

Effects of the bronchoalveolar lavage procedure on lung function in horses with clinical exacerbation of recurrent airway obstruction

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Objective—To evaluate whether bronchoalveolar lavage (BAL) alters respiratory mechanics of horses with recurrent airway obstruction (ie, heaves) over a 48-hour period.

Animals—6 horses affected with heaves.

Procedures—Horses were subjected to a complete BAL procedure, which included sedation with xylazine and butorphanol, intratracheal administration of lidocaine, and instillation and aspiration of two 250-mL boluses of saline (0.9% NaCl) solution through an endoscope (study 1). To evaluate the effects of saline solution, horses were subjected to the same procedure without saline solution instillation and aspiration (study 2). Lastly, the endoscope was similarly introduced into the lower airways, without sedation or saline instillation and aspiration (study 3). Respiratory mechanics were performed at baseline (time 0) and at 3, 6, 12, 24, and 48 hours after each procedure.

Results—In study 1, BAL induced a significant decrease in pulmonary resistance lasting up to 6 hours. This may have resulted from clearance of mucus in large airways. We also observed a significant increase in lung elastance and transpulmonary pressure at 12 hours after BAL in all 3 studies, which may be attributed to a circadian effect.

Conclusions and Clinical Relevance—Our results indicate that the temporal effects of BAL procedures on lung mechanics should be taken into account when designing research protocols involving horses with heaves. Future studies should address the immediate effects of BAL on lung function. (*Am J Vet Res* 2006;67:1929–1933)

The BAL technique is a commonly used procedure for harvesting cells representative of the alveolar population. Cytologic evaluation of BAL fluid is commonly performed in clinical practice for the diagnosis of lower airway inflammation in horses and, occasionally, in horses with recurrent airway obstruction (ie, heaves) with labored respiration. Furthermore, sequential BAL procedures are important research tools when monitoring response to drug or evaluating the effects of changes in the environment of horses with lower airway inflammation such as heaves.^{1–4} Because BAL fluid and lung function are commonly assessed jointly in research

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ABBREVIATIONS

BAL	Bronchoalveolar lavage
ΔP_L	Maximal difference in transpulmonary pressure
V_T	Tidal volume
R_L	Pulmonary resistance
E_L	Pulmonary elastance

studies, the effects of BAL on respiratory mechanics may introduce a bias in the results. To our knowledge, no data are available on the effects of BAL on lung mechanics over time and no consistent time between BAL procedures and measurements of lung mechanics has been established.

Ethical considerations are more important when BAL is to be performed in heaves-affected horses during clinical exacerbation. These horses have marked changes in their lung function, which may deteriorate when performing a BAL under sedation. Moreover, findings in a previous study⁵ indicate that BAL can induce lower airway neutrophilia, which may further worsen inflammation and lung function of horses already having respiratory difficulties. The purpose of the study reported here was thus to evaluate whether the BAL procedure alters respiratory mechanics of horses with heaves over a 48-hour period.

Materials and Methods

Animals—Six adult heaves-affected horses (5 mares and 1 gelding) from our research herd were studied. Horses were mixed breeds and 8 to 18 years of age. Criteria for inclusion were a history of chronic, recurrent periods of labored breathing at rest; maximal changes in ΔP_L of > 15 cm H_2O ; $> 15\%$ neutrophils in BAL fluid on cytologic examination when horses were stabled; and results for CBC determination and serum biochemical analysis within the reference ranges of our laboratory. Thoracic radiographs were taken prior to the stabling to exclude concurrent pulmonary conditions. Endoscopic examination of the upper airways was also performed to exclude obstructive abnormalities. None of the horses had received treatments for heaves during the 3 months preceding the study.

Prior to the experiment, horses were conditioned to stand in stocks wearing a mask. The horses were stabled in the same barn for at least 3 weeks before the experiment, and the management remained the same throughout the study period. All experimental procedures were performed in accordance with the guidelines of the Canadian Council for Animal Care and were approved by the Animal Care Committee of the Faculty of Veterinary Medicine of the Université de Montréal.

