Journal of the American Association for Laboratory Animal Science
Copyright 2006
by the American Association for Laboratory Animal Science

Vol 45, No 4
July 2006
Pages 44–48

Use of Human Nasal Cannulas during Bronchoscopy Procedures as a Simple Method for Maintaining Adequate Oxygen Saturation in Pigtailed Macaques (*Macaca nemestrina*)

Matthew J Thomas,[1,*] Leon R Flanary,[2] Bruce A Brown,[2] Michael G Katze,[1,2] and Carole R Baskin[1, 2]

Rising concerns over respiratory illnesses caused by agents such as avian influenza viruses and SARS coronavirus have prompted intensive research efforts and the resurgence of nonhuman primates as models for these human diseases. In the context of studying influenza infection and vaccine development, serial bronchoscopic procedures, including bronchial brush biopsies and bronchoalveolar lavage, were performed in pigtailed macaques (*Macaca nemestrina*). The possible need for oxygen supplementation during these procedures was anticipated because of the size of the animals relative to the 5-mm bronchoscope. We therefore monitored oxyhemoglobin saturation, a measure of arterial blood oxygen content, before and after insertion of the bronchoscope, during bronchoalveolar lavage, and after initiation of oxygen supplementation. Although more data are required to draw definitive conclusions, our findings suggested the need for oxygen supplementation during such procedures in nonhuman primates, despite the fact that human patients undergoing bronchoscopy and lavage do not routinely get oxygen unless they are already compromised. Our data also suggested that the need for supplementation could not be predicted from simple parameters such as size of the animal, presence of respiratory clinical signs, or experimental treatment. Finally, we show a simple and cost-effective method of using human nasal cannulas for delivering oxygen to pigtailed macaques during bronchoscopic procedures, and we believe that, after further testing, this method could be used safely and effectively in other nonhuman primate species.

Abbreviation: spO_2, oxygen saturation of arterial hemoglobin

Nonhuman primates have been important models of human respiratory diseases, and recent concerns about avian influenza as a zoonotic pathogen and SARS coronavirus as an emerging cause of human morbidity and mortality have intensified use of these animals in a research setting.[3,10,14-16] One relatively noninvasive method of monitoring and documenting progression of pathology in the respiratory tract of the live animal has been the use of flexible bronchoscopy.[5,7,18] Bronchoscopy also allows the use of bronchial brushes or biopsy forceps through the instrument port to sample cells and tissue from consistent anatomical locations to be processed for further analyses. One important risk of bronchoscopy is the partial but considerable obstruction of the airways during visual exploration and tissue sampling.[20] This obstruction worsens during bronchoalveolar lavage and often leads to a drop in the saturation of arterial oxyhemoglobin.[9] Such a drop is presumably indicative of hypoxemia and can lead to hypoxic hypoxia, the 1st term being defined as a drop in arterial content of oxygen and the 2nd as a decrease in tissue oxygen supply below normal levels that can be caused by airway obstruction, among other etiologies.[17] Hypoxia secondary to bronchoscopic procedures is discussed in the human medical literature, in terms of reliably predicting who will need supplementation while avoiding the cost of providing oxygen for everyone undergoing the procedure.[9,11,12,20]

Received: 12 Dec 2005. Revision requested: 31 Jan 2006. Accepted: 1 Feb 2006.
[1]Department of Microbiology, University of Washington, Seattle, Washington; [2]Washington National Primate Research Center, Seattle, Washington.
[*]Corresponding author. Email: mnkyboy@u.washington.edu

In this report, we describe a set of data that suggests the difficulty of predicting which animals will require oxygen supplementation during a bronchoscopic procedure and a simple, quick, and cost-effective technique for oxygen delivery in pigtailed macaques (*Macaca nemestrina*) involving the use of human nasal cannulas and monitoring with pulse oximetry. This method could easily be modified for other species after adequate safety testing and should be considered when performing bronchoscopic procedures in nonhuman primates.

