Introduction
In the assessment of equine lower respiratory tract disorders, an accurate and complete history is essential, and complementary to a thorough physical examination and detailed respiratory evaluation for a successful diagnosis. Thoracic auscultation and percussion are simple diagnostic techniques which are widely undervalued. Use of a rebreathing bag during auscultation is critical for the detection of subtle abnormalities, as emphasized in a study of distal respiratory tract infection in Thoroughbred foals [1]. Once clinical signs have been localized to the lower respiratory system, additional testing procedures are warranted in order to document the nature of the disease and to optimize treatment and management recommendations of each individual patient. To this end, diagnostic evaluation of the respiratory system in horses has evolved beyond the stethoscope. Semi-quantitative clinical scoring of respiratory signs [2,3], in combination with endoscopic findings, is a sensitive tool to diagnose various lung disorders in the horse. As well, a variety of pulmonary function test parameters and cytological and histological specimens are available to document the presence of inflammatory processes related to immune responses and their impact on lung function. Such tests include bronchoalveolar lavage, tracheal aspiration, ultrasonography, radiography, occasionally nuclear scintigraphy, arterial blood gases, pulmonary function testing, histamine bronchoprovocation, biopsy of lung tissue and thoracocentesis. Pulmonary function testing and the various imaging techniques are discussed more thoroughly elsewhere in this textbook. In this chapter, techniques for sampling the lower respiratory tract are discussed, including tracheal aspiration, bronchoalveolar lavage, pulmonary biopsy and thoracocentesis. Samples thus obtained may be evaluated by various methods including, but not limited to, cytological and biochemical analysis, bacterial culture, virus isolation, and histopathology. As well, these samples can be used to identify and quantify a variety of regulatory cytokines and protein constituents involved in pulmonary inflammation. Therefore, sampling of the lower respiratory tract plays a vital role both in management of clinically-diseased animals and in advancing our understanding of respiratory pathophysiology through the development of novel research tools.

Background
The development of diagnostic techniques in equine respiratory medicine has mirrored the evolution of human medicine. Historically, evaluation of lung inflammation in humans was limited to sputum samples, transtracheal aspiration, percutaneous fine needle lung aspiration, and open thoracic surgery for biopsy sample acquisition. Similarly, early diagnosis of respiratory disease in horses focused mainly on samples obtained by transtracheal aspiration and very occasionally lung biopsy techniques. Aspiration of tracheal secretions was widely used in the 1970’s and 1980’s to obtain samples from the respiratory tract for cytological analysis and bacterial culture. Values have been reported in both normal horses and horses with clinical signs of respiratory disease [4,5]. However, the advent of the flexible fiberoptic endoscope by Dr. Shigeto Ikeda in 1968 hallmarked a change in respiratory diagnostic capabilities not only in human but also veterinary medicine. With the endoscope, it became possible to obtain true samples from the lower respiratory tract with minimal invasiveness compared to previous techniques. Although the fiberoptic endoscope was initially employed as a research tool to evaluate immune-mediated inflammatory disorders such as sarcoidosis and idiopathic pulmonary fibrosis in humans, it soon became apparent that this minimally invasive technique was useful in assessing all inflammatory conditions of the human lung through the use of bronchoalveolar lavage. In the early 1980’s, bronchoalveolar lavage using fiberoptic endoscopy was first reported in horses [6]. Since that time, bronchoalveolar lavage has gained growing popularity over transtracheal aspiration for the collection of respiratory secretions, and is considered to yield a more representative sample from the lower airways.

Bronchoalveolar Lavage
Bronchoalveolar lavage (BAL) is performed in the standing animal following mild sedation. This is achieved through the administration of α₂-agonists such as xylazine hydrochloride (0.3 - 0.5 mg/kg BWt, IV) or romifidine (0.03 - 0.05 mg/kg
BWt, IV). Concurrent administration of butorphanol tartrate (0.01 - 0.03 mg/kg BWt, IV) is often useful in horses with severe clinical signs of heaves and marked airway hypersensitivity, in order to alleviate the cough response during passage of the bronchoscope. Additional restraint through the application of a nose twitch is recommended, particularly during passage of the endoscope through the nasopharynx. Cleaning the external nares with moistened gauze prior to the procedure helps to reduce contamination of the bronchoscope.

Under field conditions, bronchoalveolar lavage can be performed using a fiberoptic endoscope or can simply be done blindly using a flexible, cuffed naso-tracheal tube with an outer diameter of 8 mm (Bivona, Medical Technologies, Gary, IN). Currently in many equine referral hospitals, video endoscopy systems are preferred since this readily allows video recording or digital pictures for accurate documentation in patient medical records. The bronchoscope should be at least 160 - 180 cm in working length to allow lavage of most adult horse lungs. The depth of lung ultimately lavaged will depend greatly on the outer diameter of the bronchoscope used. In most mature horses, an outer diameter of 10 - 13 mm will result in sampling from the fourth to sixth generation bronchi.

Endoscopy offers the advantage of visual inspection of the airways prior to the bronchoalveolar lavage procedure. The nasopharynx can be carefully assessed for any evidence of anatomic abnormalities or discharges. If upper airway noise is suspected, it is important to visualize the function of the pharyngo-laryngeal structures prior to sedation and without the use of a nose twitch in order to better assess any irregularities. The trachea should be inspected for any hyperemia, as well as the quality and amount of any secretions. Multiple cough receptors exist predominantly at the laryngo-tracheal junction and at the carina (tracheal bifurcation). As a result, the normal horse will usually cough 2 - 3 times during passage of the bronchoscope, in contrast to a dramatic repetitive cough response by horses with airway hypersensitivity. Topical anesthetic often helps to alleviate such coughing in normal horses, particularly when applied to the carina after any aspirate samples have been obtained for bacterial culture. A dilute solution (0.4% w/v lidocaine in sterile saline, without epinephrine) is often effective when 60 - 120 ml is instilled at the carina prior to further advancement of the endoscope. Application of local anesthetic onto the larynx during the initial stages of endoscopy is not recommended, however, as fluid from the nasopharynx will flow down the trachea, washing upper airway contaminants to the lower airways prior to sampling for culture or cytology. Any accumulation of secretions or hemorrhage within the trachea should be recorded and described as to the location (upper, middle, lower trachea), quantity, and character/color. A gravitationally dependent region of the trachea is located at the level of the thoracic inlet, and often secretions may only be detectable in this particular site ("tracheal puddle"). More extensive secretions may be seen coursing the entire length of the trachea in advanced cases of Inflammatory Airway Disease (IAD) (Fig. 1) or severe pulmonary bacterial infection. If a pulmonary abscess is suspected, every effort should be taken to identify which bronchial segment of the lung the brownish mucopus is originating from (i.e., left, right or both) (Fig. 2). In general the most frequently affected bronchi are the cranial segments of the caudal lung lobes (Fig. 3).

