Optimization of Imaging and Bronchoalveolar Lavage Techniques to Improve Diagnostic Yield of Feline Lower Respiratory Tract Samples

by

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ABSTRACT

OPTIMIZATION OF IMAGING AND BRONCHOALVEOLAR LAVAGE TECHNIQUES TO IMPROVE DIAGNOSTIC YIELD OF FELINE LOWER RESPIRATORY TRACT SAMPLES

Kimberly Hooi
University of Guelph, 2018
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The process of investigation of feline lower respiratory tract disease can present multiple difficulties to the veterinary practitioner. Radiography is considered the first line imaging diagnostic test; however, in a proportion of cats no changes are identified. To characterize the etiology of lower respiratory tract clinical signs, sampling of the lower respiratory tract is required. Bronchoalveolar lavage (BAL) is a minimally invasive technique that is utilized to collect samples from the distal airways and alveoli. Simply, this technique involves instillation of sterile saline and re-aspiration to collect bronchoalveolar lavage fluid. In cats, two main techniques have been described but have not been compared. The purpose of this research project was to compare the two techniques for BAL in cats – bronchoscopic-BAL (B-BAL) and non-bronchoscopic-BAL (NB-BAL) and their effect on sample quality in healthy cats without respiratory tract disease and to describe a new technique for collecting BAL fluid (BALF) in cats – fluoroscopic guided BAL (F-BAL). B-BAL retrieved a higher proportion of BALF than NB-BAL but there was no difference in the sample quality in regard to cellularity and cell preservation. Using fluoroscopy, it was determined that sampling of specific lung lobes was achievable and the BALF samples retrieved were of excellent cytologic quality, cellularity and cell preservation. Either B-BAL or NB-BAL can be utilized to collect BALF in cats with signs of lower respiratory tract disease, however in order to improve diagnostic yield of bronchoalveolar lavage, a technique that allows sampling of
specific lung lobes should be considered. Therefore, where a bronchoscope is not available or feasible, F-BAL is a suitable alternative to B-BAL.
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DECLARATION OF WORK PERFORMED

I declare that, with the exception of the works listed below, all word in this thesis was performed by me, Kimberly Hooi.

With my guidance and support, Stipe Jelovecic collected data for the retrospective study for Chapter 4.

I, Kimberly Hooi, performed all writing, graphing and table formatting in this thesis with editorial comments made by Dr. Alice Defarges, Dr. Dorothee Bienzle, Dr. Stephanie Nykamp and Dr. Scott Weese.

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<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BAL</td>
<td>Bronchoalveolar lavage</td>
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<tr>
<td>BALF</td>
<td>Bronchoalveolar lavage fluid</td>
</tr>
<tr>
<td>B-BAL</td>
<td>Bronchoscopic bronchoalveolar lavage</td>
</tr>
<tr>
<td>CBC</td>
<td>Complete blood count</td>
</tr>
<tr>
<td>CRI</td>
<td>Continuous rate infusion</td>
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<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
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<tr>
<td>ELISA</td>
<td>Enzyme linked immunooassay</td>
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<tr>
<td>F-BAL</td>
<td>Fluoroscopic bronchoalveolar lavage</td>
</tr>
<tr>
<td>FNA</td>
<td>Fine needle aspiration</td>
</tr>
<tr>
<td>HRCT</td>
<td>High resolution computed tomography</td>
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<tr>
<td>IM</td>
<td>Intramuscular</td>
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<tr>
<td>IV</td>
<td>Intravenous</td>
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<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>NB-BAL</td>
<td>Non-bronchoscopic bronchoalveolar lavage</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SC</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>SpO₂</td>
<td>Blood oxygen saturation</td>
</tr>
<tr>
<td>TNCC</td>
<td>Total nucleated cell count</td>
</tr>
<tr>
<td>µL</td>
<td>Microliter</td>
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1 Literature Review

1.1 Introduction

Clinical signs of respiratory disease including coughing, sneezing, tachypnea or abnormal breathing sounds are a common presenting complaint in veterinary practices. Physical examination can be useful to localize disease to either the lower or upper respiratory tract, however as clinical signs can be reflective of many different diseases, diagnostic tests are required to better characterize the cause of clinical signs, provide an indication of a diagnosis and direct therapeutic plans.

Investigation and determination of the underlying etiology of lower respiratory tract disease in cats can be challenging for veterinarians. Non-invasive screening diagnostic tests including history, physical examination, blood work (complete blood count, serum biochemistry panel), fecal analysis (fecal flotation, Baerman test), and thoracic imaging (radiographs or computed tomography) are able to provide a suggestion of the presence of lower respiratory tract disease, however are poorly specific at determining the etiology. Diagnosis is usually achieved using a combination of imaging, blood tests and diagnostic tests that allow for samples to be obtained directly from the lower respiratory tract.

The purpose of this research was to determine the optimal bronchoalveolar lavage method for sampling the lower respiratory tract in cats, to describe a new technique for BAL in cats – fluoroscopic guided BAL and to determine the risk factors for BALF hemosiderosis in dogs and cats with respiratory disease.
1.2 Imaging of the lower respiratory tract in cats

1.2.1 Thoracic radiography

Thoracic radiographs are the most commonly used, cost effective and easily accessible diagnostic imaging modality for evaluation of a feline patient with signs of lower respiratory tract disease. General anesthesia is typically not required. Evaluation of the lungs, cardiac silhouette and other mediastinal structures that could be contributing to clinical signs can be achieved with this imaging modality. Thoracic radiographs are often the first line test to distinguish between cardiogenic and non-cardiogenic causes of lower respiratory tract signs. Pattern recognition paradigms are used to identify the compartment of the lung that is abnormal, therefore allowing clinicians to narrow down the list of possible differential diagnoses. Consideration of changes within the pulmonary vasculature and cardiac silhouette size and shape can further help clinicians to determine whether pulmonary changes are cardiogenic or non-cardiogenic in origin. Ultimately, many lower respiratory tract and cardiac diseases have overlapping radiographic findings, making this imaging modality poorly specific. Specifically, pulmonary edema secondary to cardiac disease in cats can have many radiographic patterns including but not limited to peri-bronchial or unstructured interstitial pulmonary opacities progressing to an alveolar pattern. Possible differential diagnoses that could be considered for pulmonary patterns that may arise secondary to cardiogenic pulmonary edema include non-cardiogenic pulmonary edema, bronchitis, pneumonia, bronchopneumonia, neoplasia, pulmonary hemorrhage, heartworm disease and pulmonary fibrosis. Radiographs are therefore rarely used to provide a final diagnosis, and concurrent consideration of patient history, physical examination findings and response to therapies is required to improve radiographic interpretation and the diagnostic capability of this imaging modality. Further, for feline patients, lack of radiographic abnormalities does not
completely exclude the presence of disease, as radiographs may be normal in up to 23% of patients with feline asthma.\textsuperscript{9}

1.2.2 Thoracic Ultrasonography

Ultrasound examination of the lung can be useful to evaluate disease at the peripheral surface of the lung and is invaluable for differentiation of cardiac and non-cardiac disease using echocardiography.\textsuperscript{10-13} The presence of aerated lung only allows for imaging of lesions at the visceral pleural surface; therefore, deeper lesions usually cannot be visualized or characterized.\textsuperscript{13,14} Fluid or cellular infiltrate within the pulmonary interstitium or alveolar spaces creates an acoustic window resulting in the lung parenchyma appearing as solid tissue. Lesions that can be appreciable include masses, nodules, consolidation of the lung lobe, or atelectasis.\textsuperscript{10-15} Ultrasound can guide directed transthoracic aspiration or biopsy of such lesions. Ultrasound examination and ultrasound-guided sampling of pulmonary parenchymal lesions is minimally invasive and can usually be performed with sedation.\textsuperscript{16-20} Major risks of ultrasound-guided aspiration or biopsy include hemorrhage, pneumothorax or seeding of abnormal cells along the aspiration tract, however the latter has only been reported once.\textsuperscript{19-22}

1.2.3 Thoracic computed tomography

Computed tomography (CT) is more sensitive compared to thoracic radiography due to higher contrast resolution allowing for detection of lesions as small as \textsuperscript{1mm}.\textsuperscript{5,23,24} Cross sectional imaging eliminates the presence of superimposition of thoracic structures, such that CT is considered the gold standard for pulmonary imaging in human medicine.\textsuperscript{23,24}

For cats undergoing thoracic CT, this can either be performed anesthetized, minimally sedated or non-sedated with manual restraint in a plexiglass positioning/restraining device.\textsuperscript{6,25}
Bronchospasm, laryngospasm and pulmonary atelectasis are avoided with non-sedated thoracic CT; however, motion artefact can reduce the diagnostic quality of the CT scan, requiring repeat imaging and increased radiation exposure. Iodinated contrast material can be administered during CT to highlight lesions, particularly small nodules, which may not be evident on thoracic radiographs or non-contrast CT scan. In feline patients, additional findings that can be detected using CT compared to thoracic radiography include increased lung attenuation, lung nodules, lung masses, bronchiectasis and mediastinal lymphadenopathy.  

While non-sedated thoracic CT is feasible in cats and has been demonstrated to provide additional information compared to thoracic radiographs, anesthetized, inspiratory breath-hold CT provides the most accurate discrimination between healthy cats and cats with spontaneous and experimentally induced asthma. Motion artefact encountered with non-sedated thoracic CT can cause increased lung attenuation in healthy cats and underestimate the number and severity of lesions in asthmatic cats. 

Improvements in CT technology and development of high resolution computed tomography (HRCT) has allowed for features as small as 100-200 µm to be identifiable on CT imaging. This allows for improved assessment of small airways including the ability to accurately and reproducibly measure airway thickness, luminal and small vessel diameter and to diagnose bronchiectasis, bronchiolar disease and emphysema. Bronchioles are the smallest (less than 2mm diameter), terminal airways that lack cartilage in their walls. In human medicine, particular HRCT patterns are highly suggestive of bronchiolar disease and prompt early pulmonary biopsy. Patterns corresponding to bronchiolar disease that have been described in human medicine include ‘tree in bud’ pattern, representing plugging of the centrilobular airways with
cellular material, fluid or pus, ‘centrilobular’ pattern, representing inflammation associated with peribronchiolar alveoli and ‘mosaic’ pattern, representing regional air-trapping, hypoventilation of the alveoli and hypoxic vasoconstriction.\textsuperscript{34-36} In cats, ‘tree in bud’ and ‘mosaic pattern’ on HRCT has been documented with clinical and subclinical bronchial disease.\textsuperscript{36} It was suggested that these patterns corresponded to bronchial and bronchiolar disease; however, no pulmonary biopsy was performed to localize disease to the specific airways.\textsuperscript{36} In human medicine, changes on HRCT correlate to severity of asthma with increasing number of changes negatively correlating with results of pulmonary function testing.\textsuperscript{37-40} Despite this, some asthmatic patients do not have any changes (bronchial wall thickening, bronchial dilatation, mucous impaction, emphysema, mosaic perfusion or atelectasis) on HRCT.\textsuperscript{37-40} This may be related to the fact that asthma is primarily a functional disease that causes secondary structural changes, hence in early disease where there is airway hyper-responsiveness with minimal airway inflammation and absence of airway remodeling, HRCT is still insufficiently sensitive to detect cellular changes.\textsuperscript{37} In feline patients, bronchial wall thickness to bronchial luminal diameter, and bronchial wall thickness to pulmonary artery diameter, are significantly greater in asthmatic compared to non-asthmatic cats;\textsuperscript{27} however, there is overlap in ratios obtained from asthmatic and healthy cats, and ratios may be inaccurate where there is disease affecting the bronchial luminal diameter and pulmonary arterial diameter.\textsuperscript{27,31} Although several studies report on the utility of CT for improved detection and determination of final diagnoses,\textsuperscript{6,25,27,29,32,33} no information is available that combines both CT imaging and lower airway sampling. Ultimately, although CT provides advanced information that can help to characterize the extent, severity and location of a patient’s pulmonary disease, sampling of the lesion(s) is still required to determine etiology.\textsuperscript{41,42}
1.2.4 Thoracic Magnetic Resonance Imaging

Thoracic magnetic resonance imaging (MRI) has been reported in human medicine, and is indicated where three-dimensional imaging of the lung is required and radiation exposure or iodinated contrast material associated with CT imaging are contra-indicated.\textsuperscript{43-45}

Compared to CT, advantages of MRI include enhanced soft tissue contrast and functional information, which allows for assessment of motion and perfusion of thoracic organs.\textsuperscript{44,46,47} In human medicine, MRI is reportedly superior to CT for differentiation of atelectasis and pulmonary masses, differentiation of mediastinal masses and diagnosis of pulmonary perfusion deficits.\textsuperscript{43-47} The smallest intrathoracic structure (airway or nodule) distinguishable on MRI in human medicine is reported to be 3mm, which would pose a major limitation to the use of this imaging modality in feline patients.\textsuperscript{43,44,47} Further, CT is considered to be superior to MRI in human medicine for conditions such as chronic obstructive pulmonary disease and interstitial lung disease, and for detection of small pulmonary nodules.\textsuperscript{47} To make MRI advantageous over CT, adequate equipment (at minimum 1.5T MRI) and fast imaging or respiration-gated protocols are required.\textsuperscript{48} Availability of such equipment and protocols may be limited in many veterinary facilities, precluding use of this imaging modality. No studies are available reporting the utility of MRI for evaluation of feline lower respiratory tract disease.

