How to Perform a Bronchoalveolar Lavage Using a Three-Meter Gastroscope

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1. Introduction
Bronchoalveolar lavage (BAL) is useful for the diagnosis of diffuse, non-infectious respiratory disease in the horse. Samples obtained using BAL are not suitable for bacterial culture because BAL catheters or endoscopes must be passed through the nasopharynx before entering the distal airways. For these reasons, BAL is most indicated for the diagnosis of non-infectious respiratory disease causing poor athletic performance such as recurrent airway obstruction or heaves (RAO), inflammatory airway disease (IAD), and exercise-induced pulmonary hemorrhage (EIPH). 1 There is a good correlation between BAL fluid cytology and pulmonary histopathology. 1 Only a local segment of distal airway is sampled using BAL; therefore, it must be assumed that pulmonary disease is diffuse when interpreting the results. Although horses with EIPH generally bleed from the caudodorsal lung fields, demonstration of hemosiderophages in BAL fluid is more sensitive for the diagnosis of EIPH than observing blood in the trachea after exercise. Bronchoalveolar lavage is more reliable than transtracheal aspirates (TTAs) to diagnose lower airway inflammation. However, heavy growth of fungi from a BAL sample can support the diagnosis of fungal pneumonia in suspected cases. TTAs are indicated for culture of the respiratory tract and provide a sample from the entire lower respiratory tract.

Blind passage of a BAL catheter most often results with the catheter lodging in the right caudodorsal lung field. 1 Passage of a 3-m gastroscope to perform BAL allows direct visualization of the tracheal and bronchial lumens and any respiratory secretions that may be present. Additionally, any abnormal discharges may be followed to the affected segments of lung.

This paper describes the use of a 3-m gastroscope for performing BAL in a standing, sedated horse. This technique is quick and relatively atraumatic for the horse and veterinarian and allows excellent visualization of the area of the lung sampled.

2. Materials and Methods
Horses are sedated using detomidine (0.01–0.02 mg/kg, IV) or xylazine (0.5 mg/kg, IV) and butorphanol (0.02–0.04 mg/kg, IV), and a twitch is applied as needed. 2 It is important that the horse be adequately sedated or restrained to prevent head movement during the procedure. Before the BAL, two 60-ml syringes are filled with 0.9% saline, and a third is filled with 30 ml of sterile 2% lidocaine and 30 ml of 0.9% saline. A clean 3-m gastroscope is
passed through a nostril and the pharynx into the trachea. The tracheal lumen is observed for the presence of mucopus and/or hemorrhage. The gastroscope is passed into a mainstem bronchus (Fig. 1) and advanced through progressively smaller bronchi (Fig. 2) until the endoscope can be felt to wedge into a distal bronchus. Some horses may require injection of the syringe containing the lidocaine and saline to reduce coughing and provide local analgesia before the endoscope becomes wedged within a bronchus. When the gastroscope can no longer be advanced, the remaining syringes containing isotonic saline are quickly injected through the endoscope instrument channel into the bronchus. As soon as the final syringe has been emptied, suction is applied to the syringe, and the lavage fluid is aspirated. The second and third syringes are also used to evacuate as much of the lavage fluid as possible. Generally, the yield of aspirated fluid is good (>50% of the initial volume injected). A diagnostic BAL sample will be very foamy, because it should contain much surfactant. If mucus and cellular material are visible, there is likely to be abundant material for cytologic evaluation.

The BAL fluid is filtered through a clean gauze sponge to remove surfactant and any debris from the end of the endoscope and collected into a sterile specimen cup. The second and third syringes obtained will have the best samples (appear the most foamy) for cytologic examination. The fluid in the first syringe collected is usually discarded or used to moisten the sponge before collecting the fluid from the second and third syringes.