Bronchoscopy and BAL—Bronchoalveolar lavages were performed in the morning (from 8:00 AM to 10:00 AM) by use of standard procedures for our laboratory.¹ Briefly, horses were

sedated with xylazine^a (0.3 to 0.4 mg/kg, IV) followed 10 minutes later by butorphanol^b (20 to 30 μ g/kg, IV). Bronchoscopy was performed with a fiber-optic flexible endoscope (180 cm in length, 15 mm in diameter) inserted through the nares and directed down into the left lung until its tip was wedged in the bronchus. During the progression of the endoscope through the airway, several small boluses of a 0.5% solution of lidocaine hydrochloride^c were administered (up to a maximal volume of 120 mL) to desensitize the airway mucosa. Once the endoscope was wedged in the bronchus, BAL was performed with two 250-mL boluses of sterile isotonic saline (0.9% NaCl) solution (at 37°C) rapidly instilled into the bronchus and aspirated via the endoscope's biopsy channel by use of a suction pump. The BAL fluid was collected into a siliconized glass jar and kept on ice until analysis. Total nucleated cells in BAL fluid samples were counted with a hemacytometer. Smears of the fluid were then prepared by centrifugation (at 90 \times g for 5 minutes) of 100 μ L of the BAL fluid and stained with a modified Wright solution. Differential counts were made on at least 400 cells; epithelial cells were not included in the differential count.

Respiratory mechanics measurements—Pulmonary function tests were performed on horses as described elsewhere.¹ Briefly, flow rate was obtained by use of a heated pneumotachograph^d and associated differential pressure transducer.^e The pneumotachograph was fitted to a mask placed and sealed over the nose of each horse so that the horse's nostrils were in line with the measuring equipment. During measurements, position of the head was set to minimize upper airway resistance.⁶ Electronic integration of the flow signal provided V_T . For each experiment, the system was calibrated by forcing air at known flow rates (between 0 and 9 L/s) through the pneumotachograph with blower-rotameter equipment.

Transpulmonary pressure was obtained by use of a differential pressure transducer^f that subtracted the esophageal pressure from the mask pressure. The esophageal pressure was measured with a balloon sealed over the end of a polyethylene catheter (inside diameter, 4.8 mm; outside diameter, 7.9 mm) placed in the distal third of the esophagus and distended with 3 mL of air. The pressure transducer was calibrated by use of a water manometer.

Signals from the transducers were amplified and passed through a digital-analogue converter to a computer equipped with a program for data acquisition and analysis.⁵ Values of R_L and E_L were obtained by applying the data to the multiple regression equation for the single-compartment model of the lung.⁷ The coefficients of determination for the fit of the equation to the data were calculated for each breath. Respiratory rate data were also recorded. Signals were sampled at a frequency of 120 Hz for 100 seconds, and all valid breaths were used for analysis.

Experimental protocol—The design of the study was a crossover with 6 horses subjected to 3 treatment protocols (study 1, 2, and 3). In study 1, BAL procedures were performed on the 6 horses as already described immediately following respiratory mechanic baseline recording. Lung function was then measured 3, 6, 12, 24, and 48 hours later. To test the effect of saline solution instillation, a baseline respiratory mechanics analysis was performed, followed by bronchoscopy, as in study 1, except that no saline solution was instilled (study 2). For study 2, horses were sedated and the endoscope was inserted through the lower airway with instillation of local anesthetic solution as described, but once it was wedged in a lower bronchus, the endoscope was immediately retrieved. Lastly, to test the effects of sedation and insertion of the endoscope on lung function (study 3), the endoscope was similarly inserted through the airway with

instillation of local anesthetic solution as described, but the procedure was performed on nonsedated horses. Once it reached a lower bronchus, the endoscope was withdrawn without injection of saline solution. Studies were performed in the order described, and a 2- to 4-week period elapsed between each study.

