Results of cytologic and microbiologic analysis of bronchoalveolar lavage fluid in New Zealand White rabbits

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Objective—To determine cytologic and microbiologic findings in bronchoalveolar lavage (BAL) fluid and SpO₂ values obtained during BAL in healthy rabbits.

Animals—9 rabbits.

Procedures—Bronchoscopic BAL of left and right caudal lobar bronchi (LB2 and RB4) was performed with 3 mL of sterile saline (0.9% NaCl) solution; SpO₂ was measured before, during, and after BAL. Percentage fluid recovered, total leukocyte counts, and differential cell counts were determined. Aerobic and anaerobic bacterial, mycoplasmal, and fungal cultures were performed from combined LB2 and RB4 samples.

Results—Mean ± SD percentage fluid volumes recovered from LB2 and RB4 were 53 ± 13% and 63 ± 13%, respectively. Mean ± SD total leukocyte counts from LB2 and RB4 were 422 ± 199 cells/µL and 378 ± 97 cells/µL, respectively. Macrophages were most frequently identified. There were no significant differences in volumes retrieved, total leukocyte counts, or differential cell percentages between LB2 and RB4. Microbial culture results were negative for 3 rabbits and positive for mixed aerobic and anaerobic bacterial growth in 6 and 2 rabbits, respectively. The SpO₂ was ≥ 95% in 7 of 9 rabbits after anesthetic induction, < 95% in 5 of 6 rabbits 1 minute after BAL, and ≥ 95% in 5 of 9 rabbits and > 90% in 4 of 9 rabbits 3 minutes after BAL.

Conclusions and Clinical Relevance—Bronchoscopic BAL with 3 mL of saline solution provided adequate fluid recovery for microbiologic and cytologic examination from the caudal lung lobes. Transient low SpO₂ was detected immediately after BAL. (Am J Vet Res 2008;69:572–578)

Rabbits are commonly evaluated by veterinarians for respiratory tract diseases. Bronchoalveolar lavage is a valuable tool in the diagnosis and management of respiratory conditions in humans and a number of veterinary species, including dogs, cats, horses, and non-human primates. Specific indications for BAL in rabbits include clinical signs such as coughing or wheezing, increase in respiratory rate and effort, abnormal airway sounds detected via auscultation, unexplained pulmonary infiltrates detected via diagnostic imaging, and the suspicion of an airway mass or foreign body.

Little information is available describing technique or reference range values for BAL in rabbits. Total lung lavage in healthy New Zealand White rabbits by use of instillate volumes approximating 80% of the total lung volume has been reported. However, in a clinical setting, sublobar BAL via fiberoptic or video bronchoscopy is the preferred diagnostic method because it allows direct viewing of the airways and sample collection from a lung segment or specific area of interest. To our knowledge, microbiologic and cytologic findings from sublobar BAL in healthy rabbits have not been reported. Establishment of reference intervals is critical for understanding of pathologic processes.

The objectives of the study reported here were to determine reference values for cytologic and microbiologic findings in bronchoscopic BAL fluid from healthy New Zealand White rabbits. The safety and clinical applications of the procedure were assessed by measurement of percentage fluid volume recovery and SpO₂ of the rabbits before, during, and after the procedure.

Materials and Methods

Animals—Five male and 4 female adult rabbits ranging in body weight from 2.75 to 4.25 kg were used for this study. All rabbits had been culled from a

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>BAL</td>
<td>Bronchoalveolar lavage</td>
</tr>
<tr>
<td>LB2</td>
<td>Left caudal lobar bronchus</td>
</tr>
<tr>
<td>RB4</td>
<td>Right caudal lobar bronchus</td>
</tr>
<tr>
<td>SpO₂</td>
<td>Oxygen saturation as measured by pulse oximetry</td>
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group of rabbits used previously for teaching purposes in animal-handling laboratories and had no history of illness. The rabbits were housed individually, fed a diet of pellets and hay ad libitum, and provided water ad libitum from a sipper bottle. The rabbits were assessed to be healthy on the basis of results of a physical examination. This study was approved by the Institutional Animal Care and Use Committee at the University of California, Davis.

