaphragmatic lobes but secondary pasteurella infection can distort this picture. Considerable interstitial emphysema may also be present making differentiation from interstitial pneumonia necessary.[15]

Acute pulmonary edema and congestion from heart failure due to *Hemophilus somnus* myocarditis also has to be part of the differential diagnosis with BRD complex.[16]

Table 1. Infectious agents involved in BRD complex in feedlot cattle.

Major Importance	Minor or Unknown Importance
Viruses	Viruses
Bovine Herpes 1 (IBR)	Bovine Respiratory Syncytial Virus (BRSV)
Bovine Virus Diarrhea	Parainfluenza-3 virus (PI_3)
Bacteria	Coronavirus
Pasteurella hemolytica	Adenoviruses
Pasteurella multocida	Other herpes viruses
Hemophilus somnus	Bacteria
	Actinobacillus pyogenes
	Streptococcus sp.
	E. coli
	Salmonella sp.
	Staphylococcus sp.
	Mycoplasma sp.
	Chlamydia

Summary

The first step in treatment and control of any condition is an accurate diagnosis and BRD is no exception. An accurate diagnosis has to start with astute observation and a good clinical history. For example, a good history can be helpful in that hemophilosis typically occurs a little later in the feeding period than pasteurellosis and can give the practitioner and diagnostician a clue to look for *H. somnus*. Such tools as nasal swabs can be a useful clinical aid for initiating treatment or monitoring respiratory disease on a premises. If fatalities are occurring, careful necropsies and appropriate specimens for laboratory examination can be important aids to accurate diagnosis but laboratory results have to be interpreted prudently and in the context of the overall situation. **Communication between practitioner and laboratory personnel can be extremely important in interpretation of laboratory results and help avoid misinterpretation or overinterpretation.**

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Abstract

Epidemiology of lameness in dairy cattle: description and analysis of foot lesions

R.D. Murray, D.Y Downham, M.J. Clarkson, W.B. Faull, J.W. Hughes, F.J. Manson, J.B. Merritt, W.B. Russell, J.E. Sutherst, W.R. Ward

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Information from 37 dairy farms, in four regions of England and Wales provided data on 8991 lesions and the preventive trimming of 4837 cows' feet. Of the total of 13,828 forms returned, veterinary surgeons treated 32 per cent and farmers or stockmen 46 per cent. Of the 8645 lesions associated with episodes of lameness, lesions in the hindlimbs accounted for 92 per cent, of which 65 per cent were in the outer claw, 20 per cent in the skin and 14 per cent in the inner claw. Sole ulcers (40 per cent) and white line lesions (29 per cent) were the predominant diseases of horn, and digital dermatitis (40 per cent) was the most common disease of the skin. Subjective assessments showed that sandcrack, penetration of the sole by foreign bodies and interdigital necrobacillosis were associated with the most severe cases of lameness. There was a significant seasonal effect in the reporting of lesions.