We developed this protocol to provide optimal supportive care during serial bronchoscopic procedures in pigtailed macaques used as models of influenza infection and in the context of testing a new live attenuated vaccine. Bronchoscopy was used to monitor inflammatory changes in the upper respiratory tract and to obtain bronchial brush biopsies, as well as being used during bronchoalveolar lavage.

Materials and Methods

For the purpose of this study, we obtained 7 female and 2 male pigtailed macaques (*Macaca nemestrina*), ranging in age from 3.25 to 13.6 y and in weight from 6.6 to 13.6 kg, from the Washington National Research Primate Center, a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. None of these animals had undergone any major treatments in the past. These animals were seronegative for type D simian retrovirus, simian T cell leukemia virus, and simian foamy virus. In addition, a preliminary physical exam 2 wk prior to assignment, as well

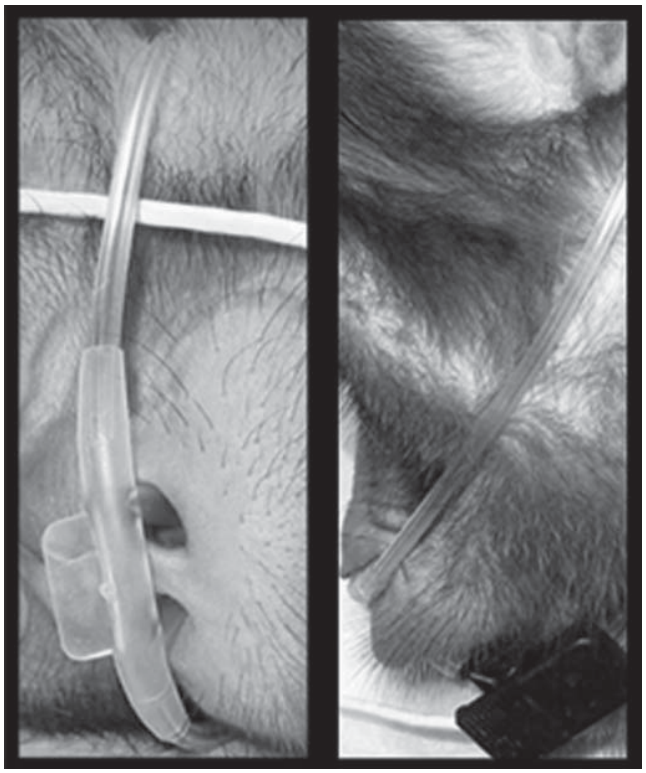

Figure 1. Placement of the human nasal cannula. The nasal cannula is inserted into both nostrils over the cord of the O_2 saturation probe, passed under both ears, and cinched behind the base of the skull to help secure it in place. The O_2 saturation probe is placed on the lip so as to not interfere with placement of the bronchoscope.

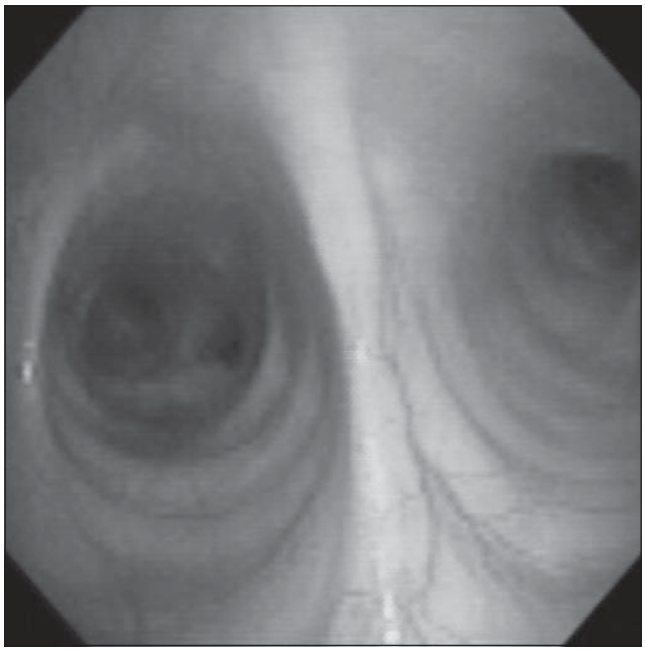

Figure 2. Photograph showing the typical placement of the bronchoscope during these procedures. For all of the O_2 saturation measurements, we placed the bronchoscope at the primary bifurcation of the trachea.

