

# 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  $\pm$  SD measurements of respiratory function were obtained in standing alpacas for FRC ( $3.19 \pm 0.53$  L), tidal volume ( $0.8 \pm 0.13$  L), and respiratory system resistance at 1 Hz ( $2.70 \pm 0.88$  cm H<sub>2</sub>O/L/s), 2 Hz ( $2.98 \pm 0.70$  cm H<sub>2</sub>O/L/s), 3 Hz ( $3.14 \pm 0.77$  cm H<sub>2</sub>O/L/s), 5 Hz ( $3.45 \pm 0.91$  cm H<sub>2</sub>O/L/s), and 7 Hz ( $3.84 \pm 0.93$  cm H<sub>2</sub>O/L/s). Mean phase angle, as a measurement of thoracoabdominal asynchrony, was  $19.59 \pm 10.06^\circ$ , and mean difference between nasal and plethysmographic flow measurements was  $0.18 \pm 0.07$  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  $58.52 \pm 12.36\%$  alveolar macrophages,  $30.53 \pm 13.78\%$  lymphocytes,  $10.95 \pm 9.29\%$  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)

A plethora of medical conditions that affect the respiratory system of camelids and that potentially alter respiratory function have been identified,<sup>1–4</sup> 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 re-

## ABBREVIATIONS

BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage fluid
FOT	Forced oscillatory technique
FRC	Functional residual capacity
PEF	Peak expiratory flow
PIF	Peak inspiratory flow
RIP	Respiratory inductive plethysmography
R <sub>L</sub>	Pulmonary resistance
R <sub>RS</sub>	Respiratory system resistance
T <sub>E</sub>	Expiratory time
T <sub>I</sub>	Inspiratory time
$\dot{V}_E$	Minute ventilation
V <sub>T</sub>	Tidal volume
X <sub>RS</sub>	Respiratory system reactance

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spiratory ailments.<sup>5</sup> 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,<sup>6</sup> cattle,<sup>7</sup> sheep,<sup>1,8,9</sup> horses,<sup>7,10,11</sup> and dogs.<sup>12–14</sup> Respiratory inductive plethysmography has been used to describe the breathing patterns of horses at rest<sup>15</sup> and during ex-

ercise,<sup>16</sup> foals with evoked obstructions of the proximal and distal portions of the airways,<sup>17</sup> healthy llamas,<sup>6</sup> and a llama with diaphragmatic paralysis.<sup>1</sup> This test is also used to diagnose inflammatory airway disease and recurrent airway obstruction in horses.<sup>5,15,18</sup> Both  $R_{RS}$  and  $X_{RS}$ , measured by FOT, are frequently used to characterize the degree of airway obstruction in horses.<sup>19–21</sup> In a study<sup>6</sup> in llamas, investigators found that  $R_L$  measured via esophageal balloon pneumotachography (criterion-referenced standard) was positively correlated with  $R_L$  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.<sup>19,20,22,23</sup> Analysis of results for cytologic examination and bacteriologic culture of BALF improves the sensitivity of the diagnosis of fungal pneumonia and tuberculosis in humans<sup>24</sup> and may also be useful in the antemortem diagnosis of several conditions that affect alpacas, such as tuberculosis,<sup>25–29</sup> nocardiosis,<sup>30</sup> viral pneumonia,<sup>31,32</sup> pulmonary neoplasia,<sup>33</sup> fungal pneumonia,<sup>2,4,34</sup> and *Rhodococcus equi*-associated pneumonia.<sup>35</sup> Reference values for BALF cytologic examination have been established for several domestic species, including horses<sup>23</sup> and dogs.<sup>36</sup> Although BAL has been performed in a llama with histoplasmosis,<sup>34</sup> no technique for the procedure or reference values for cytologic analysis of the BALF have been reported in camelids to our knowledge. Therefore, the purpose of the study reported here was to provide values for respiratory mechanical function and results of BALF cytologic examination in healthy adult alpacas, which would allow for the use of these diagnostic tools in clinical patients.