![Figure 1](https://www.ivis.org) - Endoscopic view of streams of mucopus in the trachea. - To view this image in full size go to the IVIS website at www.ivis.org . -

![Figure 2](https://www.ivis.org) - Endoscopic view of the distal trachea at the level of the carina showing serosanguinous debris originating from a right bronchus (arrow) due to a pulmonary abscess. - To view this image in full size go to the IVIS website at www.ivis.org . -

![Figure 3](https://www.ivis.org) - Equine lung diagram highlighting the most frequent sites of abscess formation. - To view this image in full size go to the IVIS website at www.ivis.org . -
In addition, the presence of edema should be determined by assessing the sharpness of the tracheal bifurcation at the carina as well as the large bronchial divisions, which will appear thickened and blunted when edema is present. Along with the appearance of mucosal edema, bronchospasm becomes evident during endoscopy, characterized by protrusion of the cartilaginous rings into the lumen of the airways and a significant reduction of the airway lumen diameter. The latter is readily observed in horses with a high degree of airway hypersensitivity such as in heaves for example. The evaluation of the airways for any of the above abnormalities is very subjective and dependent on the experience of the observer. Therefore, semi-quantitative scoring systems have been developed in order to standardize the reporting of clinical examination findings and endoscopically-determined airway abnormalities [2,3].

It is important to recognize that the strength of bronchoalveolar lavage interpretation depends on the assumption that the pulmonary pathology is diffuse in nature rather than being localized as in the case of an abscess. Further, a comparison of cytology between left and right lungs suggests that lavage of either lung would yield a representative sample of the entire lung in healthy animals and those with diffuse lung disorders [7]. A more specific site may be selected for lavage in certain cases if there is suspicion of localized lung pathology (e.g., cranioventrally for aspiration pneumonia, caudodorsally for exercise-induced pulmonary hemorrhage).

The bronchoscopic procedure consists of advancing the endoscope into a segmental bronchus until a wedge is obtained, as detected by resistance to gentle attempts at further advancement. It should be recognized that the area of lung lavaged depends greatly on the outer diameter of the bronchoscope and the technique used. Bronchoscopes with a larger outer diameter (10 - 13 mm) will generally wedge within a fourth or fifth generation branch of the respiratory tree and allow recruitment of cells from a larger number of airways and alveoli. In contrast, a bronchoscope with a very small outer diameter, or the use of a catheter passed through the biopsy channel of the bronchoscope, result in lavage of only a very limited number of small airways and alveoli since wedging occurs in a peripheral bronchiole; differential cell counts have been inconsistent between this method and traditional lavage using a larger bronchoscope [8].

Once the bronchoscope or tubing has been wedged, the bronchoalveolar lavage can be performed using sterile saline (0.9% NaCl w/v). Less bronchospasm occurs if the saline is prewarmed to 37°C prior to infusion, allowing for greater fluid recovery. The fluid is instilled via the biopsy channel of the bronchoscope, and then retrieved using either gentle suction with 60 ml syringes or using a suction apparatus set at a pressure of -5 to -15 cm H<sub>2</sub>O (-30 to -100 mmHg). Excessively negative pressure should be avoided, particularly in inflamed airways, as this will cause collapse of the airway lumen limiting the volume of fluid retrieved. This also causes trauma to the respiratory epithelium leading to a significant number of red blood cells in the fluid recovered.

The volume of infused fluid impacts on the total and differential cell counts of the resulting BAL sample [9]. Smaller volumes typically yield a bronchial wash without retrieving cells from the alveolar space, and therefore have a higher percentage of neutrophils. This is in comparison with larger volumes of infusate, which yield samples more representative of the respiratory epithelial fluid lining the alveoli. Since the volume of infusate has varied considerably in studies reported throughout the past 20 years, a standard volume of 250 - 500 ml was therefore recommended at the Michigan Workshop in order to allow for comparison between future BAL studies [10]. The fluid is infused in two or three aliquots, with suction applied between aliquots. In general approximately 40 - 60% of the infusate can be retrieved, although it is very common to obtain only very small volumes from obstructed airways (e.g., during heaves exacerbation) since airway edema and bronchospasm result in lumen collapse during suctioning.

Complications of the bronchoalveolar lavage technique are minimal. A neutrophilic inflammatory response occurs within the lavaged lung, which is detectable on subsequent lavages performed within 48 hours of the initial procedure [11]. This response is limited to the bronchus and lung segment that has been lavaged, and is not detected in adjacent bronchi or in the contralateral lung. Mild increase of rectal temperature has been observed in horses for less than 24 hours after BAL, with no adverse clinical effects. However, any persistent or exaggerated pyrexia accompanied by depression should prompt further evaluation of such cases, as these signs may indicate spread of preexisting infection as a consequence of the BAL procedure. Thus owners are advised to monitor the horse’s demeanor, appetite, rectal temperature, and to note any respiratory deterioration over the first 24 hours post-lavage.

A cuffed nasotracheal tube with an outer diameter of 9 mm will yield similar results to the above bronchoscopic method (Fig. 4). The blind technique is a suitable alternative to use of the bronchoscope when careful inspection of the lungs is not considered necessary (i.e., to determine the site of a suspected lung abscess) or when the airways have been previously
evaluated using an endoscope that was too short for bronchoalveolar lavage. With the head extended, the nasotracheal tube is passed through the nasopharynx into the trachea until a cough response is elicited, suggesting stimulation of the receptors at the carina. Topical 0.4% lidocaine is instilled (60 - 120 ml) in this region. The tube is then advanced into the lungs blindly until an adequate wedge is detected based on resistance to gentle attempts to advance the tube further. In most cases, the tube will naturally travel to the caudodorsal lung field [12,13]. The cuff is then gently inflated using 5 - 10 ml of air. This cuff assures a complete wedge to prevent the backflow of infused fluid. As above, approximately 200 - 300 ml of warmed sterile saline (5 x 60 ml) is infused using 60 ml syringes, with 120 ml first infused and withdrawn, followed by each additional 60 ml syringe being infused and withdrawn. The samples are pooled at the end of collection. The blind naso-tracheal technique is a viable alternative to fiberoptic bronchoscopy for practitioners in the field if the expense of an endoscope is not supported by their practice. It requires minimal equipment, has a low cost, and is easy to perform.

Figure 4. Tools necessary for bronchoalveolar lavage using a nasotracheal tube: Nasotracheal Bivona tube, 5 X 60 ml syringes, 10 ml syringe to inflate tube cuff, Sterile saline, lidocaine (diluted to 0.4%). - To view this image in full size go to the IVIS website at www.ivis.org . -

Tracheal Aspiration

Aspiration of tracheal secretions for cytological analysis or bacterial/viral identification may be performed percutaneously (transtracheally) or via the biopsy channel of an endoscope. Both procedures are relatively easy to perform under field conditions. Transtracheal aspiration offers the benefit of bypassing the nasal cavity and the upper respiratory tract, eliminating the risk of sample contamination by commensal microflora in the nasopharynx.