as a complete blood count and serum chemistry panel (both processed at the University of Washington Medical Center), ruled out health problems that would have rendered an animal unsuitable for the present study. All animals were moved into the room several days prior to start of the experiments, to ensure acclimation. Animals were housed in 3 experimental groups of 4, which represented a mock group and 2 groups who were administered different vaccine regimens. These groups and their experimental status were not determined relevant for the studies described in this paper. During the study, the room was categorized as Enhanced Biosafety Level 2, and all procedures were performed according to guidelines approved by the University of Washington Environmental Health and Safety Office, Occupational Health Administration, Primate Center Research Review Committee, and the Institutional Animal Use and Care Committee. Animals were fed a standard diet throughout the study (Fiber Plus Monkey Diet 5049, LabDiet, PMI Nutrition International, St Louis, MO) which was supplemented with daily fresh produce through the institutional environmental enrichment program, and they were monitored daily for appetite and general health.

Complete physical exams were performed on animals that were chemically restrained (tiletamine–zolazepam at 2 to 10 mg/kg intramuscularly[6] and atropine at 0.02 to 0.05 mg/kg intramuscularly) prior to every bronchoscopic procedure to rule out conditions that might preclude them from undergoing procedures safely. Atropine was used in all animals to reduce saliva production and subsequent risks of aspiration.[13] Atropine would have also decreased airway resistance and therefore improved lung oxygenation.[1] Although the use of atropine is contraindicated in some pulmonary patients with cough because the drug thickens bronchial secretions, this side effect did not occur in these animals.[1] Pulse oximetry measurements were made continuously with a pulse oximeter (Model 9847V, Nonin Medical, Plymouth, MN,). The pulse oximeter sensor was consistently and securely placed on the bottom lip of the animals (Figure 1), and that placement resulted in a reliable signal. Although the manufacturer recommended placing the sensor on an animal's tongue, this option was not practical during bronchoscopy.

Bronchoscopy was performed with a 5-mm video bronchoscope (model BF-P200, Olympus America, Melville, NY) with the animal in lateral recumbency. The 2.2-mm instrument port on that model was the smallest available that could accommodate the sheathed bronchial brushes that were needed to fulfill the goals of the main study. For the purpose of measuring the percentage of oxyhemoglobin saturation, the flexible bronchoscope, inserted with the help of a laryngoscope of appropriate size (WelchAllyn, Skaneateles Falls, NY), was placed immediately proximal to the primary bifurcation of the trachea (the carina; Figure 2). Bronchial lavages were done by quickly injecting and aspirating 10 ml of standard bronchoalveolar lavage fluid[4] through the bronchoscope's instrument port. This volume has provided diagnostic cytology in the past[3] and was in part chosen because the experimental protocol for the main study required serial lavages.

Oxygen was supplemented at 2 l/min through a nasal cannula of a type commonly used in human medicine (Ozone Services, Burton, British Columbia, Canada). These cannulas have a funnel-type connector and easily fit standard oxygen flow meters. The tips of the cannula were inserted into the animal's nostrils, and the tubing was passed under both ears and positioned so that the adjustment ring was behind the head (Figure 1). The cannula then was secured by sliding the adjustment ring to create a tight fit of the tubing around the skull.

Measurements of oxygen saturation of arterial hemoglobin (spO_2) were taken every 30 s for 5 min, starting prior to insertion of the bronchoscope. Oxygen delivery was initiated

