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SAMPLING THE RESPIRATORY TRACT: TECHNIQUES AND INTERPRETATION

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Summary: Sampling the respiratory tract by tracheal wash or broncho-alveolar lavage may be readily implemented in routine practice. Cytological or microbiological analysis of these samples allows sensitive diagnosis of most currently encountered equine infectious and non-infectious respiratory diseases, investigation of their pathophysiological processes and follow-up of cases after therapy. Following simple established guidelines for retrieving and processing tracheal wash (TW) and broncho-alveolar lavage (BAL) allows obtaining quality samples, essential for adequate interpretation.

Inflammatory airway diseases are highly prevalent in horses and may be associated with both environmental management and working conditions. If a detailed review of the horse’s history, together with a thorough clinical examination, is essential to lead to an accurate diagnosis, some respiratory diseases progress subclinically and call for additional investigation techniques. Respiratory samples are useful and usually sensitive techniques which may be implemented both in routine practice and research to document the underlying cause of the disease and determine its severity. They may also be used to control post-therapeutic evolution. This presentation will focus mainly on tracheal wash (TW) and broncho-alveolar lavage (BAL) samples as biopsy and nasal swabs will be dealt with in other presentations (cf. M. Venner, G. Fortier).

TRACHEAL WASH OR BRONCHO-ALVEOLAR LAVAGE?

It has now been largely recognized that both samples represent different areas of the lung and yield complementary information. Both samples may be submitted for cytological or microbiological analysis (viral or bacterial). It has been shown that results of TW and BAL cytology correlate poorly and normal reference values differ significantly (Derksen et al., 1989, Malikides 2003). Tracheal wash samples will reflect pathologic processes limited to the tracheobronchial area whereas BAL samples represent problems occurring in the more distal airways and lung. For that reason, TW cytology coupled to microbiology are indicated to diagnose infectious diseases but may be insufficient to diagnose inflammatory airway disease (IAD), early phases of recurrent airway obstruction (RAO) or exercise-induced pulmonary hemorrhage (EIPH). On the other hand, BAL fluid cytology is the exam of choice to detect low grade pulmonary inflammation or hemorrhage but unless the horse suffers from pneumonia or lung abscesses, BAL fluid is usually sterile.

TW AND BAL SAMPLING TECHNIQUES

Both sampling techniques are safe and may be readily performed in routine practise. Proper sampling and post-processing is essential as accurate laboratory interpretation closely relies on the quality of the sample obtained.

TW may be performed either by trans-endoscopical or transtracheal catheterisation. Instillation of 20-50ml of saline is useful to facilitate sample retrieval in the presence of thick mucus and improves the cytological quality of the sample. The advantages of performing a TW with an endoscope are that it is less invasive and it allows prior visualisation of the upper and central airways. A catheter is inserted through the biopsy channel of the endoscope. It may be done without prior sedation in horses with low tracheal reactivity. The use of a guarded or plugged catheter is recommended to avoid upper airway microbial contamination of the sample. Transtracheal aspiration requires sedation and surgical preparation (with local anaesthesia) of a small area at in the mid-cervical region, between two cartilage rings. There are some commercially available kits which provide a steel percutaneous introduction catheter in combination with an inner flushing catheter. Catheters should be at least 60 cm to allow reaching the tracheal inflection area, at the thoracic inlet. This
to concentrate the cells contained in the sample. After elimination of the supernatant, the pellet is collected with a pipette, spread on the glass slide and dried before being stained. In the absence of mucus, direct smears may be poorly cellular and cytospin preparations are easier to interpret. Cytocentrifugation allows concentration of cellular content in a single spot and facilitates evaluation. For regular cytology, slides may be stained using Diff-Quik, May-Gruenwald-Giemsa or Wright kits. Additional Perls staining may be useful to colour hemosiderin and Toluidine Blue for mast cells granules.

INTERPRETATION OF SAMPLE ANALYSIS

Both quantitative and qualitative approaches are required to accurately interpret TW and BAL cytology. While examining the slides, it is important estimate the cellularity of the sample, check for presence of mucus or Curschman spirals, debris, pollen, micro-organisms (bacteria, spores), etc... These elements help to reflect the horse’s environment and the timing and severity of the pathological processes involved. Horses living in dusty environments will inhale larger quantities of particles which may be found in the sample, together with richly vacuolated macrophages. Contamination from upper airway flora may be suspected in case of abundant bacteria and/or presence of large pharyngeal epithelial cells. When mucus or pus is retrieved or when the sample processing has been delayed, cells may be damaged and difficult to identify.

Quantitative differential cell counts may be influenced by several factors including exercise, cough or trauma during the procedure, sampling technique, rapidity and quality of processing and competence of the pathologist. Table 1 summarises the normal range of TW and BAL differential cell counts presented in the review by Richard et al., 2009 in laboratories using similar techniques.

