Methods of antemortem sampling for identification of microbial agents in bovine respiratory disease (BRD)

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Abstract

Although bovine respiratory disease (BRD) can often be managed satisfactorily without diagnosis of microbial agents, when presumptive management is unsuccessful, identification of viruses or bacteria may reveal unexpected contributors, or agents for which a change in vaccination strategy or antimicrobial may be appropriate. The most common and feasible techniques for field use include nasal swabs, guarded nasopharyngeal swabs (NPS), transtracheal aspiration, and non-endoscopic bronchoalveolar lavage (BAL). Nasal swabs or NPS are ideal for rapidly sampling large numbers of cattle; evidence suggests that testing these for viruses or bacteria reliably represents the agents in the lower respiratory tract at the group level. If only 1 or a small number of animals are to be sampled, the tracheal aspirate or BAL may provide a more reliable result. Recent vaccination with modified-live viral (MLV) vaccines can cause false positive results by any method, thus sampling should not be carried out within 1 month of MLV vaccination. Paired serology can be useful for diagnosis of viral agents, but serologic tests for bacterial BRD agents are not readily available in diagnostic laboratories. Paired serology can be difficult to interpret in calves with maternal antibody, or in recently vaccinated cattle.

Key words: nasal swab, nasopharyngeal swab, transtracheal aspirate, bronchoalveolar lavage, cattle, calves

Résumé

Bien que le complexe respiratoire bovin (CRB) puisse être géré de manière satisfaisante sans le diagnostic des agents microbiens, lorsque le traitement présomptif n'a pas de succès l'identification des virus ou des bactéries peut révéler des contributeurs inattendus ou des agents pour lesquelles un changement dans la stratégie de vaccination ou de l'antimicrobien est approprié. Les techniques les plus fréquemment utilisées et réalisables sur le terrain incluent les écouvillons nasaux, les prélèvements nasopharyngés, l'aspiration trachéale et le lavage broncho-alvéolaire sans endoscopie. Les écouvillons nasaux et les prélèvements na-sopharyngés conviennent le mieux pour l'échantillonnage rapide d'un grand nombre de bovins. L'évidence suggère que le test de ces prélèvements pour des virus ou des bactéries reflète bien les agents dans les voies respiratoires inférieures au niveau du groupe. Si seulement un animal ou un petit nombre d'animaux doivent être échantillonnés, l'aspiration trachéale ou le lavage broncho-alvéolaire peuvent fournir des résultats plus fiables. La vaccination récente avec des vaccins à virus vivants modifiés peut causer l'apparition de faux positifs avec chacune de ces méthodes. Ainsi, il ne faut pas échantillonner dans le mois suivant la vaccination avec des vaccins à virus vivants modifiés. Une sérologie pairée peut être utile pour le diagnostic d'agents viraux mais des tests sérologiques pour les agents bactériens associés au CRB ne sont pas toujours disponibles dans les laboratoires de diagnostic. L'interprétation de la sérologie pairée peut être difficile chez les veaux avec des anticorps maternels ou chez les bovins qui ont été vaccinés récemment.

Introduction

This review provides an overview of the strengths and limitations of various methods to identify the viruses and bacteria that are generally accepted to contribute to the development of bovine respiratory disease (BRD) in cattle, as well as a brief discussion of sample handling.

When dealing with an individual case of BRD, or a BRD outbreak, it is not always necessary to identify microbial agents. It is often perfectly reasonable to treat a case or an outbreak presumptively with 1 of the many effective anti-microbials labeled for treatment of the bacteria that commonly contribute to BRD. If an operation has repeated BRD outbreaks, it is also reasonable to first assess management practices that may underlie endemic or epidemic BRD, such as inadequate passive transfer in calves, crowded or dirty housing, poor ventilation, or inadequate nutrition. But if such factors are addressed, and disease persists, then assessment of the infectious agents involved may provide support for introduction of new vaccines, or may justify a change in routine antimicrobials used for treatment.   Regardless of when antemortem sampling is undertaken, opportunities to gain information through routine necropsy of animals that die should not be missed.

