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BRONCHOALVEOLAR LAVAGE (BAL) – EMPLOYMENT IN HORSES

Abstract

Bronchoaleolar lavage (BAL) is a valuable diagnostic method which enables collecting cytologic material from the bronchi, terminal airways and alveolar spaces (2, 13, 35). Fluid obtained in this procedure can be examined multidirectionally (cytologically, microbiologically, biochemically, immunologically). The diagnostic procedure is reliable, safe and may detect inflammation at the cytologic level (13). BAL is applied both in people and in animals (28). In veterinary medicine BAL is conducted in dogs, cats, horses, cattle and pigs. The evaluation of bronchoalveolar lavage fluid (BALF) is used in sick animals to recognize and differentiate lower airway diseases (6, 28). Bronchoalveolar lavage fluid cytology can provide a diagnosis and additional information. Practically, the most common diseases in horses, which can be diagnosed by assessment of BALF, include bronchopneumonia, exercise-induced pulmonary haemorrhage (EIPH), inflammatory airway disease (IAD) and reccurent airway obstruction (RAO) (27).

Key words: *horse, bronchoalveolar lavage, airway diseases*

Bronchoalveolar lavage (BAL) is a valuable diagnostic method, which was first used in horses in 1980, and has become increasingly popular for diagnosis of infectious and noninfectious equine airway diseases, especially disorders affecting performance (20, 27, 29, 33). The technique is inexpensive, safe and easily conducted under field conditions (22, 33). The results of bronchoalveolar lavage fluid (BALF) cytology are more consistent in healthy horses than transtracheal wash cytology and relate well to the clinical signs (13, 33). However, the correlation between clinical conditions and lung findings is closer than with the outcomes of the BALF cytology (22).

The indications for conducting BAL in horses include poor exercise performance, epistaxis, bronchopneumonia (especially chronic disease, refractory to treatment) and suspicion of recurrent airway obstruction (RAO) (14, 33). The procedure may be also useful for confirmation of specific infectious diseases, such as fungal pneumonia or *Pneumocystis carinii* (15, 18, 33). The most appropriate indication for BAL are diffuse pulmonary processes. Bronchoalveolar lavage fluid cytology represents only a pathology in the lavaged lung segments, and cannot precisely reflect the stage and severity of pulmonary disease, or may be normal in horses with a focal process. In most cases, BAL is an unreliable method for diagnosis

of bacterial respiratory tract diseases, unless the technique is sterile. The transtracheal aspiration is a more valuable method, which provides representative specimen of the bacterial population from the entire lower airway (1, 22, 33). BAL is indicated in the chronic pulmonary diseases, but may be performed in conjunction with transtracheal aspirates if a bacterial process was not excluded (1). Collecting samples for microbiological culture by bronchoscopy simplifies the technique and eliminates some of the complications, such as pneumomediastinum and cellulitis, but the samples can be contaminated by proximal airway commensal organisms (1, 14, 33). However, BALF culture may provide diagnostic and reliable information, if the endoscope is passed into the clean nares, culture results are correlated with findings of the cytologic analysis, type and number of organism recovered (14).

Culture outcomes must be evaluated in the light of the cytologic analysis and clinical examination. In some normal horses the transtracheal aspirates were culture-positive for *Klebsiella,* β-hemolytic streptococci, *Pasteurella* sp. and *Pseudomonas aeruginosa* (1). A group of bacteria of questionable pathogenicity, including *Enterobacter, Bacillus, Acinetobacter,* α-hemolytic streptococci (except for *Streptococcus pneumoniae* type 3) and *Staphylococcus epidermidis*, may be also isolated from tracheobronchial lavage fluid (1). In contrast, *S. pneumoniae* is recognized as a potential etiologic agent of the airway diseases in horses (1).