Percutaneous (transtracheal) aspiration is performed in the middle-to-lower half of the neck in the standing animal (Fig. 5). A 10 cm x 10 cm area is clipped on the ventral midline of the neck and is surgically prepared. A local anesthetic (1 -2 ml of 2% lidocaine) is infused in the skin and subcutaneous tissues. A small (0.5 cm) skin incision is made with a #10 scalpel blade if necessary, in order to facilitate penetration of a trocar through the tracheal wall in the space between two adjacent tracheal rings. Traditionally, an 8 - 10 gauge trocar was used for this procedure [4,5,14], but 12 or 14 gauge needles have also been used [15]. A 60 cm sterile polyethylene catheter (such as a 5 - 8 French dog urinary catheter) is advanced through the trocar or needle, with the tip directed towards the carina. A cough response is frequently elicited upon stimulation of the irritant receptors at the mainstem tracheal bifurcation. This is located approximately 30 - 40 cm from the site of entry in the adult horse. At that point, the trocar or needle is retracted from the skin while the catheter is maintained in place, in order to minimize the risk of severing the catheter. Approximately 30 ml of warmed, physiological saline (0.9% NaCl w/v) is instilled and immediately re-aspirated via the catheter. Saline without a bacteriostatic preservation additive should be used in order to minimize falsely negative culture results arising from inhibition of bacterial growth. In the adult horse, flushing and re-aspiration can be repeated 3 - 4 times if necessary to obtain a suitable sample. Other methods to facilitate retrieval of the infused fluid include slow retraction of the catheter during gentle suction, or lowering the horse’s head slightly to facilitate pooling of the fluid.

Figure 5. Diagram of transtracheal aspiration site. - To view this image in full size go to the IVIS website at www.ivis.org . -

Possible complications associated with the transtracheal aspirate technique include localized cellulites or abscess formation at the site of needle insertion, subcutaneous and mediastinal emphysema, and tracheal cartilage damage [4,14]. It is possible for the catheter to retroflex into the proximal trachea or oropharynx during coughing, resulting in contamination or inadvertent sampling from the upper respiratory tract. Accidental severance of the catheter, with loss into the trachea, may also occur but is generally not problematic as the catheter end is coughed out by the horse within minutes in most cases and causes no evidence of distress.

With the availability of the flexible endoscope, directly visualized tracheal aspiration has largely replaced the percutaneous technique since it can be performed less invasively [16,17]. It is very important that the endoscope be cleaned and disinfected prior to use. A sterile polyethylene catheter is passed through the biopsy channel once the endoscope has reached mid-
trachea. Plugging the distal 1 cm tip of the polyethylene catheter with sterile agar or surgical foam prior to use may help to reduce contamination of the catheter during passage through the biopsy channel; the plug is expelled into the proximal trachea prior to collection of a sample at the tracheal puddle. Being able to visualize the secretions, a sample may be collected directly for bacterial culture, viral identification and cytological analysis. In such instances, both qualitative and quantitative assessment of bacterial cultures can be obtained. However, in most cases it is necessary to instill 30 - 50 ml of sterile saline to facilitate sample collection from the tracheal puddle.

Unlike the percutaneous method of sampling, the diagnostic usefulness of transendoscopic tracheal aspiration for bacterial culture is limited due to the possibility of contamination of the endoscope tip during passage through the upper respiratory tract. A recent comparison of transendoscopic tracheal aspiration using either a guarded or non-guarded endoscope clearly showed that passage of the endoscope through the nasopharynx brings about upper airway contaminants, which are frequently responsible for many of the positive bacterial culture results from tracheal aspirate samples [18]. To circumvent this, a double-sheathed, protected catheter used transendoscopically was compared to percutaneous tracheal aspiration [19,20]. With the protected catheter, no contamination of samples was observed and the method was as sensitive as transtracheal aspiration but provided the advantage of reduced procedural complications. It should be noted that even protected collection of samples may yield false results with respect to lung pathology, as demonstrated in a comparison of transendoscopic tracheal aspirates and bronchoalveolar lavage samples performed using guarded endoscopy [18,21]. This study demonstrated that there is poor correlation between cytology findings of tracheal aspirate and BAL samples from the same horse, and that the lower trachea appears to harbor a normal bacterial flora. Hence positive bacterial culture results and observation of bacteria on the cytological smear specimens of tracheal aspirate samples should always be interpreted with caution.

**Processing of Bronchoalveolar Lavage and Tracheal Aspirate Fluid**

Following tracheal aspiration or bronchoalveolar lavage, the retrieved fluid should be examined grossly prior to processing. Initial examination involves inspection of the whole fluid for color, clarity, and the presence of flocculent debris. Normal fluid should appear clear or mildly turbid. For bronchoalveolar lavage fluid (BALF) samples, a layer of foamy surfactant indicates that the alveoli have been sampled (Fig. 6). Samples from horses with recent exercise-induced pulmonary hemorrhage (EIPH) may appear pink or red (Fig. 7), whereas more long-standing hemorrhage may result in a brown tinged color due to the presence of hemosiderin. Flocculent material reflects mucus and cellular debris within the airway lumen and alveoli.

Figure 6. Foamy white surfactant on the surface of retrieved fluid from a bronchoalveolar lavage. - To view this image in full size go to the IVIS website at www.ivis.org.

Figure 7. A normal BAL sample is shown on the left, in comparison to BAL fluid from a horse with exercise-induced pulmonary hemorrhage (right). - To view this image in full size go to the IVIS website at www.ivis.org.

Aspirated fluid can be placed in either plain glass tubes (serum tubes) or in a cellular preservative such as EDTA. Quantification of the total number of cells per milliliter of retrieved sample can be performed manually using a hemacytometer. An easier method is the use of an automated (Coulter) cell counter. The results of the Coulter counter technique should be interpreted with caution, as the broad range of cell sizes within the sample may exceed the window settings of the instrument, yielding a false underestimation of total cell count [22]. Flocculent samples should be filtered through two layers of gauze to remove excess mucus strands and other debris prior to performing a total cell count using an automated Coulter counter. However, filtering of BALF is associated with a dramatic reduction in total nucleated cell count [23], as well as a selective loss of certain cell types including epithelial cells, macrophages, and mast cells [24-26]. A recent study performed in horses comparing traditional gauze filtration to a 60 µm millipore nylon filter showed that both
Total nucleated cell count is accurate when aspirate samples are retrieved without any prior infusion of fluid. However, the diagnostic usefulness of the total cell count is lost and only an estimated cell count can be obtained if saline is infused to facilitate sample collection, since this dilutes the cell concentration. For bronchoalveolar lavage samples, previous efforts to control the effect of dilution have included calculations to estimate the relative proportion of pulmonary epithelial lining fluid (PELF) in the retrieved BAL sample. This, in turn, depends on a myriad of factors such as the total volume of infusate, the amount of fluid retrieved, the dwell time of the fluid within the alveoli, the site of lavage (bronchi versus alveoli), and the presence of pulmonary pathology. By measurement of a reference molecule present within PELF, such as albumin or urea, the amount of PELF in the sample can be theoretically calculated. Urea provides a more accurate reflection of the PELF, since albumin is a large molecule that cannot diffuse freely across the capillary-epithelial membrane into the alveoli. Using the urea dilution technique and minimizing dwell time, it was determined that the average concentration of PELF in equine BAL fluid ranges from 0.1 - 1.0 % [27]. Other methods to quantify the percentage of PELF have included measurements of potassium or calcium concentrations, as well as exogenous markers such as methylene blue, however, these methods have not been as accurate as the urea dilution technique. The major disadvantage to the urea dilution technique is that this molecule can be markedly increased in alveolar fluid if increased alveolar permeability is present due to pulmonary inflammation. As well, a prolonged dwell time of the infused fluid within the alveoli may allow excessive diffusion of urea down a concentration gradient into the saline within the alveoli, also leading to a false estimation of the total nucleated cell count. Thus even the urea dilution technique holds limitations in the quantification of molecules and cells within the BALF. However, a crude method to control for dilution is through routine use of a standard technique and site for performing the lavage, as well as ensuring that 40 - 60% of infusate is retrieved. The reference range of "normal" nucleated cell counts will vary between laboratories and between techniques; an upper limit of 0.4 x 10⁹ cells/L is used for BALF at the Animal Health Laboratory of the University of Guelph.

