Effect of a syringe aspiration technique versus a mechanical suction technique and use of N-butylscopolammonium bromide on the quantity and quality of bronchoalveolar lavage fluid samples obtained from horses with the summer pasture endophenotype of equine asthma

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OBJECTIVE
To evaluate the effect of 2 bronchoalveolar lavage (BAL) sampling techniques and the use of N-butylscopolammonium bromide (NBB) on the quantity and quality of BAL fluid (BALF) samples obtained from horses with the summer pasture endophenotype of equine asthma.

ANIMALS
8 horses with the summer pasture endophenotype of equine asthma.

PROCEDURES
BAL was performed bilaterally (right and left lung sites) with a flexible videoendoscope passed through the left or right nasal passage. During lavage of the first lung site, a BALF sample was collected by means of either gentle syringe aspiration or mechanical suction with a pressure-regulated wall-mounted suction pump. The endoscope was then maneuvered into the contralateral lung site, and lavage was performed with the alternate fluid retrieval technique. For each horse, BAL was performed bilaterally once with and once without premedication with NBB (21-day interval). The BALF samples retrieved were evaluated for volume, total cell count, differential cell count, RBC count, and total protein concentration.

RESULTS
Use of syringe aspiration significantly increased total BALF volume (mean volume increase, 40 mL [approx 7.5% yield]) and decreased total RBC count (mean decrease, 142 cells/µL), compared with use of mechanical suction. The BALF nucleated cell count and differential cell count did not differ between BAL procedures. Use of NBB had no effect on BALF retrieval.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated that retrieval of BALF by syringe aspiration may increase yield and reduce barotrauma in horses at increased risk of bronchoconstriction and bronchiolar collapse. Further studies to determine the usefulness of NBB and other bronchodilators during BAL procedures in horses are warranted. (Am J Vet Res 2018;79:348–355)

Bronchoalveolar lavage is described as a liquid biopsy and is a commonly used technique for collection of samples of respiratory secretions from the lower airways and alveoli in veterinary and human medicine. The analysis of BALF samples typically includes enumeration of erythrocytes and leukocytes. Other constituents including inflammatory mediators and drug concentrations have also been quantified. Interestingly, erythrocyte numbers that could be reflective of traumatic hemorrhage during the procedure are often not reported. In equine medicine, BAL is an essential aid in the diagnosis of inflammatory airway disease, equine asthma (previously termed recurrent airway obstruction, chronic obstructive pulmonary disease, or heaves), and exercise-induced pulmonary hemorrhage. Overall, BAL in horses is a minimally invasive, safe, and diagnostically sensitive procedure for detection of inflammation at the cytologic level in the lower airways. Cell counts in BALF samples from horses correlate well with clinical airway obstruction and responsiveness as well as exercise-induced hypoxemia and lactacidosis.

Consensus guidelines for performing BAL in horses are established. The procedure is typically...
executed either by endoscopy or blindly with a BAL catheter, with the endoscope of the catheter passed nasally into the lower airway until wedged. Lavage is then performed by instillation of warm sterile saline (0.9% NaCl) solution. Volumes of 300 to 500 mL are typically used and overcome inconsistencies in differential cell counts that arise with use of lower-volume BAL methods. Fluid is retrieved manually by syringe aspiration or mechanically with a pressure-regulated suction pump. Recent critical examination of manual versus mechanical retrieval of BAL in humans has determined that syringe aspiration improves yield, presumably by reducing bronchiolar collapse. The effects of retrieval method on yield, composition, and erythrocyte counts in BALF samples from horses have not been reported, to our knowledge. Equine asthma is a chronic condition characterized by airway hyperreactivity, reversible airway bronchoconstriction, chronic neutrophilic inflammation, and mucus accumulation. Collection of BALF samples from horses with equine asthma can be challenging because of the severity of bronchoconstriction, bronchiolar collapse, and subsequent decreases in BALF volume recovery and subsequent decreases in BALF volume recovery. Whether horses with equine asthma are at an increased risk of barotrauma during BAL is unknown. Recently, NBB, a quaternary ammonium anticholinergic parasympatholytic labeled for control of abdominal pain induced by spasmodic, flatulent, or simple impaction colic, was found to be effective in the treatment of equine asthma exacerbation. Significant clinical improvement in respiratory rate, respiratory score, and pulmonary function as a result of NBB-induced bronchodilation was noted to be rapid but short-lived, with maximal improvement achieved from at least 10 to 30 minutes after IV administration of the drug and dissipation of effect within 1 hour after treatment. Use of bronchodilators to reduce risk of bronchoconstriction and cough during BAL in horses has been recommended to facilitate retrieval of fluid; however, the effects of adjunctive use of NBB during BAL have not been described, to our knowledge.