Statistical analysis—Baseline values of ΔP_L , R_L , and E_L were compared by use of a repeated-measures ANOVA. Data were further analyzed by use of a repeated-measures ANOVA incorporating study group, horses, and time main effects as well as a group \times time interaction effect. When a significant time effect was detected, values were compared with baseline

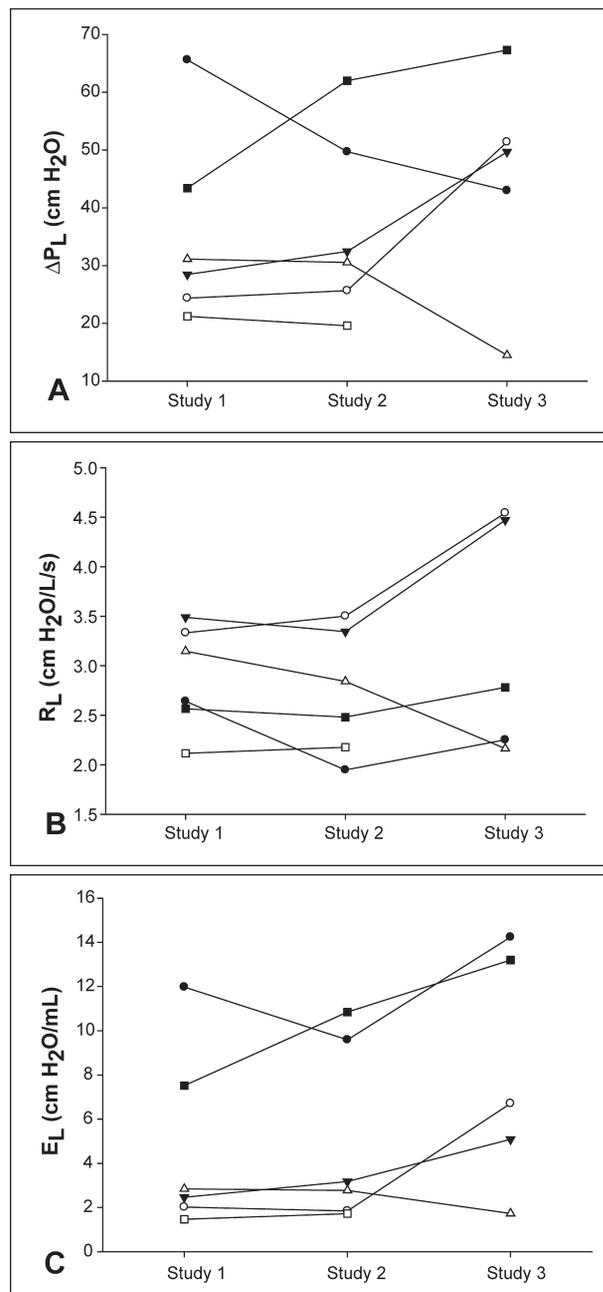


Figure 1—Individual horse values of maximal ΔP_L (A), R_L (B), and E_L (C) in study 1, 2, and 3 at baseline ($n = 6$ horses). Study 1 = Complete BAL with sedation. Study 2 = BAL without saline (0.9% NaCl) solution instillation but with sedation. Study 3 = BAL without saline solution instillation and without sedation.

Table 1—Mean \pm SEM temporal values of V_T and respiratory rate (f) in study 1 (n = 6 horses), study 2 (6), and study 3 (5).