1.3 Cardiac Biomarkers to Determine the Etiology of Respiratory Distress in Cats

Distinguishing cardiac and non-cardiac causes of respiratory distress is an important yet difficult decision in clinical practice. Radiographic patterns often overlap or are similar between cats with cardiogenic and non-cardiogenic causes of respiratory distress; therefore, radiographs
are poorly specific in determining the etiology of respiratory distress in feline patients.\textsuperscript{49} Echocardiography is considered to be the gold standard\textsuperscript{50} to determine whether there is a cardiac component to pulmonary parenchymal changes present on radiographs; however, availability of trained personnel to perform this diagnostic test is often limited particularly in a general practice setting.\textsuperscript{51} Compared to echocardiography, blood-based testing is attractive because it is minimally invasive, imparts minimal stress on the patient and does not require specific expertise or equipment to perform.\textsuperscript{52}

The most commonly reported and available biomarker in veterinary medicine for differentiating cardiogenic and non-cardiogenic causes of respiratory distress in cats is N-terminal-proBNP (NT-proBNP).\textsuperscript{51-54} Brain natriuretic peptide (BNP) is predominantly secreted by ventricular myocytes in response to myocardial stretch, volume and pressure overload.\textsuperscript{55-57} BNP is synthesized as a prohormone (proBNP), which is then cleaved and released from myocytes as active BNP and inactive N-terminal proBNP (NT-proBNP).\textsuperscript{51,55-57} NT-proBNP correlates with the amount of active proBNP; however, it has a longer plasma half-life, and is therefore a more stable marker of heart disease.\textsuperscript{51} Using echocardiography as the gold standard, a cut off of 100pmol/L NT-proBNP had high sensitivity (92.4\%) and specificity (93.9\%) for identifying patients with mild hypertrophic cardiomyopathy.\textsuperscript{58} Point-of-care NT-proBNP ELISA tests are currently available, which circumvent the need for laboratory transportation and processing to improve the accessibility and utility of this diagnostic test.\textsuperscript{52} The point-of-care test is a qualitative colorimetric test that provides clinicians with a normal or abnormal result based on a cut-off between 150-200pmol/L.\textsuperscript{52,59} The increased cut-off compared to the quantitative test performed at the laboratory reduced the sensitivity of this test; however, specificity was 100\% in distinguishing cats with cardiac disease from those without cardiac disease.\textsuperscript{52,58,59} For dyspneic feline patients, NT-proBNP
is significantly higher in cats with congestive heart failure than in cases with primary lower respiratory tract disease.\textsuperscript{51} NT-proBNP can accurately discriminate cats with congestive heart failure from those with other causes of dyspnea.\textsuperscript{51} Although considered to be an accurate and widely available test, sensitivity and specificity, depending on the cut-off value used, are unfortunately not perfect, and difficulties in discriminating the cause of dyspnea may still arise in feline patients with both pre-existing cardiac disease and primary lower respiratory tract disease.\textsuperscript{51-54,60} Consequently, where a patient has an abnormal result obtained using either the point-of-care or quantitative NT-proBNP test, or if there is still suspicion of cardiac disease despite a normal result (murmur, gallop rhythm), echocardiography should be pursued to exclude cardiac contribution to respiratory distress.\textsuperscript{51-54,58-60}

1.4 Modalities for Sampling the Lower Respiratory Tract

Several diagnostic techniques are available to obtain samples from the lower respiratory tract in cats including transthoracic fine needle aspiration, lung biopsy, transtracheal wash (TTW), non-bronchoscopic bronchoalveolar lavage (NB-BAL), or bronchoscopic BAL (B-BAL).

1.4.1 Transthoracic Fine Needle Aspiration

Transthoracic fine needle aspiration is an inexpensive, safe and accurate method for investigating the etiology of pulmonary mass lesions in dogs and cats. Comparison of cytopathology and histopathology results of samples obtained from pulmonary lesions has indicated relatively high sensitivity (70-100%) and specificity (100%) depending on the condition and utilization of ultrasound/CT for guidance of sampling.\textsuperscript{19,20,22} Sensitivity of cytopathology was 100% for fungal pneumonia (blastomycosis).\textsuperscript{22} For neoplastic disease, sensitivity is reported to be
between 70-85%.\textsuperscript{20} Accuracy of cytopathology depends on selection of appropriate sampling sites, exfoliation of the tissue, cellularity of the sample, presence of hemodilution and concurrent necrosis or inflammation.\textsuperscript{20} The latter two factors have been cited to be the most common cause of false negative results in dogs and cats undergoing transthoracic fine needle aspiration for diagnosis of intrathoracic masses.\textsuperscript{20}

Transthoracic fine needle aspiration can be performed blindly or radiographic, ultrasound or CT-guided. Cytopathology from samples collected by blind transthoracic fine needle aspiration have lower agreement (67%) to histopathologic findings compared to those collected via ultrasound guidance (86%).\textsuperscript{19} Hemothorax and pneumothorax are the most commonly expected complications. In three studies investigating complications related to ultrasound-guided fine needle aspiration of thoracic masses or focal parenchymal lesions, no complications were reported.\textsuperscript{19,22,61} Increased frequency of complications has been reported with blind fine needle aspiration, fluoroscopic-guided fine needle aspiration, when larger needles (18G) were used to collect samples, or when there was interstitial lung disease.\textsuperscript{61,62} Several studies however, have not reported complications associated with ultrasound-guided or CT-guided aspiration of intrathoracic masses.\textsuperscript{19,20,22}

1.4.2 Lung Biopsy

Lung biopsy can be performed percutaneously, thoracoscopically, bronchoscopically or via open thoracotomy. Biopsy and histopathology is indicated in patients where airway/pulmonary cytopathology (either obtained via bronchoalveolar lavage, transtracheal wash or fine needle aspiration) and radiology have failed to provide a definitive diagnosis.\textsuperscript{19,41} This technique has been utilized to diagnose bronchiolar and interstitial lung diseases (e.g. pulmonary fibrosis or
pulmonary neoplasia), where histopathology of the lung and examination of architectural changes within the pulmonary parenchyma were required to provide a definitive diagnosis for treatment and prognostication. All of these procedures require general anesthesia, post-procedural hospitalization and analgesia, and bear increased risk of pneumothorax, particularly if aerated lung is present in the area of the biopsy.

Advantages of performing lung biopsy using thoracoscopy or via open thoracotomy compared to closed pleural biopsy or bronchoscopic biopsy include the ability to perform a diagnostic and potentially therapeutic procedure under a single general anesthetic and the ability to promptly recognize and address hemorrhage if it occurs. Targeted biopsies can be obtained from gross lesions on the visceral surface of the lungs, however inspection of the internal structure of the pulmonary parenchyma is not possible. Therefore, biopsies obtained thoracoscopically or via open thoracotomy may not be representative if the lung is visibly normal on the visceral surface. Open or thorascopic techniques also allow for visualization and biopsy of structures other than the pulmonary parenchyma, including intrathoracic lymph nodes, pericardium, pleura and thoracic wall, which would not be accessible or safe using closed chest ultrasound-guided or CT-guided techniques. Thoracoscopic biopsy is minimally invasive compared to open thoracotomy and has a shorter recovery time following the procedure. Contraindications reported for this procedure in human medicine include presence of diffuse pleural adhesions that prevent formation of an adequate working space, body wall neoplasia, and lack of cardiopulmonary reserve to tolerate lung collapse, which is required to provide adequate visualization of intra-thoracic structures. Complications associated with thoracoscopy in humans vary significantly depending on the underlying disease with a reported mortality rate ranging from 0-5% and complication rate ranging from 0.6-20%. No data on complication
rates associated with this procedure are available in veterinary medicine. Complications reported in human medicine include prolonged air leak, empyema, exacerbation of interstitial pneumonitis, pulmonary edema, and development of pneumonia.\textsuperscript{70,71,73} Similar complications may occur in patients undergoing open thoracotomy.\textsuperscript{68}

Considering the morbidities relating to closed chest, thoracoscopic, or open chest pulmonary biopsy, endobronchial biopsy is a technique that has been developed in human medicine to sample airway-associated lesions, pulmonary parenchyma or tracheobronchial lymph nodes.\textsuperscript{66,74,75} Various techniques for performing this procedure have been evaluated including utilization of endobronchial cup biopsy forceps, transbronchial fine needle aspiration, transbronchial Tru-cut\textsuperscript{TM} biopsy and cryobiopsy.\textsuperscript{75-77} These procedures are either bronchoscopically guided, performed blindly or guided by ultrasound (transbronchial biopsy) or computed tomography (virtual bronchoscopy) and are usually utilized in human medicine for staging of pulmonary neoplasia and diagnosis of sarcoidosis.\textsuperscript{74-76} The major advantage of endobronchial biopsy techniques is a significantly reduced risk of pneumothorax and hemorrhage compared to other pulmonary biopsy techniques.\textsuperscript{76} Despite this, samples obtained are small, limiting diagnostic quality,\textsuperscript{66,74-77} and specialized equipment and expertise are required.\textsuperscript{66,74,75} No studies are available investigating the utility of bronchoscopic biopsy in veterinary medicine. Veterinary patient size would likely be the major limiting factor to utilization of these techniques for acquisition of pulmonary biopsy samples.

Overall, although pulmonary biopsy remains the gold standard for characterizing architectural changes within the pulmonary parenchyma, due to the invasive nature of this procedure, the relatively high risk and the small samples obtained, it is infrequently performed.
1.4.3 Tracheal Wash

Endotracheal (transoral tracheal) wash or transtracheal wash involves collection of material from the large airways (trachea and mainstem bronchi).\textsuperscript{78} Owing to their small size and inability to stimulate coughing by coupage, transtracheal wash cannot be performed in cats, thus this technique in cats is limited endotracheal wash.\textsuperscript{78,79} Compared to transtracheal wash, where the patient remains conscious and a needle is inserted percutaneously into the trachea to facilitate passage of a catheter for saline infusion and retrieval, endotracheal wash requires general anesthesia.\textsuperscript{78,79} The catheter is inserted to perform an endotracheal wash through a sterile endotracheal tube, and sterile saline is infused and collected at the level of the mainstem bronchi.\textsuperscript{78,79} Compared to bronchoalveolar lavage, tracheal wash has an increased risk of oropharyngeal contamination and reduced infusate retrieval, and does not sample distal small airways and alveoli.\textsuperscript{78-80} Consequently, cytologic samples have few leukocytes, and surfactant and alveolar macrophages are absent.\textsuperscript{80} Discordant cytologic and bacterial culture findings have been reported when comparing results of BAL and tracheal wash in dogs with lower respiratory tract disease.\textsuperscript{80} No information is available comparing the two techniques in cats. Similar results would be expected, hence leading to the recommendation that tracheal wash is inferior compared to BAL for characterizing lower respiratory tract disease in dogs and cats.

1.4.4 Bronchoalveolar Lavage

Bronchoalveolar lavage involves instillation of isotonic sterile saline into the small airways and retrieval of bronchoalveolar fluid. The cells, protein and mucus within BALF are derived from the deeper branches of the respiratory tree.\textsuperscript{81-84} Two major techniques for performing bronchoalveolar lavage have been reported in cats; nonbronchoscopic (NB-BAL) and bronchoscopic bronchoalveolar lavage (B-BAL).\textsuperscript{81,83,85,86} For both techniques, general anesthesia
is required, however for most patients undergoing this procedure, discharge from hospital can be achieved on the same day of the procedure.\textsuperscript{86}

\textbf{1.4.4.1 Non-Bronchoscopic Bronchoalveolar Lavage}

Non-bronchoscopic bronchoalveolar lavage involves collection of bronchoalveolar lavage fluid (BALF) using either a sterile urinary catheter or sterile feeding tube, which is inserted into the distal airways through a sterile endotracheal tube to facilitate infusion of aliquot(s) sterile saline and collection of BALF.\textsuperscript{78,85,87} A bronchoscope is not required for this procedure, so this technique can be performed on cats in most research and clinical settings.