The purpose of the study reported here was to compare the effect of a syringe aspiration technique versus mechanical suction technique and use of NBB on the quantity and quality of BALF samples obtained from horses with the summer pasture endophenotype of equine asthma. We hypothesized that use of syringe aspiration and administration of the bronchodilator NBB would improve yield and quality of BALF samples obtained from horses at an increased risk for bronchoconstriction and bronchiolar collapse.

Materials and Methods

Animals

Eight adult horses with a summer pasture endophenotype of equine asthma (ie, EPA) were used in the study. The horses’ mean ± SD age was 19 ± 5.4 years. There were 4 mares and 4 geldings of various breeds (3 Quarter Horses, 1 Thoroughbred, 1 Missouri Fox Trotter, 1 Tennessee Walking Horse, 1 Mustang, and 1 Appaloosa). Horses in this cohort had a well-documented ≤ 2-year history of reversible airway obstruction and neutrophilic airway inflammation while pastured in the summer in southeastern United States and were deemed otherwise healthy on the basis of results of a physical examination, CBC, and serum biochemical analysis. All protocols were approved by the Institutional Committee for Animal Use and Care of Mississippi State University.

Experimental design

A bilateral BAL procedure, in which BAL was performed sequentially (beginning with either a left or right lung site), was performed twice in each horse, once with and once without NBB premedication (0.3 mg/kg, IV) and with a 21-day washout period between each bilateral BAL session. The horses underwent BAL procedures during a 60-day period (March 15 to May 15, 2014). Although each horse had a history of EPA, all were in clinical remission or had only mild clinical signs; respiratory rates were 12 to 28 breaths/min (mean, 20.5 breaths/min; 95% confidence interval, 18.5 to 22.0 breaths/min), and clinical scores of respiratory effort were 1 to 4.5 on a scale of 0 to 8 (mean score, 2.42; 95% confidence interval, 2.0 to 2.8). Horses remained on pasture for the entire study period with the exception of a 48-hour monitoring period immediately following each BAL procedure, during which horses were kept in a stall and observed hourly for any adverse effects.

Bilateral BAL procedure

Each horse was placed in stocks and sedated with detomidine (0.02 to 0.04 mg/kg, IV) and butorphanol tartrate (0.02 mg/kg, IV). A flexible videoendoscope (length, 300 cm; end external diameter, 10.4 mm) was passed through the right or left nasal passage into the trachea and wedged in the distal aspect of 1 lung (left or right side). To decrease cough during passage of the videoendoscope, 50 to 80 mL of 2% lidocaine hydrochloride was instilled in small boluses as the videoendoscope was advanced to anesthetize the airway mucosa. A total of 300 mL of warm (37°C) sterile isotonic saline (0.9% NaCl) solution was infused manually with five 60-mL syringes. The BALF was retrieved with mechanical suction or syringe aspiration until fluid was no longer available. The syringe aspiration procedure used was that described by Costa et al and involved a 60-mL syringe and gentle manual aspiration. The mechanical suction procedure used was that described by Jean et al. Briefly, a pressure-regulated suction pump was applied with incrementally increasing pressures so that the closing pressure of a wedged bronchus (15 to 60 mm Hg or 1.10 to 4.40 mm Hg) was not exceeded until fluid was no longer retrieved. Immediately following BAL in 1 lung location, the endoscope was withdrawn to the carina and advanced into the contralateral lung and a second BAL was performed with the alternate...
fluid retrieval technique. For the first bilateral BAL session in each horse, the order of the fluid retrieval techniques and lung side lavaged with each technique was determined with a random number table.

Use of NBB premedication (0.3 mg/kg, IV) for the first bilateral BAL session in each horse was assigned by a coin toss. If assigned, NBB was given twice during the bilateral BAL procedure, with the first dose administered just prior to passing the endoscope into the naris and the second dose administered at the time the endoscope was withdrawn to the carina. Mean time for completion of a single BAL procedure was 30 minutes, and doses of NBB were given approximately 8 to 12 minutes prior to achieving each wedge and beginning the instillation of saline solution. After a 21-day washout period, the second bilateral BAL session was performed for each horse with the alternate first-session factors regarding NBB use, the order of fluid retrieval technique, and the lung side lavaged with each technique.