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Differential diagnosis is always made by confronting information gained from a detailed history, a thorough clinical examination of the horse and relevant ancillary diagnostic techniques, including respiratory samples. TW and BAL samples are particularly well indicated for the diagnosis of acute or chronic inflammatory airway diseases. Cases in which TW and BAL may yield disappointing results are those resulting in focal lesions (abscesses), pulmonary neoplasia or interstitial pneumonia.

In cases of viral infection, abundant epithelial cells, single or in tufts, showing signs of degeneration and ciliocytophtoria may be seen in the TW and several isolated epithelial cells may also be found in the BAL. It may be associated with neutrophilia in the TW or lymphocytosis of the BAL.

PROCESSING SAMPLES FOR CYTOLOGY

After retrieval of TW or BAL, samples should be examined macroscopically: is the sample clear or turbid, does it contain mucus, debris, food or blood? To best preserve cells, samples should ideally be processed without delay or placed in EDTA tubes and refrigerated at 4°C if processing is impossible within 8 hours. For cytological analysis, direct smears and/or cytospin slides may be prepared. For direct smears, samples should be either centrifuged or left to sediment

reactions and improve sample quality. A transient and mild local neutrophilia may occur after BAL sampling, but adverse reactions have been reported with this procedure.

CONCLUSIONS

The BAL procedure should be performed in a sedated horse as the procedure induces cough and may be painful in horse with airway inflammation. BAL samples may be retrieved either through an endoscope or blindly through a specific balloon-cuffed catheter. The use of an endoscope allows inspection of the lower airways and choice of the sampled area: sampling of the ventral lung is indicated when infectious or aspiration pneumonia is suspected whereas sampling of the caudo-dorsal region is recommended when searching for chronic inflammatory diseases or EIPH. There may also be some differences between left and right lung samples. The endoscope or catheter is advanced beyond the bronchial carina until wedged in a 4th or 5th generation bronchus (a length of at least 240 cm is required for adult horses). Pre-warmed 0.9% saline is then infused and retrieved through sequential syringe boluses or via a vacuum pump. There is no consensus as to the quantity of saline to instil and retrieve, but a volume of at least 250 ml of fluid has been recommended (Robinson 2001). The volume of fluid infused will influence the outcome of the differential cell count: a higher percentage of neutrophils and fewer mast cells and lymphocytes are recovered from infusion of small volumes (50 mL) in comparison to larger volumes (300 ml) (Richard et al., 2009). The retrieved BAL should be foamy, indicating the withdrawal of alveolar surfactant content. In horses with inflamed airways, prior instillation of a diluted local anaesthetic (xylocaïne or lidocaïne) may be used to reduce cough, subsequent pain and contamination of the sample by calls resulting from mechanical trauma (epithelial cells, erythrocytes). In horses suffering from RAO, administration of a bronchodilator before the procedure will help to alleviate bronchospastic

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Horses with very high eosinophil counts both in BAL and TW may have parasitic infiltration of the lungs by *Dictyocaulus arnfieldi*. Parasites are seldom seen macroscopically. More moderate eosinophil elevations may be seen in horses with type I hypersensitivity.

BAL cytology is considered as the reference technique for the definitive diagnosis of IAD. The cytological profile is characterised by an increase in total nucleated cell count and inflammatory cell populations such as a mild to moderate increase in neutrophils, mast cells and/or eosinophils (Couëtil et al., 2007). Horses with concurrent positive bacteriology of TW are at higher risk of showing IAD.

In some circumstances IAD may be difficult to distinguish from RAO (or “heaves”). Heaves-affected horses develop significant respiratory neutrophilia in response to inhalation of specific allergens. Neutrophil counts may rise up to 80-100 % in TW and BAL when the horse encounters a phase of exacerbation of the disease. It is associated with presence of dense mucus and Curschman spirals resulting from mucus plugging of the smaller bronchioles.

Respiratory samples will also be modified in horses displaying EIPH. Samples obtained post-exercise may show haemorrhagic content and cytology slides will indicate presence of high amounts of erythrocytes. If the sample is performed belatedly after an episode of EIPH, erythrocytes may be phagocytised by macrophages (erythrophages). After some time, the erythrocytes are broken down and hemosiderin content is progressively concentrated within the cytoplasm of these macrophages, which are then termed hemosiderophages. The aspect of hemosiderophages evolves with time and they may persist several months within the lung. Consequently, it is possible through BAL to determine if the horse has had single or recurrent episodes of EIPH according to the stage of hemosiderophage differentiation.

REFERENCES


HEWSON & VIÉL, 2002: Sampling, microbiology and cytology of the respiratory tract. In / Lekeux PM (ed) Equine respiratory disease