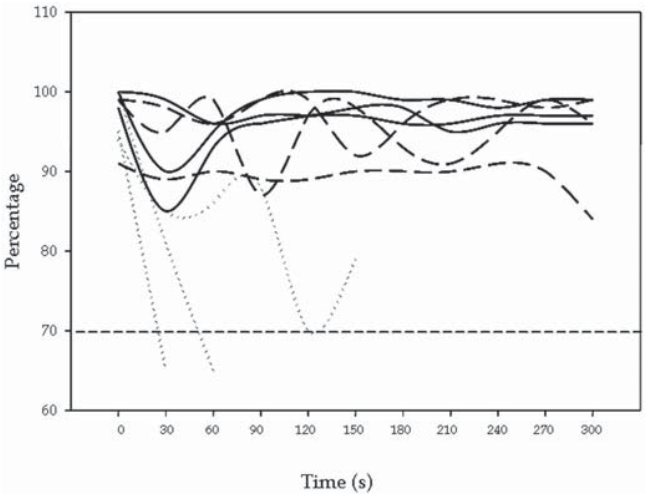

Figure 3. Percentage saturation of oxyhemoglobin for all 9 animals during bronchoscopy without O_2 supplementation, with the bronchoscope placed as shown in Figure 2. There was a wide range of responses to the procedure without discernable influence of age or weight. Several of the animals (solid lines) had an initial drop but quickly recovered on their own. Others (dashed lines) had more variations but did not require oxygen supplementation. Animals that dropped below 70% saturation (dotted lines) received O_2 at 2 l/min without delay. All other animals were given oxygen after 300 s.

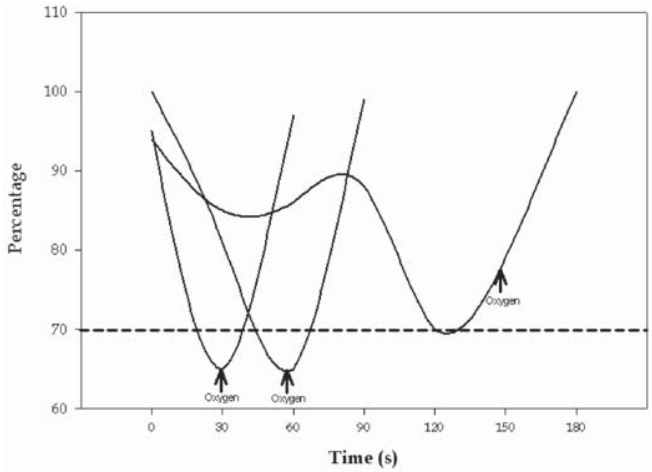

Figure 4. Percentage saturation of oxyhemoglobin before and after O_2 supplementation of the 3 animals whose oxyhemoglobin saturation dropped below 70% during bronchoscopy alone (Figure 3). Once oxygen was administered at 2 l/min (arrows), oxyhemoglobin returned to 100% O_2 saturation within 30 s. Likewise, all animals that did not need O_2 supplementation reached 100% oxyhemoglobin saturation within a few seconds once O_2 was administered after 300 s (not shown).

whenever spO_2 dropped below 70% for 30 s or 2 consecutive measurements. After 5 min, all animals were supplemented with oxygen regardless of their spO_2 value. On a different day, measurements were performed according to the same protocol, but a bronchoaveolar lavage was added after 5 min, and all animals were supplemented with oxygen, regardless of their spO_2 value, 2 min after the lavage was initiated.

Statistical calculations were made using the statistical cutoffs described in the results section with SigmaStat for Windows (Systat Software, Point Richmond, CA) and figures were made with SigmaPlot for Windows (Systat Software, Point Richmond, CA).

Results

Effect of bronchoscopy on oxyhemoglobin saturation. All baseline spO_2 measurements were greater than 90%, indicative of adequate lung oxygenation. After insertion of the bronchoscope, spO_2 values showed wide variation that could not be attributed to their respective experimental groups or to other physical parameters (Figure 3); although all animals exhibited a drop in spO_2 within the first 90 s after placement of the bronchoscope, the magnitude of this change varied from a few percent to almost 30%. Those animals that demonstrated a very modest drop in oxyhemoglobin saturation recovered quickly on their own to their baseline level or even higher. Other animals showed wide fluctuation in spO_2 during bronchoscopy but never dropped to a level that would be cause for concern. In these animals, spO_2 rose to 100% almost instantly when oxygen administration was initiated after 5 min. However, 3 animals exhibited dramatic decreases in spO_2 below 70% and had to be supplemented with oxygen almost immediately. These animals quickly recovered when oxygen was administered (Figure 4). We confirmed that spO_2 measurements were significant ($P \leq 0.02$, 2-way repeated measures analysis of variance) between these identified groups. Among all animals, however, time was the only significant ($P = 0.05$) factor in accounting for spO_2 variations. Finally, time, age, and weight all were unsuccessful in predicting spO_2 in best-subset regression analysis.