Samples should be processed for cytological analysis in a timely fashion in order to prevent deterioration of cellular morphology and bacterial proliferation, both of which can wrongfully influence the cytological results. Pickles et al., [28] identified an appreciable deterioration in BALF cell readability with time, depending on the temperature used for storage. Based on their findings, all samples should be processed before 8 hours post-collection if the sample is stored at room temperature, in order to minimize cellular deterioration. However, this time can be extended to 24 hours if the sample is refrigerated at 4°C. Under field conditions it is preferable to prepare direct smears as early as possible following the fluid collection. The dried slides can be kept at room temperature and sent to a laboratory at the convenience of the clinician. Bacterial overgrowth in the fluid sample occurs by 8 hours post-collection at room temperature and by 24 hours in samples stored at 4°C [28]. Fixation of samples using an equal volume of 40% ethanol at the time of collection eliminates the occurrence of bacterial overgrowth, but makes interpretation of cell type difficult due to poor cellular morphology and thus is not recommended [28,29].

For cytological interpretation of the sample, cytopsins (if available) and direct smears should be prepared. Cytopsin smears are generally preferred by the clinical pathologist since many cells are concentrated into one area of the microscope slide, making cell counting easier to perform. However, it is also very important to examine a concurrently prepared direct smear of the sample since large clumps of mucus containing cellular and non-cellular material are frequently not found on the cytopsin preparation.

Cytopsins can be made with a microcentrifuge using 100 - 200 µl of whole sample depending on the degree of sample cellularity. Cytocentrifugation results in a decrease in the proportion of lymphocytes in the sample [30]. The use of high-speed cytocentrifugation (90 g) may produce less cellular damage and lymphocyte loss than cytocentrifugation at lower speeds (23 g) [25]. In general, the decrease in lymphocyte population holds questionable clinical significance, but as a research tool it will make comparison of differential cell counts difficult between studies in healthy and sick horses. The most accurate determination of lymphocyte numbers when cell concentration techniques are used to produce cytology slides is the filter preparation technique developed by Saltini [31]. However, this technique is technically demanding and time consuming to perform. Therefore, Laviolette developed a method of centrifugation onto microscope glass covers, which yields accurate lymphocyte estimates and is simpler to perform [32]. As well, a new method using a Perspex block and cell sedimentation was shown to yield slightly greater lymphocyte numbers than cytocentrifugation, produced excellent quality slides for interpretation of cell morphology, and was more applicable to field use by veterinarians for sample preparation [33].
Following preparation of cytospins, the remaining sample (10 - 50 ml) is centrifuged at 600 g to 800 g for 10 minutes, which will yield a pellet at the bottom of the tube and overlying supernatant (Fig. 8). The fluid supernatant is decanted completely by gently inverting the tube, and the remaining pellet is resuspended by gently tapping on the tube or through the use of a clean pipette. This concentrated sample is used to make direct smears by placing a drop at the base of a clean glass slide and then tipping the slide in several directions to spread the concentrated fluid around on the slide. Smears that are too thin will yield insufficient cells for identification, whereas thick smears with excessive mucus will make cell differentiation very difficult or impossible to perform. Smearing the fluid with a second slide held at a 45º angle may facilitate smears of more concentrated samples, as is done for smears on whole blood. Placing two slides flat together to perform the smear is not recommended, however, as larger components of the fluid (macrophages, multinucleated giant cells, thick mucus) are frequently lost from the smear in this process. It is essential that the smears be rapidly air-dried to prevent alterations in cell morphology. A small desk-top fan is invaluable in the preparation of excellent quality smears (Fig. 9), and greatly improves the diagnostic efficacy of respiratory cytology.

Both cytospins and direct smears can be routinely stained using Wright-Giemsa, Diff-Quik, May-Grunwald or Leishman’s stains. In general, 6 - 8 slides should be made from each sample, although only one cytospin and one direct smear are stained in most cases. Occasionally the extra slides will be needed if staining is improper (too light or too dark) or if slides are broken during transit to the laboratory. Additional stains can also be applied to the remaining slides if needed for further cellular identification, such as Gram staining for bacterial identification, non-specific esterase (alpha-naphthyl acetate esterase) to differentiate immature macrophages from large lymphocytes, Toluidine Blue for metachromatic (mast cell) granules, and Perl’s Prussian blue for hemosiderin. Following staining, a coverslip is applied to the slide. Slides processed in this manner can be stored for very long periods of time for future reference, and are useful if repeated sampling is used to monitor response to treatment in the patient.

**Interpretation of Cytology**

The accuracy of cytological interpretation will vary with the method of sample collection and the processing techniques utilized. Undoubtedly research work has demonstrated that there are significant differences between the cell differential reference range of bronchoalveolar lavage and tracheal/transtracheal aspirate samples. Respiratory epithelial cells are typically present to varying degrees in tracheal aspirate samples, depending on the degree of trauma induced by the collection procedure. As well, a higher percentage of neutrophils are noticeable in tracheal aspirate samples in comparison with BALF from normal horses. Due to these differences, much controversy exists over the appropriate site for sample collection. Although it has been long believed that aspirate samples collected from the lower trachea are more representative of the inflammatory status of whole lung, a poor correlation exists between tracheobronchial aspirates and BAL from the same animal [21,34] and between histopathology findings on postmortem [35]. In fact, tracheal aspirate samples may instead represent a collection of local inflammatory cells keeping check of natural bacterial inhabitants of the lower trachea rather than being strictly a population of cells swept from the bronchi and terminal airways by the muco-ciliary apparatus. In contrast, good agreement exists between BAL cytology and histopathology results [12,36]. As well, elevations in various cell populations in BALF has been positively correlated with the degree of airway hyperreactivity as measured by histamine bronchoprovocation testing [2,37]. This suggests that BALF appears to be a more sensitive method to evaluate the cellular milieu within the segmental bronchi, bronchioles and alveoli of the lung in horses, and therefore is the method of choice for assessing the peripheral airways. An exception to this is in cases of pleuropneumonia, where tracheal aspiration is superior to bronchoalveolar lavage if the bronchus of the affected lung area cannot be readily identified. Rossier demonstrated that
BALF cytology may be normal in some horses with confirmed pleuropneumonia, indicating that BAL samples obtained from areas other than the affected bronchus will not be representative in such localized lung pathology [38].