Bronchoalveolar lavage is routinely conducted in standing, sedated horse (xylazine hydrochloride 0.2-0.8, in foals 1.0 mg/kg, i.v. or romifidine 0.01 mg/kg i.v. in combination with butorphanol tartrate 0.02, in foals 0.07 mg/kg i.v.), alternatively restrained with a nose twitch (1, 7, 9, 10, 14, 16, 22, 33, 37). The procedure can be performed using a BAL catheter or a flexible fiberoptic endoscope (1.8-2 meters) (1, 22, 33). Equine BAL catheters have on the end an inflatable cuff. The flexible tube, after being lubricated with viscous 2% lidocaine hydrochloride, is introduced into nasal ductus and through larynx, trachea and lower airway until it becomes wedged in a third or fourth generation bronchus, and the cuff is inflated with air (1, 22, 33). The technique can be a reason of bronchial mucosal pressure necrosis or overdistension of the distal airways with fluid. The procedure is limited to diffuse pulmonary processes, because the catheter is passed blindly and the person performing the examination cannot choose an appropriate pathologically changed lung segment. A blindly introduced BAL catheter enters mostly the right caudodorsal lung (22, 33).

Bronchoalveolar lavage via bronchoscopy is more reliable in horses with focal or regional pulmonary diseases (33, 34). Bronchoscope is passed into the wiped with a gauze nares and 60 ml of 0.2% lidocaine solution is sprayed into the larynx, trachea and carina to reduce cough reflex (10, 14). Endoscopy allows the examiner to visualize airway and select a particular pathological changed bronchus (hyperemia, mucopurulent exudates) for sampling, but it is difficult to obtain a seal in the airway without an inflatable cuff and the end of endoscope may traumatize the bronchial wall. It can be a cause of iatrogenic hemorrhage (14, 33).

In bronchoalveolar lavage is used sterile warm (body temperature) isotonic crystalloid solution in volume 100-300 ml or 360 ml phosphatebuffered saline (PBS), which is infused by the syringe 50 ml (100-120 ml) under pressure through the

bronchoscope biopsy channel (1, 10, 14, 16, 26, 33, 34). The technique is repeated until exudates are almost removed (14). About 50-80% of the introduced volume using manual aspiration is recovered (1, 16, 33). Macroscopic examination should be first performed: evaluation of color and detection of flocculent debris. BALF cytology should include total and differential cell counts, vitality and morphologic description of cells and identification of pharyngeal contamination. If the samples cannot be instantly subjected cytologic analysis, they should be chilled (33).

The quality estimation is performed in the microscopic specimens prepared from the precipitate (34). The technique using cytocentrifugation may result in lower lymphocyte counts. Differential cell evaluation is performed by examination of 200 consecutive nucleated cells on each slide (31, 33, 36). In most cases, total nucleated cell counts are less than 300 cells in 1 µl of BALF (33). Cellular composition in cytologic preparations can be changed in pathological conditions and bronchoalveolar lavage fluid may be determined as neutrophilic, eosinophilic, lymphocytic, macrophagic or mixed (33). In BALF fungi, bacteria, eggs and larvae of parasites (in horses: *Dictyocaulus arnfieldi, Parascaris equorum*) can also be found (1, 34). Fungal hyphae and bacterial organisms can occur free or engulfed within mononuclear phagocytic cells (1).

The predominant fraction in BALF in adult normal horses are macrophages (30-70%) and lymphocytes (18-70%) (1, 25, 33, 34).

Alveolar macrophages are usually uniform, with minimal cytoplasmic vacuolization (33). The large cells with vacuolizated cytoplasm- active cells- can also be observed (34).

Lymphocytes are round or oval and have a large nucleus (34). An increase in their number in chronic and viral diseases is observed (34).

Neutrophils constitute less than 5% of the total cell counts (25, 29, 33). An elevated neutrophil percentage appears in the bacterial processes, such as pneumonia, and RAO (50-80%) (1, 9, 22, 24, 32, 33, 34). Therefore, if neutrophilic BALF is culture-negative, recurrent airway obstruction is most likely the diagnosis (22, 24). However, bacteria can be result of primary pneumonia or secondary infection of a lung afflicted by RAO (22). The estimation of cytologic specimens allows to detect early inflammatory processes, but is not specific for RAO (8). The horses are classified as RAO-susceptible when neutrophil percentage exceeds 20% (17). A very useful additional, mark in horses with RAO is significantly higher concentration of myeloperoxidase (MPO) which is a product of neutrophils (29).