First: Confirm that Respiratory Disease is Present

It is important to understand that **the sampling methods reviewed in this paper are only appropriate for use in cattle confirmed to have BRD by clinical assessment, with or without additional tests to confirm the presence of lung disease**. The bacteria that most commonly contribute

to the development of BRD in North America–*Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, Mycoplasma bovis*, and *Trueperella pyogenes* (the last associated with chronic abscessing pneumonia)–are all organisms that can also be commensals on the respiratory mucosa of healthy cattle. Thus, a diagnosis of BRD cannot be made simply by isolating any of these bacteria from the respiratory tract of 1 or more cattle. This is why **testing for bacterial BRD pathogens should not be undertaken without also testing to confirm the presence of lung disease** via clinical assessment, necropsy, ultrasound, radiography, and/or cytologic evaluation of tracheal aspirates or bronchoalveolar lavage (BAL). However, when isolated from respiratory materials collected from cattle with evidence of lung pathology, any or all of the agents can be presumed to contribute to disease. In other words, identification of the common bacterial pathogens in the presence of clinical signs and other diagnostic test results consistent with BRD is meaningful; identification of the bacteria alone is not necessarily meaningful. For example, it would be inappropriate and possibly incorrect to make a diagnosis of pneumonia in a calf with no evidence of respiratory disease simply because *Mannheimia haemolytica*--or any of the other bacteria listed above--was identified in materials collected from the calf's respiratory tract.

The interpretation of respiratory virus identification in cattle is a little more complicated, because we do not currently understand any viruses to be "normal flora". This may change in coming years as more study of the respiratory "virome" (all viruses that can be identified by sequencing DNA and RNA in respiratory tissue or secretions) is undertaken.[12] It may be that some viruses are essentially normal flora, but that has not yet been confirmed. However, while many viruses have been identified in respiratory tissues or secretions from cattle with respiratory disease, it has not been possible to induce respiratory disease by experimental challenge of immunologically naïve cattle with some of these, which has been traditionally defined by Koch's postulates as necessary to "prove" an agent causes disease. Another approach to assigning "causation" is epidemiologic: agents more often identified in cattle or operations with BRD than from cattle or operations without signs of BRD are considered to contribute to the development of BRD. However, some agents are significantly associated with BRD in some epidemiologic studies, but not in others. Moreover, shedding of BRD agents by untreated animals in a group where others have been treated may be due to subclinical BRD in the untreated animals.

The preceding information is meant to emphasize that **microbiologic testing should only be undertaken when the presence of BRD is confirmed; microbiologic testing should not be used to confirm the presence of BRD**. Moreover, confirmation of the presence of BRD by clinical signs alone may be misleading. Given that the sensitivity of clinical diagnosis has been estimated to be as low as 27%,[15] and the specificity has been estimated to be as low as 63%,[19] confir-

mation of the presence of lung pathology in 1 or more animals in an affected group, by necropsy, transthoracic ultrasound, or other methods, is advised before microbiologic testing is undertaken. For valuable individuals, thoracic radiographs and/or cytologic evaluation of fluid collected by tracheal aspiration and/or BAL can also be used to confirm changes consistent with pulmonary inflammation and pathology.

While cattle should not be sampled for microbiologic testing before the presence of BRD is confirmed, in groups where BRD has been confirmed in some cattle but not others, it can be useful to sample some cattle that have not yet been identified to have signs of BRD. Particularly in the case of outbreaks suspected to be due to viral agents that are not shed for more than a few days (e.g. BHV-1 or BRSV), cattle in the early stages of infection, when only fever is evident, may be more likely to yield virus. Thus, when sampling during an outbreak, it is advised to measure the rectal temperature of several cattle, with the objective of sampling some cattle that are febrile but have not yet been identified with signs of disease leading to treatment.

Effects of Modified-live Viral Vaccination on Test Results

Before sampling cattle for microbiologic diagnosis, confirm that the cattle have not recently been vaccinated with modified-live virus (MLV) vaccines. While vaccines delivered intranasally are most likely to be shed for at least several days post-vaccination,[18] some MLV vaccines given by injection can also be identified in respiratory secretions collected from recently vaccinated cattle, particularly when PCR is used for diagnosis.[18] While precise guidelines are not available for all agents and all vaccines, given the available information, it is advised to wait at least 1 month after vaccination with MLV vaccines before attempting microbiologic diagnosis to identify agents that may be causing BRD in an animal or group.