**BALF sample processing and evaluation**

Fluid from each lung side was collected separately into chilled siliconed Erlenmeyer flasks. The total volume of BALF recovered and macroscopic assessment findings (color, turbidity, and presence of foam) were recorded. Separate 10-mL samples of BALF from each lung side were placed in tubes containing EDTA and were submitted for analysis within 1 hour after completing the bilateral BAL procedure. Total protein concentration in the BALF was determined with a refractometer. Total nucleated cells and RBCs were counted in undiluted samples by use of a hemocytometer. For each BALF sample, 2 direct smears and 2 cytocentrifuged (90 X g for 10 minutes) smears were prepared and all 4 slides were stained in a slide stainer with a modified Wright stain. Differential cell counts were obtained by counting 400 cells of each BALF sample; epithelial cells were not included in the differential cell count. Differential cell counts (subsequently converted to percentages) were performed by a single board-certified pathologist (MEJ) who was unaware of the specifics details of sample collection.

**Statistical analysis**

Summary statistics (means, SEs, and minimum and maximum values) of BALF volume and each of the BALF cell counts were calculated as combinations of the 2 variables used in the statistical analysis (treatment groups), namely mechanical suction with NBB, mechanical suction without NBB, syringe aspiration with NBB, and syringe aspiration without NBB. There were 8 values for each treatment group. The summary statistics of each BALF volume and cell counts were also calculated for each level of the variables used in the statistical analysis (statistical groups), namely mechanical suction, syringe aspiration, procedure without NBB, and procedure with NBB. For the summary statistics, values from the right and left sides at each BAL procedure were considered as unique measurements, independent of horse identity and procedure day; therefore, the summary statistics were calculated by the overall groupings, not by horse.

The BALF cell counts were not normally distributed and were transformed by taking the log₁₀ value of the count plus 1. Statistical inferences for the BALF cell counts were based on the transformed data, but summary statistics were reported for the nontransformed data. Generalized linear mixed models were fit for each outcome (volume of retrieved BALF and log-transformed values of total nucleated cells/µL, RBCs/µL, and differential WBC percentages [ie, percentages of monocytes, neutrophils, lymphocytes, eosinophils, and mast cells]). The NBB status—retrieval method were the fixed effects in the models. The order of retrieval methods and the NBB status—retrieval method interaction were also considered as fixed effects in each model, but were not significant and were removed from each model. Lung side within horse and horse were included as the random effects.
with variance components covariance structure. The distribution of the conditional residuals was evaluated for each outcome to ensure the assumptions of the statistical method had been met. An α level of 0.05 was used to determine significance for all comparisons. Results are reported as mean, SE, and minimum and maximum values (or range) unless otherwise reported as the median with the IQR.

Results

Each bilateral BAL procedure, with or without NBB administration, was well tolerated by all 8 horses with EPA, and no adverse effects were encountered. The order of retrieval method (P ≥ 0.394) and NBB status-retrieval method interaction (P ≥ 0.105) were not significant for any variable.

All 32 BALF samples had a total protein concentration < 2.0 mg/dL. Relative to findings for the mechanical suction technique (n = 16), the syringe aspiration technique (16), regardless of NBB use, yielded a significantly (P = 0.001) increased volume of retrieved BALF (Figure 1). The mean increase in BALF volume achieved with syringe aspiration was 7.5% (40 mL). For the mechanical suction technique, the median BALF volume was 58 mL (IQR, 56 to 85 mL). For the syringe aspiration technique, the median BALF volume was 95 mL (IQR, 58 to 145 mL). The volumes of BALF retrieved when horses had or had not received NBB did not differ significantly (P = 0.38). Without NBB administration (n = 16), the median BALF volume was 80 mL (IQR, 44 to 121 mL). With NBB administration (n = 16), the median BALF volume was 60 mL (IQR, 47 to 118 mL).