**Induction and instrumentation**—Food was withheld for no more than 4 hours prior to induction of anesthesia. Anesthesia for bronchoscopic examination was induced with xylazine and ketamine hydrochloride (3 mg/kg, IM, and 30 mg/kg, IM, respectively), and anesthesia was maintained with 1% to 2% isoflurane in 95% to 100% oxygen via a nosecone mask throughout the procedure. The rabbits were positioned in sternal recumbency, and a rolled towel was placed under the ventral aspect of the neck to gently stretch the neck for ease of bronchoscopic placement. Rodent cheek dilators and an incisor speculum were fitted in place, and the oral cavity was cleaned of food material prior to insertion of the bronchoscope. The SpO2 was monitored with a probe placed over the central auricular artery.

**Bronchoscopy**—All bronchoscopic examinations and BAL were performed by the same clinician (LRJ). Bronchoscopy with BAL was performed by use of a 2.5-mm × 70-cm flexible endoscope. The bronchoscope was sterilized prior to each procedure by immersion in glutaraldehyde. After complete airway inspection lasting approximately 5 minutes in duration, the bronchoscope was withdrawn from the airways. The exterior sheath of the endoscope was wiped with sterile saline (0.9% NaCl) solution, and the biopsy channel was rinsed with sterile saline solution to remove any contaminants. On entry into the airways for lavage, care was taken to avoid contaminating the bronchoscope in the oropharynx and large airways. The bronchoscope was advanced into the most distal aspect of the LB2 and RB4 and wedged gently in place. Sterile saline solution (3 mL) was instilled through the biopsy port followed by 1 mL of air to clear the channel. Gentle hand suction was then applied to recover fluid from the bronchoalveolar space. Percentage fluid volume recovery was recorded, and BAL was performed distally in the RB4 in a similar fashion.

The SpO2 was recorded when the bronchoscope was positioned at the carina, during each BAL, and at 1 and 3 minutes after BAL. The BAL fluid samples were submitted immediately for cytologic evaluation and for aerobic and anaerobic bacterial, mycoplasmal, and fungal cultures.

**Cytologic examination**—All cytologic samples were evaluated by the same clinical pathologist (WV). Differential cell counts and microscopic evaluation were performed on the right and left samples separately for each rabbit. All samples were analyzed within 30 minutes of receipt in the laboratory. A 200-µL aliquot of well-mixed BAL fluid was added to a 1.5-mL Eppendorf tube along with 200 µL of isotonic PBS solution. The diluted sample was mixed with an applicator stick to remove any particulate matter or mucus aggregates. A 40-µL aliquot of the diluted sample was placed in a hematology analyzer in predilute mode, and RBC and nucleated cell counts were determined. Subsequently, an additional 250-µL aliquot of diluted sample was cytocentrifuged for 5 minutes by use of clean, reusable chambers and glass slides. Cytocentrifuged slides were air-dried and processed in an automated slide stainer. A 100-cell differential cell count was completed on a Wright-Giemsa stained slide by a licensed medical technologist and verified by a board-certified clinical pathologist (WV). Morphologic assessment of cells was also performed.

**Microbiologic examination**—Aliquots from LB2 and RB4 from each rabbit were combined for microbiologic examination. Samples for microbiologic culture were centrifuged at approximately 5,000 × g at 25°C for 5 minutes. A portion of the sediment was inoculated onto aerobic blood agar plate (5% sheep RBGs) and incubated at 37°C in an atmosphere of 10% CO2 in air. For *Mycoplasma* spp culture, a sample of sediment was inoculated on *Mycoplasma* medium containing 15% horse serum, 15% yeast extract, 2 × 10^6 units of penicillin G, and 10% thallium acetate and incubated at 37°C in an atmosphere of 10% CO2 in air. Another portion of sediment was inoculated onto an anaerobic blood agar plate (prereduced, anaerobically sterilized 5% laked sheep RBGs and vitamin K1 in a *Brucella* agar base), placed into an anaerobic chamber, and incubated at 37°C in an atmosphere of 90% N2, 5% CO2, and 5% H2 in the presence of a palladium catalyst. Fungal culture was performed by inoculation onto an inhibitory mold plate (containing chloramphenicol) and incubated aerobically at 30°C in air and in trypticase soy broth at 37°C for up to 4 weeks.

Standard laboratory methods were used to provide semi quantitative assessment of microbial growth. Small numbers of bacteria were defined by the growth of few colonies of organisms in the first quadrant of the culture plate.