Antemortem Sampling Methods

Nasal and Nasopharyngeal Swabbing
Traditionally, nasal swabs have been used to sample cattle for respiratory viruses, but not bacteria, because identification of bacteria which can be commensals from the nasal passages has been of uncertain relevance in the diagnosis of BRD. Nasal swabs are collected with a cotton- or polyester (Dacron)-tipped swab approximately 6 inches (15 cm) in length. Cotton swabs may be superior for bacterial culture, while some evidence suggests that polyester swabs are superior for identification of at least some viruses. Although the value of this practice has not been confirmed in a controlled study, it is advised to wipe out the nostril to be sampled with a single-use disposable paper towel before swabbing, in order to remove dirt and excessive secretions that may be heavily contaminated with irrelevant bacteria

or fungi, which may interfere with an accurate test result. This is particularly important if virus isolation or bacterial culture is to be attempted. The swab should be inserted into the ventral meatus of the nostril for nearly the entire length of the swab, rubbed back and forth on the mucosa, then withdrawn. Obviously, restraint of the animal's head will be necessary. Do not touch the swab to the planum of the muzzle or other areas before or after sampling; transfer the swab immediately into the tube to be used for transport. If multiple animals are to be sampled, consider wearing examination gloves and changing gloves between animals sampled in order to prevent transfer of agents from 1 animal to the swab collected from another animal.

Although nasal swabs have not traditionally been used for identification of bacterial BRD pathogens, in 1 study, the results of nasal swab culture for identification of bacterial BRD pathogens were found to agree well with results of transtracheal aspiration and culture, in dairy calves with clinical signs of acute undifferentiated BRD.[7] This suggests that nasal swabs may be adequate to identify bacteria associated with BRD in clinically affected animals. Nasal swabs have been used in multiple studies to identify *Mycoplasma bovis* and other mycoplasmas.[2,14,20] However, some investigators have found overgrowth of contaminants to limit the value of unguarded swabs for identification of bacterial pathogens.[16] This observation led to the widespread adoption of guarded nasopharyngeal swabbing (NPS) as a method to obtain a sample thought to be more representative of agents causing BRD with less contamination.

Guarded NPS is completed with a long (26 – 33 inches, or 66 – 83 cm) single or double-sheathed swab, of the type typically used for mare uterine culture. Versions that can be used include the Double Guarded Uterine Culture Swab (MAI Animal Health, Item #64300) or the Double Guarded Culture Swab (Jorgensen Labs Inc., Item #J0273). The available swabs are longer than needed for bovine nasopharyngeal swabbing, which makes them unwieldly for use, especially in small calves. To collect a guarded nasopharyngeal swab, the head is restrained, and the nostril is wiped clean with a single-use disposable paper towel. The guarded swab with swab inside the guarding sheath is advanced into the ventral meatus to approximately 1 inch below the medial canthus of the eye. The inner sheath is then advanced approximately 1 inch, then the swab is advanced a further 0.5 to 1 inch, rolled 3 – 4 times, then withdrawn into the inner sheath. The inner sheath is then withdrawn into the outer sheath, and the entire guarded swab is removed from the nostril. The swab is withdrawn from the distal aspect of the swab (that is, not pushed through the contaminated end), inserted into the transport tube, and the handle of the swab is cut with scissors or broken to a length that allows the tube to be capped. The swab should be moist but not bloody. Blood may be seen on the swab if the animal throws its head during sampling, or if the swab is advanced too far. If sampling multiple cattle and multiple swabs are bloody even if cattle are not struggling,

then the swabs have likely been inserted too far, and subsequent swabs should not be inserted quite as far. The value of sampling with a guarded NPS is that the swab is protected from contamination with bacteria from the rostral nasal passages, thus fewer contaminants should be present on the plate if swabs are submitted for bacterial culture. As described for nasal swabbing, if multiple cattle are to be sampled it is advised to wear disposable examination gloves and change gloves between animals sampled to prevent transfer of agents from 1 animal onto a swab collected from another animal.

In calves or cattle with clinical signs of BRD, guarded NPS have been shown to agree moderately well to very well with the results of samples collected from the lower respiratory tract by tracheal aspiration, tracheal swabbing, or bronchoalveolar lavage.[1,6,7,11] The agreement between NPS and lower airway of cattle without BRD has not been evaluated extensively, so the results of NPS culture should not be expected to reliably indicate whether bacteria are present in the lower airways of healthy cattle. In cattle or calves with BRD, the agreement between guarded NPS and lower airway culture is generally better at the level of the group than at the level of the individual. Therefore, if only 1 or 2 calves or cattle with BRD are to be sampled, a transtracheal aspirate or BAL is likely to provide a sample that more reliably represents agents in the lower airways. However, when sampling multiple calves or cattle with signs of respiratory disease, the composite results of NPS culture of the entire group can be expected to represent the genus and species of bacteria that would be found with more invasive lower airway sampling. The agreement between NPS and lower airway samples for assessment of antimicrobial susceptibility of isolated bacteria has not been studied extensively. More information is needed to clarify how well NPS swabs represent clinically relevant antimicrobial susceptibility information. In 1 study, antimicrobial sensitivity testing of bacteria isolated from NPS cultures from a small number of cattle agreed moderately well with antimicrobial susceptibility testing of bacteria isolated from the lower respiratory tract for most antimicrobials tested.[6]

