A plethora of medical conditions that affect the respiratory system of camelids and that potentially alter respiratory function have been identified, yet functional analysis has rarely been performed in this species. Therefore, there is a lack of understanding of physiologic pulmonary function and the effect various diseases would have on respiratory mechanical function in camelids.

Diagnostic modalities that provide information regarding the function of the respiratory system can improve the identification of respiratory diseases that are not detected by conventional imaging techniques as well as aid in the characterization, localization, and objective assessment of response to treatment of many respiratory ailments. For example, measurement of FRC is helpful in identifying trapped gas within the alveoli, and values have been established for clinically normal animals of several domestic species, including llamas, cattle, sheep, horses, and dogs. Respiratory inductive plethysmography has been used to describe the breathing patterns of horses at rest and during exercise.

Respiratory mechanics and results of cytologic examination of bronchoalveolar lavage fluid in healthy adult alpacas

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Objective—To evaluate respiratory mechanical function and bronchoalveolar lavage (BAL) cytologic results in healthy alpacas.

Animals—16 client-owned adult alpacas.

Procedures—Measurements of pulmonary function were performed, including functional residual capacity (FRC) via helium dilution, respiratory system resistance via forced oscillatory technique (FOT), and assessment of breathing pattern by use of respiratory inductive plethysmography (RIP) in standing and sternally recumbent alpacas. Bronchoalveolar lavage was performed orotracheally during short-term anesthesia.

Results—Mean ± SD measurements of respiratory function were obtained in standing alpacas for FRC (3.19 ± 0.53 L), tidal volume (0.8 ± 0.13 L), and respiratory system resistance at 1 Hz (2.98 ± 0.70 cm H2O/L/s), 2 Hz (2.98 ± 0.77 cm H2O/L/s), 3 Hz (3.84 ± 0.93 cm H2O/L/s), 5 Hz (3.45 ± 0.91 cm H2O/L/s), and 7 Hz (3.14 ± 0.77 cm H2O/L/s). Mean phase angle, as a measurement of thoracoabdominal asynchrony, was 19.59 ± 10.06°, and mean difference between nasal and plethysmographic flow measurements was 0.18 ± 0.07 L/s. Tidal volume, peak inspiratory flow, and peak expiratory flow were significantly higher in sternally recumbent alpacas than in standing alpacas. Cytologic examination of BAL fluid revealed 58.52 ± 12.36% alveolar macrophages, 30.53 ± 13.78% lymphocytes, 10.95 ± 9.29% neutrophils, 0% mast cells, and several ciliated epithelial cells.

Conclusions and Clinical Relevance—Pulmonary function testing was tolerated well in nonsedated untrained alpacas. Bronchoalveolar lavage in alpacas yielded samples with adequate cellularity that had a greater abundance of neutrophils than has been reported in horses. (Am J Vet Res 2012;73:146–152)
ercise, foals with evoked obstructions of the proximal and distal portions of the airways, healthy llamas, and a llama with diaphragmatic paralysis. This test is also used to diagnose inflammatory airway disease and recurrent airway obstruction in horses. Both $R_{ns}$ and $X_{ns}$ measured by FOT, are frequently used to characterize the degree of airway obstruction in horses. In a study in llamas, investigators found that $R_{c}$ measured via esophageal balloon pneumotachography (criterion-referenced standard) was positively correlated with $R_{c}$ measured via FOT, with the latter having the advantage of being less invasive and time-consuming.

Analysis of BALF can replace the need to obtain lung biopsy specimens in many patients and is frequently used to diagnose diffuse pulmonary disease such as inflammatory airway disease in horses, and may also be useful in the ante mortem diagnosis of several conditions that affect alpacas, such as tuberculosis and nocardiosis, viral pneumonia, pulmonary neoplasia, fungal pneumonia, and Rhodococcus equi-associated pneumonia. Reference values for BALF cytologic examination have been established for several domestic species, including horses and dogs.

Although BAL has been performed in a llama with diaphragmatic paralysis. This test is also referenced standard) was positively correlated with RL, via RIP, via monosinusoidal FOT, and via evaluation of BALF. Respiratory inductive plethysmography and FOT were performed in alpacas that were standing and sternal recumbent. All tests of pulmonary mechanical function were completed in unsedated alpacas, and short-term anesthetics were subsequently administered IM to alpacas prior to BAL.