Total nucleated cell counts and differential WBC counts by treatment grouping (ie, data for each retrieval method with or without NBB administration), for each aspiration method, and for treatment or no treatment of horses with NBB were summarized (Tables 1 and 2). Administration of NBB was associated with an increase (P = 0.03) in the mean percentage of neutrophils in BALF samples of 7.1%. In the absence of NBB treatment (n = 16), the median percentage of neutrophils in BALF samples was 10% (IQR, 5% to 44.5%). With NBB treatment (n = 16), the median percentage of neutrophils in BALF samples was 25% (IQR, 13.3% to 45%). Other than the increase in neutrophils associated with NBB treatment, nucleated cell counts and percentages of monocytes, lymphocytes, eosinophils, and mast cells in BALF samples were not altered by the method of fluid retrieval (syringe aspiration or mechanical suction) or by treatment of horses with NBB.

Compared with findings for the syringe aspiration method, the number of RBCs in BALF samples was significantly (P = 0.04) increased by use of the mechanical suction method, with a mean increase of 142 RBCs/µL (Figure 2). With syringe aspiration (n = 16), the median RBC count in BALF samples was 240 RBCs/µL (IQR, 150 to 280 RBCs/µL). With mechanical suction (n = 16), the median RBC count in BALF samples was 285 RBCs/µL (IQR, 230 to 615 cells/µL). When horses did or did not receive NBB treatment, RBC counts in BALF samples were not significantly different.

Other than the increase in neutrophils associated with NBB treatment, nucleated cell counts and percentages of monocytes, lymphocytes, eosinophils, and mast cells in BALF samples were not altered by the method of fluid retrieval (syringe aspiration or mechanical suction) or by treatment of horses with NBB.

Table 1—Total nucleated cell count and differential WBC counts (%) in BALF samples obtained from 8 horses with EPA by use of 2 fluid retrieval techniques with or without administration of NBB and grouped on the basis of retrieval technique and NBB status.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>Retrieval method–treatment grouping</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of nucleated</td>
<td></td>
<td>SA–NBB</td>
<td>SA–No NBB</td>
</tr>
<tr>
<td>cells/µL</td>
<td>Mean (SEM)</td>
<td>220.63 (91.68)</td>
<td>155.25 (67.33)</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>Mean (SEM)</td>
<td>30.50 (5.04)</td>
<td>41.13 (8.37)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>12–47</td>
<td>4–71</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>Mean (SEM)</td>
<td>26.00 (5.07)</td>
<td>25.13 (10.75)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>10–41</td>
<td>4–91</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>Mean (SEM)</td>
<td>42.00 (5.81)</td>
<td>31.50 (7.15)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>13–70</td>
<td>4–57</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>Mean (SEM)</td>
<td>0 (0)</td>
<td>0.38 (0.38)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0–4</td>
<td>0–3</td>
</tr>
<tr>
<td>Mast cells (%)</td>
<td>Mean (SEM)</td>
<td>1.50 (0.46)</td>
<td>1.88 (0.91)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0–4</td>
<td>0–6</td>
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</tbody>
</table>

For each horse, BAL was performed bilaterally once with and once without premedication with NBB (21-day interval). Bronchoalveolar lavage was performed bilaterally (right and left lung sites) with a flexible videointubendoscope passed through the left or right nasal passage. During lavage of the first lung site, a BALF sample was collected by means of either gentle syringe aspiration (SA) or mechanical suction (MS) with a pressure-regulated wall-mounted suction pump. The endoscope was then maneuvered into the contralateral lung site and lavage was performed with the alternate fluid retrieval technique. A total of 300 mL of warm (37°C) sterile isotonic saline (0.9% NaCl) solution was infused manually with five 60-mL syringes. The endoscope was withdrawn to the carina. Total nucleated cells were counted in undiluted samples by use of a hemocytometer. Differential cell counts were obtained by counting 400 cells of each BALF sample; epithelial cells were not included in the differential cell count. Differential cell counts were performed by a single board-certified pathologist who was unaware of the specific details of sample collection.
different. In the absence of NBB treatment (n = 16), the median RBC count in BALF samples was 282 RBCs/µL (IQR, 225 to 586 cells/µL). With NBB treatment (n = 16), the median RBC count in BALF samples was 265 RBCs/µL (IQR, 216 to 385 cells/µL).

Discussion

In equine medicine, assessment of BALF is an essential tool for diagnosis of diseases characterized by airway hyperresponsiveness, including asthma and inflammatory airway disease. Collection of BALF samples can prove to be challenging in horses with hyperresponsive airways because of provoked bronchiolar collapse, which decreases recovery volumes and increases the risk of iatrogenic airway trauma. Identification of a modified BAL technique that improves BALF yield and quality for use in horses with airway hyperresponsiveness would enhance the diagnostic usefulness of BAL in a subset of horses in which BALF analysis is commonly performed.