The samples obtained with this technique have reduced cellularity and increased ratio of bronchial epithelial cells to macrophages compared to B-BAL, indicating increased larger airway sampling (trachea and main stem bronchi) as opposed to alveolar sampling.\textsuperscript{88} With this technique it is not possible to know the precise source of the sample or acquire samples from focal pulmonary lesions, but it is more likely that the sample originates from the dependent caudal lung lobe when the cat is in lateral recumbency.\textsuperscript{78,85,87} Examination of the airway mucosa is also not possible but bronchoscopic airway mucosal changes have not been demonstrated to predict diagnosis in cats, implying that this technique would likely yield similar cytopathology samples to B-BAL with comparable cytologic quality and diagnostic accuracy.\textsuperscript{89} As multi-segment bronchoalveolar lavage has been determined to yield variable cytologic results in cats,\textsuperscript{90} utilization of an imaging modality such as fluoroscopy to guide sampling of focal lesions using NB-BAL might be an advantageous modification of this technique.
1.4.4.2 Bronchoscopic Bronchoalveolar Lavage

Bronchoscopic bronchoalveolar lavage involves collection of BALF using a flexible endoscope that is inserted into the lower airways. Isotonic saline is infused and retrieved via the endoscope. This technique allows for direct visualization of the airways and sampling of directed lesions where focal respiratory disease has been identified. Examination of the mucosal surface of the airways and assessment of mucus accumulation, erythema, stenosis, epithelial irregularity, bronchiectasis and airway collapse can also be performed. These findings can provide an indication of the disease process that is likely to be present in dogs. However, specific changes visualized have not been determined to be correlated with particular cytologic diagnoses in cats. In cats, it has been proposed that gross bronchoscopic changes may be useful in describing the severity of a cat’s respiratory disease. No studies have been performed to monitor progression of gross bronchoscopic changes in relation to progression of disease or in response to medical therapy. Therefore, the clinical significance of such findings is questionable. There are no studies following the progression of bronchoscopic scores of individual patients from the time of diagnosis and after institution of therapy.

In summary, bronchoalveolar lavage is considered to be a minimally invasive method for sampling the lower airways cats. It has good diagnostic yield and low patient risk, which justifies its use as the primary diagnostic test for sampling canine and feline lower airways.

1.4.5 Complications of Bronchoalveolar Lavage

Complication rate is reported to be less than 5% in human patients undergoing bronchoalveolar lavage, and most commonly reported complications relate to changes in respiratory function following the procedure. No significant difference in alteration of
respiratory function was appreciable in patients with underlying respiratory disease when undergoing bronchoalveolar lavage in conjunction with bronchoscopy compared to bronchoscopy alone.\textsuperscript{94} The most common minor complications reported include transient fever, coughing, bronchospasm, and transient infiltrates, chills, myalgias, hemoglobin desaturation, and reduction in lung function.\textsuperscript{95} Major complications are considered rare but may include pneumothorax, pneumonia, respiratory failure and requirement for mechanical ventilation, cardiac arrest and death.\textsuperscript{95}

For cats with respiratory disease undergoing NB-BAL, the complication rate has not been reported. In clinically healthy cats, the major complication found was transient hemoglobin desaturation.\textsuperscript{83,87} Pneumothorax and prolonged anesthetic recovery could also be possible complications of this technique.

For cats with clinical signs of respiratory disease undergoing B-BAL, the reported complication rate was 38\%, which is much higher compared to human medicine.\textsuperscript{86} Most of the reported complications (24\%) were considered to be minor with transient hemoglobin desaturation most commonly reported.\textsuperscript{86} The major complication rate is much lower in comparison (6\%) and similar as in human medicine, including pneumothorax, failure to restore ventilation following anesthesia, or need for prolonged oxygen supplementation and hospitalization following bronchoalveolar lavage.\textsuperscript{86} In a study investigating various aspects of B-BAL in 68 cats with respiratory disease, underlying disease process or the volume of fluid instilled was not associated with an increased complication rate.\textsuperscript{86} Significantly lower complication rate was reported following implementation of pre-treatment with injectable bronchodilators (terbutaline) prior to bronchoscopy and bronchoalveolar lavage.\textsuperscript{78,86} Pre-treatment with inhaled bronchodilators prior to
bronchoalveolar lavage in allergen-sensitized cats reduced the risk of bronchospasm associated with bronchoalveolar lavage.\textsuperscript{96} Consequently, it is recommended that all cats undergoing bronchoalveolar lavage are pre-treated with bronchodilators.\textsuperscript{78,86,96}

**1.4.6 Bronchoalveolar Lavage Technique**

**1.4.6.1 Volume Instilled and Proportionate Fluid Return**

Volume of fluid instilled, amount of suction applied and location of sampling can affect cytology results. Currently, there is no standardized volume for feline BAL, and the endoscopist usually chooses a volume based on personal preference.\textsuperscript{86} In human BAL, increased sampling of the lower airways and alveoli is achieved with greater proportionate return of infusate.\textsuperscript{97,98} Initial aliquots retrieved contain different cellular and protein components compared to later aliquots and represent ‘bronchial washings’ with minimal return of alveolar macrophages.\textsuperscript{97-100} Several aliquots are often instilled, particularly where interstitial disease is being investigated.\textsuperscript{100,101} In dogs, weight-adjusted volumes increased the amount of epithelial lining fluid sampled compared to fixed infusate volumes.\textsuperscript{102} For feline BAL, reported fixed infusate volumes range from 3-20mL\textsuperscript{81,85-87,89,103} and reported weight-adjusted volumes range from 3-5mL/kg.\textsuperscript{87} The number of aliquots can range between 1-5 aliquots per site,\textsuperscript{81,87} however 2-3 aliquots per site is most commonly reported.\textsuperscript{86,89,103} Ideal infusate volumes, number of aliquots per site, and number of sampling sites, has yet to be established for feline BAL. However, compared to dogs, there is less weight variation between cats, so there may not be a pronounced effect between weight-adjusted aliquots and fixed volume aliquots. In one retrospective study of cats undergoing B-BAL, number of aliquots, volume of infusate and volume infused per kilogram of weight were not associated with presence or severity of complications.\textsuperscript{86} Overall, when comparing all feline NB-BAL and B-BAL studies, regardless of volume of infusate utilized, the proportionate volume of BALF
retrieved was similar between studies (55%-80%).\textsuperscript{81,85-87,89,103} A more recent retrospective study investigating B-BAL found that lower volume per kilogram instilled resulted in greater proportionate return of fluid, and provided samples of good diagnostic quality\textsuperscript{86}. The ability to utilize a lower volume per kilogram of infusate could have been accomplished by more directed alveolar sampling as a thinner diameter bronchoscope can be wedged into a more distal bronchus.\textsuperscript{86} Some earlier studies involved instillation of aliquots directly into the endotracheal tube via a syringe adapter.\textsuperscript{83,84,87} This would have inherently required an increased volume of infusate to sample the alveoli. In human and canine patients, a minimum of 40% of fluid return is required to obtain a diagnostic sample.\textsuperscript{82,104} Further, in canine patients increased proportionate return of BALF was associated with improved sample quality, but did not affect rate of diagnosis.\textsuperscript{105-107} The effects of volume instilled on proportionate fluid return and sample quality have yet to be prospectively investigated for feline BAL.

1.4.6.2 Suction Technique

The two major methods of BALF retrieval reported in human literature, dogs and horses are suction utilizing a handheld syringe or suction using a mechanical unit.\textsuperscript{100,101,105-107} The negative pressure created by the device utilized to aspirate BALF can cause bronchospasm reducing BALF return.\textsuperscript{100} In dogs, greater return of BALF was achieved using a mechanical suction unit compared to aspiration with a syringe.\textsuperscript{105-107} In feline patients, the use of a mechanical suction unit has not been investigated and the effect of suction using differently sized syringes is unknown. Pressure generated is likely to vary depending on equipment factors including the inner diameter, length and rigidity of the bronchoscope or catheter used to perform BAL, and patient factors such as size and morbidity.\textsuperscript{100} Considering the predisposition of feline patients for bronchospasm and bronchoconstriction, the clinician may prefer to adjust the pressure on a case-
by-case basis through manual aspiration. No studies are available to compare the utilization of mechanical suction and manual aspiration for retrieval of fluid when performing feline BAL.

1.4.6.3 Bronchoscope Diameter

The diameter of the endoscope affects sampling depth, amount of fluid retrieved, cytologic quality of the fluid retrieved and complications. In a retrospective study of 68 cats with respiratory diseases undergoing B-BAL with different endoscopes the volume of fluid instilled and proportion of fluid retrieved did not contribute to an increased complication rate. In that study, 3 bronchoscopes were utilized (2.5 mm x 100 cm [1.2mm working channel], 3.8 mm x 55 cm [1.2 mm working channel] and 5.0 mm x 55 cm [2.0 mm working channel]) and significantly more complications were recorded for the 2.5 mm diameter bronchoscope compared to the 3.8 mm and 5.0 mm bronchoscopes. The proposed reasons for the increased complication rate with the thinner bronchoscope were not discussed. The thinner working channel and increased length associated with the 2.5 mm bronchoscope could have contributed to increased suction pressure required to retrieve BALF, causing increased bronchospasm and complication rate. Although a significantly reduced number of complications were recorded with the larger diameter bronchoscopes, use of larger diameter bronchoscopes to perform BAL in cats increases the risk of tracheal obstruction due to the narrow diameter of their trachea, and limits the ability to deliver oxygen to the patient during the procedure. Further, larger diameter bronchoscopes require patient extubation to facilitate passage of the bronchoscope. Repeated extubation increases the risk of laryngospasm, which further increases the risk of complication. In human medicine, two studies have been performed comparing standard bronchoscopy to ultrathin bronchoscopy. Conflicting results have been reported regarding complication rate. One study demonstrated no significant difference in complication rate between the two techniques, whereas another study
demonstrated significantly more complications with ultrathin bronchoscopy.\textsuperscript{109} Hence, larger studies are required to determine the effect on bronchoscope diameter and complication rate in both human and veterinary patients.

1.4.6.4 Location of Sampling

The diameter of bronchoscope or catheter used and location for sampling is currently not standardized in feline patients. All techniques described in the literature thus far mention advancing the catheter or bronchoscope until ‘wedge’ position is reached.\textsuperscript{81,85} This results in bronchoscopes or catheters of wider diameter being wedged in more proximal airways compared to bronchoscopes or catheters of thinner diameter.\textsuperscript{81} Different epithelial surface areas would consequently be washed resulting in inherent differences in cellularity of a sample based on the instrument selected to perform bronchoalveolar lavage.\textsuperscript{81,111} In human patients with diffuse disease it is recommended to sample from the right middle lung lobe or lingula\textsuperscript{100} and to advance the bronchoscope into the third or fourth generation bronchus.\textsuperscript{100} This location has been found to achieve greater BALF recovery compared to other sites.\textsuperscript{100} Even if radiographic changes are diffuse, cytologic findings can still be discordant between cat lung lobes (total nucleated cell count, differential cell count)\textsuperscript{90} leading to different final diagnoses. For cats with pulmonary disease of allergic or sterile inflammatory etiology, this could be explained by differences in exposure to particulate matter and subsequent differing inflammatory responses in different lung fields.\textsuperscript{90} Further, radiographs lack sensitivity in characterizing location, distribution and severity of pulmonary disease.\textsuperscript{41,90} Consequently, a single sample taken from one lung field may not provide a complete assessment of an individual cat’s clinical disease. To our knowledge there are no studies evaluating inherent cytologic differences between lung segments in healthy cats. Computed
tomography may be a more sensitive tool to consider prior to BAL to select the appropriate lung segment to sample.  

1.4.6.5 Patient Factors

Disease nature, distribution and size of lesions can affect proportion of fluid retrieved and quality of BALF in feline patients. Similar findings are reported for humans, where cigarette smokers are known to have increased alveolar macrophages, and patients with chronic obstructive pulmonary disease tend to have increased airway collapse and reduced fluid return. This can lead to difficulties in interpreting some BAL samples due to poor sample return or poor sample quality due to the patient’s underlying disease. For this reason in human medicine it is recommended that patient factors be considered in the interpretation of BAL results.

1.4.7 Bronchoalveolar Lavage Fluid Processing

Following collection of BALF, several processing aspects can alter cellular and protein contents of BALF. Samples that are not immediately processed (within 2 hours) and stored either in the refrigerator or at room temperature may have unreliable neutrophil and eosinophil counts due to cellular apoptosis or rupture, potentially leading to misdiagnosis. Prompt analysis is therefore recommended.