**Histologic examination**—At the completion of the bronchoscopic procedure, rabbits were euthanatized by IV administration of pentobarbital and lung tissue from lavaged regions of the caudal lungs lobes was collected. Pulmonary tissue was inspected grossly for abnormalities, placed into neutral-buffered 10% formalin, processed for 5 minutes. A portion of the sediment was inoculated onto an anaerobic blood agar plate (prereduced, anaerobically sterilized 5% laked sheep RBGs and vitamin K1 in a *Brucella* agar base), placed into an anaerobic chamber, and incubated at 37°C in an atmosphere of 90% N2, 5% CO2, and 5% H2 in the presence of a palladium catalyst. Fungal culture was performed by inoculation onto an inhibitory mold plate (containing chloramphenicol) and incubated aerobically at 30°C in air and in trypticase soy broth at 37°C for up to 4 weeks. Specimens were sectioned at a thickness of 5 µm and stained with H&E. Histologic sections were microscopically examined independently by 2 pathologists.

**Statistical analysis**—Percentage fluid volume recovery from LB2 and RB4, total leukocyte cell counts, and differential leukocyte percentages are expressed as mean ± SD values. The BAL fluid volume retrieved, total leukocyte cell counts, and differential leukocyte cell percentages from LB2 and RB4 were compared by use of the Wilcoxon signed rank test. Significance was defined as a value of P ≤ 0.05.

**Results**

No lesions were identified in the airways of any of the rabbits during bronchoscopic examination or via histologic examination. Eighteen fluid samples were
macrophages of—

Cells/

µ—Photomicrograph. Multinucleate, Eosinophils small

57 4

were detected in 6 samples were alveolar macrophages samples—

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centages from LB2 and RB4 were determined

13%) and from RB4 was 1.9

obtained from the 9 rabbits. Fluid volume (percentage

volume recovered) from LB2 was 1.6 ± 0.4 mL (53 ±

13%); and from RB4 was 1.9 ± 0.4 mL (63 ± 13%); the
difference was not significant.

Total leukocyte cell count and differential cell per

percentages from LR2 and RB4 were determined (Table 1).

All samples contained some RBCs. Rare to low numbers of RBCs were detected in most samples, whereas moderate to numerous RBCs were detected in 2 of 18 samples (1 sample each from 2 rabbits). The total nucleated cell count included only leukocytes because the epithelial cells were present in variably sized clusters that were excluded from the nucleated cell count by the gate settings in the hematology analyzer. Moderate to numerous variably sized clusters of these cytologically normal ciliated epithelial cells were detected in 17 samples (Figure 1). Greater than 60% of all leukocytes were alveolar macrophages (Figure 2). Multinucleate macrophages were detected in 6 samples (Figure 3), and occasional mitotic macrophages were identified in 3 samples. Rare macrophages with intracellular debris, plant material, or hemosiderin were also detected in 4 samples. Lymphocytes were the second most numerous cell type. Typically, macrophages comprised ≥ 80% and lymphocytes comprised ≤ 11% of the total leukocyte cell counts. In 5 samples, scattered plasma cells and occasional reactive lymphocytes were also seen, regardless of the percentage of lymphocytes in the sample (Figure 4). Heterophils and eosinophils comprised variable numbers of the remainder of the total nucleated cell counts (generally < 5% for each; Figure 5). There were no significant differences in total leukocyte cell counts or differential cell percentages between LB2 and RB4.

Table 1—Leukocyte cell counts (mean ± SD [range]) from BAL samples retrieved via sublobar bronchoscopy from the LB2 and RB4 of 9 healthy New Zealand White rabbits.