## 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  $\pm$  SD,  $3.5 \pm 2.1$  years). Body weight ranged from 49 to 109 kg (mean body condition score, 4 on a scale<sup>37</sup> of 1 to 9). The alpacas belonged to 3 farms that used similar housing conditions. Alpacas were housed on grass pastures and were fed 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. 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,<sup>13</sup> via RIP,<sup>1,15</sup> via monosinusoidal FOT,<sup>38</sup> and via evaluation of BALF. Respiratory inductive plethysmography and FOT were performed in alpacas that were standing and sternally 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,<sup>13,14</sup> horses,<sup>11</sup> and llamas.<sup>6</sup> 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 (120°) stopcock<sup>a</sup> and nondiffusible gas collection bag containing a breathable test gas (0.3% CO, 10% He, 20% O<sub>2</sub>, and 69.7% N<sub>2</sub>) at a volume of 40 mL/kg. The initial and final concentrations of He and the final concentration of CO<sub>2</sub> were measured following a rebreathing period of 90 seconds for use in calculating FRC at equilibrium. Three consecutive measurements were obtained for each alpaca, and the mean was calculated.

**RIP and pneumotachography**—Respiratory inductive plethysmography was performed in accordance with the methods previously described for llamas.<sup>6</sup> Modifications implemented included placement of the thoracic band overlying 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  $R_{RS}$  and  $X_{RS}$  as described elsewhere.<sup>6,39</sup> The  $R_{RS}$  comprises the additive effects of airway resistance, chest wall resistance, and  $R_L$ . 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<sup>b</sup> (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 bronchiole. 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

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.<sup>40</sup> Cells were classified by one of the authors (MRM) as the percentage of macrophages, lymphocytes, neutrophils, eosinophils, and mast cells by counting a minimum of 400 cells (magnification, 630X).

**Statistical analysis**—Descriptive analyses of respiratory mechanical variables (FRC,  $V_T$ ,  $Ti$ ,  $Te$ ,  $\dot{V}_E$ , PIF, PEF,  $R_{RS}$ ,  $X_{RS}$ , and RIP-derived variables) were reported as mean  $\pm$  SD. Correlations between FRC and body dimensions (body weight, height, body length, and chest circumference) were evaluated by use of the Spearman  $\rho$ . 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, with values of  $P < 0.05$  considered significant. All statistical analyses were performed by use of commercial software.<sup>6</sup>

## Results

**Physical characteristics, hematologic evaluation, and radiographic examination**—Mean  $\pm$  SD measurements of body weight ( $70.50 \pm 15.74$  kg), height ( $92.35 \pm 4.99$  cm), body length ( $84.83 \pm 8.48$  cm), and chest circumference ( $103.83 \pm 10.72$  cm) were within anticipated limits for healthy adult alpacas.<sup>41</sup> Similarly, results of the CBC and serum biochemical analysis were within the respective reference ranges reported for alpacas.<sup>42</sup> 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  $\pm$  SD FRC in alpacas was  $3.19 \pm 0.53$  L, which translated to  $46.29 \pm 7.50$  mL/kg. A significant positive correlation ( $r = 0.645$ ;  $P = 0.003$ ) between FRC and body weight was

detected in the study animals. However, FRC was not significantly correlated with body length, height, or chest circumference.

Respiratory inductive plethysmography could not be performed in 2 of 16 alpacas (1 was vocalizing throughout the test, and the other did not tolerate handling 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 sternal recumbency (13/13) and in most of the alpacas evaluated while standing (8/12). Mean phase angle, a measurement of thoracoabdominal asynchrony, was  $19.59 \pm 10.06^\circ$  in standing alpacas and  $17.56 \pm 9.12^\circ$  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.

All measured variables of respiratory mechanical function obtained via FOT in 13 standing and 12 sternally recumbent alpacas were summarized (Table 2). Values of  $R_{RS}$  with acceptable coherence ( $\geq 0.9$ ) were not obtained for all frequencies in several alpacas. Poor coherence is the result of an increase in the noise signal that can be caused by vocalization, closure of the glottis, and eructation. Significant differences based on body position were not observed for  $X_{RS}$  and  $R_{RS}$ .