Prior to slide preparation for cytologic evaluation, total nucleated cell count is typically performed using either a single cell impedance counter or manually in a hemocytometer. Low total nucleated cell counts can provide an early indication of poor sample quality related to poor collection technique or delayed sample processing. Filtration of the BALF to remove mucus
has been described,\textsuperscript{81,86} however this removes an unpredictable amount of cells resulting in various
degrees of under-estimation of total nucleated cell count.\textsuperscript{100}

Cytocentrifugation of the sample to concentrate cells is recommended to provide
homogenous cellular distribution and better cell preservation.\textsuperscript{117} Performing differential cell
counts on smears of pelleted cells may result in underestimation of neutrophil count.\textsuperscript{117} On manual
smears of pelleted cells there is poorer preservation of leukocytes and uneven distribution of cells,
rendering appropriate identification of cells more challenging.\textsuperscript{117} Compared to manual smears of
pelleted cells, slides made from cytocentrifugation of BALF have improved cell preservation,
facilitating consistent cell identification.\textsuperscript{117} Cytocentrifugation of human BAL samples increased
sensitivity for detection of infectious agents and neoplastic cells.\textsuperscript{118,119} During the
cytocentrifugation, the speed and duration of the process can cause variable loss of the lymphocyte
population within a BALF sample, reiterating the need to employ consistent cytocentrifugation
speed and duration for every sample.\textsuperscript{100}

Following cytocentrifugation, inadequate adherence during fixation can result in
preferential loss of lymphocytes, which is exacerbated when aqueous stains are used.\textsuperscript{120} This was
found to be negated by use of spray fixation and use of alcohol-based stains, however it was
recommened that differences in BAL cytology results should also take into account differences
in slide preparation, specifically fixation and staining.\textsuperscript{120}

Once the slide is prepared the number of cells counted to provide a differential cell count
has varied tremendously between different studies evaluating feline bronchoalveolar
techniques.\textsuperscript{81,83,84,89,103,117,121} Counting larger numbers of cells to obtain the differential cell count
leads to increased sensitivity for detection of rarer cells such as mast cells and greater reliability.
In human medicine it is recommended that a minimum of 500 nucleated cells are counted to establish differential cell counts.  

1.4.7.1 Urea Dilution

To estimate the amount of epithelial lining fluid sampled and allow more accurate and comparable total nucleated cell counts and absolute cell counts, urea dilution has been proposed. Urea diffuses freely throughout the body including through the alveolar wall. Quantification of urea in plasma and BALF allows for determination of the amount of epithelial lining fluid recovered, which is calculated by simple dilution principles. This has been described to be possible in feline patients, however, has only been described for NB-BAL technique and requires further investigation to validate repeatability for B-BAL technique. Urea dilution is utilized in human medicine, however its relevance is limited because of the presence of disease that increases epithelial permeability, variable dwell time of fluid during bronchoalveolar lavage, and variability in overall duration of bronchoalveolar lavage procedure.

1.4.8 Cytologic Findings

Normal cytologic findings have been described independently for both B-BAL and NB-BAL techniques but have not been directly compared in cats. Normal feline bronchoalveolar lavage fluid consists predominantly of alveolar macrophages (60-78%). Eosinophils can represent up to 20-25% of cells in cats, however as in dogs and humans, the remaining cell types (neutrophils, lymphocytes, epithelial cells) should represent small proportions of the differential cell count. Colony-housed cats will normally have a greater proportion of eosinophils on BAL cytology compared to pet cats. No investigation has been performed to determine the cause of increased BALF eosinophilia in colony-housed cats. Environmental factors
such as exposure to allergens may play a role. In a study of 24 clinically normal cats, the increased proportion of eosinophils in feline BALF was not associated with histopathologic evidence of lower airway inflammation. As only a small number of cats were included in this study, relatively high proportion of eosinophils on BALF cytology may be part of normal variation among healthy cats, represent an increased population of ‘inactive’ eosinophils, or, alternately represent subclinical disease.

1.4.9 Factors Indicating Poor Sample Quality

Presence of Simonsiella bacteria and squamous epithelial cells provides indication of oropharyngeal contamination. This could happen easily in feline patients, particularly for B-BAL technique, since the bronchoscope is often passed through the pharynx directly into the trachea without the protection of a sterile endotracheal tube. Excessive suction can lead to iatrogenic hemorrhage demonstrated by red blood cells as well as large numbers of columnar epithelial cells. The latter may also arise from sampling of the upper airways as opposed to the lower airways or due to underlying patient disease leading to excessive exfoliation. Low proportion of macrophages can help to distinguish location of sampling since sampling from the alveoli should produce high proportion of alveolar macrophages.

1.4.10 Bronchoalveolar Lavage Respiratory Biomarkers in Cats

Aside from differentiation of cardiac and non-cardiac causes of respiratory distress in cats, ideal utilization of biomarkers in respiratory disease could also be extended to determination of the etiology of lower respiratory tract signs, such that appropriate therapy could then be initiated.

Biomarker measurement in BALF has been investigated to provide early detection of asthma in cats and to distinguish cats with chronic bronchitis from cats with asthma. Asthma
is primarily considered to be a T-helper-2 lymphocyte driven hypersensitivity reaction to inhaled aeroallergens, and cytokines produced by these lymphocytes drive eosinophilic airway inflammation.\textsuperscript{129,132} Human asthmatic patients have increased interleukin (IL)-4, tumour necrosis factor (TNF)-\(\alpha\), and nitric oxide metabolites with decreased interferon (IFN)-\(\gamma\) which is a cytokine produced by the T-helper-1 pathway.\textsuperscript{131,133,134} When measuring these cytokines in BALF, concentrations of IL-4 and IFN-\(\gamma\) were below the limits of detection in cats with naturally occurring asthma, experimentally-induced asthma, and naturally occurring chronic bronchitis.\textsuperscript{129,132} No significant difference was detected in TNF-\(\alpha\) concentrations between the groups.\textsuperscript{129} These results indicate the lack of benefit of measurement of these cytokines for diagnosis and determination of long-term prognosis and response to therapy in asthmatic cats. Nitric oxide metabolites were increased in cats with naturally-occurring chronic bronchitis compared to those with non-septic suppurative inflammation or research cats with asthma.\textsuperscript{129} Although a significant difference was found, the sample size of this study was small and methods for measurement of these biomarkers are not readily available, making the measurement of this biomarker not clinically useful.\textsuperscript{129} Endothelin-1 (ET-1) is implicated in airway inflammation, bronchoconstriction and structural remodelling of the airways in humans.\textsuperscript{135} Cats with experimentally induced asthma have significantly higher concentrations of BALF ET-1 compared to healthy controlled cats.\textsuperscript{130} However, no investigation of BALF ET-1 concentration was performed in cats with naturally occurring asthma or other causes of lower respiratory tract disease, thus the ability of this biomarker to determine the etiology of a cat’s lower respiratory tract disease is unknown at this time. Further, progression in relation to the clinical course of disease is unknown; therefore, information regarding the utility of this biomarker in guiding treatment and long-term prognosis is not available at this time.
1.4.11 Hemosiderophages

Hemosiderin is an iron storage complex consisting of ferritin, denatured ferritin, iron and other material.\textsuperscript{136} Although an iron storage complex, the presence of hemosiderin in macrophages (hemosiderophages) is not thought to be reflective of a defect in iron metabolism. Prior hemorrhage or increased erythrocyte migration or diapedesis secondary to vascular congestion or pulmonary hypertension cause increased quantity of stainable iron within tissues due to increased red blood cell degradation by pulmonary macrophages.\textsuperscript{137,138}

The major differential diagnoses for airway hemosiderophages in feline patients include feline asthma, heartworm disease, traumatic injury, infection, foreign body migration, lung lobe torsion, embolism or infarction, neoplasia, bleeding disorders and congestive heart failure.\textsuperscript{139} A retrospective study investigating the associated etiologies with pulmonary hemosiderosis demonstrated that hemosiderosis was a common finding associated with pulmonary neoplasia, asthma or cardiac disease.\textsuperscript{139,140} Samples collected in that study were only from cats with clinical signs of respiratory tract disease and sample collection was limited to tracheal wash as opposed to bronchoalveolar lavage; thus a low proportion of alveolar macrophages were obtained. In human adults, and children with idiopathic pulmonary hemosiderosis, an association between presence of hemosiderosis and increased exposure to moulds and platelet function defects has been found, however the true underlying etiology and reasons for development of more severe hemosiderosis in some patients compared to others continues to be unknown.\textsuperscript{141,142} Two cats with a history of mold exposure were reported to have acute pulmonary hemorrhage during general anesthesia; however, the occurrence of exposure to this inhaled toxin is rare in most household cats, so this is unlikely to be the cause of pulmonary hemosiderosis for most feline patients. Mild to moderate hemosiderosis has been found to be associated with increased eosinophil count in asthmatic cats.\textsuperscript{140}
Inhaled aeroallergens and particulate matter may be the trigger for subclinical pulmonary hemorrhage causing hemosiderosis in these patients. Further investigation of the incidence of hemosiderosis in cats without signs of respiratory disease is required, and ideally this would be performed on bronchoalveolar lavage fluid, which includes alveolar macrophages.

1.5 Improving the Diagnostic Investigation of Lower Respiratory Tract Disease in Cats

At present, there are no studies in the literature that directly compare samples obtained using the two major BAL techniques described in cats. Individually, both NB-BAL and B-BAL have been documented to be safe, feasible and minimally invasive techniques to obtain samples from the lower respiratory tract in cats. Further, it has been demonstrated that both techniques can provide diagnostic samples representative of the cells and proteins within the alveoli and distal airways. While B-BAL allows the clinician to examine the airway mucosa and sample focal lesions, the possible requirement for extubation can pose a significant risk to a feline patient with respiratory disease. On the other-hand, while NB-BAL can be performed with equipment readily available in most general practices via an endotracheal tube, ‘blind sampling’ may result in normal or non-representative cytologic findings, reducing the diagnostic utility of the procedure. It could be possible to modify the NB-BAL technique by using fluoroscopy and guide-wires, which would allow for selective catheterization of specific lung lobes to sample focal lesions, however this has not been documented in human or veterinary medicine. To obtain the most diagnostically accurate sample, the appropriate site for BAL must be selected. Thoracic radiographs are known to be poorly sensitive for characterizing the presence and location of lesions in the lower respiratory tract of cats. Thoracic CT is known to be superior to thoracic
radiography for characterizing the presence and location of lower respiratory tract lesions in cats. Several studies have documented the utility of non-sedated CT in the investigation of lower respiratory tract disease in cats.\textsuperscript{6,25} Computed tomography does not yield cytologic or microbiologic information, however, combining the use of thoracic CT with a BAL technique that allows for samples to be obtained from focal lesions would provide the most diagnostically useful information for cats with lower respiratory tract disease.

We hypothesized that there would be no significant difference in the quality of BALF samples retrieved by B-BAL and NB-BAL techniques. We further hypothesized that it would be feasible using fluoroscopy and guidewires to modify the NB-BAL technique such that selective catheterization of lung lobes can be performed. This would allow sampling of focal lesions and would provide an alternate option for BAL in cats where a bronchoscope was not available. The purpose of this research project was to compare B-BAL and NB-BAL techniques in healthy cats, and to develop a minimally invasive technique for performing BAL (fluoroscopic guided BAL) in cats with respiratory disease.
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2 Comparison of Bronchoscopic and Non-Bronchoscopic Bronchoalveolar Lavage in Healthy Cats

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2.1 Abstract

Objective

To compare bronchoalveolar lavage (BAL) accomplished by use of a bronchoscopic (B-BAL) and non-bronchoscopic (NB-BAL) technique in healthy cats.

Animals

12 healthy cats.

Procedures

Two BALs were performed in a randomized order 2 weeks apart in each cat. Cats were anesthetized, and a 2.9-mm fiberoptic bronchoscope (B-BAL) or 8F red rubber catheter (NB-BAL) were wedged in bronchus. Two 5-mL aliquots of saline (0.9% NaCl) solution were infused into the left and right caudal lung fields and aspirated manually with a 20-mL syringe. Proportion of BAL fluid (BALF) retrieved, depth of wedging, and anesthetic complications were recorded. Total nucleated cell count, differential cell count, and semiquantitative scores of cytologic slide quality were determined for all BALF samples. Results were compared with ANOVAs and Wilcoxon signed-rank tests.
Results

Proportion of retrieved BALF and depth of wedging was significantly greater for B-BAL than NB-BAL. Differential cell counts and cytologic slide quality did not differ significantly between techniques. Complications included transient hemoglobin desaturation (24/24 [100%] BALs) and prolonged anesthetic recovery time (4/24 [17%] BALs). Anesthetic recovery scores did not differ significantly between techniques.