Interpretation of cytology from respiratory tract secretions is based on the percentage of the various cell populations present in the sample. This is determined by performing a differential cell count on at least 200 - 300 consecutive cells in order to obtain an accurate representation of the cell types present. Prior to performing the cell count, the entire slide should be examined under both low and high power in order to identify regional discrepancies within the slide. These commonly occur in association with mucus strands (trapped inflammatory cells within mucus may not be detected elsewhere on the slide) and between the base and tip of a slide. If such discrepancies are detected, an effort should be made to include each unique region of the slide during the differential cell count. The cytospin and direct smear should also be compared for similarities or differences, as it is often easier to perform the differential count on the cytospin but this may lead to underestimation of certain cell types. The final differential cell count should be performed under oil immersion (1000X) in order to assure the specific morphologic characteristics of each cell.

Reference ranges for each cell population in BALF have been determined by many studies in the past 20 years. As mentioned previously, comparison between studies has been difficult due to differences in collection technique, sample processing, and the target population studied. In general, however, the normal BAL differential cell count of clinically healthy horses shows predominantly macrophages and lymphocytes (Table 1). Note that smaller volumes of instillate typically yield a higher percentage of neutrophils in the cell differential, due to primarily bronchial sampling. Non-cellular features of BALF which should be recognized and considered in the overall interpretation of the sample include quantity and quality of mucus strands, Curschmann’s spirals (casts of inspissated mucus plugs in the terminal airways) (Fig. 10), fungal spores (Fig. 11), and extracellular or intracellular bacteria. A description of each cell type is included here for reference.

Table 1. Reference values for BAL cytology of clinically normal horses of increasing ages, using similar BAL collection techniques.

<table>
<thead>
<tr>
<th>Author</th>
<th>Number of horses (Age in years)</th>
<th>Volume of Infused Saline</th>
<th>Macro (%)</th>
<th>Lymph (%)</th>
<th>Neut (%)</th>
<th>Mast (%)</th>
<th>Eosino (%)</th>
<th>Epith (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rush Moore [62]</td>
<td>n = 6 (2.7 +/- 1.1)</td>
<td>300 ml</td>
<td>65 +/- 5</td>
<td>28 +/- 3</td>
<td>4 +/- 0.3</td>
<td>0.3 +/- 0.3</td>
<td>1.2 +/- 0.8</td>
<td>NE</td>
</tr>
<tr>
<td>McKane [39]</td>
<td>n = 62 (2.8 +/- 1.4)</td>
<td>65 ml</td>
<td>59 +/- 10</td>
<td>31 +/- 9</td>
<td>9 +/- 6.3</td>
<td>NE</td>
<td>0.5 +/- 3.1</td>
<td>0.4 +/- 0.8</td>
</tr>
<tr>
<td>Hare [3]</td>
<td>n = 12 (3.1 +/- 0.9)</td>
<td>500 ml</td>
<td>60 +/- 5</td>
<td>37 +/- 5</td>
<td>2 +/- 1.5</td>
<td>0.4 +/- 0.4</td>
<td>0.03 +/- 0.1</td>
<td>0.4 +/- 0.8</td>
</tr>
<tr>
<td>Fogarty [52]</td>
<td>n = 11 (3.2 +/- 1.2)</td>
<td>120 ml</td>
<td>65 +/- 6</td>
<td>28 +/- 6</td>
<td>7 +/- 3.3</td>
<td>0.2 +/- 0.6</td>
<td>0 +/- 0</td>
<td>NE</td>
</tr>
<tr>
<td>Clark [63]</td>
<td>n = 10 (4 to 6)</td>
<td>200 ml</td>
<td>56 +/- 1</td>
<td>37 +/- 2</td>
<td>6 +/- 0.5</td>
<td>0.8 +/- 0.2</td>
<td>0.5 +/- 0.1</td>
<td>NE</td>
</tr>
<tr>
<td>Derksen [34]</td>
<td>n = 10 (6.9 +/- 2.1)</td>
<td>300 ml</td>
<td>45 +/- 3</td>
<td>43 +/- 3</td>
<td>9.0 +/- 1.2</td>
<td>1.2 +/- 0.3</td>
<td>&lt;1.0 +/- 0.7</td>
<td>3.5 +/- 0.7</td>
</tr>
<tr>
<td>Traub-Dargatz [40]</td>
<td>n = 9 (4 - 14)</td>
<td>180-500 ml</td>
<td>31 +/- 6</td>
<td>60 +/- 6</td>
<td>5 +/- 1.6</td>
<td>2.7 +/- 0.7</td>
<td>1.2 +/- 0.4</td>
<td>0.9 +/- 0.6</td>
</tr>
<tr>
<td>Sweeney [11]</td>
<td>n = 7 (8.8 +/- 5.2)</td>
<td>300 ml</td>
<td>46 +/- 11</td>
<td>47 +/- 11</td>
<td>2.3 +/- 2.2</td>
<td>4.4 +/- 2.0</td>
<td>0</td>
<td>NE</td>
</tr>
<tr>
<td>Lapointe [30]</td>
<td>n = 6 (12.5 +/- 5.1)</td>
<td>500 ml</td>
<td>26 +/- 6</td>
<td>69 +/- 3</td>
<td>2.2 +/- 1.9</td>
<td>0.2 +/- 0.3</td>
<td>0.0 +/- 0</td>
<td>NE</td>
</tr>
</tbody>
</table>

Macro = alveolar macrophages, Lymph = lymphocytes, Neut = neutrophils, Mast = mast cells, Eosino = eosinophils, Epith = epithelial cells, NE = not evaluated.

Figure 10. A Curschmann spiral (arrow) in mucus from BAL fluid. Wright-Giemsa stain. Magnification: 400X. - To view this image in full size go to the IVIS website at www.ivis.org . -
**Cell Differential**

**Macrophages** (Fig. 12) - Macrophages are variably-sized cells (≈ 15 - 40 µm diameter) with a high cytoplasm:nucleus ratio (approximately 3:1). These cells may demonstrate vacuolation, and may contain phagocytosed debris (e.g., erythrocytes, apoptotic cells, fungal spores, hemosiderin, pollen particles, etc.) within the cytoplasm (Fig. 13). Although in human and laboratory animals, there is some difficulty in differentiating between small, immature macrophages and lymphocytes of the same size, this situation is rarely problematic in equine BAL cell differentials. However, for the purposes of investigational studies, specific staining such as alpha-naphthyl-acetate esterase will produce a brick-red stain in macrophages and cause lymphocytes and neutrophils to appear blue-green [22,23]. Hemosiderophages are macrophages containing phagocytized hemosiderin in a granule form, seen as amber, green or brown pigment present within vacuoles in the cytoplasm (Fig. 14). The Perl’s Prussian blue stain is the stain of choice to demonstrate and accentuate the presence of hemosiderin in the cytoplasm of alveolar macrophages, confirming positive staining of ferric iron. Good correlation has been reported between the presence of hemosiderophages in BALF and the presence of pulmonary hemorrhage in the dorsocaudal lung lobes at necropsy [12]. Hemosiderophages can be detected for several months after an episode of EIPH, and were detected to varying degrees from 90% of horses in training in a study by McKane et al., [39]. Additionally, it is common to observe multinucleated giant cells laden with hemosiderin in race horses suffering from recurrent episodes of pulmonary hemorrhage.

