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Bronchoalveolar lavage fluid in Standardbred racehorses: Influence of unilateral/bilateral profiles and cut-off values on lower airway disease diagnosis

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ABSTRACT

The aim of this study was to determine whether the lung side being sampled would significantly influence bronchoalveolar lavage (BAL) cytological profiles and subsequent diagnosis in Standardbred racehorses. One hundred and thirty-eight French Trotters in active training and racing were included in a prospective observational study. BAL was performed using videoendoscopy in both right and left lungs during summer meetings in 2011 (64 horses) and 2012 (74 horses). Cytological data performed 24 h later from right and left lungs were compared and specifically used to classify horses as affected with exercise-induced pulmonary haemorrhage (EIPH), inflammatory airway disease (IAD), or were 'controls'. For IAD, cytological definition was based on two different cut off values.

Neutrophil percentages, haemosiderophage percentages and the haemosiderophage/macrophage (H/M) ratios were significantly higher in the right compared to the left lung. Measures of intra-class correlation coefficients revealed a fair agreement between left and right lungs for percentages of mast cells, eosinophils, and for the H/M ratio, and a moderate agreement for neutrophil percentages. Fair to moderate agreements were observed between left and right lungs for the diagnosis of IAD and/or EIPH based on kappa coefficients. When sampling one lung only, the risk of incorrectly classifying a horse as a 'control' increased with the use of the restraint cut-off values for IAD. As BAL from one lung is not representative of the other lung in the same horse, both lungs should be sampled for a better assessment of lung cellularity and for a precise diagnosis of lower airway diseases.

Introduction

Lower respiratory tract diseases such as exercise-induced pulmonary haemorrhage (EIPH) and inflammatory airway disease (IAD) are common causes of poor performance in racehorses, reportedly due to impaired pulmonary gas exchange (McKane et al., 1995; Couetil and Denicola, 1999; Couroucé-Malblanc et al., 2002; Hinchcliff et al., 2005b; Sanchez et al., 2005).

IAD has been defined as a neutrophilic and/or mastocytic and/or eosinophilic mild lower airway inflammation affecting horses of any age, which might exhibit signs of intermittent cough and increased mucoid airway secretions, without evidence of severe respiratory signs at rest (Robinson, 2003; Couetil et al., 2007). Although endoscopic and cytological evaluations of tracheal secretions have been commonly used in the diagnosis of IAD (Martin et al., 1999; Newton and Wood, 2002; Sanchez et al., 2005; Wood et al., 2005; Durando et al., 2006), a lack of agreement between tracheal and bronchoalveolar (BAL) cell populations (Derksen et al., 1989; Malikides et al., 2003) has led to BAL fluid (BALF) cytology or pulmonary function testing being recommended as the only accurate diagnostic methods in horses (Couetil et al., 2007).

EIPH is another common disease of racehorses resulting from stress failure of pulmonary capillaries occurring during maximal or sub-maximal exercise (Birks et al., 1997). Firstly described by the presence of post-exertional epistaxis in severe cases (Cook, 1974), different diagnostic methods for EIPH have been used. These include post-exercise tracheobronchoscopic evaluation of blood (Pascoe et al., 1981; MacNamara et al., 1990; Lapointe et al., 1994; Birks et al., 2002; Hinchcliff et al., 2005a; Costa et al.,

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2012), or detection/quantification of erythrocytes (Langsetmo et al., 2000; Epp et al., 2006; McKane and Slocombe, 2010) and/or haemosiderophages (Fogarty and Buckley, 1991; McKane et al., 1993; Langsetmo et al., 2000; Doucet and Viel, 2002; Newton and Wood, 2002; Sanchez et al., 2005) in respiratory fluids.

Recommendations still differ regarding the method of choice for diagnosing EIPH, and no methods reported so far allow for an accurate evaluation of the severity of bleeding (Derksen et al., 2011). However, quantification of haemosiderophages in BALF seems to be a more sensitive technique for the detection of EIPH. Furthermore, this method does not necessitate an examination soon after strenuous exercise or multiple examinations as for tracheal endoscopic evaluation (Fogarty and Buckley, 1991; McKane et al., 1993; Meyer et al., 1998; Doucet and Viel, 2002).

Even when considering BAL as the gold standard method for diagnosing lower airway diseases, standardisation of sampling procedure and cytological interpretation is still required. Previous reports described different techniques for BAL collection regarding lavage volume, site of sampling, choice of aliquot submitted to analysis and procedures for conservation and preparation of samples (Sweeney et al., 1992; McGorum et al., 1993; Pickles et al., 2002a,b; Jean et al., 2011). The International Workshop on Equine Chronic Airway Disease recommended a volume of 250–500 mL of fluid to be instilled as one to three bolus (Robinson, 2001), and general guidelines recommended pooling all syringes for analysis (Hoffman, 2008).

Only a few studies have tried to identify regional variations in BALF cytology, including the comparison between left and right lungs. McGorum et al. (1993) found no difference in BALF from different regions of the right and left lung in six healthy and six heaves affected horses, as did Meyer et al. (1998) from both lungs in six healthy racehorses and Deniau et al. (2010) on seven IAD-affected horses. Sweeney et al. (1992) compared BAL from both lungs in 23 healthy horses and found mast cells to be significantly higher in the left lung, whereas they were significantly higher in the right lung in the study by Jean et al. (2011) who looked at five healthy and five recurrent airway obstruction (RAO)-affected horses.

All of these studies concluded that a single BAL sample was representative of the entire lung, and that results obtained were unlikely to be influenced by the site of collection (Jean et al., 2011). The number of horses included in these previous studies might however be insufficient to reach a definitive conclusion that both lungs are equivalent when diagnosing lower airway diseases in racehorses. Furthermore, as BAL is often described as an expensive and invasive procedure, only a few epidemiological studies have used this method for the detection of inflammatory and/or haemorrhagic conditions of lower airways in horses (Newton and Wood, 2002; Wasko et al., 2011).