Variable	Time before (0 h) and after BAL					
	0 h	3 h	6 h	12 h	24 h	48 h
V_T (L)						
Study 1	4.4 \pm 0.2	4.2 \pm 0.3	4.2 \pm 0.3	4.3 \pm 0.2	4.0 \pm 0.2	4.5 \pm 0.3
Study 2	4.5 \pm 0.4	4.3 \pm 0.4	4.0 \pm 0.3	4.5 \pm 0.4	4.0 \pm 0.3	4.0 \pm 0.4
Study 3	3.9 \pm 0.5	4.2 \pm 0.5	4.2 \pm 0.5	4.2 \pm 0.4	3.9 \pm 0.4	3.9 \pm 0.3
f (min ⁻¹)						
Study 1	20.4 \pm 5.2	24.3 \pm 5.1	25.1 \pm 5.8	24.0 \pm 5.5	20.5 \pm 4.9	21.1 \pm 5.0
Study 2	23.0 \pm 6.2	25.9 \pm 7.4	26.8 \pm 7.7	24.5 \pm 7.3	24.3 \pm 7.5	24.1 \pm 7.1
Study 3	25.6 \pm 7.6	28.4 \pm 8.0	27.5 \pm 7.9	30.7 \pm 7.7	25.9 \pm 6.3	29.2 \pm 8.2

Study 1 = Complete BAL with sedation. Study 2 = BAL without saline (0.9% NaCl) solution instillation but with sedation. Study 3 = BAL without saline solution instillation and without sedation.

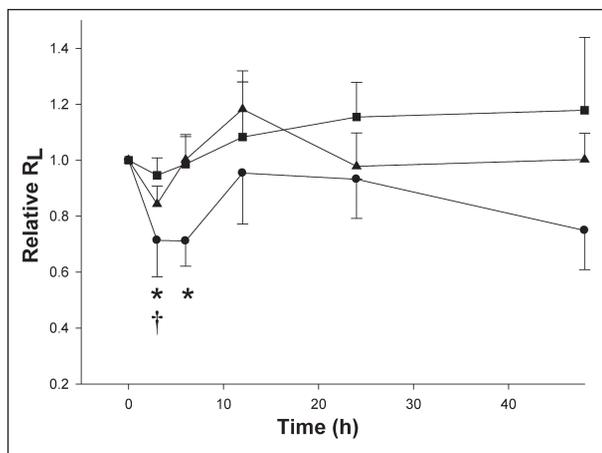


Figure 2—Mean \pm SEM values of relative changes in R_L at baseline (0 hours) and 3, 6, 12, 24, and 48 hours after the BAL or control procedures in study 1 (n = 6 horses; circles), study 2 (6; triangles), and study 3 (5; squares). *Significant ($P < 0.05$) difference between group mean value and group mean baseline value in study 1. †Significant ($P < 0.05$) time main effect at 3 hours. See Figure 1 for remainder of key.

by use of linear contrast of group means. When a significant group main effect or when group \times time interaction was observed, values were compared with baseline value by use of contrasts for each group separately. Values of $P \leq 0.05$ were considered significant.

Results

Animals—Bronchoalveolar lavage and respiratory mechanics procedures were well tolerated in all but 1 horse, which had intense coughing during bronchoscopy in study 3. One horse was removed from study 3 because it did not tolerate lung mechanics measurements and was at risk of injury to itself. Cytologic examination of BAL fluid samples revealed a high percentage of neutrophils in all horses (18% to 44%), confirming lower airway inflammation and the diagnosis of heaves in these horses.

Respiratory mechanics measurements—Consistent with the finding of labored breathing at rest, all horses had high values of ΔP_L , R_L , and E_L at baseline. No significant difference was found in lung mechanics parameters between the baseline values of the 3 study periods (Figure 1). Values of ΔP_L increased in 3 of the 6 horses

over the 6-week study period, whereas lung mechanics improved in 2 horses. The lung function of 1 horse remained unchanged throughout the study period (Figure 1). No significant variation was found in V_T and respiratory rate with time in the 3 studies (Table 1).

A significant time main effect was observed in R_L (Figure 2) when data from all groups were analyzed by use of a repeated-measure ANOVA. This finding was caused by a significant ($P = 0.04$) decrease in R_L at 3 hours, compared with baseline values. Interestingly, when groups were analyzed separately, significant changes in R_L over time were observed only in study 1 at 3 and 6 hours. Compared with baseline, 3 of 6 horses from study 1 had a decrease in R_L ranging from 46% to 78%, whereas it improved mildly in 4 of 6 horses of study 2 (19% to 34%) and 2 of 5 horses of study 3 (13% to 25%; Figure 3). The improvement in R_L was still present 6 hours after BAL for 4 horses in study 1 (range, 29% to 59%), whereas only 2 (18% and 26%) and 1 horses (27%) kept values of R_L below baseline in study 2 and 3, respectively (Figure 3).