**Lymphocytes** (Fig. 15) - Morphologically, lymphocytes have a densely-dark-blue staining nucleus, and very minimal cytoplasm. A mixed population of both small (approximately 6 µm diameter, dense nucleus) and large-type (approximately 10 - 15 µm diameter, less dense nucleus) lymphocytes is observed in normal horse BALs. The reference interval for BALF lymphocytes of clinically healthy horses has varied considerably between reports, ranging from 28 - 68% of the cell differential [6,11,30,40]. This might reflect differences in staining techniques and interpretation, but may also reflect significant differences in the type of environment where the horses are kept such as pasture versus stabling, or more likely a failure to identify horses with subclinical inflammatory airway disease. Monoclonal antibody staining has demonstrated that the majority of lymphocytes in equine bronchoalveolar lavage samples from normal horses are T lymphocytes, with a predominance of CD3+ (T-cell receptor complex) and CD8+ (cytotoxic T cell) surface molecules [41]. Only a very low proportion (< 10%) of lymphocytes in equine BALF are B cells. The role of lymphocytes in the lungs of healthy and heavey horses has been, and continues to be, ill defined. In recent years, there has been a growing interest in defining the equine equivalent of murine Th, or Th, subpopulations of lymphocytes in the lung, as well as determining the role of these populations as orchestrators of airway inflammation in heavey horses through the local generation and release of specific cytokines [42-44].
Neutrophils (Fig. 16) - Neutrophils contain a multi-segmented nucleus, and range from 10 - 12 µm in diameter. Although an increased neutrophil population in BALF was traditionally associated with bacterial infection, it is now recognized and accepted that significant neutrophil influx also accompanies non-infectious inflammatory airway disease (IAD). Thus, neutrophil morphology should be carefully assessed to determine the presence or absence of a bacterial etiology. Signs of neutrophil degeneration may be detected in septic pneumonia, characterized by karyolysis (nuclear swelling), loss of segmentation, or karyorrhexis (nuclear rupture). In contrast, BAL neutrophils from horses with heaves or IAD typically show reasonably good preservation of cellular morphology. This is not applicable to tracheal aspirate samples in which the neutrophils typically have a more degenerative appearance even in heavy horses. With cases of heaves or IAD, neutrophil apoptosis may be evident, as characterized by a very condensed nucleus and minimal cytoplasm (Fig. 17).

Mast Cells (Fig. 18) - Mast cells are variably-sized cells that contain multiple metachromatic granules within the cytoplasm. Variable states of degranulation may be noted, depending on the presence of airway hyperreactivity as well as BALF processing techniques. Proper identification of mast cells may be difficult if substantial degranulation has occurred. Therefore, further identification of mast cells may be assisted by the use of special stains such as Carnoy’s fixation plus Toluidine blue stain to accentuate the metachromatic granules [45]. Examining smears on low power prior to high power magnification may also give a subjective (but more representative) interpretation of the prevalence of mast cells; these cells are surrounded by a bright purple halo of released granules at low power which is often not appreciable at higher magnifications. Mast cells release pro-inflammatory mediators such as histamine [46], leukotrienes, and platelet activating factor. An increased percentage of mast cells in BALF has been correlated with increased hyperreactivity to histamine bronchoprovocation in horses with decreased exercise tolerance [37].

Globule Leukocytes (Fig. 19) - Globule leukocytes, also referred to as "basophiloid cells", are cells with a very strong metachromatic appearance when stained with Wright-Giemsa stain. They are very rarely observed in BALF of healthy horses. However, these cells may occur in high numbers in horses with IAD or heaves. They appear as variably sized, round-to-ovoid cells containing large, basophilic, grape-like granules within the cytoplasm, and an eccentric nucleus often partially obscured by the cytoplasmic granules. Individual granules are about ten-to-twenty times larger than mast cell granules and can often be observed free throughout mucus strands on direct smear preparations. At present, the functional significance of globule leukocytes is unknown. They are considered by some scientists to represent migratory cells from the lamina propria, which pass through the respiratory epithelium into the lumen of the airways as a non-specific defence mechanism [47]. Hare [45] reported that specific cell staining using formalin and Toluidine blue distinctively detected globule leukocytes. The clinical impression is that increased globule leukocyte numbers in BALF are generally observed concurrent with an increase of mast
cells in the BALF of young horses with IAD, and that treatment with mast cell stabilizing drugs in such cases often yields disappointing results.

Figure 19. Globule leukocytes (arrows) trapped in mucus from BAL. Wright-Giemsa stain. Magnification: 1000X. - To view this image in full size go to the IVIS website at www.ivis.org.

Eosinophils (Fig. 18) - Eosinophils (10 - 15 µm diameter) are easily identified in equine BALF by the cluster of large, refractile, acidophilic granules within their cytoplasm. An increased eosinophil percentage typically occurs concurrent with an elevated presence of mast cells, and is felt to reflect chemotaxis of eosinophils in response to mast cell degranulation. Thus an elevated population of eosinophils in BAL fluid of horses is generally a transient finding, and such an increase is seldom repeatable despite performing a second BAL within 24 hours of the first sample collection. Increased airway hyperresponsiveness at rest has been correlated with an elevated eosinophil population in BALF of young racehorses with poor performance [2]. Although eosinophils are the hallmark of human asthma, their role in equine heaves or non-septic IAD needs further study with respect to the generation and release of specific cytokines relevant to allergic airway inflammation.

Epithelial Cells (Fig. 20) - Ciliated epithelial cells are occasionally identified in BALF. These columnar cells are characterized by their basilar nucleus and apical plate of cilia. A thin, cone-shaped basilar pedicle may also be apparent, particularly in epithelial cells removed due to mucosal trauma during the sampling procedure. In contrast, a non-traumatic increase in epithelial cell population in BALF is likely to be observed as the result of respiratory viral infection. A characteristic sign of epithelial damage from viral infection is the presence of ciliocytophtheria (loss of ciliary plates), pyknosis of epithelial cell nuclei and intracytoplasmic inclusions in basilar cell fragments. BALF of heavey horses or IAD young horses in an acute state of exacerbation may also demonstrate epithelial cells with loss of cilia and cytoplasmic damage, however, separation of the entire ciliary plate is rarely evident in such cases.