Conclusions and clinical relevance

Results indicated that NB-BAL was noninferior to B-BAL in regard to ease of performance, anesthetic variables, and cytologic slide quality for cats without clinical respiratory disease.
2.2 Introduction

Bronchoalveolar lavage is a minimally invasive technique used to obtain luminal cells from terminal bronchi, bronchioles, and alveoli for investigating pulmonary disease.\(^1\) In human, canine, and equine medicine, certain aspects of BAL techniques can affect the quality of BALF samples and therefore the diagnostic and clinical use of those samples.\(^2,3\)

Two common techniques for performing BAL in cats have been described.\(^1,4\)–\(^6\) One involves a sterile catheter that is blindly wedged into a terminal bronchus (NB-BAL),\(^4,5\) and the other technique consists of a flexible bronchoscope wedged into a bronchus in the lung lobe of interest (B-BAL).\(^1,6\) Non-bronchoscopic BAL can be performed without a bronchoscope, which may be useful when such equipment is unavailable. However, blindly wedging the catheter in NB-BAL precludes visual examination of the airways and restricts the ability of clinicians to collect samples from multiple lung lobes or focal lesions, which may reduce the diagnostic value of the procedure for cats with focal small airway or pulmonary parenchymal disease.\(^7\) In contrast, B-BAL allows for examination of the airways, including visual examination of airway collapse, determination of the amount of mucus and erythema, and retrieval of foreign bodies.\(^8\) Samples may be collected from multiple lung lobes or focal lesions during B-BAL; however, depending on the diameter of the bronchoscope, extubation may be required, which can cause respiratory compromise and anesthetic complications.\(^1,6,9\) The purpose of the study reported here was to compare the quality of BALF samples obtained by NB-BAL and B-BAL techniques in healthy cats. We hypothesized that NB-BAL would be noninferior to B-BAL for procedural variables, anesthetic complications, and cytologic quality of samples.
2.3 Materials and Methods

Animals

Twelve healthy domestic shorthair cats were included in the study. Sample size calculation was based on data that indicated BALF retrieval in healthy cats of 56%⁵ and 79%¹ for NB-BAL and B-BAL, respectively. Therefore, it was estimated that 12 cats undergoing both techniques would be required to detect a difference of 10% in BALF retrieval between B-BAL and NB-BAL (power, 98%; α, < 0.05). Data regarding differences in quality of cytologic preparations related to different BAL protocols were unavailable.

The 12 cats were deemed healthy on the basis of results of a physical examination, CBC, serum biochemical analysis, fecal flotation, Baermann test, and ELISA to detect antibodies against FIV and FeLV antigen; measurement of total thyroxine concentration; and evaluation of 3-view thoracic radiographs by a board-certified veterinary radiologist (SGN). Cats had regularly received vaccinations and anthelmintic preventatives, and they did not have signs of respiratory tract disease during the 6 months preceding enrollment. Cats were cared for in a facility accredited by the Canadian Council on Animal Care. The study protocol was approved by the University of Guelph Animal Care Committee.

Study design

A randomized crossover study was conducted. Each cat underwent both B-BAL and NB-BAL; there was a 2-week interval between procedures. The order of the techniques and side on which the initial BAL was performed were determined by use of a random number table.
Anesthesia

Beginning approximately 28 hours before induction of anesthesia, each cat received 4 doses of terbutaline sulfate (0.01 mg/kg, SC, q 8 h); the last injection was administered 2 to 4 hours before induction of anesthesia. Cats were sedated by an IM injection of dexmedetomidine (5 µg/kg) and butorphanol tartrate (0.3 mg/kg). Anesthesia was induced by IV administration of propofol titrated to effect; anesthesia was maintained with a constant rate infusion of propofol that provided an adequate plane of anesthesia. To facilitate intubation, lidocaine was topically applied on the larynx; each cat then was intubated with a sterile endotracheal tube. All cats received IV fluids, and supplemental oxygen was provided via a 3.5F sterile feeding tube inserted through the endotracheal tube. Cats were monitored during anesthesia by means of a continuous ECG, pulse oximetry, and physical assessments. Adequate SpO2 was defined as > 95%. Procedures were terminated when SpO2 was < 90% for > 10 minutes or < 85% for > 5 minutes or a cat was bradycardic (heart rate < 80 beats/min) and unresponsive to standard interventions. The anesthesiologist scored the quality of anesthetic recovery after each BAL was completed (Appendix 1). The scoring system involved the required duration of oxygen supplementation after extubation, development of pneumothorax, requirement for ventilation, and potential procedure-related fatality, which are the most commonly reported complications related to BAL.

BAL

An esophageal marking catheter was inserted before each BAL procedure to measure the wedging depth of the inserted catheter and bronchoscope. For the NB-BAL technique, cats were placed in lateral recumbency, and the dependent lung was lavaged first. To lavage the opposite
lung lobe, cats were repositioned in the alternate lateral recumbency. An 8F soft red rubber catheter with 0.035 hydrophilic guide wire was wedged into a terminal bronchus. Location of placement was confirmed with fluoroscopy; fluoroscopic images were acquired in a dorsoventral projection. For the B-BAL technique, cats were placed in sternal recumbency. A 2.9-mm flexible fiber-optic endoscope was advanced through the endotracheal tube into the lung lobe of interest and wedged into a terminal bronchus; location of the endoscope was confirmed with fluoroscopy.

The right and left caudal lung lobes were lavaged with both techniques. Two 5-mL aliquots of sterile saline (0.9% NaCl) solution, each of which was followed by 2 mL of air, were infused, and then sequentially retrieved via gentle pulsatile manual aspiration with a 20-mL syringe attached to a conical plastic adapter connected to the end of the feeding tube (NB-BAL) or to the working channel of the endoscope (B-BAL). Dwell time (time from infusion of saline solution to the first attempted aspiration) was < 20 seconds during each collection. Aspiration was continued until fluid was no longer recovered. The bronchoscope was cleaned and sterilized with a standard cold sterilization method between successive uses.

Amount of fluid retrieved, proportion of fluid retrieved, lowest SpO₂, depth of wedging, and anesthetic recovery score were recorded. Fluoroscopic still images obtained during BAL were used to calculate depth of wedging by measuring the length of the inserted bronchoscope (B-BAL) or guide wire (NB-BAL) from the cranial aspect of the second rib to the point of wedging. Measurements were performed on images captured at peak inspiration.

**BALF analysis**
The BALF samples were immediately placed in sterile tubes on ice; they were delivered within 60 minutes after collection to a laboratory for processing. Each BALF cytology sample was labeled with a unique identification code. Cytologic evaluation was performed on each sample collected from each lung lobe. A separate small portion of each sample was pooled from both lung lobes in each cat and submitted for aerobic bacterial culture, *Mycoplasma* culture, and *Mycoplasma* PCR assay. Total nucleated cell counts were determined by electrical impedance with an automated cell counter. A 200-µL aliquot of each sample was cytocentrifuged (180 X g for 6 minutes) and used to prepare 2 slides. Two additional slides were prepared from fluid centrifuged at 500 X g for 5 minutes. Slides were stained with Wright stain, and differential cell counts of a minimum of 500 nucleated cells of all slides were performed at 400X magnification by a board-certified veterinary pathologist (DB) who was unaware of the source of each sample. For each slide, 7 variables associated with sample and slide quality were scored (Appendix 2). Mean scores ≥ 2 for cellularity and cell preservation were required for a BALF sample to be considered of adequate diagnostic quality. Samples were considered excellent when mean cellularity and cell preservation scores were ≥ 3. Inflammation in BALF was defined as a total nucleated cell count > 0.5 X 10⁹ cells/L or ≥ 17% eosinophils, ≥ 7% neutrophils, or ≥ 5% lymphocytes, as reported in another study.

CT

Seven days after the second BAL procedure, noncontrast CTs of the thorax of each cat was performed to further investigate potential causes of abnormal BALF cytologic findings. Cats were sedated by an IM injection of dexmedetomidine (5 µg/kg) and butorphanol tartrate (0.3 mg/kg) and placed in sternal recumbency in a transparent positioning device as described elsewhere.
During CT, cats received supplemental oxygen (fraction of inspired O\textsubscript{2}, 40%) via tubing that delivered oxygen into the positioning device. The CT protocol was as follows: pitch, 1.375; kV, 120; mA, 140; slice thickness, 0.625 mm; increment, 0.625; gantry rotation speed, 1 second; and a detail algorithm. Motion artifacts during CT scanning were noted, and CT was repeated when motion artifact was considered to interfere with interpretation.

*Dirofilaria immitis* immunoassay, NT-proBNP assay, and assessment of coagulation variables

After CT was completed, a jugular blood sample (5 mL) was obtained from each cat and used for coagulation tests (prothrombin and activated partial thromboplastin times), a *D immitis* lateral-flow immunoassay,\textsuperscript{t} and an NT-proBNP assay.\textsuperscript{u} Blood collected for detection of antibodies against *D immitis* was allowed to sit undisturbed at room temperature (21°C) for 20 minutes to clot; it then was centrifuged to separate the serum. Serum was harvested and stored frozen (–80°C) until used for testing.

Statistical analysis

Data distributions were assessed for normality with Shapiro-Wilk tests. Nonparametric data were logit transformed, and pair-wise comparisons between BAL techniques were made with the Wilcoxon signed rank test. A multivariate ANOVA was used to compare the effect of BAL technique and lung lobe of sample collection. Analyses were performed with a statistical software program.\textsuperscript{v} Values were considered significant at $P < 0.05$. 
2.4 Results

Animals

The 12 cats enrolled in the study consisted of 7 castrated males and 5 spayed females. Mean ± SD age was 6.2 ± 0.87 years (range, 4.5 to 6.9 years), and mean body weight was 5.3 ± 1.2 kg (range, 3.1 to 7.0 kg). All cats had unremarkable findings for physical examination and results of a CBC, serum biochemical analysis, fecal flotation, and ELISA for FIV and FeLV; measurement of total thyroxine concentration; and thoracic radiography.

Bronchoscopy and BAL

Bronchoscopic evaluation did not detect gross changes in any of the cats. Duration of BAL did not differ significantly \((P = 0.35)\) between B-BAL (median, 28 minutes; range, 12 to 50 minutes) and NB-BAL (median, 28 minutes; range, 21 to 47 minutes; Table 2.1). A significantly \((P = 0.01)\) greater number of attempts was required to catheterize the lung lobe of interest when performing NB-BAL (median, 1.5 attempts; range, 1 to 6 attempts) than when performing B-BAL (median, 1 attempt; range, 1 to 2 attempts). For NB-BAL, successful catheterization of the lung lobe was achieved on the first attempt in only 6 of 12 catheterizations. Median total volume of infusate was 1.89 mL/kg (range, 1.43 to 3.18 mL/kg). Depth of wedging was significantly \((P = 0.01)\) greater for B-BAL (median, 90.25 mm; range, 72.80 to 122.00 mm) than for NB-BAL (median, 81.60 mm; range, 67.20 to 92.90 mm). The proportion of BALF retrieved was significantly \((P = 0.01)\) greater for B-BAL (median, 70%; range, 10% to 100%) than for NB-BAL (median, 55%; range, 20% to 80%).

-55-
Impact of BAL on anesthetic recovery

Anesthetic recovery scores did not differ significantly \((P = 1.00)\) between B-BAL (median, 1; range, 1 to 2) and NB-BAL (median, 1; range, 1 to 2). Anesthetic recovery score for 1 cat was 2 for both techniques, and anesthetic recovery scores for 2 other cats was 2 for one technique but not for the other technique. For these 3 cats, a prolonged period of oxygen supplementation was required after extubation to maintain \(\text{SpO}_2 > 95\%\). For the remaining BAL procedures, all cats recovered without complications and did not require oxygen supplementation for > 10 minutes after extubation to maintain \(\text{SpO}_2 > 95\%\). The lowest \(\text{SpO}_2\) recorded during the BAL procedures did not differ significantly \((P = 0.46)\) between B-BAL (median, 83%; range 58% to 92%) and NB-BAL (median, 78%; range, 54% to 87%).