While guarded NPS can also be used to sample cattle for identification of viruses, it is not clear that this approach is superior to nasal swabbing. Because nasal swabbing is less cumbersome, and short nasal swabs cost less than the long, guarded swabs used for NPS, in most cases short swabs are likely to be more efficient for identification of viruses. However, in 1 study comparing the agreement between agents found on nasal swabs, NPS, or in BAL to the agents found in transtracheal aspirates in dairy calves with undifferentiated BRD, for BRSV the agreement between nasal swabs and tracheal aspirates was only moderate, and for respiratory coronavirus the agreement was even worse. When calves were positive for BRSV it was more often found in tracheal aspirates and BAL, while when calves were positive for coronavirus, it was more often found on nasal swabs and NPS.[7] In that study no other viruses were found, so it is not possible to make similar comparisons for other viruses. While

these results suggested that tracheal aspirates or BAL are more reliable than nasal swabs or NPS for the identification of BRSV in individual calves, nasal swabs have been used to identify BRSV in experimentally infected calves[10] and in natural outbreaks,[8] so when multiple calves are to be sampled in an outbreak, nasal swabs can be used to reliably identify BRSV in at least some of the animals.

Tonsillar Washing

Tonsillar washing has been used to identify *Mannheimia haemolytica*,[9] which appears to persist in the tonsillar crypts when the bacteria may not be present in high enough numbers to identify by routine nasal swabbing or NPS. The technique requires some practice, and is likely most useful for research applications. When testing cattle during outbreaks of respiratory disease, nasal swabs or NPS are likely to be adequate and logistically more simple to collect, as swabs are more often positive during outbreaks than when cattle are healthy.

Tracheal Aspiration

Secretions from the bronchioles and bronchi are ultimately expelled from the respiratory tract by movement up the trachea by the mucociliary apparatus. Thus, aspiration of fluid from the trachea is used to collect a sample of material that originated in the lower respiratory tract, while avoiding contamination by bacteria and fungi in the nasal passages and nasopharynx. While it can be argued that tracheal aspiration is the best antemortem approach for retrieving a composite, uncontaminated sample from the lower airways, it is also the most invasive of the methods commonly used to sample airways. The technique requires some practice for proficiency, and of the commonly used sampling methods, it takes the most time to complete. Thus tracheal aspiration may best be reserved for sampling individuals or small numbers of animals, when a sample certain to represent the lower airways is desired.

Tracheal secretions can be collected either by transtracheal aspiration or endotracheal aspiration. In the field, transtracheal aspiration is likely to be most feasible. Kits are available for transtracheal aspiration (for example the Large Animal Trans-Tracheal Wash Kit, MILA International, Inc., Item #TW1228, or the JorVet Tracheal Wash Kit, Jorgensen Laboratories Inc., Item #J0283). An advantage of such kits is that they include a trochar to insert through the tracheal wall instead of a needle, which greatly decreases the chance that the catheter used to instill and recover fluid will accidentally be cut off inside the trachea. Alternatively, materials can be purchased individually and used to create homemade kits; a 12- or 14-gauge 2-inch needle and a polypropylene tube of appropriate diameter and length to insert through the needle can be used satisfactorily. For very small ruminants (such as small calves, lambs, or kids) a through-the-needle intravenous catheter with a small-gauge needle can be feasible for transtracheal aspiration. The catheter to be threaded

through the needle that is passed into the trachea should be long enough to reach the thoracic trachea, where the trachea is horizontal and instilled fluid can be aspirated more easily.

The basic approach to perform a transtracheal aspirate is:

1) Restrain the animal with the head in extension, with nose pointing upward. For large cattle it can be helpful to place 2 halters on the animal, so that the head and neck stay centered in the chute.

2) Clip and aseptically prep a site approximately 6 inches long by 4 inches wide, directly over the ventral aspect of the trachea, about one-third of the distance from the larynx to the thoracic inlet.

3) Instill 3 to 5 ml of 2% lidocaine subcutaneously at the site where the trochar or needle will be placed.