Figure 20. Sheet of ciliated columnar epithelial cells from a BAL. Wright-Giemsa stain. Magnification: 1000X. - To view this image in full size go to the IVIS website at www.ivis.org.

Bacteriology

Samples collected by nasopharyngeal swabs are not representative of pulmonary disease since they reflect bacterial commensals of the upper respiratory tract. Thus they offer minimal diagnostic usefulness in the evaluation of equine lower respiratory tract disease. The exception to this is the use of nasopharyngeal swabs to isolate Streptococcus equi subspecies equi, the causative organism of "strangles", since this is primarily an upper respiratory tract infection.

Sampling of the lower respiratory tract for bacterial culture may be accomplished by tracheal aspiration (percutaneous or transendoscopic) or bronchoalveolar lavage. Each technique offers advantages and limitations with respect to diagnostic efficacy in bacterial culture. Respiratory secretions obtained by either percutaneous or transendoscopic tracheal aspiration may culture positive for both pathogenic and non-pathogenic ("transient") bacteria in 32 - 92% of samples from clinically healthy horses and foals [17,48-51]. Of positive bacterial cultures in a study of transendoscopic tracheal aspiration [50], only half were accompanied by the presence of an inflammatory cytological profile. Growth of a mixed population of bacteria, in particular Staphylococcus epidermidis, Bacillus sp., or Enterobacter sp. in percutaneous tracheal aspirates is highly suggestive of skin contamination of the sample during collection, as these organisms are common commensals of the skin or are ubiquitous in the environment [49].

Positive bacterial culture results may also be obtained from bronchoalveolar lavage samples in clinically normal horses, and typically yield only low numbers of colony-forming units (CFUs) per sample [52]. The presence of squamous epithelial cells indicates upper respiratory tract contamination of the sample (Fig. 21), and in such cases bacterial culture results should be ignored. Positive predictive value of culture results may be enhanced by protection of the bronchoscope from pharyngeal contamination during passage; this technique has demonstrated efficacy in studies in calves, foals, and mature horses [18,53,54]. Another method of sampling for bacterial culture is the use of a protected catheter brush via the biopsy channel of the endoscope. However, this technique is less sensitive than both tracheal aspiration and bronchoalveolar lavage for the
isolation of bacterial organisms [19,54].

Figure 21. Squamous epithelial cells (black arrows) with attached rod-shaped bacteria (red arrow) occasionally observed in the BAL. Wright-Giemsa stain. Magnification: 1000X. - To view this image in full size go to the IVIS website at www.ivis.org.

Common bacteria recognized as potential pathogens of the lower respiratory tract are listed in Table 2. Any positive culture results should be interpreted with caution and in consideration of the clinical signs and cytological profile of the animal. Significant culture results should be primarily limited to those obtained from clinically-affected horses (e.g., fever, depression, tachypnea, abnormal respiratory sounds on auscultation, presence of nasal discharge, abnormal radiographic findings, ultrasonographic evidence of lung consolidation). As well, significant bacterial culture results should be limited to situations where the bacteria isolated are recognized equine respiratory pathogens, heavy bacterial growth is obtained, and the cytological profile of the sample reflects suppurative inflammation. Quantification of bacteria may assist in the determination of culture significance, but is only useful in aspirates obtained without the instillation of saline during the collection procedure. In such cases, bacterial growth of a pure culture in excess of $10^6$ colony forming units (CFU) per milliliter likely reflects a true state of infection, whereas culture of less than $10^4$ CFU/ml is likely of minimal significance, particularly if a mixed culture is obtained [55].

| Table 2. Common pathogenic isolates from bronchopneumonic lungs in horses and foals [49,51]. |
|-------------------------------------------------|-------------------------------------------------|
| **Mature horses**                                | **Foals**                                       |
| Streptococcus zooepidemicus                      | *Rhodococcus equi*                             |
| Actinobacillus suis                              | *Streptococcus zooepidemicus*                  |
| Streptococcus equi var equuli                   | *Klebsiella pneumoniae*                        |
| Pseudomonas aeruginosa                          | *Bordetella bronchiseptica*                    |
| Escherichia coli                                | *Pseudomonas aeruginosa*                       |
| Bordetella bronchiseptica                       | *Escherichia coli*                             |
| Streptococcus equi var equi                     | *Streptococcus equi var equi*                  |
| Streptococcus pneumoniae                        | *Streptococcus pneumoniae*                     |
| Klebsiella pneumoniae                           | *Staphylococcus epidermidis*                   |
| Enterobacter spp.                               | *Enterobacter spp.*                            |
| *Staphylococcus aureus*                         | *Staphylococcus aureus*                        |

**Percutaneous Lung Biopsy**

Percutaneous lung biopsy is useful in evaluating pulmonary pathology for conditions that are diffuse within the lung, but offers limited value for evaluating focal or localized conditions unless ultrasound guidance is used to select the site of biopsy. Most frequently, lung biopsy is utilized as a complementary diagnostic aid to bronchoalveolar lavage and pulmonary function testing in order to determine the degree of airway remodeling such as inflammatory cell infiltrate, smooth muscle hypertrophy, as well as collagen formation and deposition.

Minimal equipment is required for percutaneous lung biopsy (Fig. 22). A 14-gauge soft tissue core biopsy needle of 15 cm length (minimum 10 cm) generally yields suitable samples for histological evaluation [56]. Automated biopsy instruments may be preferable over manual instruments for ease of cutting during the procedure. However, both types of instruments are commonly employed in the hospital setting. Sedation of the animal with xylazine hydrochloride (0.3 - 0.5 mg/kg, IV) or romifidine (0.03 - 0.05 mg/kg, IV) is recommended to facilitate safe collection of the biopsy sample.

Figure 22. Tools needed for percutaneous lung biopsy: Clippers, sterile preparation solutions and gauze, sterile lidocaine, sterile gloves, 12 ml syringe, 20 gauge 1.5” needle, #15 scalpel blade, manual or automatic (not shown) biopsy needle, skin suture material, buffered formalin. - To view this image in full size go to the IVIS website at www.ivis.org.

Lung biopsy is performed transthoracically in the 7th or 8th intercostal space on either the left or right side of the chest, at a
level approximately 10 cm dorsal to a horizontal line through the olecranon. This location obtains samples from the ventral third of the right or left middle lung lobe [6,56]. A 4 X 4 cm area of skin is clipped and surgically prepared. Using aseptic technique, approximately 10 - 15 ml of sterile local anesthetic (e.g., 2% lidocaine) is infiltrated subcutaneously and within the intercostal muscle layers down to the level of the parietal pleura. It is crucial that the parietal pleura be effectively desensitized in order to minimize a painful response during insertion of the biopsy needle. Once the local block has taken effect, a small stab incision is made through the skin with a #15 scalpel blade to facilitate introduction of the biopsy needle. Although it is recommend that the site of insertion be at the cranial margin of the rib in order to avoid the intercostal vessels and nerves that travel along the caudal border of each rib, the thickness of the chest wall and the intercostals space does not always practically allow the avoidance of these structures. The concern of inducing a painful reaction if an intercostal nerve is touched is largely eliminated when proper intercostal local anesthetic has been injected.