**BAL and evaluation of BALF**—Examination of lateral and dorsoventral radiographs obtained with the BAL catheter in place revealed that the collection tube was located in the right caudodorsal lung field in 12 alpacas and in the left caudodorsal lung field in 4 alpacas. Cytologic analysis of 16 samples (1 sample/alpaca) of BALF revealed a mean  $\pm$  SD of  $58.52 \pm 12.36\%$  alveolar macrophages,  $30.53 \pm 13.78\%$  lymphocytes,  $10.95 \pm 9.29\%$  polymorphonuclear cells (neutrophils),  $0\%$  eosinophils, and  $0\%$  mast cells (Figure 2). Furthermore, several ciliated epithelial cells per hpf were observed in 10 samples during cytologic analysis. Minimal numbers of RBCs were observed in the samples. Results of cyto-

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

Variable	Standing	Sternally recumbent	<i>P</i> value*
Respiratory rate (breaths/min)	$30.89 \pm 10.27$	$29.14 \pm 8.76$	0.514
$V_T$ (L)	$0.80 \pm 0.13$	$0.92 \pm 0.20$	0.016
$V_T$ (mL/kg)	$12.13 \pm 2.54$	$14.02 \pm 3.19$	0.010
$\dot{V}_E$ (L/min)	$23.97 \pm 6.81$	$26.18 \pm 8.18$	0.168
PIF (L/s)	$1.30 \pm 0.37$	$1.54 \pm 0.53$	0.009
PEF (L/s)	$1.13 \pm 0.30$	$1.34 \pm 0.38$	0.016
PEF-to-PIF ratio	$0.88 \pm 0.12$	$0.88 \pm 0.13$	0.314
$Ti$ (s)	$0.93 \pm 0.26$	$0.97 \pm 0.22$	0.485
$Te$ (s)	$1.23 \pm 0.46$	$1.29 \pm 0.56$	0.940
$Te$ -to- $Ti$ ratio	$1.32 \pm 0.25$	$1.33 \pm 0.39$	0.532
Difference in flow (L/s)†	$0.18 \pm 0.07$	$0.27 \pm 0.19$	0.058
Phase angle ( $^\circ$ )	$19.59 \pm 10.06$	$17.56 \pm 9.12$	0.470

\*Represents results of a paired-samples  $t$  test; values were considered significant at  $P < 0.05$ . †Difference between nasal and plethysmographic flow measurements.

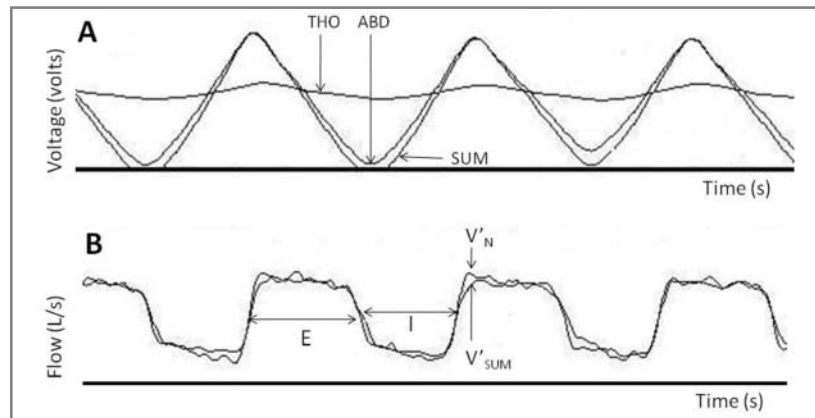


Figure 1—Diagrams depicting RIP and pneumotachographic signals in a representative clinically normal alpaca. A—Thoracic (THO) and abdominal (ABD) contribution to breathing in a healthy alpaca; the abdominal contribution (reflecting diaphragmatic motion) led the thoracic contribution (reflecting thoracic motion) in all alpacas, which yielded a mean  $\pm$  SD minimal phase angle of  $19.59 \pm 10.06^\circ$  in standing alpacas and  $17.56 \pm 9.12^\circ$  in sternally recumbent alpacas. B—The nasal flow ( $V'_N$ ) measured by use of a pneumotachograph and sum of the flow ( $V'_{SUM}$ ) signal derived from elastic band measurements in a healthy alpaca. The beginning of expiration (E) and beginning of inspiration (I) were defined by upward- and downward-directed zero crossings (regions encompassed by the double arrowheads) of flow signals, respectively. The delta flow represents the difference between the external flow ( $V'_{SUM}$ ) and nasal flow ( $V'_N$ ) signals during the first 25% of exhaled volume.

Table 2—Mean  $\pm$  SD  $R_{RS}$  and  $X_{RS}$  obtained by use of FOT in standing and sternally recumbent adult alpacas.