A significant change was also found in E_L (Figure 4) and ΔP_L (Figure 5) over time in the 3 groups of horses. This was caused by a significant increase in E_L and ΔP_L at 12 hours after BAL or control procedures.

Discussion

The main finding of our study is that BAL was associated with a decrease in R_L for up to 6 hours following the procedure. This effect was associated with saline solution instillation, which suggests that saline solution may clear mucus secretions in the middle-sized and larger airways of horses affected with heaves. These results are of importance for clinicians when performing a BAL to confirm the diagnosis of heaves in horses with labored breathing at rest. Furthermore, these data should be taken into account when designing research protocols that combine BAL and lung mechanics measurements. This would be particularly critical when the temporal effects of drugs are being investigated in horses with heaves, as in the absence of proper placebo control horses, a beneficial effect could be wrongly attributed to the medication being evaluated.

The increase in E_L at 12 hours was observed in all 3 studies and thus was not attributed to the BAL pro-

cedure. While a larger study would be needed to thoroughly evaluate the safety of the BAL, our results indicate that lung mechanics of heaves-affected horses do not deteriorate after a BAL is performed. In agreement with this finding, BAL has been found to be safe in

humans with asthma and chronic obstructive pulmonary disease.⁸⁻¹⁰ However, the immediate effects of the BAL procedure on the respiratory mechanics and other vital parameters were not evaluated in our study and warrant future investigations.

The beneficial effect of the BAL procedure on R_L observed at 3 and 6 hours in horses from study 1 appears to be the result of saline solution administration, as bronchoscopies performed with or without sedation were not associated with a decrease in R_L . This improvement was likely the result of removal of mucus from the larger airway after saline solution instillation, thus decreasing resistance to airflow where it has the greatest velocity and is the most likely to become nonlaminar. In support of this hypothesis, changes were of a greater magnitude for R_L than for E_L , which are believed to reflect primarily the central and peripheral airways mechanics, respectively. The improvement in R_L at 3 hours may also have been attributable, in part, to the expulsion of mucus from larger airways during the coughing episodes commonly observed while injecting the lidocaine solution or when the endoscope is withdrawn at the end of a BAL procedure. We did not record the number of coughs in the 3 studies; thus, we do not know if horses coughed more in study 1 than without saline solution instillation. Furthermore, results of previous studies indicate that xylazine and butorphanol have effects that can alter respiratory mechanics in horses. In fact, xylazine administration decreases R_L for 30 minutes in ponies affected with heaves,¹¹ butorphanol inhibits mucus secretion and plasma extravasation,^{12,13} and xylazine and butorphanol have an inhibitory effect on cholinergic and noncholinergic airway constriction.¹⁴⁻¹⁷ However, we did not measure any effect of sedation on lung function