<table>
<thead>
<tr>
<th>BAL sample</th>
<th>Total cells/L</th>
<th>Heterophils</th>
<th>Lymphocytes</th>
<th>Macrophages</th>
<th>Eosinophils</th>
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<tr>
<td></td>
<td>Cells/L</td>
<td>%</td>
<td>Cells/L</td>
<td>%</td>
<td>Cells/L</td>
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<tr>
<td>LB2</td>
<td>422 ± 199 (200–700)</td>
<td>6 ± 9 (0–28)</td>
<td>1 ± 1 (0–4)</td>
<td>50 ± 33 (15–98)</td>
<td>12 ± 7 (6–30)</td>
</tr>
<tr>
<td>RB4</td>
<td>378 ± 97 (300–500)</td>
<td>7 ± 4 (1–15)</td>
<td>2 ± 1 (1–5)</td>
<td>34 ± 18 (16–75)</td>
<td>9 ± 4 (4–16)</td>
</tr>
<tr>
<td>Combined</td>
<td>400 ± 153 (200–700)</td>
<td>6 ± 6 (0–28)</td>
<td>2 ± 1 (0–5)</td>
<td>42 ± 27 (15–98)</td>
<td>11 ± 6 (4–30)</td>
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</table>

Figure 1—Photomicrograph of a medium-sized cluster of ciliated respiratory epithelial cells in BAL fluid from a rabbit. Other cells are macrophages. Wright-Giemsa stain; bar = 20 µm.

Figure 2—Photomicrograph of macrophages and small lymphocytes (small, dark cells) in BAL fluid from a rabbit. Notice a small cluster of ciliated respiratory epithelial cells (top left) and a plasma cell (bottom center). Wright-Giemsa stain; bar = 20 µm.

Figure 3—Photomicrograph of a large multinucleated macrophage in BAL fluid from a rabbit. Wright-Giemsa stain; bar = 20 µm.

Single or small clusters of anucleate squamous epithelial cells, some with adherent bacteria of mixed types, were also observed in 6 samples. Large rod-shaped bacteria with stacked striations resembling Simonsiella sp were identified with squamous epithelial cells in 4 samples.

Fluid samples from 3 rabbits did not yield growth on any culture. Small numbers of nonmotile, nonfermenting, oxidase-positive, gram-negative aerobic bacteria, designated by the laboratory as nonfermenter group 3 organisms, were the most commonly isolated aerobic bacterial species and were identified in 4 of 9 samples. This group of organisms is commonly isolated...
along with other mixed bacteria and cannot be classified via routine phenotypic characteristics and commercial identification methods. Further methods of identifying these bacteria, including molecular sequencing of the 16S rRNA gene, were not performed. Two of the 6 samples with aerobic growth had mixed growth of 2 or more bacterial species including *Bordetella bronchiseptica*, *Brevundimona dimunta*/*olegella urethralis*, and *Enterobacter* spp. Results of culture for *Mycoplasma* spp were negative for all 9 samples. Growth of anaerobic bacterial species was detected in 2 of 9 samples. In 1 sample, growth of small numbers of *Prevotella heparinolyticus*—like organism, *Propionibacterium acnes*, and *Bacteroides fragilis* was detected, and in the second sample, small numbers of *B. fragilis* were detected. Two of 9 samples yielded positive results for fungi that were considered contaminants (*Candida tropicalis* in 1 sample and a single colony of *Aspergillus* spp in the second).

The SpO₂ values were determined via pulse oximetry during bronchoscopy (Figure 6). Prior to bronchoscopy, SpO₂ was within reference range (≥93%) in 7 rabbits and >90% in all rabbits. In 2 rabbits, SpO₂ was <95% at induction and throughout the entire procedure. During BAL, SpO₂ decreased to <95% (range, 85% to 93%) in 5 rabbits, and at 1 minute after BAL, SpO₂ was ≤95% in 5 rabbits (range, 78% to 94%; n = 6 rabbits). By 3 minutes after BAL, SpO₂ was ≥95% in 5 rabbits and >90% in the remaining 4 rabbits.

**Discussion**

Bronchoscopic BAL in the healthy rabbits examined in the present study resulted in collection of a directed airway sample and allowed determination of cell counts and of cell types. Total nucleated cell counts were similar to those obtained in studies of healthy dogs and cats, in which total nucleated cell counts of 500 and 400 nucleated cells/µL, respectively, are most commonly cited. In addition, the mean total leukocyte cell counts were similar to the total leukocyte cell counts established from previous rabbit studies in which either total lung washes or multiple lavage procedures were performed as terminal procedures, suggesting that lower volumes of lavage fluid can be used in this species to achieve equivalent results. Further studies would be necessary to evaluate whether total leukocyte cell counts differ with single versus multiple lavages in the same rabbit.