FRC via helium dilution—Functional residual capacity was measured by use of a rebreathing method described in dogs, horses, and llamas. Briefly, all alpacas were fitted with a clear plastic face mask with a small dead space (100 mL) and sealed with a latex shroud. The mask was connected to a 3-way angled tap [ie, withers], body length from point of shoulder to ischial tuberosity, and chest circumference over the eighth intercostal space) were obtained by use of a flexible measuring tape with the alpacas in a standing position. The respiratory tract of each alpaca was evaluated via a rebreathing technique that involved the use of helium in room air as a test gas for the measurement of FRC, via RIP, via monosinusoidal FOT, and via evaluation of BALF. Respiratory inductive plethysmography and FOT were performed in alpacas that were standing and sterno recumbent. All tests of pulmonary mechanical function were completed in unsedated alpacas, and short-term anesthetics were subsequently administered IM to alpacas prior to BAL.

RIP and pneumotachography—Respiratory inductive plethysmography was performed in accordance with the methods previously described for llamas. Modifications implemented included placement of the thoracic band overlapping the eighth intercostal space, which corresponded to the center of the thorax and point of greatest thoracic excursion in alpacas. The thoracic and abdominal bands were placed in a vertical position.

Monosinusoidal FOT—The monosinusoidal FOT (1 to 7 Hz) was used to determine $R_{ns}$ and $X_{ns}$ described elsewhere. The $R_{ns}$ comprises the additive effects of airway resistance, chest wall resistance, and $R_{c}$. All alpacas were lightly restrained by a handler to ensure that their head position remained neutral with the mandible positioned parallel to the ground.

BAL and evaluation of BALF—All alpacas were anesthetized by IM injection of a combination of xylazine hydrochloride (0.5 mg/kg), butorphanol tartrate (0.05 mg/kg), and ketamine hydrochloride (5 mg/kg) and maintained in sternal recumbency with the head and neck extended vertically. A protective mouth gag was placed to allow inspection of the larynx with the aid of a laryngoscope. An enteral feeding tube (250 cm in length and 6 mm in diameter) that contained a guide wire was inserted into the trachea of each alpaca and advanced until it wedged into a bronchus or bronchule. The guide wire was then removed, and an aliquot of 30 to 50 mL of sterile saline (0.9% NaCl) solution was instilled and immediately aspirated for sample collection. One or 2 additional aliquots of saline solution

Materials and Methods

Animals—Sixteen healthy client-owned adult alpacas (9 males and 7 females) were included in the study. Alpacas were between 2 and 9 years of age (mean ± SD, 3.5 ± 2.1 years). Body weight ranged from 49 to 109 kg (mean body condition score, 4 on a scale of 1 to 9). The alpacas belonged to 3 farms that used similar housing conditions. Alpacas were housed on grass pastures and were led supplemental grass hay and commercially available concentrate for alpacas. No signs of respiratory disease were reported, and none of the alpacas had been subjected to pulmonary function testing or a laboratory environment. The alpacas were admitted to our veterinary hospital at least 1 day prior to testing; they were housed in a well-ventilated stall bedded with straw and provided ad libitum access to grass hay. All lung function testing was performed in a temperature-controlled environment. Written client consent was obtained for all alpacas prior to enrollment in the study. All procedures were approved by the Clinical Sciences Research Committee at the Cummings School of Veterinary Medicine at Tufts University.

Design—A complete history and results of physical examination, lateral thoracic radiography, a CBC, and serum biochemical analysis were obtained prior to study enrollment to exclude alpacas with any evidence of preexisting respiratory disease. Body measurements (height measured at the highest point of the shoulders [ie, withers], body length from point of shoulder to ischial tuberosity, and chest circumference over the eighth intercostal space) were obtained by use of a flexible measuring tape with the alpacas in a standing position.
were instilled on the basis of the volume and turbidity of the retrieved sample. A lateral and a dorsoventral thoracic radiograph were obtained at the end of the procedure to verify the location of the collection tube. The obtained BALF was subsequently submitted for cytocentrifugation preparation. Slides were stained with modified Wright stain for cytologic analysis and differential cell count. The presence of mast cells was verified by use of toluidine blue. Cells were classified with values of