The objective of the present study was to determine whether the lung side being sampled would significantly influence BALF cytological profiles and the diagnosis of sub-clinical lower airway disease in French Standardbred racehorses.

Materials and methods

Horses

One hundred and thirty-eight French Trotter horses (76 geldings, 58 females and 4 males), aged 3–9 years (mean 4.7 ± 1.6 years old), were included in the study. Horses came from 11 different training stables, and were sampled during summer race meetings in 2011 (64 horses) and 2012 (74 horses). They were all involved in active training and had raced within 1 month of sampling.

Prior to any procedure, each horse was submitted to a thorough clinical examination in order to ensure that no obvious clinical abnormality was present. Venous blood samples were collected in the morning between 0600 and 0730 h and before feeding, for a complete haematological and biochemical assessment to rule out any systemic disease.

The study was approved by the regional Animal Ethic Committee (CEEA.2012.179) and all owners signed a consent form.

Bronchoalveolar lavage

Horses that were not racing within 10 days following the sampling were sedated with IV romifidine, 0.04 mg/kg (Sedivet, Boehringer Ingelheim). For other horses racing within 10 days BAL was achieved with a nose twitch and without sedation because of drug testing considerations.

BAL was performed using a flexible 3.2 m long, 12.8 mm tip diameter videoendoscope (Optomed). The endoscope was first introduced without sedation in the ventral meatus of the left nostril to assess laryngeal function according to the Havermeyer grading system (Dixon et al., 2003). Pharyngitis was also scored using a grade 1–4 scale (Raker and Boles, 1978). If sedation was necessary and feasible based on above considerations, the endoscope was then removed and romifidine was injected IV. Following sedation, the videoendoscope was reintroduced through the left nostril, via the pharynx into the trachea, and randomly directed into the left or right main stem bronchus until wedged in a distal bronchus. When sedation was not used, this procedure was conducted immediately following laryngeal assessment. Tracheal mucus accumulation (grade 1–5 scale) and tracheal septum thickness (grade 1–2 scale) were also recorded (Gerber et al., 2004; Koch et al., 2007).

A total of 250 mL of sterile isotonic saline solution was instilled into the bronchus via the endoscope biopsy channel, which was previously pre-filled with 20 mL of saline. A first 125 mL bolus was instilled, using two 60 mL pre-filled syringes. Immediately after instillation of the second syringe, aspiration was manually performed with the same syringe used for injection. The first 20 mL of aspired liquid, corresponding to the volume of the endoscope biopsy channel that did not reach the lung, was discarded. The residual liquid was then collected in one or two syringes, depending on the volume harvested. The second 125 mL bolus of isotonic saline was similarly injected and collected. At the end of lavage in the first lung side, the endoscope instrument channel was cleaned with a 30 mL bolus of isotonic saline. Then, the endoscope was moved back to the carina and introduced in the contralateral lung and lavage procedure was repeated.

The volume of liquid collected and macroscopic assessment (colour, turbidity, and presence of foam) were recorded, and syringes were pooled in a metallic bowl for each lung side. A sample was taken from both pools and kept into EDTA tubes.

BAL fluid analysis

BALF samples were preserved in EDTA and kept at room temperature. The volume recovered was sufficient for analysis of all 276 samples (138 horses × 2), which were then investigated. At reception in the laboratory, within 24 h of collection, 200 μL of fluid were immediately cytocentrifuged (80 *g*, 10 min) (Shandon Cytospin, Thermo Scientific) and stained with May-Grünwald-Giemsa (MGG). Left and right samples were then processed simultaneously for each horse. Differential cell count was performed on 300 cells, and the number of each cell type was expressed as a percentage of total nucleated cells. Epithelial cells were not included in the differential count.

Case definition

Horses were considered as 'controls' according to the formal definition of inflammatory airway disease (IAD) when BALF cytological profiles were ⩽5% neutrophils, ⩽2% mast cells and <1% eosinophils (Robinson, 2003; Couetil et al., 2007). Horses with values above the cut-off for any of these three cell types in at least one lung were considered to have evidence of IAD. An alternative definition of 'control' horses, based on higher cut-off values of neutrophils (⩽10%), mast cells (<5%) and eosinophils (<5%) was also used in this study (Hare and Viel, 1998; Hughes et al., 2003; Richard et al., 2010a; Beekman et al., 2011; Koblinger et al., 2011; Wasko et al., 2011).

The ratio of BALF haemosiderophage/macrophage (H/M) > 20% was considered as evidence of previous episode of EIPH (Richard et al., 2010a,b).

Statistical analysis

Normality of continuous data distribution was evaluated using the Shapiro–Wilk *W* test. The different variables were not normally distributed with data presented as 'median, 1st–3rd quartile', unless stated otherwise. Because of the large number of horses, data were log 10 transformed to normalise distribution. Multiple comparisons were performed using one-way ANOVA with Tukey post hoc test, and independent Student's *t* test. A paired *t* test was used when comparing left to right lungs. Associations between cell percentages from both lungs were evaluated using Pearson's correlation coefficient and linear regression analysis (equation: $y = ax + b$), respectively. For this, 95% confidence interval (CI) of the slope (a) and intercept (b) of the regression line should include the ideal regression ($y = 1x + 0$), if the investigated parameters are equivalent. Measures of agreement for numerical and categorical ('control', 'IAD', 'EIPH') variables were evaluated using the intra-class correlation coefficient (ICC) and the Cohen's kappa coefficient (κ), respectively. Both