Alveolar macrophages represented the predominant cell population in lavage fluid of rabbits evaluated in this study. Alveolar macrophages were also reported as the most abundant cell type (>95%) in studies that did not use a bronchoscope. In rabbits examined here, macrophage percentage was slightly lower at 69% to 94% possibly because of the low instillate volume or single-lavage procedure. In humans, increases in the percentage of alveolar macrophages followed by increases in the percentage of lymphocytes were identified cytologically as sequential lavages were performed. In horses, single small-volume lavage yielded a lesser percentage of mast cells and a greater percentage of neutrophils than did single instillate volumes 7 times as great, suggesting that the single instillate volume could...
alter the differential cell percentages. It is possible that if sequential lavages or larger fluid volumes had been used in the rabbits in this study, the differential cell percentages may have been more similar to those of previous rabbit studies. Further sublobar bronchoscopy studies in rabbits evaluating larger-volume lavage or sequential lavages would be necessary to determine whether the differential cell percentage is affected by either of these techniques.

Occasional binucleate and multinucleate macrophages were observed, and these cells have been seen with similar frequency in BAL fluid from healthy individuals of other species, particularly horses. Similarly, occasional reactive lymphocytes and plasma cells were also observed in the BAL fluid of the rabbits reported here in the absence of any other evidence of inflammation.

Differential cell counts in healthy rabbits examined here revealed a paucity of inflammatory cells in comparison to percentages reported in healthy dogs and cats. Therefore, it is anticipated that BAL fluid will be a clinically useful tool in identifying infectious and inflammatory diseases of the lower portion of the respiratory tract in rabbits. Similarly, research into drug disposition or inhalation-induced injury would be facilitated by documentation of an inflammatory response. Differential cell counts were similar between lobes in healthy rabbits; however, this may not be true in diseased rabbits. Hawkins et al determined that 37% of BAL fluid samples had different results between lobes regarding differential cell percentages in dogs with respiratory tract disease.

The large number of morphologically normal epithelial cells and the various numbers of RBCs in BAL samples in this study were dissimilar to those reported in other species. Therefore, it is anticipated that BAL fluid will be a clinically useful tool in identifying infectious and inflammatory diseases of the lower portion of the respiratory tract in rabbits. Similarly, research into drug disposition or inhalation-induced injury would be facilitated by documentation of an inflammatory response. Differential cell counts were similar between lobes in healthy rabbits; however, this may not be true in diseased rabbits. Hawkins et al determined that 37% of BAL fluid samples had different results between lobes regarding differential cell percentages in dogs with respiratory tract disease.

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Six of 9 rabbits in this study had aerobic bacterial growth, but most of the cultures had only small numbers of mixed bacterial species. This was not unexpected because results of aerobic cultures are frequently positive in BAL fluid samples from healthy small animals. In studies performed in healthy cats, 44% to 77% of cats had positive results of aerobic cultures. Given the low numbers of organisms cultured, the lack of bacteria identified via cytologic and histologic examinations, and the lack of clinical respiratory tract signs in these rabbits prior to BAL, it is probable that these bacteria represent normal flora of the respiratory tract but they may also indicate oral contamination of the lavage fluid. In this study, the bronchoscope was wiped with sterile saline solution before the BAL was performed to minimize contamination from the oropharynx, but the oropharynx is narrow in rabbits, which might make minimizing oropharyngeal contamination more difficult. To our knowledge, the normal oropharyngeal flora of rabbits have not been reported. However, whenever a mixed bacterial population is present, pharyngeal contamination should be considered. Bacteria resembling Simonsiella spp, a known pharyngeal inhabitant of dogs and cats, were identified in 4 of 18 of the rabbit samples in this study. Rabbits are a coprophagic species, so it is possible that cecal flora may also be found in their oropharynx. B fragilis is the most common cecal microflora in rabbits and was identified in anaerobic cultures from the lower airways of 2 of 9 rabbits in this study.