4) Wearing sterile gloves or exam gloves, make a full-thickness stab incision with a scalpel blade at the site where the trochar/needle will be inserted.

5) While using the non-dominant hand to stabilize the trachea (to prevent it from moving side-to-side), use the dominant hand to firmly push the trochar/needle through the tracheal wall. This may take some force in large cattle. Once the needle pops through the tracheal wall, air may be heard moving through the trochar/needle.

6) Push the hub of the trochar/needle up so the end of the trochar/needle that is inside the trachea is directed downward, then thread the catheter through the needle to the thoracic trachea. Depending on the size of the animal, instill 20 to 60 ml of sterile isotonic saline as quickly as possible, then aspirate back repeatedly, moving the catheter in and out while aspirating. Often no more than 10% of the fluid instilled is recovered. The rest of the fluid will be coughed out or absorbed.

7) Transfer the fluid to a sterile tube. Consider dividing the sample into 2 aliquots, 1 for culture and virus identification, and 1 for cytologic evaluation.

8) Remove the cannula/needle and catheter. Ideally a pressure wrap is placed over the site for 24 hours to limit subcutaneous emphysema, but this is not absolutely necessary.

Instillation of fluid may induce some coughing, which can help to increase the volume of fluid recovered. However, forceful repeated coughing can cause the cannula to be flipped up into the pharynx. If this occurs, fluid may come out of the mouth when instilled, and the sample will not be useful for diagnosis as it will be contaminated with oral bacteria. If this occurs, remove the cannula/needle and catheter and repeat the process. Administration of an intravenous dose of butorphanol may decrease paroxysmal coughing.

The fluid recovered from a transtracheal aspirate is transferred aseptically to a sterile tube and submitted for identification of viruses and/or bacteria as described below. Ideally, a subsample of the fluid is placed into a second

tube for submission for cytologic evaluation. Because the trachea is not a sterile site, simply culturing bacteria from a tracheal aspirate does not indicate pneumonia. The presence of cytological signs of inflammation (large proportion of neutrophils which may be toxic, with mucus and intracellular bacteria) confirms the presence of an inflammatory response and supports a diagnosis of infectious pneumonia. Alternatively, if clinical signs or ultrasound evaluation indicate lung disease, the cytologic evaluation can be omitted. If fluid collected by transtracheal aspiration is to be submitted for cytologic evaluation, contact the laboratory to determine how the sample should be prepared before shipment. Cells deteriorate quickly in saline, and so it may be advisable to make some direct smears onto glass slides to send with the tube of fluid for cytologic evaluation. Alternatively, slides can be made and stained with Diff-Quik and read in-house.

To accomplish endotracheal aspiration, the head is restrained and an endoscope is advanced through the nose and into the trachea, and a small bore catheter of adequate length (available for purchase from companies that supply accessories for endoscopes, or from some companies that sell medical supplies) is threaded through the biopsy channel of the endoscope and into the trachea to the level of the thoracic trachea, where the trachea is horizontal, and fluid instilled will pool for collection. Sterile saline (similar volume as described for transtracheal aspiration) is instilled through the catheter and aspirated and then submitted as described for transtracheal aspiration. An alternative approach to endotracheal aspiration is to extend the head horizontally, place a speculum into the oral cavity, and then advance a small length of custom-made tubing (for example, the guard from a guarded NPS) into the larynx. A smaller gauge catheter for instilling saline can then be threaded through the tube inserted into the larynx and into the trachea. Endotracheal aspiration through an endoscope is relatively straightforward, but the endoscope biopsy channel must be disinfected between animals, so it is not feasible for sampling a large number of animals. Obviously, this approach also requires an endoscope of sufficient length to enter the trachea. Endotracheal aspiration through a custom-made tube inserted through the larynx requires substantial practice and is not widely used, though if the approach can be mastered it is less invasive than transtracheal aspiration.

Although tracheal aspiration is invasive, complications are uncommon. The trachea is well equipped to clear small amounts of fluid and bacteria, so it is unlikely that respiratory infection will be induced. More common complications include 1) abscessation at the site where the needle was introduced (particularly if the trochar/needle is removed before the catheter, so that bacteria in the trachea are pulled through the subcutaneous space as the catheter is removed); 2) subcutaneous emphysema on the neck and thorax; this is typically seen for 24 to 48 hours after the procedure and should resolve without intervention; 3) cutting or breaking the catheter during the procedure, so that the distal aspect

of the catheter is left in the trachea. This is alarming, but the animal should eventually cough the catheter out in the hours after the procedure. If the animal is placed in a stall or pen without bedding, the end of the catheter can often be found lying on the ground several hours later, after it is coughed out (although the animal may also chew and swallow the catheter after it reaches the pharynx). The catheter fragment can also be removed with an endoscope if available. Hypoxia and exacerbation of respiratory distress can be induced in patients with severe lung disease and respiratory compromise, and the procedure should be avoided in such patients.