Eosinophils are occasionally observed in normal horses (<2%) (25, 33). An increase in eosinophil number appears in parasitic diseases, allergy, EIPH and neoplastic processes (10, 33). Eosinophilic granulocytes can be a marker of underlying allergic pulmonary diseases or could secondarily migrate to the sites of bleeding (10). However, pulmonary eosinophilia, abated without treatment and not caused by deworming, was also described in standardbreds in training (30).

Mast cells are occasionally observed in BALF in normal horses (<2%) (25, 33). An increase in mast cells appears in allergic processes and in RAO in horses (9, 34).

Erythrocytes in BALF are observed in the bleeding (EIPH) and lung hemorrhage (trauma, disorders of coagulation, error during the bronchoscopy) (29, 34). During EIPH in BAL fluid can be observed an increase in erythrocytes and haemosiderophages number (11, 19, 23). However, there is still no 'gold standard' method to confirm EIPH, because very little is known whether the lavaged lung segment is representative of pulmonary region which is bleeding (29).

Neoplastic cells can come from all types of cells in the respiratory tract. The specific marks of neoplasmatic cells include polymorphy, changed structure of the chromatin, an increased number of micronuclei and partition of the nucleus (34).

Detection of squamous epithelial cells or feed material in cytologic specimens indicates pharyngeal contamination (33).

In BALF are also evaluated total protein, phosphorus and phospholipid concentrations, which are components of pulmonary surfactant, synthesized and secreted by alveolar type II epithelial cells. Abnormalities in surfactant quality and quantity are observed in acute lung injury and RAO (5, 14). In older horses age-related alterations in pulmonary tissue result in a decreased content of phospholipids (4).

BAL in conjunction with histamine bronchoprovocation (HBP) is a very useful method for diagnosis of inflammatory airway disease (IAD) which was defined as nonseptic inflammation affecting horses of any age . Abnormalities in BALF cytology are various and include an increase in the total nucleated cells number and evaluated neutrophil, lymphocyte, mast cell or eosinophil percentage (3, 21, 25, 29). BAL fluid findings in horses affected IAD differ from the results of cytologic analysis in horses with RAO, which suggests other pathogenesis (8, 21).

Bronchoalveolar lavage is used not only in diagnosis but also in the early treatment of pneumonia, especially serious cases of pneumonia associated with transport which appear within 24 hours of long distance travel. The hospitalized horses are subjected to BAL at intervals of several days according to clinical conditions and the results of bronchoscopy. The procedure improves the outcomes of treatment and shortens the period of antibiotic application (14).

In France were performed the screening studies on occurrence of equine herpesvirus 5 (EHV-5) in BAL fluid. PCR was used in the isolation of EHV-5. Further research is needed to determine a potential pathogenic role of EHV-5 in airway disease in horses (12). Probably, EHV-5 may be an etiologic agent in the development of equine multinodular pulmonary fibrosis (EMPF) (38).

The most common complications after BAL in horses include cough, an increase in the body temperature, rales and crepitus above lavaged lung segments (34). It is not necessary to add antibiotics to the lavage solution. Local inflammation characterized by an increase in neutrophils number appears in the lavaged areas for at least 48 hours after the procedure (14, 33). Long-term focal inflammation following BAL is not observed in horses (33).

Bronchoalveolar lavage in horses is a very useful valuable method to recognize and characterize lower respiratory tract disease in horses, especially in disorders for which BAL is the reference method for diagnosis. The procedure creates a lot of

possibilities, not only of the confirmation but also in the treatment of airway disorders in horses. The technique is safe, simple and inexpensive and each veterinarian can perform BAL, even under field conditions.

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Стаття надійшла до редакції 11.03.2010