Bronchoscopic BAL is challenging in rabbits because of the small diameter of the trachea, which precludes intubation during the procedure. Nevertheless, in this study, BAL performed in anesthetized New Zealand White rabbits during bronchoscopic examination was quick and provided adequate sample recovery volume for cytologic and microbiologic evaluation. Percentage fluid recovery in the present study was lower than that reported from a previous rabbit study (68% to 79%) in which instillates volumes approximating 80% of the total lung volume were used. Total fluid volumes of 25 to 38 mL (12 to 15 mL/kg) used in that study were > 10 times the total fluid volumes of 6 mL (1.4 to 2.2 mL/kg) used in our study. In dogs and cats, greater percentage volumes have been recovered during bronchoscopic BAL than in our study; however, greater total instillate volumes, multiple lavage procedures, or both were commonly used. For example, in healthy dogs, approximately 65% of the fluid was recovered when 6.6 to 11.6 mL/kg were instilled. The percentage recovery for healthy cats ranged from 50% to 79% when instillate volumes of 10.9 to 15 mL/kg were used, and greater volumes were recovered after repeated lavage. In a recent study in cats with respiratory disease, 51% to 73% of the fluid was recovered when only 3 to 5 mL/kg was instilled. Percentage recovery may have been higher if larger volumes had been instilled or multiple lavage procedures had been performed. However, in the clinical setting, minimizing anesthesia and procedural time for a compromised patient with respiratory tract disease is often critical for survival. Results determined here suggested that acceptable fluid recovery can be obtained after a single administration of a small volume of fluid in New Zealand White rabbits when bronchoscopic BAL is performed.

The quality of patient recovery from the BAL procedure is an important clinical objective when performing BAL in pet rabbits and in rabbits used in research. In a study of nonendoscopic collection of distal airway fluid, rabbits recovered from the BAL procedure with only a slight increase in respiratory rate during the first 24 hours after the procedure but no other variables were evaluated. In the study reported here, hemoglo-
bin saturation decreased during and immediately after BAL but postprocedural recovery was not evaluated. However, pulse oximetry data revealed that hemoglobin saturation returned to preprocedure values in 8 of the 9 rabbits. Pulse oximetry is an important noninvasive tool for the evaluation of arterial oxygenation, which has been termed the fifth vital sign.\textsuperscript{17,38} Current pulse oximetry techniques should not result in systematic errors when used in rabbits\textsuperscript{28,40} and are widely used in rabbit clinical medicine and research. Pulse oximetry in rabbits provides an accurate estimate of arterial oxygen saturation exceeding 85%.\textsuperscript{39} Although this has not been specifically evaluated in rabbits, these readings are considered to be accurate to within 4% (95% confidence intervals) when arterial oxygen saturation exceeds 70%.\textsuperscript{41}

In vitro measurements of absorption characteristics of hemoglobin measured at the wavelengths of interest revealed no differences between rabbit and human hemoglobin.\textsuperscript{37} and in vivo comparisons of SpO\textsubscript{2} and arterial oxygen saturation in Swedish Landrace rabbits revealed good correlation.\textsuperscript{39} Ideally, arterial blood gases would be evaluated in the event of low SpO\textsubscript{2} to determine arterial oxygenation. On the basis of decreased hemoglobin saturation in rabbits examined here, intubation and oxygen supplementation would be recommended before BAL when technically possible. Alternately, jet ventilation could be used throughout the procedure to improve oxygenation.\textsuperscript{33} Minimally, the rabbit should be maintained in sternal recumbency with oxygen provided by chamber or BAL when technically possible. Alternately, jet ventilation and oxygen supplementation would be recommended after arterial oxygenation. On the basis of decreased hemoglobin saturation during and immediately after BAL, it is likely that the rabbit is fully recovered. Slow anesthetic face mask and respiratory rate and effort carefully monitored until the rabbit is fully recovered. Slow anesthetic face mask and respiratory rate and effort carefully monitored during and immediately after BAL when technically possible. Alternately, jet ventilation and oxygen supplementation would be recommended after arterial oxygenation. On the basis of decreased hemoglobin saturation in rabbits examined here, intubation and oxygen supplementation would be recommended before BAL when technically possible. Alternately, jet ventilation could be used throughout the procedure to improve oxygenation.\textsuperscript{33} Minimally, the rabbit should be maintained in sternal recumbency with oxygen provided by chamber or BAL when technically possible. Alternately, jet ventilation and oxygen supplementation would be recommended after arterial oxygenation. On the basis of decreased hemoglobin saturation during and immediately after BAL, it is likely that the rabbit is fully recovered. Slow anesthetic face mask and respiratory rate and effort carefully monitored until the rabbit is fully recovered.