Effects of bronchoscopy and bronchoalveolar lavage on oxyhemoglobin saturation. A similar procedure was performed on a different day to measure spO_2 during bronchoalveolar lavage in addition to bronchoscopy without concurrent oxygen supplementation. The lavage was initiated 5 min into the procedure to allow for spontaneous recovery of oxyhemoglobin saturation in some animals, as previously described. One animal did not undergo lavage because its hemoglobin oxygen saturation quickly dropped to 70% during bronchoscopy, and this animal had to be supplemented with oxygen. Of note, this animal did require oxygen supplementation during the 1st set of measurements. The placement of the flexible bronchoscopy was at the same location as described earlier, and it was apparent that the lavage fluid was causing physical obstruction of the airway (Figure 5). All remaining animals exhibited a clinically significant drop in spO_2 during and immediately after delivery of the lavage solution (Figure 6): although the oxyhemoglobin saturation did not warrant oxygen supplementation according to our preset standards, it did reach levels as low as 75%. At 2 min into the lavage procedure, all animals were given oxygen, and all quickly returned to preprocedural levels, although 2 of the 9 animals took as long as 2 min to fully recover. Neither of these 2 animals had required oxygen supplementation during the 1st set of measurements. We confirmed that spO_2 measurements were significant ($P \leq 0.01$, 2-way repeated-measures analysis of variance) between these 2 groups. Among all animals, however, time was the only significant ($P \leq 0.01$) factor in accounting for spO_2 variations. Again, time, age, and weight were unsuccessful in predicting spO_2 in best-subset regression analysis.

Discussion

Oxygen supplementation is not used during routine bronchoscopic procedures in human patients, although it is recommended.[11] Pigtailed and other macaques are significantly smaller than adult humans, but human-size bronchoscopes are commonly used in these animals because of the lower cost and greater availability of refurbished models; therefore, the need for oxygen supplementation is likely to be much higher. The present data suggest that clinically significant obstruction of the airways

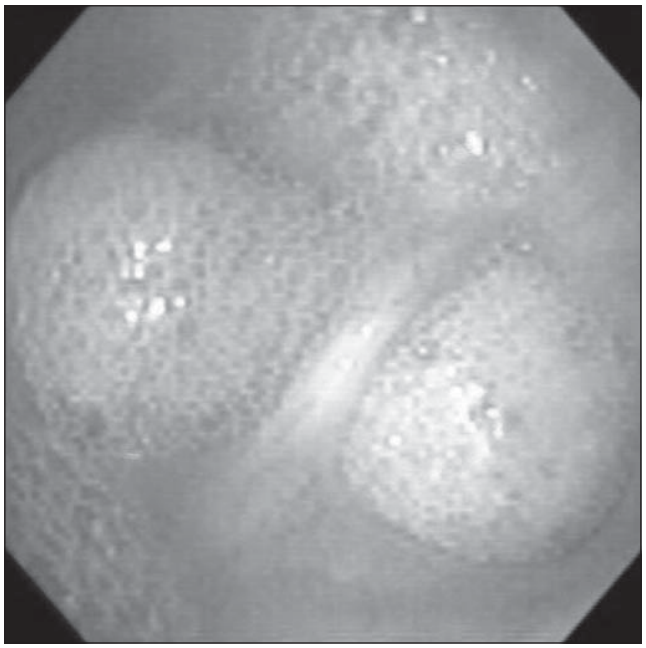

Figure 5. Extent of airway obstruction during bronchoalveolar lavage. Although the fluid is absorbed within a few seconds, impairment of effective airway oxygenation may last longer in some animals, as our data suggest.