BALF analysis

Total or differential cell counts for macrophages, neutrophils, eosinophils, lymphocytes, and mast cells did not differ significantly between BAL techniques (Table 2.1). Cytologic quality of slide preparations also was not significantly different between the 2 techniques. Median cellularity score for samples obtained by both techniques was 3 (range, 1 to 4). One sample obtained by use of B-BAL and 3 samples obtained by use of NB-BAL had a cellularity score of 1. The median cell preservation score did not differ significantly for samples obtained by use of B-BAL (4; range, 2 to 4) and NB-BAL (4; range, 0 to 4). One sample obtained by use of NB-BAL yielded poor cell preservation. There were no significant differences in median scores for RBCs, epithelial cells, or mucus between B-BAL and NB-BAL.
Dark gray-blue cytoplasmic granules were detected in macrophages of BALF samples from all cats. Examination of specimens stained with Prussian blue stain indicated that these granules contained iron, which confirmed that these macrophages were hemosiderophages. A moderate to marked number of hemosiderophages was present in all samples, and the median score did not differ significantly between BAL techniques (Table 2.1).

Differential cell counts did not differ significantly between the 2 techniques. On the basis of a total nucleated cell count > 0.5 X 10^9 cells/L or an increased proportion of eosinophils or neutrophils, results for 12 BAL samples were consistent with findings for cats with asthma,^{10,12} and results for all 24 BAL samples were suggestive of bronchitis.^{10,12}

Aerobic bacterial culture and *Mycoplasma* PCR assay results were negative for all BALF samples. *Mycoplasma* cultures yielded positive results for 2 samples each obtained by B-BAL and NB-BAL (1 sample each *Mycoplasma felis* and *Ureaplasma* with each technique). From the same individuals, the B-BAL sample was *Mycoplasma* culture positive and the NB-BAL sample was *Mycoplasma* culture negative; conversely, NB-BAL samples were *Mycoplasma* culture positive and B-BAL samples negative. Three cats with positive results for culture of *Mycoplasma* spp or *Ureaplasma* spp had eosinophilic inflammation evident on cytologic examination, and 1 cat with positive results for culture of *Ureaplasma* spp had neutrophilic inflammation evident on cytologic examination.
Table 2.1 Values for procedure variables and results of cytologic analysis for NB-BAL and B-BAL in 12 cats

<table>
<thead>
<tr>
<th>Variable</th>
<th>NB-BAL median (range)</th>
<th>B-BAL median (range)</th>
<th>Median difference (NB-BAL – B-BAL)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL Duration (minutes)</td>
<td>28 (21-48)</td>
<td>28 (12-50)</td>
<td>0</td>
<td>0.35</td>
</tr>
<tr>
<td>Lowest SpO2 (%)</td>
<td>78.0 (54-87)</td>
<td>82.5 (58-92)</td>
<td>-4.5</td>
<td>0.46</td>
</tr>
<tr>
<td>Infusate retrieved (%)</td>
<td>55 (20-80)</td>
<td>70 (10-100)</td>
<td>-15</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Length of catheter at wedging (mm)</td>
<td>81.6 (67.2-92.9)</td>
<td>90.3 (72.8-22.0)</td>
<td>-8.65</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Attempts to wedge (number)</td>
<td>1.5 (1-6)</td>
<td>1 (1-2)</td>
<td>0.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TNCCc (x10⁹/L)</td>
<td>1.00 (0.37-2.80)</td>
<td>1.04 (0.31-3.80)</td>
<td>-0.04</td>
<td>0.80</td>
</tr>
<tr>
<td>Cytology Score²,12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellularity</td>
<td>3 (1-4)</td>
<td>3 (1-4)</td>
<td>0</td>
<td>0.72</td>
</tr>
<tr>
<td>Cell preservation</td>
<td>4 (0-4)</td>
<td>4 (2-4)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>0.5 (0-4)</td>
<td>0 (0-4)</td>
<td>0.5</td>
<td>0.20</td>
</tr>
<tr>
<td>Epithelial cells</td>
<td>1 (1-3)</td>
<td>1 (0-4)</td>
<td>0</td>
<td>0.58</td>
</tr>
<tr>
<td>Bacteria</td>
<td>0 (0-1)</td>
<td>0 (0-0)</td>
<td>0</td>
<td>0.50</td>
</tr>
<tr>
<td>Hemosiderophages</td>
<td>3 (2-4)</td>
<td>3 (1-4)</td>
<td>0</td>
<td>0.71</td>
</tr>
<tr>
<td>Mucus</td>
<td>1 (0-4)</td>
<td>2 (0-4)</td>
<td>-1</td>
<td>0.42</td>
</tr>
<tr>
<td>Cell type (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
<td>68.5 (24-90)</td>
<td>70.0 (41-91)</td>
<td>-1.5</td>
<td>0.85</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>5.0 (1-17)</td>
<td>5.5 (1-23)</td>
<td>-0.5</td>
<td>0.85</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>13.0 (0-73)</td>
<td>10.0 (0-28)</td>
<td>3</td>
<td>0.20</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>8.5 (0-36)</td>
<td>9.5 (2-36)</td>
<td>-1</td>
<td>0.27</td>
</tr>
<tr>
<td>Mast cells</td>
<td>0 (0-1)</td>
<td>0 (0-0)</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

a Non-bronchoscopic bronchoalveolar lavage
b Bronchoscopic bronchoalveolar lavage
c Total nucleated cell count

See Appendix 2 for scoring description.
CT findings

Despite the lack of clinical or radiographic signs of respiratory tract disease, CT changes were identified in 9 of 12 cats. Four cats had ground-glass opacities in the right cranial lung lobe (n = 1), right cranial and caudal lung lobes (2), and right caudal lung lobe (1); 2 cats had focal soft tissue opacities in the left cranial lung lobe; 2 cats had ground-glass opacities and soft tissue opacities in the right and left cranial lung lobes; and 1 cat had a diffuse reticular pattern affecting all lung lobes. Four cats had lesions in the right caudal lung lobe. Of these cats, 3 also had CT lesions in another lung field where BAL had not been performed (cranial lung lobe). Eight cats had evidence of thickened bronchi on thoracic CT that had not been evident on thoracic radiographs. Seven of these 8 cats had BALF with higher eosinophil (n = 3), neutrophil (2), or eosinophil and neutrophil (2) counts in BALF samples obtained with both techniques.

Dirofilaria immitis immunoassay, NT-proBNP assay, and assessment of coagulation variables

All cats had negative results for antibodies against D immitis, and coagulation times were within reference intervals. One cat had a high NT-proBNP concentration (175 pmol/L). Echocardiography of that cat revealed hypertrophic cardiomyopathy. The NT-proBNP concentration for all other cats (median, 61 pmol/L; range, 24 to 85 pmol/L) was within the reference interval (<100 pmol/L).
2.5 Discussion

In the study reported here, both B-BAL and NB-BAL were relatively easily performed and yielded samples with no significant differences in quality. Both techniques allowed for collection of samples from specific lung lobes, which is useful in cats with small airway or pulmonary parenchymal disease because cytologic findings can differ among lung lobes.\(^7\) Samples were obtained from only the caudal lung fields, but it would be of interest to compare the feasibility of collection of samples from other lung fields with these techniques. Use of fluoroscopy to guide the catheter for NB-BAL could be useful when a small-diameter bronchoscope is not available.

Maintenance of an intubated airway throughout the entire BAL procedure in each cat likely contributed to the low complication rate. Laryngospasm from repeated intubation and insertion of the bronchoscope during bronchoscopy was avoided,\(^13\) and the risk of bronchospasm was reduced by premedication with bronchodilating agents.\(^9\) Therefore, NB-BAL may constitute a safe procedure in intubated cats when bronchoscopy is not available. A limitation of NB-BAL is the inability to visually examine the airway mucosa, but the appearance of the mucosa did not contribute important information for cats with respiratory tracts disease in another study.\(^8\)

To allow for retrieval of BALF from the same lung lobes and comparison of BALF cytologic findings between the 2 techniques, fluoroscopy was used to determine catheter location and to guide sample collection for NB-BAL. Therefore, the NB-BAL technique described was a modification of the blind NB-BAL technique. For the blind NB-BAL technique, the catheter is wedged in the distal portion of a bronchus where lavage is initiated, and the location of sample collection is unknown. Compared with results for the blind NB-BAL technique, use of fluoroscopy
to confirm the location of sample collection for NB-BAL would have increased the procedural time and number of attempts required to wedge the catheter in the desired location.

The proportion of BALF retrieved ranged from 55% to 80%, which is consistent with other reports\textsuperscript{1,4-6} that involved highly variable infusion volumes. A significantly greater proportion of BALF was retrieved with B-BAL than with NB-BAL, which likely was attributable to differences in instrumentation, depth of wedging, and body position.

Outer diameter of the bronchoscope and catheter was similar for B-BAL and NB-BAL; however, rigidity, internal diameter, and length of tube differed and could have affected the pressure required to aspirate and retrieve fluid. To minimize pressure differences between instruments, a mechanical suction unit could have been used to provide consistent continuous aspiration of BALF. Use of such an aspiration technique for cats has not been evaluated, but improved retrieval of BALF in dogs has been reported.\textsuperscript{2,3,14}

Because the bronchoscopic technique allowed for direct visual examination of the airways, it was not surprising that it resulted in a greater wedging depth. Visual examination was not possible during NB-BAL; therefore, incomplete wedging of the catheter may have led to leakage of infusate into other lung lobes and less fluid retrieval, compared with fluid retrieval for B-BAL.

Differences in body position also may have contributed to differences in the depth of wedging and percentage of fluid retrieved, as described elsewhere.\textsuperscript{15} Although samples were collected from the caudal lung lobes for both techniques, differences in body position may have influenced the portion of the lung field that was infused and the location where the infused fluid pooled.
Surprisingly, positioning the cats in lateral recumbency did not result in the NB-BAL catheter readily entering the dependent lung lobe, as was suggested in another report.\textsuperscript{4} Rather, the catheter was not in the correct location on the first attempt for half of the NB-BAL procedures. Fluoroscopy was useful for visual evaluation of the location and the passage of the catheter. If fluoroscopy were to be used to guide sample collection for NB-BAL, it would likely be feasible to perform this technique on cats positioned in sternal recumbency, which would be beneficial for visual evaluation of both the left and right lung lobes with minimal superimposition and facilitate access to and sample collection from all lung lobes.

Discrepant results for \textit{Mycoplasma} culture and PCR assay were found for 4 BALF samples. It has been suggested in some, but not all, studies that PCR assay is more sensitive than culture.\textsuperscript{16,17} In the present study, samples that had positive results for \textit{Mycoplasma} culture yielded only low numbers of \textit{Mycoplasma} spp or \textit{Ureaplasma} spp, and the \textit{Mycoplasma} PCR assay had a higher limit of detection than did \textit{Mycoplasma} culture. Therefore, it was feasible that a PCR assay may yield negative results, despite positive results for culture. Discordant \textit{Mycoplasma} culture results between the 2 BAL techniques could have arisen from transient nonpathogenic colonization of the small airways or pulmonary parenchyma with \textit{Mycoplasma} spp or \textit{Ureaplasma} spp or failure of growth after transient storage in saline solution.\textsuperscript{18,19} \textit{Ureaplasma} spp were cultured from 1 NB-BAL sample with neutrophilic inflammation. That same cat had neutrophilic inflammation in the sample collected by use of B-BAL, but \textit{Ureaplasma} spp were not identified. Considering the absence of clinical or radiographic signs suggestive of \textit{Ureaplasma} infection, it was most likely that a positive culture result reflected nonpathogenic colonization of the small airways or pulmonary parenchyma. In cats with clinical signs of small airway or pulmonary parenchymal
disease, results of *Mycoplasma* culture should be considered in conjunction with findings of thoracic imaging and BALF cytologic examination to determine the potential clinical relevance.\(^{19}\)

In the study reported here, 43 of 48 samples had cellularity and cell preservation scores \(\geq\) 2, which were considered to be of adequate diagnostic quality.\(^{20,21}\) Five samples had low cellularity scores (3 NB-BAL and 1 B-BAL) or excessive cell lysis (1 NB-BAL). Potential causes of low cell yield on glass slides despite adequate cell counts include excessive mucus content of samples that prevents cell deposition on slides during cytocentrifugation, inclusion of a large number of epithelial cells in the BALF, inadequate mixing of samples, and processing errors.\(^{22-26}\) Of the 4 samples with low cellularity scores, 2 were from BAL with limited fluid retrieval (approx 30\%), and 2 were from BAL with adequate fluid retrieval (50\% to 80\%).\(^{21,27}\) For those samples, there was no evidence of admixture of epithelial cells or excessive mucus content, and the nucleated cell count was within the reference interval. Therefore, it was most likely that samples were not adequately mixed to provide a homogeneous cell distribution prior to cytocentrifugation. Cell lysis can result from excessive suction applied during BALF retrieval, excessive centrifugal force during cytocentrifugation, or exposure to hypotonic fluids. The cause of the limited quality of the 5 slides was not investigated.