Results

Physical characteristics, hematologic evaluation, and radiographic examination—Mean ± SD measurements of body weight (70.50 ± 15.74 kg), height (92.35 ± 4.99 cm), body length (84.83 ± 8.48 cm), and chest circumference (103.83 ± 10.72 cm) were within anticipated limits for healthy adult alpacas. Similarly, results of the CBC and serum biochemical analysis were within the respective reference ranges reported for alpacas. Examination of thoracic radiographs did not reveal any changes consistent with respiratory disease in the alpacas.

Respiratory mechanics—Statistical analysis revealed a normal distribution for all data in standing and sternally recumbent alpacas. The mean ± SD FRC in alpacas was 3.19 ± 0.53 L, which translated to 46.29 ± 7.50 mL/kg. A significant positive correlation (p = 0.045; P = 0.003) between FRC and body weight was detected in the study animals. However, FRC was not significantly correlated with body length, height, or chest circumference.

Statistical analysis—Descriptive analyses of respiratory mechanical variables (FRC, V̇e, Ti, Te, V̇e, PIF, PEF, Ṙe, Ẋe, and RIP-derived variables) were reported as mean ± SD. Correlations between FRC and body dimensions (body weight, height, body length, and chest circumference) were evaluated by use of the Spearman ρ. Outliers were detected via extreme values analysis. The effect of position (standing vs sternally recumbent) was analyzed by use of a paired-samples t test, and the other tests did not tolerate associated with placement of elastic chest and abdominal bands). Of the remaining 14 alpacas, 2 did not remain standing for the duration of the test, and 1 did not become sternally recumbent. All RIP-derived variables in standing and sternally recumbent alpacas were summarized (Table 1). The abdominal contribution to respiration was greater than the thoracic contribution (Figure 1) in all of the alpacas evaluated in standing recency (13/13) and in most of the alpacas evaluated while standing (8/12). Mean phase angle, a measurement of thoracoabdominal asynchrony, was 19.19 ± 10.06° in standing alpacas and 17.56 ± 9.12° in sternally recumbent alpacas. Values did not differ on the basis of body position (sternally recumbent vs standing; P = 0.470) and were characterized by the abdomen leading inspiration and expiration in all alpacas.

Table 1—Mean ± SD RIP-derived variables in healthy alpacas that were standing (n = 12) and sternally recumbent (n = 13).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standing</th>
<th>Sternally recumbent</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory rate (breaths/min)</td>
<td>30.89 ± 10.27</td>
<td>29.14 ± 8.76</td>
<td>0.514</td>
</tr>
<tr>
<td>V̇e (L)</td>
<td>0.80 ± 0.13</td>
<td>0.92 ± 0.20</td>
<td>0.016</td>
</tr>
<tr>
<td>V̇e (mL/kg)</td>
<td>12.13 ± 2.54</td>
<td>14.02 ± 3.19</td>
<td>0.010</td>
</tr>
<tr>
<td>V̇e (L/min)</td>
<td>25.97 ± 6.81</td>
<td>26.18 ± 8.18</td>
<td>0.188</td>
</tr>
<tr>
<td>PIF (L/s)</td>
<td>1.30 ± 0.37</td>
<td>1.54 ± 0.53</td>
<td>0.009</td>
</tr>
<tr>
<td>PEF (L/s)</td>
<td>1.13 ± 0.30</td>
<td>1.34 ± 0.38</td>
<td>0.016</td>
</tr>
<tr>
<td>PEF-to-PIF ratio</td>
<td>0.88 ± 0.12</td>
<td>0.88 ± 0.13</td>
<td>0.314</td>
</tr>
<tr>
<td>Ti (s)</td>
<td>0.93 ± 0.26</td>
<td>0.97 ± 0.22</td>
<td>0.485</td>
</tr>
<tr>
<td>Te (s)</td>
<td>1.23 ± 0.46</td>
<td>1.29 ± 0.56</td>
<td>0.940</td>
</tr>
<tr>
<td>Te-to-Ti ratio</td>
<td>1.32 ± 0.25</td>
<td>1.33 ± 0.39</td>
<td>0.532</td>
</tr>
<tr>
<td>Difference in flow (L/s)†</td>
<td>0.18 ± 0.07</td>
<td>0.27 ± 0.19</td>
<td>0.058</td>
</tr>
<tr>
<td>Phase angle (°)</td>
<td>19.59 ± 10.06</td>
<td>17.56 ± 9.12</td>
<td>0.470</td>
</tr>
</tbody>
</table>