Variable	Oscillation frequency (Hz)	n	Standing	n	Sternally recumbent	P value*
$R_{RS}$ (cm H <sub>2</sub> O/L/s)	1	13	$2.70 \pm 0.88$	8	$3.14 \pm 0.66$	0.304
	2	13	$2.98 \pm 0.70$	12	$3.10 \pm 0.75$	0.534
	3	13	$3.14 \pm 0.77$	10	$3.08 \pm 0.81$	0.419
	5	13	$3.45 \pm 0.91$	12	$2.99 \pm 0.60$	0.229
	7	12	$3.84 \pm 0.93$	12	$3.36 \pm 0.48$	0.104
$X_{RS}$ (cm H <sub>2</sub> O/L/s)	1	13	$-2.59 \pm 1.42$	7	$-2.53 \pm 1.00$	0.328
	2	13	$-1.39 \pm 0.49$	11	$-1.40 \pm 0.43$	0.850
	3	13	$-0.87 \pm 0.54$	9	$-0.97 \pm 0.63$	0.675
	5	13	$-0.40 \pm 0.62$	11	$-0.44 \pm 0.67$	0.417
	7	12	$-0.43 \pm 0.60$	11	$-0.35 \pm 1.21$	0.978

n = Number of alpacas for which data were available (inclusion criteria for FOT, coherence  $\geq 0.9$ ).  
See Table 1 for remainder of key.

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<sup>7</sup> have the lowest FRC relative to body weight (39.4 mL/

kg), followed by llamas<sup>6</sup> (40 mL/kg), alpacas (46 mL/kg), and sheep<sup>43</sup> (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.<sup>5</sup> 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.<sup>7,13,43</sup> In alpacas, FRC was significantly correlated ( $r = 0.645$ ;  $P = 0.003$ ) with body weight, which is similar to observations in horses<sup>11</sup> and dogs of the same breed.<sup>13</sup> In contrast, FRC was only correlated with body length in llamas,<sup>6</sup> whereas positive associations between FRC and circumference, body weight, and length have been reported in sheep.<sup>9</sup> 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<sup>12–14</sup>



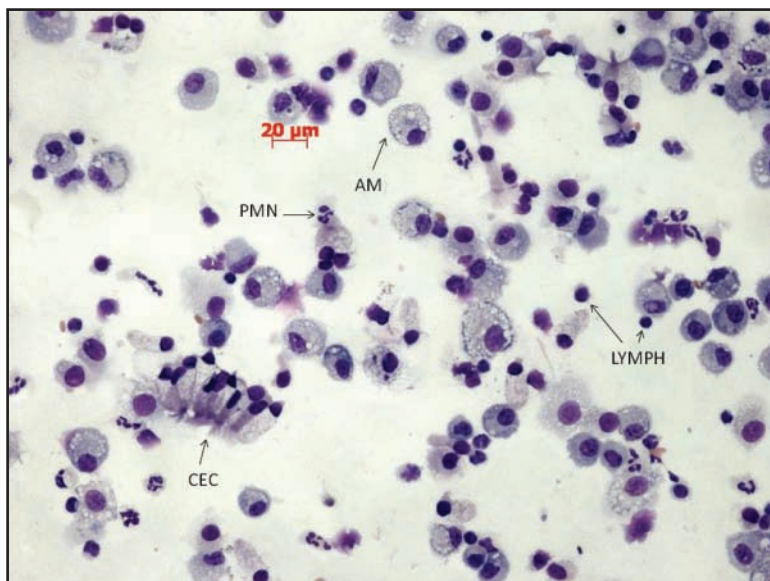


Figure 2—Photomicrograph of BALF collected from a healthy alpaca and prepared by cytocentrifugation. Most cells are alveolar macrophages (AM), followed by lymphocytes (LYMPH) and neutrophils (PMN). Several ciliated epithelial cells (CEC) per hpv were observed. Modified Wright stain; bar = 20 µm.

(FRC, 34.8 to 87.9 mL/kg). However, relative changes in FRC may serve as an important index of pulmonary dysfunction. For example, reduced lung compliance (eg, pulmonary fibrosis) and loss of lung volume related to pneumonia or pulmonary infiltrates may reduce FRC.<sup>6</sup> The helium dilution technique for determination of FRC was uncomplicated and tolerated well by all untrained and nonsedated alpacas in the present study.