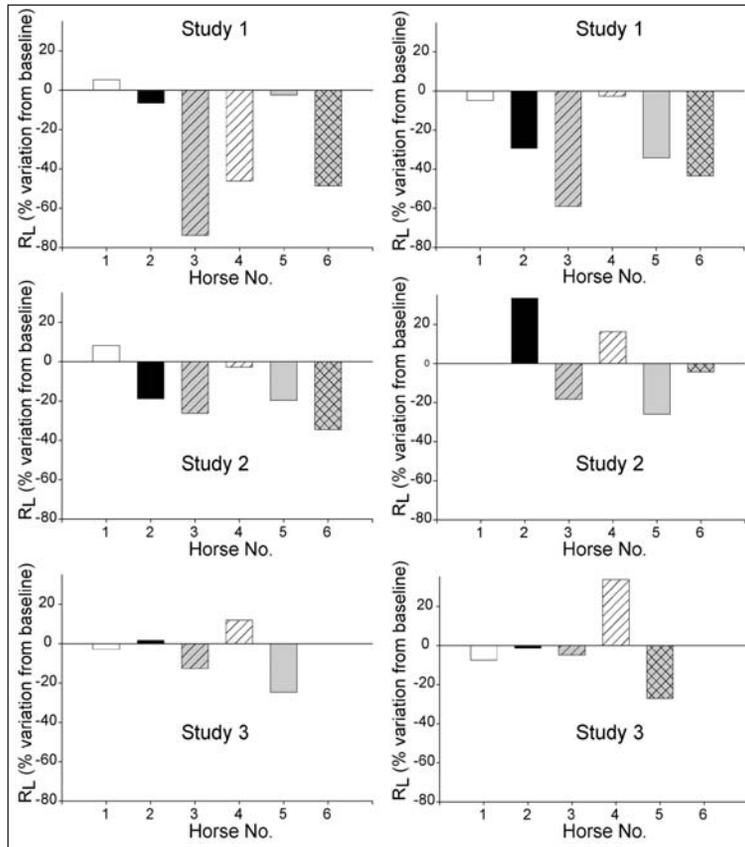


Figure 3—Bar graphs of individual horse values of the percentage variation in R_L , compared with baseline, at 3 (graphs on the left) and 6 (graphs on the right) hours in study 1 ($n = 6$ horses), 2 (6), and 3 (5). See Figure 1 for remainder of key.

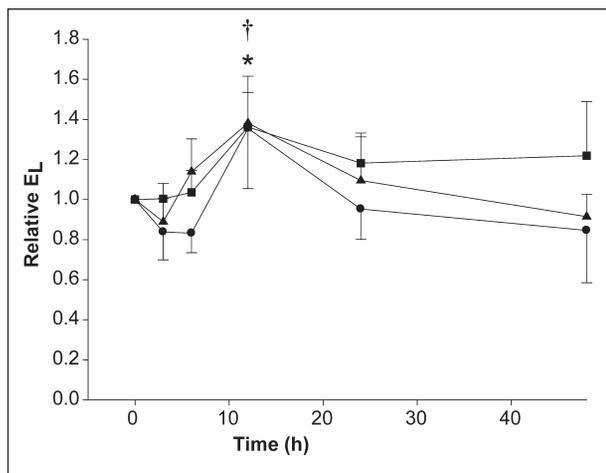


Figure 4—Mean \pm SEM values of relative changes in E_L at baseline (0 hours) and 3, 6, 12, 24, and 48 hours after the BAL or control procedures in study 1 ($n = 6$ horses; circles), study 2 (6; triangles), and study 3 (5; squares). *Significant ($P < 0.05$) difference between group mean value and group mean baseline value in all studies. †Significant ($P < 0.05$) time main effect at 12 hours. See Figure 1 for remainder of key.

noncholinergic airway constriction.¹⁴⁻¹⁷ However, we did not measure any effect of sedation on lung function

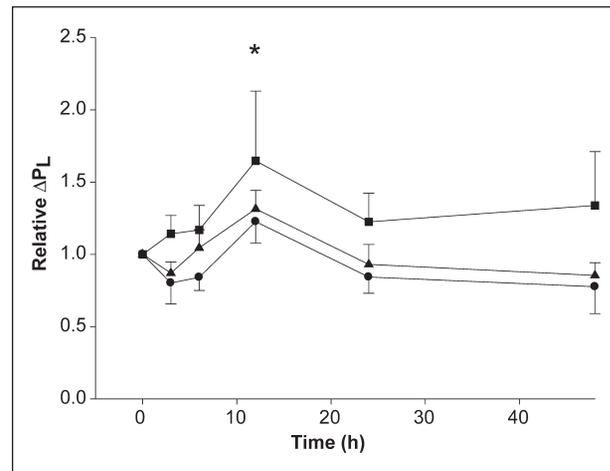


Figure 5—Mean \pm SEM values of relative changes in ΔP_L at baseline (0 hours) and 3, 6, 12, 24, and 48 hours after the BAL or control procedures in study 1, 2, and 3. See Figures 1 and 4 for key.

