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Tracheal wash and bronchoalveolar lavage: sampling technique and fluid interpretation

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Introduction

Once respiratory disease is identified it is important to determine the cause, but many of the clinical signs of respiratory disease are common to multiple causes. Examining the cytological characteristics of the respiratory secretions can provide both an aetiology and, with repeated sampling, an indication of treatment response.

Tracheal aspirates

Most horses with pulmonary disease, accumulate excess respiratory secretion in the tracheal pool, approximately 15 cm proximal to the carina. There are 2 methods for collecting tracheal aspirates. Percutaneous transtracheal aspiration offers the benefit of bypassing the upper respiratory tract, almost eliminating the risk of sample contamination by commensal microflora of the nasopharynx. Transnasal aspiration is performed in the mid trachea, where the tracheal rings are palpable ventrally. The area is clipped, disinfected and then 2 ml of local anaesthetic is injected subcutaneously. Next a large bore (14 to 10 gauge) catheter is passed between 2 tracheal rings into the lumen of the trachea, the stylette removed and narrow sterile tubing passed down into the lumen. Twenty to 50 ml of sterile saline is then infused into the trachea and aspirated from the tracheal pool.

Transendoscopic tracheal aspiration has largely replaced percutaneous aspirates because it is less invasive and requires no sedation or hair clipping. Also reports suggest that the quantity of endoscopically visible tracheal mucus is more closely correlated with poor performance than neutrophil percentages. The endoscope must be disinfected and a sterile polyethylene catheter is passed through the biopsy channel once the endoscope has reached the mid-trachea. Bacterial culture of transendoscopic aspirates has been criticised due to possible contamination from the nasopharynx. However, studies have shown contamination is less problematic than previously thought. Using a protected double lumen catheter or sterile agar plug can further reduce the risk of contamination. The ease and reliability of transendoscopic sampling outweighs the disadvantage of possible bacterial contamination.

Transient contaminants may be present in normal tracheal secretions. Therefore, culture results must be viewed with respect to the presence of intracellular bacteria and large numbers of bacteria in Gram stains. Semi-quantitative bacteriological techniques can be used. Normal secretions usually contain low numbers (<1000 colony forming units/ml) of mixed bacteria. Secretions from horses with bacterial infections usually contain larger numbers (>1,000,000 colony forming units/ml) of usually one bacterial species.

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) gives a more accurate representation of lung pathology than tracheal aspiration, except when pathology is localised to the cranioventral lung fields. BAL is invaluable in the investigation of pulmonary diseases, particularly recurrent airway obstruction and exercise-induced pulmonary haemorrhage. The volume of saline reported for BAL sampling ranges from 60–500 ml. While some research studies suggest that differential cell counts may be biased by small volume lavages, the published results do not appear significantly different between most studies. Clinically, 60–180 ml of saline is easier to handle in the field situation.

BAL is performed using a 2–2.5 m endoscope or a BAL catheter. The advantages of transendoscopic sampling are visualisation of the sample site and instillation of the local anaesthetic (20 ml) into the carina and major branches of the bronchi to reduce coughing. The advantages of the BAL catheter are that it is considerably cheaper and that the catheter procedure is faster and often requires no sedation or local anaesthesia.

Processing BAL and tracheal aspirate fluid samples

Following tracheal aspiration or BAL, the sample fluid should be examined for colour and the presence of mucus. Normal fluid should appear clear or mildly turbid. For BAL samples, a layer of foamy surfactant indicates that the alveoli have been sampled.

Samples should be processed within 8 h to minimise cellular deterioration and bacterial overgrowth. Fixation of samples using an equal volume of 40% ethanol eliminates bacterial overgrowth, but results in poor cellular morphology. Under field conditions it is best to prepare smears as soon as possible after sampling. Dried slides can then be sent to a laboratory.

The reference range of ‘normal’ nucleated cell counts is 300–800 cells/µl. Cytospin preparations are generally best for dilute samples, since many cells are concentrated into one area of the microscope slide, making cell counting easier. Alternatively, 5–10 ml of sample can be centrifuged, the supernatant poured off and the cell pellet smeared. Slides can be stained using Diff-Quik, Gram stain, Toluidine Blue (mast cells) and Perl’s Prussian blue (hemosiderin).

Tracheal wash and BAL cytology

BAL samples of normal horses contain mostly macrophages (60%), lymphocytes (35%), few neutrophils (<5%), and very few mast cells, eosinophils and epithelial cells (<1% each). Tracheal aspirates are mostly, macrophaghes (40–60%), a smaller percentage of lymphocytes (10–20%), a larger percentage of epithelial cells (10–20%) and similar percentages of neutrophils (5–10%), mast cells and eosinophils (<1% each) as BAL samples.

Neutrophils

Horses with symptomatic recurrent airway obstruction always show a respiratory secretion neutrophilia. However, RAO horses in remission have normal respiratory cytology. Respiratory viruses can induce a transient pulmonary neutrophilia. Thus it may not be possible to differentiate acute viral airway disease from RAO using cytology alone. However, horses with chronic post viral airway disease usually have normal respiratory neutrophil numbers and so can be differentiated from cases of RAO. Respiratory secretions from horses with bacterial bronchopneumonia generally have increased numbers of neutrophils containing intracellular bacteria.

Eosinophils

Respiratory secretion eosinophilia is uncommon and usually attributed to Dictyocaulus arnfieldi. However, other allergic causes should be considered in horses with a good worming history.
Haemosiderophages and erythrocytes
Free erythrocytes may be identified in respiratory secretions collected within 3 days of significant pulmonary haemorrhage. These erythrocytes are phagocytosed by macrophages giving rise to haemosiderophages. Haemosiderin is easily recognised as brown or blue/black pigment granules. The clearance of haemosiderin from the lungs may take several months.

Further reading

NOTES