Bronchoalveolar lavage (BAL)

Bronchoalveolar lavage (BAL) yields a sample of fluid from a single bronchoalveolar unit. Thus, BAL provides a sample from the deepest region of the lung, but from only 1 relatively small area of the lung. Therefore BAL is most appropriate when lung disease is diffuse as the sample collected may not represent the diseased area of lung if the process is localized. Consistent with this, a recent report found poor correlation between the presence of lung consolidation identified by transthoracic ultrasound evaluation and cytologic evidence of inflammation in BAL fluid in calves.[17] In spite of this, BAL has been used by some investigators to identify evidence of inflammation, and/or microbial agents associated with BRD, in calves with bronchopneumonia,[13] which is not necessarily diffuse.

A BAL can be collected by a tube inserted blindly through the nose, into the trachea, and then into the bronchoalveolar unit to be sampled, or it can be collected through an endoscope of sufficient length to wedge into a bronchus. It is unlikely that a 1-meter-long endoscope will be adequate for a BAL for any but the smallest calves. Therefore endoscopic BAL will most often require a 3-meter-long endoscope, which is unlikely to be available to many practicing veterinarians. A BAL collected by a tube inserted blindly through the nasal passage is feasible for collection in the field. The procedure requires a tube long enough and of small enough diameter to be passed through the nostril, into the trachea, and advanced until it wedges into a bronchus. It is helpful if the tube has an inflatable cuff, which can facilitate wedging the tube into the bronchus. It is necessary to wedge the tube in place so instilled saline can be retrieved back through the tube; if the tube becomes displaced the saline will flow out around the tube and the sample will not be collected. Tubes for BAL can be purchased (for example, Large Animal Broncho-Alveolar Lavage Catheter, MILA International, Inc., Item #BAL240, or Broncho-Alveolar Lavage Catheter, Jorgensen Laboratories, Inc., Item #J0639). Because it is not usually feasible to sterilize tubes quickly in the field, if multiple animals are to be sampled, a separate tube will be required for each animal. The tubes can be reused, but it will be necessary to clean and sterilize the tubes between uses.

The basic approach to perform a BAL is:
1) Place a halter on the animal to be sampled and re-

strain the head in extension, with the nose pointed upward.

2) Wipe the nostril clean with a disposable paper towel. While not necessary, it can be helpful to instill 2 to 3 mL of 2% lidocaine into the nostril to facilitate passage of the tube.

3) Advance the tube into the nasopharynx and through the larynx and into the trachea. This can be challenging; if the tube is swallowed it will enter the esophagus and perhaps rumen and be contaminated. It can be helpful to advance the tube quickly at the time of inspiration, when the larynx is open most widely. Occasionally in difficult cases it can also be helpful to instill 5 to 10 ml of 2% lidocaine through the tube into the nasopharynx at the level of the larynx. Once the tube is advanced into the trachea, coughing may occur. The placement of the tube in the trachea can be confirmed by shaking the trachea side-to-side, which can cause the tube to palpably rattle in the trachea. Once in the trachea, the tube should be advanced rapidly until it is wedged into place, and can be advanced no further. If the tube is cuffed, the cuff should then be inflated.

4) Depending on the size of the animal, 60 to 180 ml of sterile isotonic saline is instilled through the tube, and then the fluid is withdrawn. A good sample will be clear to cloudy with grossly visible foam due to surfactant from the alveoli. Hemorrhage is not expected. Approximately half the volume instilled is typically recovered; the remainder of the fluid will be coughed out or absorbed.

5) If inflated, the cuff should be deflated, and then the tube is removed.

6) The sample should be transferred to tubes as described for tracheal aspirate. As described for tracheal aspiration, if cytologic evaluation is to be completed the fluid should be processed the same day, as advised by the laboratory where cytologic evaluation will be performed, as the cells will deteriorate quickly in saline.

Complications of BAL are uncommon. These include introduction of infection, trauma to the pharynx, hemorrhage, and induction of bronchospasm and/or hypoxia in patients with severe lung disease and respiratory compromise. These can be avoided by using clean and careful technique. The procedure should be avoided in animals with significant respiratory distress.