*Represents results of a paired-samples t test; values were considered significant at P < 0.05. †Difference between nasal and plethysmographic flow measurements.
logic examination of BALF did not differ significantly among alpacas from the different farms.

Discussion
To our knowledge, the study described here is the first application of respiratory mechanical function tests and evaluation of BALF in adult alpacas. The ease with which pulmonary function testing was performed in conscious alpacas may provide an opportunity to use these noninvasive tests as ancillary diagnostic tools in clinical patients. Analysis of BALF will allow further characterization of respiratory disease conditions in camelids.

The FRC obtained in alpacas was comparable to results reported in other standing nonintubated foregut fermenters, with a relatively greater FRC per kilogram of body weight in species with lower body mass. Cattle,\(^7\) have the lowest FRC relative to body weight (39.4 mL/kg), followed by llamas\(^6\) (40 mL/kg), alpacas (46 mL/kg), and sheep\(^9\) (52 mL/kg). Functional residual capacity is the passive balance between the inward elastic recoil of a lung and outward elastic recoil of the chest wall.\(^5\) Thus, species-associated differences in FRC may be related to the chest wall and lung compliance, abdominal volume, posture, and shape of the chest and diaphragm.\(^7,13,43\) In alpacas, FRC was significantly correlated (\(r = 0.645; P = 0.003\)) with body weight, which is similar to observations in horses\(^11\) and dogs of the same breed.\(^13\) In contrast, FRC was only correlated with body length in llamas,\(^6\) whereas positive associations between FRC and circumference, body weight, and length have been reported in sheep.\(^9\) Variation in body condition and type may partly explain the failure of allometric scaling to predict FRC on the basis of body weight within and between species. For example, body weight fails to be useful for predicting FRC in dogs, in which body shape differs substantially with a breed.\(^12-14\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Oscillation frequency (Hz)</th>
<th>n</th>
<th>Standing</th>
<th>n</th>
<th>Sternaly recumbent</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>R(_{ms}) (cm H(_2)O/L/s)</td>
<td>1</td>
<td>12</td>
<td>2.70 ± 0.88</td>
<td>8</td>
<td>3.14 ± 0.66</td>
<td>0.304</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>13</td>
<td>2.98 ± 0.70</td>
<td>12</td>
<td>3.10 ± 0.75</td>
<td>0.534</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>13</td>
<td>3.14 ± 0.77</td>
<td>10</td>
<td>3.08 ± 0.81</td>
<td>0.419</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>13</td>
<td>3.45 ± 0.91</td>
<td>12</td>
<td>2.99 ± 0.60</td>
<td>0.229</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>12</td>
<td>3.84 ± 0.93</td>
<td>12</td>
<td>3.36 ± 0.48</td>
<td>0.104</td>
</tr>
<tr>
<td>X(_{ms}) (cm H(_2)O/L/s)</td>
<td>1</td>
<td>13</td>
<td>–2.59 ± 1.42</td>
<td>7</td>
<td>–2.53 ± 1.00</td>
<td>0.328</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>13</td>
<td>–1.39 ± 0.49</td>
<td>11</td>
<td>–1.40 ± 0.43</td>
<td>0.850</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>13</td>
<td>–0.67 ± 0.54</td>
<td>10</td>
<td>–0.97 ± 0.63</td>
<td>0.675</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>13</td>
<td>–0.40 ± 0.62</td>
<td>11</td>
<td>–0.44 ± 0.67</td>
<td>0.417</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>12</td>
<td>–0.43 ± 0.60</td>
<td>11</td>
<td>–0.35 ± 1.21</td>
<td>0.978</td>
</tr>
</tbody>
</table>

n = Number of alpacas for which data were available (inclusion criteria for FOT, coherence \(\geq 0.9\)). See Table 1 for remainder of key.
In humans, however, the thoracic contribution (Figure 1), which is similar to the abdominal contribution, compared with the signal for the rest. This was indicated by the larger signal for the abdominal (ie, intercostal muscle) contribution to ventilation at rest. In trained and nonsedated alpacas in the present study.