Hemosiderophages were present in all samples. Hemosiderophages are not considered a typical finding in BALF of clinically normal cats, but they are common in cats with conditions such as asthma, neoplasia, heartworm disease, coagulopathies, and cardiac disease.\(^{15,28}\) Considering that hemosiderophages were present in the first BAL samples, it was unlikely that they were the result of hemorrhage during the procedure because of the short procedure time and the time required for acute hemorrhage to manifest as hemosiderophages. Heartworm disease,
coagulopathies, cardiac disease, and bronchopneumonia were ruled out on the basis of negative antibody test results, coagulation times within reference intervals, NT-proBNP concentrations within the reference interval, and negative results for bacterial culture, respectively. One cat had hypertrophobic cardiomyopathy as indicated by echocardiographic findings and a high NT-proBNP concentration, but there was no evidence of congestive heart failure. Therefore, the BAL cytologic findings for 10 cats of the study were consistent with asthma or chronic bronchitis, despite the fact the cats were clinically normal at the time of enrollment. The CT examination revealed that thick bronchial walls were more common in cats with eosinophilic than neutrophilic inflammation, which is also consistent with asthma.29–32 The proportion of eosinophils in BAL obtained from clinically normal cats was < 7%33,34 and < 17%.1,33 Such discrepancies may result from differences in antigenic exposure, animal housing density, air quality, and other factors associated with group housing. For the purposes of the present study, we used reference limits listed in another study.12 Pulmonary changes were identified with thoracic CT, but not radiography, in all cats, except for 3, at study entry, which is consistent with reports7,15 of the relative insensitivity of thoracic radiography for the diagnosis of structural changes in cats with disease of the small airways or pulmonary parenchyma. Ideally, thoracic CT would have been performed on all cats before the start of the study, but because we did not anticipate that cats would have asthma, radiography was considered sufficient to rule out major respiratory tract disease. Lesions identified by use of CT were considered to indicate true lung disease because the lung lobes that were not lavaged also were affected. Furthermore, considering there was evidence of eosinophilic or neutrophilic (or both) inflammation, it was likely that most cats had subclinical asthma or bronchitis (or both). Histologic or cytologic examinations might have further characterized the lesions,7,15 but they were not performed.
The study had several limitations. The 2 BAL techniques were evaluated in research cats deemed to be healthy on the basis of results of physical examination, screening hematologic evaluation, and thoracic radiography. The low anesthetic and procedural complication rates for both BAL techniques may have been attributable, in part, to the health status of the subjects, and these rates might be higher in cats with respiratory compromise. Cytologic findings for BALF were abnormal in most cats. Although cats were found to have subclinical asthma or bronchitis (or both), these conditions were considered unlikely to have impacted comparing the results for the BAL techniques and might even be more relevant for patients requiring BAL for diagnostic investigation. Furthermore, the crossover design should have limited the impacts of any procedure-associated effects. Cats in research colonies are housed in environments that differ from those for client-owned cats, and research cats may be exposed to a greater density of inhaled allergens, which would account for subclinical asthma. Another limitation was the relatively small sample size, which may have resulted in type II errors.

In the present study, NB-BAL was not inferior to B-BAL regarding ease of performance, patient anesthetic stability, and quality of cytologic preparations for cats with no clinical signs of respiratory tract disease. Although B-BAL allowed for greater depth of wedging, visual examination of the bronchial mucosa, and greater fluid retrieval, there were no significant differences in cytologic quality or differential cell counts between techniques. Future studies may incorporate fluoroscopy in NB-BAL to guide placement of catheters to reach specific lung lobes or focal lung lesions.
2.6 Footnotes

a. Chiron Compounding Pharmacy, Guelph, ON, Canada.

b. Dexdomitor, Zoetis Canada Inc, Kirkland, QC, Canada.

c. Torbugesic, Zoetis Canada Inc, Kirkland, QC, Canada.

d. Fresenius Kabi Canada Ltd, Richmond Hill, ON, Canada.

e. Lidodan Endotracheal, Odan Laboratories, Point-Claire, QC, Canada.

f. Sheridan/CF endotracheal tube, Teleflex Medical Canada Inc, Markham, ON, Canada.

g. Kangaroo polyvinyl chloride feeding tube with radioopaque line, Covidien, Saint Laurent, QC, Canada.

h. Sizing catheter, Infiniti Medical, Menlo Park, Calif.

i. Rusch soft rubber bladder catheter, Teleflex Medical Canada Inc, Markham, ON, Canada.

j. Weasel Wire, Infiniti Medical, Menlo Park, Calif.

k. BV Endura Mobile C-arm, Philips Healthcare, Markham, ON, Canada.

l. Flex X, Karl Storz Endoscopy Ltd, Mississauga, ON, Canada.

m. Monoject 20-mL syringe with Leur-lock tip, Covidien, Mansfield, Mass.

n. Plastic multipurpose tubing adapter, Cook Inc, Bloomington, Ind.

o. Animal Health Laboratory, University of Guelph, Guelph, ON, Canada.

p. Universal mycoplasma detection kit (ATCC 30-1012K), ATCC, Manassas, Va.

q. Z2 Coulter counter, Beckman Coulter, Mississauga, ON, Canada.


s. GE Bright Speed, General Electric Healthcare, Milwaukee, Wis.

t. Heska Solo Step FH, Heska, Barrie, ON, Canada.

u. Cardiopet proBNP test–feline, IDEXX Laboratories Inc, Markham, ON, Canada.
2.7 Reference


3 Use of Fluoroscopic-Guided Bronchoalveolar Lavage to Sample The Lower Respiratory Tract in Two Cats with Respiratory Disease

3.1 Abstract

Case Description

Two cats (0.75 and 3.0 years of age) were examined because of spontaneous abnormal respiration.

Clinical Findings

Thoracic radiography demonstrated diffuse respiratory disease in both cats. Computed tomography confirmed radiographic findings in one out of two cats.

Airway Sampling and Outcome

Cats underwent fluoroscopic-guided bronchoalveolar lavage (BAL) under general anesthesia. Using a red rubber soft catheter and guide wire, selective catheterization and lavage of the radiographically most severely affected lung lobes (left and right caudal) was successful in Cat 1. Selective catheterization of the right cranial lung lobe failed in Cat 2; however, similar radiographic lesions were visible in the left caudal and right middle lung lobes, and these were lavaged successfully. Analysis of the BAL fluid samples showed excellent cytologic quality. The eventual diagnoses were bronchopneumonia and bronchitis in cats 1 and 2, respectively. Both cats were discharged the same day that fluoroscopic-guided BAL was performed.

Clinical Relevance
Fluoroscopic-guided BAL can be used to collect bronchoalveolar lavage fluid in cats with respiratory disease. Bronchoalveolar lavage was able to be performed in the caudal lung lobes and right middle lung lobe using this technique in two cats. Attempts to catheterize and perform bronchoalveolar lavage in the cranial lung lobes was unsuccessful in one cat. Further studies are required to determine in which patients fluoroscopic-guided BAL is most appropriate, and to refine the technique.
3.2 Case 1

A 0.75-year-old, male castrated domestic long hair cat (Cat 1) weighing 4.6 kg [10 lb] was presented with chronic (8 months) increased respiratory sounds (i.e., gurgling) and intermittent moist, coughing. Results of a complete blood count (CBC) were within reference limits. Feline leukemia virus antigen test, feline immunodeficiency virus antibody test, Baermann and fecal flotation tests were negative. Thoracic radiographs revealed a mild interstitial pulmonary pattern in the left caudal lung lobe and soft tissue opacity within the region of the (right) accessory lung lobe.

To further characterize changes on thoracic radiographs, bronchoalveolar lavage was recommended. Cat 1 underwent fluoroscopic-guided bronchoalveolar lavage (F-BAL), an investigational procedure approved by the University of Guelph Animal Care Committee. The cat was sedated with butorphanola and acepromazineb and placed in sternal recumbency. General anesthesia was induced with propfolec (25 mg, IV) and the cat was intubated with a sterile, size 4 endotracheal tube.d The cat received salbutamole at induction to decrease the risk of bronchoconstriction.1 The patient was maintained under general anesthesia with continuous rate infusion of propfolec at 150-350 µg/kg/min [68-159 µg/lb/min]. Fluoroscopic-guided bronchoalveolar lavage was performed. A 5 Fr feeding tubef as placed in the endotracheal tube and connected to an anesthetic machine to provide continuous flow by oxygen supplementation at 3L/min during the procedure. An 8 Fr red rubber soft catheterg was cut at the distal tip and a Christmas tree adapterh was inserted into the proximal end. A 0.035” angled hydrophilic wirei was inserted via the Christmas tree adaptor into the red rubber catheter with 0.5 cm of the soft angle tip extending out of the distal end of the red rubber catheter (Figure 3.1). A mosquito hemostat
was placed on the wire at the proximal end of the red rubber catheter to prevent further advancement of the wire inside the lungs. The combination of wire and red rubber catheter was then inserted into the endotracheal tube and selective catheterization to facilitate BAL of the affected lung lobes was performed using fluoroscopy in vertical beam (dorsoventral view). At each BAL site, two aliquots of 5 mL of warmed (37°C [98.6°F]) sterile, 0.9% sodium chloride (saline) solution followed by 2 mL of air were injected sequentially and immediately retrieved by gentle hand suction with a 20 mL syringe. The left caudal and right caudal lobes were catheterized and lavaged successfully in this manner.

Figure 3.1 Standard angle hydrophilic weasel wire inserted into an 8Fr feeding tube and a Christmas tree adaptor. Mosquito forceps placed at the proximal end of the feeding tube to prevent further movement of the wire.
The percentage of volume of BALF retrieved was approximately 50% from each side. The duration of general anesthesia (from intubation to the extubation) was 30 minutes. Based on pulse oximetry, oxygen saturation of hemoglobin was 100% within 5 minutes after the last lavage. Extubation occurred uneventfully 10 minutes after end of the F-BAL procedure.

Specimens from each site were placed in individual tubes on ice following collection and cytological analyses were conducted within 1 hour of collection at the Animal Health Laboratory (Guelph, Ontario, Canada). Nucleated cell concentrations were determined by electrical impedance. A 200 µL aliquot of each sample was cytocentrifuged for 6 minutes at 180Xg and additional slides were prepared from fluid sedimented for 5 minutes at 500Xg.Slides were Wright-stained and differential cell counts of ≥400 nucleated cells were performed at 400X magnification on cyto centrifuge preparations by a board-certified pathologist (DB) blinded to the origin of samples. The same pathologist also semi-quantitatively assessed 5 variables representative of sample and slide quality: cellularity, number of clusters and sheets of epithelial cells, cell preservation and number of bacteria (Appendix 2). A small aliquot derived from the 2 BALF sample sites was pooled and submitted for bacterial and Mycoplasma culture.

Cytologic evaluation of BALF revealed suppurative inflammation and intra- and extracellular bacteria of rod and coccoid morphology. Cellularity of both washes (left and right) was scored as 4 (highest possible score is 4). Cell preservation was scored as 3/4 (right side) and 4/4 (left side). Culture of the BALF yielded positive results for Mycoplasma felminutum and Pasteurella multocida. A diagnosis of bronchopneumonia was made, and the cat was discharged the same day as the F-BAL procedure. The cat received antibiotics (doxycycline 5mg/kg [11
mg/lb] orally twice daily for 21 days) and improvement was noted by day 2. Recurrent lower respiratory tract signs were again noted 8 months later, and the cat was empirically treated again with antibiotics (amoxicillin-clavulanic acid\(^a\) 13.5mg/kg [30mg/lb] orally twice daily for 14 days). No diagnostic tests were performed at that time.

### 3.3 Case 2

Cat 2 was a 3.0-year-old, female, spayed, domestic short hair cat weighing 5.6 kg [12.3 lb] with a 3-month history of coughing and wheezing. Results of a CBC showed no abnormalities, and results of feline leukemia virus antigen, feline immunodeficiency virus antibody, Baermann and fecal flotation tests were negative. Cardiomegaly and multifocal bronchocentric pulmonary opacities were present on thoracic radiographs, but there was no evidence of pulmonary edema. N-terminal-pro brain natriuretic peptide\(^d\) was 24 pmol/L; a value inconsistent with cardiac disease.\(^2\) Thoracic computed tomography (CT)\(^f\) revealed diffuse, ground glass lesions in the caudodorsal lung lobes; focal, hyperdense structures in the right cranial, right middle and left caudal lung lobes; left caudal lobar bronchial wall thickening and irregularity; and mild sternal lymphadenomegaly.