after baseline in study 2 (horses with sedation and bronchoscopy but no saline solution administration). Unfortunately, because of the short-term effects of xylazine on vital functions, previous studies^{11,18,19} on the respiratory effects of xylazine in horses did not perform measurements after > 30 minutes following drug administration, so we cannot compare our results with the measurements previously reported. A likely explanation for the lack of residual effects of sedative administration in study 2 is that the described effects of xylazine and butorphanol were probably gone by 3 hours after administration.

We also observed a significant increase in E_L and transpulmonary pressure at 12 hours in the 3 studies, without associated changes in respiratory rate and V_T . These changes may be the result of a circadian effect on lung mechanics, similar to the increase in E_L measured at night in heaves-affected horses in a previous study.²⁰ Alternative explanations for the changes in E_L may be related to the trauma and subsequent inflammation created by the passage of the endoscope or local anesthetics on the airway epithelium. In support of this explanation, results of previous studies indicate that airway neutrophilia follows BAL procedures in horses,⁵ humans, and other species,²¹⁻²³ but this effect is short-lived, and values returned to baseline 12 hours later, suggesting no damage ensued from the airway neutrophilia, which is in agreement with what has been described in other species.²⁴ However, because an endoscopic examination was performed in all 3 groups, the design of our study does not allow for the distinction of a circadian effect from the effect of the passage of the endoscope to explain the observed increase in E_L . Although unlikely, the attribution of this increase in E_L to the passage of the endoscope would suggest that bronchoscopy might directly result in the deterioration of lung function in horses.

In conclusion, the BAL procedure resulted in a transient improvement in R_L lasting at least 6 hours in some horses. This effect may have resulted from clearance of mucus in large airways and was followed by an increase in E_L that could be the result of a circadian effect or of the passage of the endoscope into the lower airway. These findings should be taken into account in the design of research protocols when sequential lung function measurements are performed following a BAL procedure in horses with heaves.

- a. Rompun, Bayer Animal Health, Toronto, ON, Canada.
- b. Torbugesic, Wyeth Canada, Guelph, ON, Canada.
- c. Xylocard 1, Astra Pharma Inc, Mississauga, ON, Canada.
- d. Fleisch No. 4, Oem Medical, Richmond, Va.
- e. Model 143PC03D, Micro switch, Honeywell, Scarborough, ON, Canada.
- f. Model HCXPM005D6V, Sensor Technics, Newport News, Va.
- g. Anadat and Labdat 5.1, RHT Infodat, Montreal, QC, Canada.

References

1. Leguillette R, Desevaux C, Lavoie JP. Effects of pentoxifylline on pulmonary function and results of cytologic examination of bronchoalveolar lavage fluid in horses with recurrent airway obstruction. *Am J Vet Res* 2002;63:459-463.

2. Jackson CA, Berney C, Jefcoat AM, et al. Environment and prednisone interactions in the treatment of recurrent airway obstruction (heaves). *Equine Vet J* 2000;32:432-438.

3. Robinson NE, Jackson C, Jefcoat A, et al. Efficacy of three corticosteroids for the treatment of heaves. *Equine Vet J* 2002;34:17-22.

4. Giguere S, Viel L, Lee E, et al. Cytokine induction in pulmonary airways of horses with heaves and effect of therapy with inhaled fluticasone propionate. *Vet Immunol Immunopathol* 2002;85:147-158.

5. Sweeney CR, Rossier Y, Ziemer EL, et al. Effect of prior lavage on bronchoalveolar lavage fluid cell population of lavaged and unlavaged lung segments in horses. *Am J Vet Res* 1994;55:1501-1504.

