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Short Communication

Influence of bronchoalveolar lavage volume on cytological profiles and subsequent diagnosis of inflammatory airway disease in horses

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ABSTRACT

The aim of the study was to determine whether instillation of either 250 mL or 500 mL of saline for bronchoalveolar lavage (BAL) would influence cytological confirmation of inflammatory airway disease (IAD). Thirty client-owned Standardbred racehorses were sampled via endoscopy with 250 mL of saline in one lung and 500 mL in the contralateral lung. The procedure was repeated 72 h later, reversing the volume per lung. The proportions of BAL fluid (BALF) recovered were significantly higher and neutrophil percentages significantly lower with the larger volume. A poor agreement was found between methodologies in terms of final diagnosis, when based on proportions of neutrophils (>10% from at least one lung). Within the recommended range (250–500 mL), the instilled volume significantly influenced cytological profiles. Establishing specific BALF reference values is warranted.

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Inflammatory airway disease (IAD) is characterised clinically by poor-performance, coughing and excess tracheal mucus (Robinson, 2003). The final diagnosis is confirmed by cytological examination of bronchoalveolar lavage (BAL) fluid (BALF) (Couëtil et al., 2007), which reveals moderately increased proportions of neutrophils and/or mast cells and/or eosinophils.

Few data are available about the volume of fluid instilled and the subsequent BALF cytology obtained. Higher proportions of neutrophils and lower proportions of mast cells were found following instillation of small (50 mL) compared to larger (350 mL) volumes (Sweeney et al., 1992). A total volume of 250–500 mL of BALF has been recommended for performing BAL (Robinson, 2001), but this is a wide range. Also, BALF samples collected simultaneously from both lungs of the same horses were recently found to differ; neutrophil percentages were significantly higher in the right compared to the left lung (Depecker et al., 2014). The aim of the present study was to determine whether instillation of the highest volume of the recommended range (250–500 mL) in both lungs would influence BALF cytological profiles and the subsequent diagnosis of IAD in racehorses.

Thirty client-owned Standardbred racehorses (aged 3–9 years), located in three different training centres (10 per centre) and actively involved in training or racing, were randomly included whatever their level of performance. Horses were free of any clinical evidence of respiratory disease at rest. The study was approved by the Animal Ethic Committee (CEEA-PdL 2015.70; 15 July 2015), and informed consent was provided by all owners.

Samples were performed by the same operator at 72 h interval, both in the left and right lungs according to previously published procedures (Depecker et al., 2014). On Day 1, the endoscope was randomly introduced within one lung and 250 mL (two boluses of 125 mL) was manually instilled and immediately aspirated. The channel was flushed and the endoscope introduced in the contralateral lung, where 500 mL (two boluses of 250 mL) was instilled and aspirated. On Day 4, the procedure was repeated, reversing the volume instilled per lung. Samples were kept in EDTA tubes at +4 °C for cytological preparation within 12 h.

All laboratory analyses were conducted in a blinded manner. Total cell count was performed automatically (ADVIA 120, Siemens Healthcare Diagnostics); 300 µL of BALF samples was cytocentrifuged (80 *g*, 10 min), stained with May–Grünwald–Giemsa, and differential cell counts performed on 300 leukocytes. Horses exhibiting BALF samples with >10% neutrophils were classified as 'IAD'. Any BALF samples with ≤10% neutrophils were classified as 'control' (CTL) cytology.

Continuous data that were not normally distributed, as assessed by Shapiro–Wilk *W* test, were log-transformed (Prism, GraphPad). The effect of instilled volume on cytological profiles was evaluated by ANOVA and Tukey–Kramer post-hoc test (General Linear Model), with sampling day and training centre as covariates (NCSS). Agreement among methodologies for IAD diagnosis was

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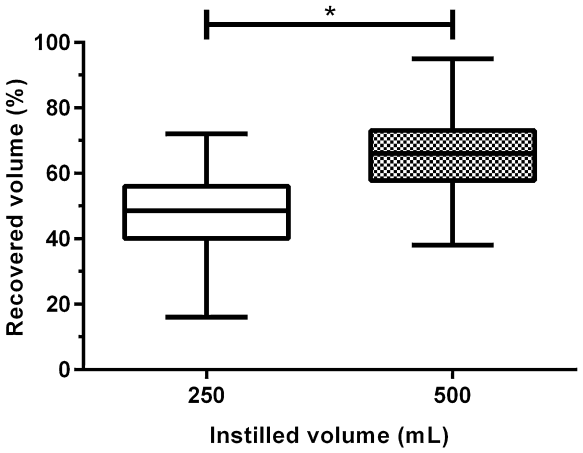

Fig. 1. Proportions of bronchoalveolar lavage fluid recovered from both lungs of 29 horses after instillation of either 250 mL or 500 mL of saline. *Significantly different ($P < 0.05$).

measured using Cohen's kappa (κ) coefficient; the corresponding proportions were compared by McNemar test with continuity correction. Values of $P < 0.05$ were considered significant.