In the present study, BALF samples were collected by syringe aspiration or mechanical suction methods, each after horses had or had not been treated with the bronchodilator NBB. The intent was to evaluate and compare the effects of each method and bronchodilator treatment on yield, total nucleated cell count, differential WBC percentages, and RBC count of BALF samples. Bronchoalveolar lavage was performed on horses with EPA. This disease is characterized by airway hyperreactivity and bronchoconstriction, factors that increase the risk of bronchiolar collapse during BAL. Interestingly, there was no significant NBB status–retrieval method interaction for any variable of interest. On the basis of current human medical literature regarding increased BALF yields achieved with syringe aspiration and anecdotal reports of increased BALF yields from horses premedicated with a bronchodilator, we anticipated the combination of the syringe aspiration method and administration of NBB to be associated with the highest total volumes and lowest RBC counts in BALF samples obtained from the horses of the present study. However, the study results indicated that no inference regarding the interaction between fluid retrieval method and the use of NBB could be made.

Relative to the mechanical suction method, the syringe aspiration method significantly increased total volumes of and decreased total erythrocyte counts in BALF samples but did not alter total nucleated cell counts or differential WBC percentages. Premedication with NBB increased the percentage of neutrophils in BALF samples but did not significantly change sample volumes, total nucleated

<table>
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<tr>
<th>Table 2—Total nucleated cell count and differential cell counts in BALF samples obtained from the 8 horses in Table 1 and grouped on the basis of fluid retrieval technique or NBB status.</th>
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</thead>
<tbody>
<tr>
<td><strong>Variable</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td>No. of nucleated cells/µL</td>
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<tr>
<td>Range</td>
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<tr>
<td>Monocytes (%)</td>
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<td>Range</td>
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<td>Neutrophils (%)</td>
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<td>Eosinophils (%)</td>
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<td>Range</td>
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<tr>
<td>Mast cells (%)</td>
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<tr>
<td>Range</td>
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</table>

*Within a variable, value in the NBB treatment group differs significantly (P = 0.03) from that in the no NBB treatment group. See Table 1 for key.

Figure 2—Box-and-whisker plots of the RBC counts in BALF samples obtained from the 8 horses in Figure 1. Median RBC counts in BALF samples collected by SA and MS differed significantly (P = 0.04). See Figure 1 for key.

(P = 0.33) different. In the absence of NBB treatment (n = 16), the median RBC count in BALF samples was 282 RBCs/µL (IQR, 225 to 586 cells/µL). With NBB treatment (n = 16), the median RBC count in BALF samples was 265 RBCs/µL (IQR, 216 to 385 cells/µL).
cell counts, differential WBC percentages, or RBC counts.

In the present study, total volumes of BALF sample obtained by use of the syringe aspiration method were greater than those of samples obtained by use of the mechanical suction method (mean increase, 40 mL [approx 7.5%]), regardless of whether horses were or were not treated with NBB. Total nucleated cell counts and differential WBC counts in BALF samples did not differ significantly between the 2 fluid retrieval methods. These results were in agreement with increased BALF yields of 7% and 8% associated with syringe aspiration, relative to yields achieved with mechanical suction, in children and adults, respectively. In those investigations, more patients in the syringe aspiration group met American Thoracic Society criteria for an optimal BAL characterized by a total BALF sample volume ≥30% of the total fluid volume instilled. Total BALF volumes <30% of the instilled fluid volume may provide misleading differential WBC counts, an effect that is profound when the total retrieved fluid volume is <10% of the total instilled volume. Interestingly, in the present study, the syringe aspiration method was associated with a greater number of BAL procedures that yielded adequate total sample volume (≥30% of the total instilled fluid volume). Nine of 16 procedures performed with the syringe aspiration method yielded sample volumes ≥30% of the total instilled fluid volume. In comparison, 4 of 16 procedures performed with the mechanical suction method yielded adequate total sample volumes. Furthermore, all BAL procedures performed with the syringe aspiration method met the criteria of retrieval of ≥10% of the fluid volume instilled, whereas 3 procedures performed with the mechanical suction method retrieved total sample volumes less than this threshold.