Thoracocentesis

Infection with *Mannheimia haemolytica* or *Histophilus somni* or, less commonly, other agents, may cause pleuropneumonia, leading to pleural inflammation and effusion. Other disease processes, such as neoplasia, can also cause pleural effusion. A sample collected from inside the airways is, in most cases, adequate for microbiologic diagnosis of pleuro-

pneumonia. However, in unusual cases of pleuropneumonia, or in other disease processes, it may be desired to collect a sample of pleural effusion for microbiologic and/or cytologic evaluation via thoracocentesis.

The basic approach to thoracocentesis is:

1) Clip and aseptically prep a site over the fifth to seventh intercostal space, at the lowest point in the fluid (typically approximately 2 to 3 inches above the point of the olecranon). On the left side, take care to avoid the heart. If an ultrasound machine is available the location of the fluid can be easily confirmed, but it is not absolutely necessary to use ultrasound to perform thoracocentesis. The disappearing art of percussion may also be used to identify the location of pleural effusion.

2) Instill 2 to 4 ml of 2% lidocaine subcutaneously at the site to be sampled. Remember that the needle or teat cannula that will be inserted into the pleural space should be inserted right off the front of the rib, to avoid hitting the blood vessels and nerves that run behind the rib.

3) Apply sterile gloves and use a scalpel blade to make a full-thickness stab incision through the skin. It can be helpful to have sterile gauze available to dab away excess blood.

4) Insert the male end of a sterile extension set into the female end of a sterile teat cannula, then attach a 10 to 30 ml syringe into the female end of the extension set. This will prevent air from entering the pleural space when the teat cannula is inserted into the pleural space. A 2-inch (5 cm) 14-gauge needle could be used instead of the teat cannula to enter the pleural space, but a needle is more likely to lacerate the lung, possibly inducing serious pneumothorax.

5) Using firm pressure, push the teat cannula through the thoracic wall, entering the thorax right off the front of the rib. Once the teat cannula has been advanced to the hub, aspirate back with the syringe to withdraw fluid. Handle fluid as described for tracheal aspirate.

Complications of thoracocentesis are uncommon, but include abscess or hematoma formation at the site of centesis, or pneumothorax due to entrance of air through the teat cannula, or due to laceration of the lung. Abscess or hematoma at the site of centesis can be managed symptomatically. If care is taken to minimize entrance of air into the pleural space, the degree of pneumothorax induced is negligible. The most serious complication is lung laceration, which can be avoided with proper restraint and by using a blunt-ended teat cannula instead of a needle for aspiration.

Transthoracic Lung Biopsy

Transthoracic lung biopsy may be considered the antemortem sampling approach of last resort for the diagnosis of lung disease. This technique is invasive, and complications

that can be life-threatening or fatal are not rare. The procedure is most appropriate when diffuse lung disease is present, or when ultrasound can be used to confirm the exact site of abnormal lung to be sampled, because the sample collected is quite small relative to the size of the lung. Transthoracic lung biopsy is most appropriately used to collect a sample of lung tissue for histopathologic evaluation +/- microbiologic testing to confirm the etiology of lung disease when etiologic diagnosis by other methods has not been possible. Approaches have been described.[3,4] A biopsy is collected using a Tru-Cut or similar biopsy instrument through a stab incision at a site over an intercostal space that has been clipped, aseptically prepped, and blocked. Biopsies are usually collected from the dorsocaudal lung, where blood vessels and airways are smaller, and damage to the structures is less likely to lead to severe complications. Complications include pneumothorax or pulmonary/pleural hemorrhage that can be severe. In 1 study,[5] transthoracic lung biopsy was evaluated for identification of early lung disease in feedlot cattle, but the technique did not identify information in enough cattle to be considered useful.

In referral institutions, lung biopsy can also be performed via thoracoscopy, which may be safer and which may yield a larger and thus more diagnostic sample. However, this would be relatively expensive, and thus likely appropriate for only valuable individuals with lung disease that cannot be confirmed by any other method.

Serology

Serologic testing can be used to confirm infection with agents associated with BRD. Most diagnostic laboratories offer serologic testing to identify serum antibodies to viruses but not bacteria. Antibody titers to the bacteria that commonly cause BRD can be difficult to interpret, and are generally reserved for research applications.