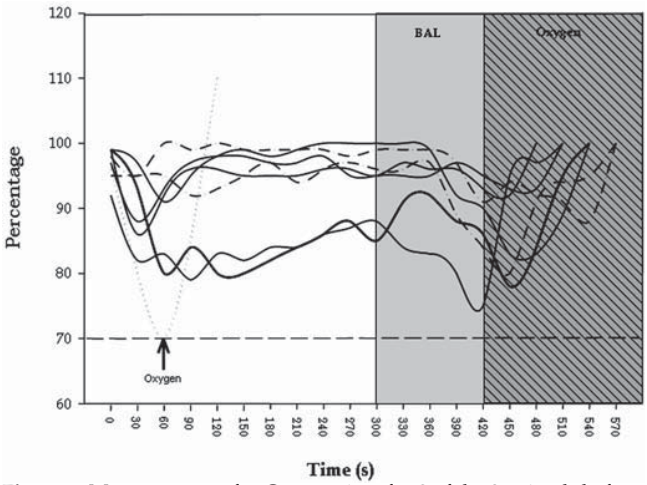

Figure 6. Measurement of spO_2 over time for 8 of the 9 animals before and during bronchoalveolar lavage (BAL). One animal was excluded because it was wheezing slightly during the physical exam. Most animals experienced an initial drop in spO_2 after the lavage fluid was injected, and they recovered to different extents during the following 120 s (light shading). All animals benefited from having oxygen administered after that time (diagonal shading); most recovered completely within 50 to 110 s of oxygen administration, although 2 animals took as long as 150 s (dashed lines). The animal (dotted line) that had dropped below 70% spO_2 after 60 s and prior to the lavage was given oxygen at 2 l/min arrow), after which its spO_2 returned to 100% within 30 s.

occurs frequently and unpredictably in pigtailed macaques undergoing bronchoscopy, even without bronchoalveolar lavage. Indeed, in all cases, the need for oxygen supplementation did not depend on size, age of the animal, or experimental group (Figures 3, 4, and 6). The role of gender was more difficult to gauge because of the disproportionate numbers of females used in the study, but it would not be expected to play a significant role. The fact that 2 of the animals that had difficulties during the 2nd set of measurements and during the lavage were different from the ones who did require oxygen during the 1st session also suggests the unpredictability of the need for supplementation. Interestingly, both of these animals belonged to the same experimental group, which could have been a factor.

Assessing oxyhemoglobin saturation by measuring light absorbance of pulsating vascular tissue at 2 different wavelengths (650 and 805 nm) provides only an estimate of arterial blood oxygenation, but this estimate is considered to be accurate, and pulse oximetry is regarded as a time-tested and reliable clinical tool.[2] Although bronchoscopy has been used increasingly in veterinary medicine, the question of oxygen supplementation in small animals and nonhuman primate species has not been pursued, to the best of our knowledge, except in patients with respiratory compromise.[8,19] In contrast, the subject has been fairly extensively discussed in human medicine, even when respiratory distress due to a pre-existing condition is not a concern.[11,20] After the relatively modest initial investment in the pulse oximeter, nasal cannulas and oxygen use for these short procedures constitute a nominal additional investment with potentially great benefits, considering the costs of the animals and bronchoscopic apparatus. More data is needed to ascertain the safety and efficacy of supplementing oxygen during all bronchoscopic procedures in this and other nonhuman primate species, but all animals in this small study responded positively, even though their responses were very variable among different individuals and from one day to the next. Therefore, we recommend that oxygen supplementation should be further tested for routine use during bronchoscopic procedures in nonhuman primates and other species.

Acknowledgments

This work was supported by the National Institutes of Health and by the staff at The Washington National Primate Research Center. In particular, we would like to thank Kay Larson, MS, for her skilled technical assistance. We also would like to credit Leon R Flanary for medical photography.