The biopsy needle is slowly advanced through the intercostal muscles until increased resistance is detected as it encounters the parietal pleura. A marked drop in resistance indicates that the pleura has been penetrated. The biopsy needle is advanced into the lung parenchyma for approximately 2 - 3 cm. The inner stylet is then advanced to "open" the needle, and the stylet is carefully held immobile as the outer sheath is rapidly advanced to accomplish a cutting action. Once the biopsy has been done, the needle is removed and the tissue section is gently transferred to a container of 10% neutral buffered formalin or gluteraldehyde solution for fixation, with effort taken to avoid crushing the tissue architecture as it is removed from the biopsy needle. In this respect, a 20-gauge needle is often helpful to remove the lung tissue from the biopsy needle. Care should be taken to not contaminate the biopsy stylet with formalin, since the biopsy needle can be reused to obtain further samples during the procedure if necessary. A single biopsy needle can make several tissue cuts before it becomes blunt and should be replaced. In addition to histopathology, lung tissue can be submitted for bacterial or fungal culture, or for electron microscopy depending on the working diagnosis prompting the biopsy procedure. A single skin suture or tissue glue may be required to close the stab incision following the biopsy procedure.

Although most biopsy samples obtained using the percutaneous method will yield excellent pulmonary tissue samples for histological evaluation, other tissues such as liver and diaphragm muscle may inadvertently be sampled, particularly if the sample is taken beyond the 8th intercostal space. Complications associated with lung biopsies are minimal, and are generally restricted to self-limiting hemoptysis not requiring medical intervention. Focal subpleural hemorrhage is detectable at the biopsy site during postmortem examination within several days of the biopsy procedure [6,56]. However, rare complications such as tachycardia, tachypnea, pneumothorax, respiratory distress, epistaxis, pulmonary hemorrhage, pale mucous membranes, great vessel hemorrhage, collapse, and peritonitis from inadvertent intestinal biopsy have been reported historically in association with the procedure [57]. Therefore, lung biopsy should not be considered an innocuous procedure and warrants post-biopsy monitoring for at least 24 hours. Lung biopsy is contraindicated in animals experiencing dyspnea, a marked elevation in respiratory rate, or paroxysmal coughing, since these patients are at greater risk for complications due to the potential for pulmonary tissue lacerations by the biopsy needle in the presence of increased chest excursions.

Transendoscopic Biopsy
Biopsy of the respiratory mucosa can be performed via an endoscope under standing sedation of the horse. Topical anesthetic (0.4% w/v lidocaine in sterile saline, without epinephrine) is recommended prior to mucosal biopsy. Forceps are passed through the biopsy channel of the bronchoscope and samples are collected by closing the forceps over a septum between two bronchi. Once the tissue is grasped firmly, the biopsy instrument is sharply tugged to detach the tissue. Tissue samples obtained using this method are much smaller than with percutaneous lung biopsy, which can limit their diagnostic usefulness. Orientation of the tissue during paraffin embedding is also important in the ability to obtain transverse sections for histological assessment. It is not uncommon for repeat sectioning of the paraffin-embedded tissue to be necessary before a suitable transverse section is obtained. The transendoscopic biopsy technique samples mucosa from the large bronchi, and thus may not accurately reflect pathology of the peripheral airways where many of the chronic diffuse lung disorders predominate. As well, many biopsy instruments designed for use with human colonoscopes are ineffective at obtaining adequate samples from the respiratory system due to the firm fibrous adhesions of the respiratory epithelium. Recent improvements in this technique have been achieved through the use of a McGregor Biopsy instrument which allows for collection of full-thickness mucosal samples [58].

Thoracentesis
The indication for thoracentesis is to evaluate the bacteriological and cytological content of fluid accumulating within the pleural space. Pleuritis is defined as an infection of the pleural space with the resulting accumulation of fluid due to increased capillary permeability and decreased pleural lymphatic drainage. When pleuritis is accompanied by an underlying condition such as pneumonia, lung abscess or neoplasm, it is defined as a parapneumonic effusion [59]. The latter is the most
frequently encountered in horses and the incidence has been reported to represent two thirds or more of all cases presented with pleural effusion [60,61]. The presence of pleural effusion can be suspected based on the findings of thoracic auscultation and percussion. Today with the more frequent use of ultrasonography in the field, pleural effusion can be confirmed by visualization prior to performing thoracocentesis.

The technique involved in performing thoracocentesis is easy and simple. A 4 X 4 cm area is surgically prepared (i.e., clipped and scrubbed) between the 6th and 7th intercostal space, 3 to 4 cm above the costochondral junction or one to two centimeters above the lateral thoracic vein. It is crucial that an adequate amount of sterile local anesthetic (10 - 15 ml or more) be infiltrated in the subcutaneous tissue, intercostal muscles and the parietal pleura. It is advisable while infiltrating the intercostal muscle and the parietal pleural to clearly identify the position of the intercostal space where the needle will be inserted. Then a 14 gauge, 13.3 cm Teflon catheter (Angiocath, Becton Dickinson Vascular Access, Sandy, VT, USA.) can be used for the procedure. Alternatively, a sterile teat cannula may be used, which does, however, necessitate a stab incision of the skin and the muscle to facilitate its insertion. Multiple punctures of the parietal pleura must be avoided as this will result in leakage of pleural fluid into the muscle and subcutaneous tissues causing localized cellulitis.

Pleural effusion should be collected into EDTA tubes for cytological analysis, as well as serum tubes for biochemical analysis and bacterial culture. At least one of the serum tubes should be filled completely, in order to remove all air from the tube for the purpose of anaerobically culturing the fluid. The fluid should be assessed grossly for its color, odor and consistency. A putrid smell to the fluid is often associated with anaerobic infection and bares a very poor prognosis. Cytological smears and cytospins (if available) can be made in the same manner as described for bronchoalveolar lavage fluid samples earlier in this chapter. Cytological examination of the fluid is performed to determine the differential cell count, as well as the detection of bacteria if present. Gram staining of the sample should also be performed to guide initial antibiotic therapy until culture and sensitivity results become available. Biochemical analysis may be used as an ancillary test to heighten suspicion of bacterial infection while awaiting culture results; low pH, low glucose concentration, and high lactate are generally affiliated with the presence of bacterial growth within the effusion.

The information obtained from thoracocentesis has several benefits largely pertaining to case management. First, it will guide the clinician in the selection of an appropriate antibiotic therapy. Second, it will dictate the decision to initiate tube drainage of the pleural space. Although it has not been a common practice for veterinary clinicians in the field to place a chest drain in horses diagnosed with pleuritis or parapneumonic effusion, adequate drainage of the fluid in the pleural space is as important in resolution of the condition as antimicrobial therapy. The technique of thoracic drain placement and management is discussed elsewhere in this textbook.

References

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