Identification of infection with respiratory viruses commonly associated with BRD is best accomplished using paired serology. A one-time test can be difficult to interpret, because vaccination or past exposure may lead to the presence of antibodies that do not necessarily indicate recent exposure. Identification of a 4-fold increase (that is, two 2-fold dilutions) in antibody titer between an acute and a convalescent sample (collected 2 to 4 weeks after the acute sample) suggests recent infection. Note that a 4-fold *fall* (or decrease) in titer also suggests recent infection. Because the significant change in titer may be missed in a single individual, paired serology is most useful for identifying group-level infections by sampling multiple animals. The acute samples should be collected at the time of the respiratory outbreak, and the serum should be stored in the freezer until the convalescent sample is collected, so both samples can be tested at the same time. This is important, because day-to-day variations in serologic tests could lead to misleading differences between the acute and convalescent samples if they are run on different days.

The results of serologic testing of calves that are likely to have maternal antibodies are particularly difficult to interpret. This is because maternal antibodies may prevent calves from seroconverting, even when they are infected. Thus, failure to seroconvert in calves with maternal antibodies does not rule out infection. Moreover, it is not possible to differentiate maternal antibodies from the calf's own endogenously generated antibodies. Recent vaccination in cattle of any age can also make interpretation of serologic testing difficult. Given all of the factors that can influence the interpretation of serologic testing, it is important to carefully consider how the results are likely to look, and whether the results are likely to be helpful, before submitting samples for serologic testing to identify the infectious case of BRD outbreaks

Sample Handling Before and During Transport to the Diagnostic Laboratory

Whether collecting samples for identification of viral or bacterial pathogens, it is best to contact the laboratory where the samples will be tested in advance to determine the preferred transport medium to be used, and to determine the best temperature for sample storage. The information may be available on the laboratory's website. Identification of *Histophilus somni, Mycoplasma bovis,* or other mycoplasmas are particularly likely to require special medium for transport. The laboratory staff can provide good advice regarding the best sample-handling methods for the agents to be identified. Note that the laboratory will most likely not attempt to identify *Mycoplasma bovis* or other mycoplasmas unless this is specifically requested. In many cases the laboratory can provide transport media, or can provide information about where transport medium can be purchased.

In general, swabs collected for identification of viruses should be collected into transport medium that is specifically formulated for virus identification. Such media will usually contain antibiotics to inhibit growth of bacteria which could interfere with viral identification. This is particularly important if swabs are to be tested by virus isolation or fluorescent antibody testing, which generally require a cell culture step, in which bacterial overgrowth can be a particular problem. Transport tubes for bacterial culture should not be used to transport swabs for virus identification as overgrowth of unwanted bacterial contaminants is more likely in medium designed for bacterial transport.

When either viruses or bacteria are to be identified by PCR, inserting swabs into dry tubes is likely acceptable. Swabs in dry tubes can then be stored on ice packs, or in the refrigerator or freezer, before sending to the laboratory for PCR. The exact storage temperature to be used is more critical if virus isolation or bacterial culture is to be attempted because some viruses and bacteria do not tolerate freezing, while others do not tolerate refrigeration, or storage at room temperature. Communicate with the diagnostic laboratory to determine the best storage and

shipping temperatures before collecting samples for virus isolation or bacterial culture.

Conclusions

Effective management of BRD does not always require microbiologic diagnosis. However, when logical management practices have not been effective, or when it is desired to determine whether new vaccines or antimicrobials may be appropriate to introduce, a variety of approaches can be used for identification of the viruses and bacteria associated with BRD. Microbiologic testing should only be conducted in animals with BRD confirmed by physical examination, necropsy, transthoracic ultrasound, or other tests; the mere presence of infectious agents in respiratory materials should not be interpreted to confirm BRD. When multiple animals with signs of BRD are tested, nasal swabs or guarded NPS for bacterial culture, virus isolation or diagnosis by PCR reliably represent the agents found in the lower respiratory tract. In other words, nasal swabs and guarded NPS provide representative information about the lower respiratory tract at the group level, though not always at the individual level. Thus, if only 1 or a small number of animals are to be tested, the methods that directly sample the lower respiratory tract, tracheal aspiration or BAL, are more likely to yield representative results. Tracheal aspiration provides a composite sample of all airways, while BAL results in a sample from a single bronchoalveolar unit, therefore tracheal aspiration may provide the most representative sample of the lower airways. However, transtracheal aspiration is the most invasive and time-consuming test. Before collecting any samples for the first time, contact the diagnostic lab where samples will be tested to confirm the best media and temperature for sample transport.

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