Alpacas had a greater abdominal (ie, diaphragmatic and abdominal muscle) contribution than the ribcage (ie, intercostal muscle) contribution to ventilation at rest. This was indicated by the larger signal for the abdominal contribution, compared with the signal for the thoracic contribution (Figure 1), which is similar to observations in llamas.<sup>6</sup> In humans, however, the contribution of ribcage motion predominates over abdominal movement during quiet breathing.<sup>44</sup> Healthy horses have an approximately equal thoracic and abdominal contribution to ventilation at rest.<sup>45,46</sup> 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.<sup>45</sup>

In addition to the larger contribution from the abdomen, the timing was such that abdominal contribution (reflecting diaphragmatic motion) led thoracic (reflecting thoracic motion) movement in all alpacas, thus resulting in a minimal phase angle of  $19.59 \pm 10.06^\circ$  in standing alpacas and  $17.56 \pm 9.12^\circ$  in sternally recumbent alpacas. In healthy horses, the abdominal compartment can initiate respiration in a subset of animals,<sup>46</sup> with a mean phase angle of  $8.3^\circ$  to  $17.3^\circ$ . The phase angle is a measurement of thoracoabdominal synchrony with values  $> 90^\circ$  indicating paradoxical respiration and values  $> 180^\circ$  representing complete thoracoabdominal asynchrony. In healthy llamas, the movement of the thorax and abdomen reportedly is

synchronous<sup>6</sup>; however, the phase angle has not been measured in this species.<sup>6</sup> Diseased camelids may have major abnormalities in the breathing pattern, which are easily determined by use of RIP.<sup>1</sup> In horses, for example, RIP was used to verify that patients with recurrent airway obstruction have a significantly greater phase angle ( $62 \pm 18.6^\circ$ ), compared with the phase angle in control animals, and that the thorax is leading respiration in most diseased animals.<sup>46</sup> Lower airway obstruction leads to an increase in the difference between plethysmographic and nasal flow during the first 25% of expiration. The mean difference in flow recorded in the healthy alpacas of the present study ( $0.18 \pm 0.07$  L/s) was similar to values obtained in llamas<sup>6</sup> ( $0.04 \pm 0.14$  L/s).

In the present study, mean  $V_T$  in standing alpacas was 12 mL/kg, which is similar to values reported in horses<sup>7,47,48</sup> and dogs<sup>14</sup> but higher than values in llamas (7 mL/kg),<sup>6</sup> cattle (8 mL/kg),<sup>7,47</sup> and sheep (6 to 7 mL/kg).<sup>43</sup> Compared with llamas,<sup>6</sup> alpacas have a larger  $V_T$ , longer  $T_i$  and  $T_e$ , and lower  $\dot{V}_E$  and respiratory frequency. The observed species differences may relate to absolute differences in lung volume, dynamic compliance, size or shape of the abdomen, or relative abdominal mass.

Because some alpacas have a tendency to become sternally recumbent when nervous or intimidated, RIP results were obtained in standing and sternally recumbent animals. A significantly higher  $V_T$ , PIF, and PEF were observed in the sternally recumbent alpacas, compared with values in standing alpacas, without alteration in respiratory frequency. We speculate that a shift in abdominal organs during sternal recumbency may have allowed for a larger  $V_T$  and consequently a higher PIF and PEF.

Values for  $R_{RS}$  were higher in the alpacas of the present study than in llamas<sup>6</sup> across all impulse frequencies. This finding was expected because smaller animals are likely to have a higher respiratory resistance as a result of their overall smaller airway diameter. In another study,<sup>6</sup> investigators validated the use of noninvasive FOT in healthy llamas by detecting a positive correlation between  $R_L$  measured via esophageal balloon pneumotachography and  $R_L$  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  $R_{RS}$  at 1 Hz, which predominantly reflects lower airway function, with  $R_{RS}$  at higher impulse frequencies that characterize the central and upper airway diameter. This feature is particularly desirable for the evaluation of camelids because their narrow nasal passages contribute substantially to total  $R_{RS}$ . Upper airway obstruction has been observed in alpacas with congenital disease (partial choanal atresia), nasal bots, allergic

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  $R_{RS}$  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%).<sup>23</sup> 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.<sup>42</sup> 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.<sup>40</sup> 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 smoke inhalation<sup>49</sup> or viral infections, which did not pertain to 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, non-invasive, 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 camelids. 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.

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- b. Mila International Inc, Erlanger, Ky.
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