Healthy horses and mammals,46 with a mean phase angle of 8.3° to 17.3°. The compartment can initiate respiration in a subset of animals.45 In healthy llamas, the thoracoabdominal asynchrony. In healthy llamas, the thoracic contribution to ventilation at rest.

In addition to the larger contribution from the abdomen, the timing was such that abdominal movement during quiet breathing.44 Healthy horses have an approximately equal thoracic and abdominal contribution to ventilation at rest.45 These differences may be explained by the relative smaller chest size, greater percentage of the thorax located underneath the forelimb, and possibly lower active excursion of the ribcages of alpacas during respiration in comparison with these factors in adult horses.45

In contrast to conventional techniques, FOT also allows for the rapid differentiation of upper airway versus pulmonary disease. This delineation is obtained by comparing Rs measured via esophageal balloon pneumotachography and Rs measured via FOT. Although the conventional esophageal balloon technique serves as a criterion-referenced standard, it is a time-consuming and moderately invasive procedure and therefore not ideal for the assessment of compromised animals. In contrast to conventional techniques, FOT also allows for the rapid differentiation of upper airway versus pulmonary disease. This delineation is obtained by comparing Rs measured via esophageal balloon pneumotachography and Rs measured via FOT. Although the conventional esophageal balloon technique serves as a criterion-referenced standard, it is a time-consuming and moderately invasive procedure and therefore not ideal for the assessment of compromised animals.

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rhinitis, and pharyngeal collapse as well as laryngeal disease and dysfunction. Thus, use of the FOT may allow for rapid, noninvasive localization of dysfunction to the upper airway in these patients. Accurate placement of the face mask, with a latex shroud secured over the bony portion of the nasal passages, is instrumental to avoid inadvertent narrowing of the airways during pulmonary function testing. The Rsc as determined by the use of FOT was not significantly different between sternally recumbent and standing alpacas.

Cytologic evaluation of the BALF revealed percentages of alveolar macrophages and lymphocytes similar to those reported in horses, but the mean percentage of neutrophils was higher in the alpacas of the present study (11%) than in healthy horses (< 5%). This finding may mimic the higher peripheral neutrophil count in alpacas, with an apparently greater leukocyte response to bacterial infection than is evident for horses. Artificial blood contamination was considered an unlikely cause of the higher BALF neutrophil percentages because a minimal number of RBCs were identified during cytologic examination. Theoretically, cell populations could have been impacted on the basis of sampling location if BALF were partially obtained from bronchi versus terminal bronchioles in some alpacas. No mast cells were identified with the use of toluidine blue, a staining technique that can improve the recognition of mast cells in equine BALF. In contrast to results for clinically normal horses, BALF of alpacas also contained numerous ciliated epithelial cells that line the trachea and bronchi in vivo. In humans, the presence of large numbers of ciliated epithelial cells in the BALF has been related to in vivo. In humans, the presence of large numbers of ciliated epithelial cells in the BALF has been related to smoking inhalation or viral infections, which did not enter the alpacas of our study. We speculate that BALF sampling in alpacas may have induced minor airway trauma and thus led to the recovery of ciliated epithelial cells. The described BAL technique yielded samples with adequate cellularity and resulted in no complications in these healthy alpacas, but its use may be limited in clinical patients because short-term injectable anesthesia was required.

Pulmonary function testing may be a rapid, noninvasive, well-tolerated modality for use in improving the characterization and localization of respiratory dysfunction in alpacas. In contrast, collection of BALF for cytologic examination may not be successful without the use of chemical restraint in smaller camels. The study reported here revealed pertinent differences in results of cytologic evaluation of BALF and respiratory mechanical function of healthy alpacas, compared with values reported for other domestic species.

References

24. Baughman RP, Dohn MN, Loudon RG, et al. Bronchoscopy with injectable anesthesia was required.