One horse was excluded because of reluctance during BAL procedure on Day 4. A total of 29 horses (116 BALF samples) were investigated. Total cell count ($P = 0.02$), as well as lymphocyte, macrophage and neutrophil proportions ($P < 0.001$) were significantly influenced by the horse's training centre of origin. Also, BALF samples collected on Day 4 yielded a higher proportion of neutrophils than on Day 1 ($P = 0.03$), mainly for horses from one training centre (Appendix: Supplementary Fig. S1). Controlling for these confounding factors, proportions of recovered BALF were significantly higher ($P < 0.001$; Fig. 1) and neutrophil percentages were significantly lower ($P = 0.02$; Table 1) when instilling 500 mL compared with 250 mL. No significant differences were observed in total cell count or in proportions of other cell types (Table 1).

When 250 mL was instilled, five horses were diagnosed as CTL (both lungs) and 24 exhibited BALF cytology from at least one lung that was consistent with IAD. Similarly, 11 horses were CTL (both lungs) and 18 were compatible with IAD (≥1 lung) when instilling 500 mL. Overall, 25 horses were classified as IAD from at least one lung. Among these, the final diagnosis matched for 17 horses and conclusions were divergent among methodologies for 8/25 (32%) horses. A poor agreement only ($\kappa = 0.30$; CI 0.02–0.70) was found between volumes. The proportions of horses classified as CTL or IAD according to volume were however not significantly different ($P = 0.08$).

Table 1
Total and differential cell counts (median; 1st to 3rd quartile) in bronchoalveolar lavage fluid from both lungs of 29 horses after instillation of either 250 mL or 500 mL of saline.

BALF cell type	Instilled volume	
	250 mL	500 mL
Total cell count (/mm^3)	430; 323–585	340; 265–505
Neutrophils (%)	14.0; 7.8–20.3*	10; 6.8–16.0*
Eosinophils (%)	0.0; 0.0–0.0	0.0; 0.0–0.0
Mast cells (%)	2.0; 1.0–2.0	2.0; 1.0–3.0
Lymphocytes (%)	43.0; 28.8–52.5	35.0; 26.0–52.0
Total macrophages (%)	38.5; 31.8–56.0	47.5; 34.8–55.3
Haemosiderophages (%)	2.0; 0.0–4.3	2.0; 0.0–10.3

* Significantly different from each other ($P < 0.05$), controlling for training centres and day of sampling.

This is, to our knowledge, the first study in which the influence on BALF cytology of two different volumes of lavage fluid within the recommended range (250–500 mL) has been investigated with a subsequent diagnosis. A significant influence of the sampling day on BALF neutrophil proportions was observed, in accordance with previous data describing a transient (48 h) inflammation induced by the procedure (Sweeney et al., 1994). A 72 h washout period between both samples might be insufficient when investigating horses in training.

Neutrophil proportions in BALF were significantly lower when instilling a large volume, according to the previous study by Sweeney et al. (1992). Very small volumes (50 mL) might however represent bronchial lavage rather than bronchoalveolar lavage (Pickles et al., 2002). A total of 250 mL or 500 mL was instilled in the present study, as recommended for BAL (Robinson, 2001). Also, we selected a higher cut-off value (10%) than has been previously recommended to define neutrophilic IAD (5%; Robinson, 2003). Higher cut-off values were shown to increase the agreement between left and right lung BALF cytology for IAD diagnosis, when instilling 250 mL (Depecker et al., 2014).

Despite poor agreement between methodologies, the volume instilled did not significantly influence the proportions of horses, in terms of final diagnosis. Such a result should however be interpreted with caution. Sampling one lung only is enough for the cytological confirmation of IAD, while sampling both lungs is required to definitively classify the horse as CTL (Depecker et al., 2014). Our findings highlight the relevance of standardising the BAL procedure, which should be performed consistently with the same volume of fluid. Furthermore, BALF cytology for confirmation of IAD should always be interpreted within its clinical context, including racing performances.

In conclusion, the volume of lavage fluid administered, even complying with the previously recommended range, significantly influenced BALF cytological profiles. Establishing BALF reference values for each of the extreme volumes (250–500 mL) is warranted for cytological confirmation of IAD.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.tvjl.2015.09.027.

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