6. Lavoie JP, Pascoe JR, Kurpershoek CJ. Effect of head and neck position on respiratory mechanics in horses sedated with xylazine. *Am J Vet Res* 1992;53:1652-1657.

7. Bates JH, Shardonofsky F, Stewart DE. The low-frequency dependence of respiratory system resistance and elastance in normal dogs. *Respir Physiol* 1989;78:369-382.

8. Humbert M, Robinson DS, Assoufi B, et al. Safety of fiberoptic bronchoscopy in asthmatic and control subjects and effect on asthma control over two weeks. *Thorax* 1996;51:664-669.

9. Elston WJ, Whittaker AJ, Khan LW, et al. Safety of research bronchoscopy, biopsy and bronchoalveolar lavage in asthma. *Eur Respir J* 2004;24:375-377.

10. Hattotuwa K, Gamble EA, Jeffery PK, et al. Safety of bronchoscopy, biopsy, and BAL in research patients with COPD. *Chest* 2002;6:1909-1912.

11. Broadstone RV, Gray PR, Robinson NE, et al. Effects of xylazine on airway function in ponies with recurrent airway obstruction. *Am J Vet Res* 1992;53:1813-1817.

12. Belvisi MG, Rogers DF, Barnes PJ. Neurogenic plasma extravasation: inhibition by morphine in guinea pig airways in vivo. *J Appl Physiol* 1989;66:268-272.

13. Rogers DF, Barnes PJ. Opioid inhibition of neurally mediated mucus secretion in human bronchi. *Lancet* 1989;1:930-932.

14. LeBlanc PH, Eberhart SW, Robinson NE. In vitro effects of alpha-2-adrenergic receptor stimulation on cholinergic contractions of equine distal airways. *Am J Vet Res* 1993;54:788-792.

15. Grundstrom N, Andersson RG. In vivo demonstration of alpha-2-adrenoceptor-mediated inhibition of the excitatory non-cholinergic neurotransmission in guinea pig airways. *Naunyn Schmiedeberg Arch Pharmacol* 1985;328:236-240.

16. Belvisi MG, Stretton CD, Barnes PJ. Modulation of cholinergic neurotransmission in guinea-pig airways by opioids. *Br J Pharmacol* 1990;100:131-137.

17. Belvisi MG, Chung KF, Jackson DM, et al. Opioid modulation of non-cholinergic neural bronchoconstriction in guinea-pig in vivo. *Br J Pharmacol* 1988;95:413-418.

18. Reitemeyer H, Klein HJ, Deegen E. The effect of sedatives on lung function in horses. *Acta Vet Scand Suppl* 1986;82:111-120.

19. Lavoie JP, Pascoe JR, Kurpershoek CJ. Effects of xylazine on ventilation in horses. *Am J Vet Res* 1992;53:916-920.

20. Jean D, Vrins A, Lavoie JP. Monthly, daily, and circadian variations of measurements of pulmonary mechanics in horses with chronic obstructive pulmonary disease. *Am J Vet Res* 1999;60:1341-1346.

21. Cohen AB, Batra GK. Bronchoscopy and lung lavage induced bilateral pulmonary neutrophil influx and blood leukocytosis in dogs and monkeys. *Am Rev Respir Dis* 1980;122:239-247.

22. Kazmierowski JA, Gallin JI, Reynolds HY. Mechanism for the inflammatory response in primate lungs. Demonstration and partial characterization of an alveolar macrophage-derived chemotactic factor with preferential activity for polymorphonuclear leukocytes. *J Clin Invest* 1977;59:273-281.

23. Von Essen SG, Robbins RA, Spurzem JR, et al. Bronchoscopy with bronchoalveolar lavage causes neutrophil recruitment to the lower respiratory tract. *Am Rev Respir Dis* 1991;144:848-854.

24. Krombach F, Fiehl E, Burkhardt D, et al. Short-term and long-term effects of serial bronchoalveolar lavages in a nonhuman primate model. *Am J Respir Crit Care Med* 1994;150:153-158.