The BAL fluid is transferred into clean 10- to 15-ml centrifuge tubes and centrifuged for 5 min at ~3000 rpm. If considerably higher speeds are used, the sample will be difficult to resuspend. With slower speeds, the cellular material will remain suspended rather than settling into a pellet. All but ~1 ml of the supernatant should be removed. The pellet is resuspended in 1 ml of saline. A small amount of the mixture is transferred onto a glass microscope slide closer to one end of the slide (about one third of the way along the slide) using a plastic transfer pipette, wooden applicator stick, or a needle and syringe. The drop should be ~0.25–0.33 cm in diameter. Another clean glass slide is placed on top of the first slide, allowing the drop of material to start to spread, and the two slides are pulled apart horizontally, making certain to have the two slides remain perfectly parallel to each other. The resultant smeared material should end before the end of the slide, such that there are edges on all sides of the material (Fig. 3). If the collected BAL fluid does not appear cellular, cloudy, or frothy, centrifuge as above but resuspend in 0.5 ml of saline or less and prepare squash preparations. Ideally, if the sample is cell-poor, a cytocentrifuged (cytospin) preparation should be made from the centrifuged material such that additional concentration is achieved.

The smears should be completely air-dried. Make certain to avoid excessive heat (do not heat fix!) and formalin fumes (do not prepare or store near containers of formalin). Both of these cause major problems with proper preservation and staining of the cells. Slides are stained using Wright-Giemsa–type stains. If EIPH is suspected, Prussian blue type stains are useful to identify hemosiderophages.

Ship prepared slides (make two to four slides) to the laboratory of your choice in a small box or have pickup by the laboratory courier service. Do not

Fig. 1. Endoscopic view of the tracheal carina and the mainstem bronchi.

Fig. 2. Endoscopic view of distal bronchi.

Fig. 3. Preparation of BAL slides using the squash technique.
put glass slides in cardboard slide mailers in unpad-
ded envelopes and send through mail because auto-
mated canceling machines will break glass slides.
Additional padding is needed to assure safe transit
in the mail.

3. Results
The authors have used this technique exclusively for
the last 6 yr to obtain BAL fluid from horses sus-
pected of RAO or EIPH without major complica-
tions. Most horses are very tolerant of the
procedure, and it can be completed in <5 min.
Some horses may cough vigorously as the gastro-
scope is passed into the distal airways. Thus, it is
important to have the syringe containing lidocaine
and saline ready when passing the endoscope so that
it can be injected when needed. Most horses be-
come more comfortable quickly after injection of li-
docaine. If an adequate BAL sample is not
obtained, it is most often caused by not having the
gastroscope properly wedged into a small bronchus.
Horses are placed back into their stalls or dis-
charged as soon as the effects of sedation have dis-
appeared. Horses may be returned to work 24–48 h after BAL.

4. Discussion
Advantages of using a 3-m gastroscope to perform
BAL is that the airways can be visualized while they
are sampled and that the procedure can be com-
pleted in a short period of time with minimal dis-
comfort for the horse. Tracheal mucopus is usually
observed in horses affected with RAO or IAD.
Hemorrhage in the lumen of the trachea may be
observed in some horses with EIPH, depending on
when they are examined after exercise.1 Allergic
airway disease such as RAO or IAD may predispose
some horses for EIPH by causing inflammation of
the bronchioles. Some iatrogenic hemorrhage may
occur using an endoscope for BAL; therefore, careful
passage and wedging of the endoscope are important
to reduce mucosal trauma.

The procedure can be rapidly performed in a
standing, sedated horse by passing the 3-m gastro-
scope into the trachea and successive bronchi until it
becomes wedged. Three 60-ml syringes filled with
isotonic saline are injected through the gastroscope
and rapidly aspirated to obtain the BAL sample.
Direct visualization of the airways speeds the pro-
cedure and allows accurate guidance of the endo-
scope through the airway to the desired location in a
terminal bronchus.

Endoscopic BAL allows another clinically valu-
able use of the 3-m gastroscope. The technique is
straightforward and quick and provides much infor-
mation for the diagnosis of lower airway disease
such as RAO, IAD, and EIPH.

References
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Effects of antitussive agents administered before bronchoal-