Although the gross appearance of all BALF samples retrieved in the present study was not blood-tinted, retrieval by the syringe aspiration method significantly decreased total RBC counts (mean decrease, 142 RBC/µL), compared with findings for the mechanical suction method. The presence of erythrocytes without signs of erythropagocytosis in BALF samples is primarily a result of minor trauma to the epithelium that occurs during the BAL procedure in horses. Evidence of acute hemorrhage is rarely found in human BALF samples unless iatrogenic trauma from the BAL procedure results in hemorrhage. The clinical relevance of differences in the BALF erythrocyte counts in the present study remains unknown because RBC counts in collected samples are not routinely reported when BAL is performed diagnostically in horses. However, iatrogenic barotrauma leading to hemorrhage also results in contamination of BALF with other constituents of vascular origin such as circulating leukocytes, protein, and immunoglobulins, thereby reducing pulmonary specificity of BALF results. Bronchoalveolar lavage methods that significantly reduce iatrogenic trauma and hemorrhage are inherently laudable.

Failure to identify an increase in collected BALF volumes following administration of the bronchodilating anticholinergic NBB in the present investigation was not anticipated. This effect was conserved for both the syringe aspiration and mechanical suction methods. Although anecdotally recommended, reports of formal investigations of the diagnostic usefulness of premedication with bronchodilators to facilitate BAL in horses are limited. Conversely, premedication with nebulized albuterol is the standard of care for performing BAL in people in asthmatics specifically, additional parenteral premedication with the anticholinergic agent, atropine sulfate, is recommended. The immediate effects of bronchodilators used to facilitate BAL in humans have not been reported, to our knowledge. This likely reflects the fact that most asthmatics have a preexisting bronchodilator treatment regimen. Absence of an effect of NBB on BALF volumes obtained from horses in the present study could have been influenced by the timing of the investigation, given that study horses were in clinical remission from EPA or were only mildly affected. The mean neutrophil percentage in the BALF samples from the study population was 27.7%, which met the criteria of moderate to severe neutrophilia (≥25%). Another possibility was that the effect of NBB on airways of different caliber is variable, and any attenuation of antimuscarinic effect could lead to failure to prevent bronchiolar collapse in smaller airways. Differences in the responsiveness of upper versus lower airway smooth muscle to several agents that modify airway caliber have been documented. Further investigation of the adjunct use of bronchodilators, and specifically NBB, during BAL in horses is warranted.

Interestingly, BALF samples obtained when horses received NBB had neutrophil percentages that were mildly increased (7%), compared with BALF samples retrieved without bronchodilator use. These results met the criteria for significance, but there was a large overlap in the data ranges (Table 1) for the samples collected with and without NBB, and the clinical or biological importance of this finding is unknown. Furthermore, prior to log transformation of the data, a significant difference in neutrophil percentages of samples collected when horses did or did not receive the bronchodilator was not found (data not shown; P = 0.1).

Results of the present study indicated that, compared with a mechanical suction method, use of a manual syringe aspiration method to retrieve fluid samples during BAL provides an advantage for horses with airway hyperreactivity by increasing the volume of BALF retrieved and decreasing RBC contamination. These outcomes indicated that syringe aspiration may limit airway collapse and appears to enhance the diagnostic usefulness of BAL in this patient population. Although pretreatment of horses with the bronchodilator NBB in this study yielded no beneficial effect on
BALF volume retrieved or RBC contamination, investigation of bronchodilator treatment prior to BAL in horses with more severely exacerbated disease may reveal beneficial outcomes on volume and quality of collected BALF samples.

Acknowledgments

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Footnotes

a. Buscopan injectable solution, Boeringher-Ingelheim Vetmed-ica Inc, St. Louis, Mo.
b. Karl Storz, Veterinary Endoscopy America Inc, Goleta, Calif.
g. PROC MIXED, SAS for Windows 9.4, SAS Institute, Inc, Cary, NC.

References

34. Summary and recommendations of a workshop on the inves-

Correction: Effects of stacked wedge pads and chains applied to the forefeet of Tennessee Walking Horses for a five-day period on behavioral and biochemical indicators of pain, stress, and inflammation

In the report "Effects of stacked wedge pads and chains applied to the forefeet of Tennessee Walking Horses for a five-day period on behavioral and biochemical indicators of pain, stress, and inflammation" (*Am J Vet Res* 2018;79:21–32), the name of the sixth listed author was misspelled. The correct spelling is Peter Krawczel.