References

1. **Aiello SE, Mays A, Amstutz HE,** editors. 2002. The Merck veterinary manual. Whitehouse Station (NJ): Merck & Co.
2. **Barker SJ, Tremper KK.** 1987. Pulse oximetry: applications and limitations. Int Anesthesiol Clin **25:**155–175.
3. **Baskin CR, Garcia-Sastre A, Tumpey TM, Bielefeldt-Ohmann H, Carter VS, Nistal-Villan E, Katze MG.** 2004. Integration of clinical data, pathology, and cDNA microarrays in influenza virus-infected pigtailed macaques (*Macaca nemestrina*). J Virol **78:**10420–10432.
4. **Bielefeldt Ohmann H, Babiuk LA.** 1986. Bovine alveolar macrophages: phenotypic and functional properties of subpopulations obtained by Percoll density gradient centrifugation. J Leukoc Biol **39:**167–181.
5. **Coffman RL, Hessel EM.** 2005. Nonhuman primate models of asthma. J Exp Med **201:**1875–1879.
6. **Cohen BJ, Bree MM.** 1978. Chemical and physical restraint of nonhuman primates. J Med Primatol **7:**193–201.
7. **Croix DA, Board K, Capuano S III, Murphey-Corb M, Haidaris CG, Flynn JL, Reinhart T, Norris KA.** 2002. Alterations in T lymphocyte profiles of bronchoalveolar lavage fluid from SIV- and *Pneumocystis carinii*-coinfected rhesus macaques. AIDS Res Hum Retroviruses **18:**391–401.
8. **Dye TL, Teague HD, Poundstone ML.** 1998. Lung lobe torsion in a cat with chronic feline asthma. J Am Anim Hosp Assoc **34:**493–495.
9. **Golpe R, Mateos A, Jones AM, O'Driscoll BR.** 2002. Supplemental oxygen during flexible bronchoscopy. Chest **121:**664–665.

10. **Haagmans BL, Kuiken T, Martina BE, Fouchier RA, Rimmelzwaan GF, van Amerongen G, van Riel D, de Jong T, Itamura S, Chan KH, Tashiro M, Osterhaus AD.** 2004. PEGylated interferon-alpha protects type 1 pneumocytes against SARS coronavirus infection in macaques. Nat Med **10:**290–293.
11. **Jones AM, O'Driscoll R.** 2001. Do all patients require supplemental oxygen during flexible bronchoscopy? Chest **119:**1906–1909.
12. **Kvale PA.** 2002. Prevention and management of hypoxemia during fiberoptic bronchoscopy. Chest **121:**1021–1022.
13. **Martin DP, Darrow CC II, Valerio DA, Leiseca SA.** 1972. Methods of anesthesia in nonhuman primates. Lab Anim Sci **22:**837–843.
14. **Rimmelzwaan GF, Claas EC, van Amerongen G, de Jong JC, Osterhaus AD.** 1999. ISCOM vaccine induced protection against a lethal challenge with a human H5N1 influenza virus. Vaccine **17:**1355–1358.
15. **Rimmelzwaan GF, Kuiken T, van Amerongen G, Bestebroer TM, Fouchier RA, Osterhaus AD.** 2001. Pathogenesis of influenza A (H5N1) virus infection in a primate model. J Virol **75:**6687–6691.
16. **Rimmelzwaan GF, Kuiken T, van Amerongen G, Bestebroer TM, Fouchier RA, Osterhaus AD.** 2003. A primate model to study the pathogenesis of influenza A (H5N1) virus infection. Avian Dis **47:**931–933.
17. **Stedman TL.** 2004. The American Heritage Stedman's medical dictionary. Boston: Houghton Mifflin.
18. **Tate MK, Rico PJ, Roy CJ.** 2004. Comparative study of lung cytologic features in normal rhesus (*Macaca mulatta*), cynomolgus (*Macaca fasicularis*), and African green (*Chlorocebus aethiops*) nonhuman primates by use of bronchoscopy. Comp Med **54:**393–396.
19. **Tseng LW, Waddell LS.** 2000. Approach to the patient in respiratory distress. Clin Tech Small Anim Pract **15:**53–62.
20. **Yildiz P, Ozgul A, Yilmaz V, Jones AM, O'Driscoll BR.** 2002. Changes in oxygen saturation in patients undergoing fiberoptic bronchoscopy. Chest **121:**1007–1008.