This cat also underwent F-BAL, was sedated with butorphanol\(^a\) combined with dexmedetomidine\(^e\) and placed in sternal recumbency. General anesthesia was induced with propofol\(^e\) (25 mg, IV). The cat was intubated with a sterile endotracheal tube\(^d\) (size 5.0) and received salbutamol\(^e\) at induction to decrease risk of bronchoconstriction.\(^1\) The cat was maintained with continuous rate infusion of propofol\(^e\) at 150-350 \(\mu\)g/kg/min [68-159 \(\mu\)g/lb/min], and F-BAL was performed in the same manner as described for Cat 1.
Using fluoroscopic guidance, the right cranial lung lobe could not be selectively catheterized using the guide wire and red rubber catheter as described above. However, the left caudal and right middle lung lobes had similar radiographic lesions and were selectively catheterized and lavaged (Figure 3.2). The percentage of BALF volume retrieved was 50% and 40% for the left and right sides, respectively. The duration of general anesthesia (from intubation to the end of the procedure) was 45 minutes. Oxygen saturation of hemoglobin (measured as in Cat 1) was 100% within 5 minutes after the last wash. Extubation occurred uneventfully 10 minutes after the F-BAL procedure ended. The cat did not need oxygen supplementation after F-BAL.

Figure 3.2 Fluoroscopic image of catheterization of the right middle lung lobe using an 8Fr feeding tube combined with a hydrophilic weasel wire Cat 1.
Samples of BALF were processed as described above. Cellularity and cell preservation in both samples had scores of 4 (Appendix 1). Cytologic evaluation revealed mixed, non-suppurative inflammation with hemosiderin pigment and intra- and extracellular bacteria. The BALF culture result was negative. The cat was diagnosed with bronchitis and was discharged on the day of the F-BAL procedure. The cat received prednisolone (5mg orally once daily) and improvement was noted by day 2. Long-term follow-up was unavailable.

3.4 Discussion

Bronchoalveolar lavage is rarely performed in cats because of the risk of complications such as bronchoconstriction and laryngospasm. Unless a small endoscope is available (≤4 mm diameter), extubation is required in most cases, which increases the risk of bronchoconstriction and laryngospasm, and compromises recovery. Similar to feline medicine, the bronchoscopic BAL technique also has complications in human pediatric patients because patients are usually intubated with small endotracheal tubes and bronchoscopy is difficult to perform whilst maintaining a patent airway. Non-bronchoscopic BAL has been considered a good alternative since it allows the clinician to keep the endotracheal tube in place. In human pediatric intensive care units, this technique has been developed over the last 20 years and is the technique of choice for sampling the lower airways of pediatric patients. The major drawback of non-bronchoscopic BAL is that specific affected lung lobes cannot be sampled as the catheter used for BAL is inserted blindly.

Selecting and lavaging the most affected lung fields in cats is important to better characterize disease and appropriately treat patients. We recently reported on 12 healthy cats that
underwent modified, non-bronchoscopic BAL. In all cats, the left and right caudal lung lobes were lavaged using a red rubber tube with a hydrophilic weasel wire guided by fluoroscopy. The BALF samples were of excellent quality, and the procedures were uneventful. The 2 cases reported here are the first patients with clinical respiratory disease in whom F-BAL was used.

In the described cases, selective catheterization of the right caudal, right middle and left caudal lung lobes was possible, while retaining an endotracheal tube in place during the entire procedure. In one cat, the cranial lung lobe was not successfully catheterized. However, the contralateral caudal lung lobe with similar lesion was successfully catheterized and lavaged. Right cranial lung lobes may be difficult to catheterize because of the sharp angle of the right cranial bronchus. Another type of catheter (eg, Berenstein or Cobra) with greater flexibility and/or wider potential angle might increase success with catheterizing the cranial lung lobes. Contrast resolution of fluoroscopy is less than that of radiography. It is difficult to localize the carina on fluoroscopic images. However, the location of the carina was determined in relation to the ribs on thoracic radiographs. Knowledge of the location of the carina facilitated the efficient manipulation of the wire for insertion into the desired bronchus (left vs. right).

In Cat 1 the catheter seemed to be in the region of the accessory lung lobe (Figure 3.3). However, due to superimposition, the accessory lung lobe is difficult to distinguish from the right caudal lung lobe both radiographically and fluoroscopically. An orthogonal view of the chest might be helpful, but depending on the fluoroscopic equipment used, this may be an easy or difficult step to add to the procedure.
The percentage of fluid retrieved was comparable to that of other methods, and the cytologic quality of samples was excellent. Analysis of BALF yielded diagnosis and therapeutic options that improved clinical outcomes in both patients. Until a more suitable catheter or wire to catheterize the cranial lung lobe in cats is identified, performing bronchoalveolar lavage using F-BAL may not be feasible in this location.

There were no complications associated with F-BAL in these two patients. However, caution is indicated for manipulation of the wire since advancement beyond the feeding tube poses
a risk of bronchial perforation and pneumothorax. Mosquito hemostats were applied to the guide wire at the proximal end of the red rubber catheter. These were used to prevent advancement of the guide-wire more than 0.5cm beyond the distal cut end of the soft red rubber catheter. Ability to retain the endotracheal tube in place throughout the BAL is one of the biggest advantages of this BAL technique compared to the bronchoscopic technique. Unless endoscopes <4 mm in diameter are used, intermittent extubation is required to insert the endoscope. Multiple extubations and intubations increase the risk of laryngospasm and bronchoconstriction, and complicate recovery in cats.3,14

One limitation of F-BAL is that the airway mucosa cannot be evaluated. However, in a recent study of 48 cats with various respiratory diseases (i.e., inflammatory, neoplastic and infectious) gross bronchoscopic abnormalities did not differ significantly between different disease categories.15,16 Moreover, 25% of cats with respiratory disease had airway collapse, which can complicate treatment and worsen prognosis. Fluoroscopy is a reliable method to diagnose airway collapse,17 hence F-BAL could be used to concurrently evaluate airway collapse and collect BALF.

This case report has limitations in addition to small sample size. Ideally, F-BAL should be compared to bronchoscopic-BAL regarding sample quality, recovery rates, and frequency of complications. Overall, fluoroscopic-guided BAL appears to be a safe technique that allows lavage of specific lung lobes; however, cranial lung lobes may be more difficult to catheterize than other lung lobes. Further study and refinement of the technique in a larger population of cats with clinical respiratory disease is warranted.
3.5 Footnotes

a. Torbugesic, Zoetis Canada Inc, Kirkland, QC Canada

b. Atravet, acepromazine maleate injection, 10mg injectable, Boehringer Ingelheim, Burlington, Ontario, Canada

c. Propofol Injectable, Fresenius Kabi Canada Ltd, Richmond Hill, ON Canada

d. Sheridan/CF Endotracheal Tube, Teleflex Medical Canada Inc, Markham, ON Canada

e. Ventolin HFA, 100mcg, Glaxo Smith Kline Inc, Mississauga, Ontario, Canada

f. Kangaroo Polyvinyl Chloride Feeding Tube with Radioopaque Line, Covidien, Saint Laurent, QC Canada

g. Rusch Soft Rubber Bladder Catheter, Teleflex Medical Canada Inc, Markham, ON Canada

h. Plastic Multipurpose Tubing Adapter, Cook Inc, Bloomington, IN

i. Weasel Wire, Infiniti Medical, Menlo Park, CA

j. General Electric Precision 500D, GE Medical System SA, Buc, France

k. Monoject 20 mL Syringe Regular tip, Covidien, Mansfield, MA

l. Nonin Model 8500 Handheld Pulse Oximeter, Nonin Medical Inc, Plymouth, MN

m. Z2 Coulter counter, Beckman Coulter, Mississauga, ON Canada

n. Shandon Cytospin 4, Thermo Fisher Scientific Inc, Waltham, MA

o. Apo-Doxy, Apotex Inc, Toronto, ON Canada

p. Clavamox, Zoetis Inc, Kalamazoo, MI

q. Cardiopet proBNP Test – Feline, IDEXX Laboratories Inc, Markham, ON Canada

r. GE Bright Speed, General Electric Healthcare, Milwaukee, WI

s. Dexdomitor, Zoetis Canada Inc, Kirkland, QC Canada
t. Novo Prednisolone, Novopharm Ltd, Toronto, ON Canada

3.6 Reference


4 Bronchoalveolar Lavage Hemosiderosis in Dogs and Cats with Respiratory Disease

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4.1 Abstract

Background

Hemosiderophages can be found in bronchoalveolar lavage samples and have been reported in association with a wide range of respiratory and cardiovascular disorders in cats and humans.

Objectives

To retrospectively evaluate the presence of hemosiderin in canine and feline bronchoalveolar lavage (BAL) samples and the association with signalment, clinical signs prior to bronchoalveolar lavage, historic disease prior to bronchoalveolar lavage, prior transthoracic fine needle aspiration, and bronchoalveolar lavage procedure time and cytologic interpretation.

Methods

Medical records of dogs and cats with respiratory disease that underwent bronchoalveolar lavage between 2007 and 2016 were reviewed. Appropriate medical information and bronchoalveolar lavage results were available from 171 dogs and 34 cats. Cases were assigned to 4 disease categories: pneumonia, inflammatory disease, neoplasia or normal airways based on BAL cytological findings. Degree of hemosiderosis was classified based on a semi-quantitative scoring
scale. Exact logistic regression was used to evaluate the relationship between risk factors and presence of BAL hemosiderosis on cytology.

**Results**

Hemosiderin was identified in 13/171 (7.6%) canine samples and 18/34 (52.9%) feline samples. Cats were 13.33 times more likely to have pulmonary hemosiderosis on bronchoalveolar lavage cytology compared to dogs (p < 0.001). Increased respiratory rate, prolonged bronchoalveolar lavage time, concurrent transthoracic fine needle aspiration, and cytologic diagnosis were associated with an increased risk of hemosiderosis in dogs. No specific risk factors associated with pulmonary hemosiderosis in cats were identified.

**Conclusions**

Hemosiderosis is more common in BAL samples from cats than from dogs and is associated with a diverse range of disease conditions.
4.2 Introduction

Increased stainable iron within alveolar macrophages, or pulmonary hemosiderosis, is a relatively common finding on cytologic evaluation of bronchoalveolar lavage (BAL) samples in dogs and cats.\textsuperscript{1,2} Although hemosiderin is an iron storage complex, pulmonary hemosiderosis does not refer to a defect in iron metabolism, but rather it is thought to arise from previous hemorrhage or erythrocyte diapedesis secondary to vascular congestion or pulmonary hypertension.\textsuperscript{1-3} Hemorrhage from leakage of pulmonary capillaries can arise secondary to direct mechanical trauma from inhaled particulate matter, allergens, blunt trauma, stress-related injury of endothelium due to inflammatory disease (infectious or immune-mediated), upper airway obstruction, increased blood flow or blood viscosity, coagulopathy or vasculopathy.\textsuperscript{1,2,4,5} Although procedures such as BAL, fine needle aspiration or biopsy of the pulmonary parenchyma can result in acute iatrogenic hemorrhage, which usually manifests with free erythrocytes rather than hemosiderin.\textsuperscript{2} In dogs and cats, hemosiderin-containing macrophages traditionally were thought to be associated with congestive heart failure; however, hemosiderosis has also been described in a large number of primary respiratory or cardiovascular conditions, and in systemic disease.\textsuperscript{1,4} High frequency of hemosiderosis in feline tracheal wash samples, and association with underlying disease, has previously been reported,\textsuperscript{1} but conditions related to BAL hemosiderosis in dogs and cats are unknown. The objectives of this study were to determine the frequency of pulmonary hemosiderosis in cats and dogs undergoing BAL, to characterize the cytologic findings in BAL specimens, and to determine whether there was an association between pulmonary hemosiderosis and specific respiratory diseases.
4.3 Methods

Medical records of dogs and cats with respiratory disease that underwent BAL between 2007-2016 at the Ontario Veterinary College were searched. From each medical record signalment, disease prior to BAL, treatment prior to BAL, clinical signs and vital parameters prior to BAL, hematological and biochemistry parameters, collection of adjunctive samples of the lower respiratory tract (specifically transthoracic fine-needle aspiration [FNA]), BAL procedure time, and final diagnoses were recorded.

Conditions prior to BAL that specifically recorded were presence of gastrointestinal disease, autoimmune disease, neoplasia and osteoarthrosis. These diseases have been reported to be associated with pulmonary hemosiderosis in human medicine.6-8 A previous diagnosis of gastrointestinal disease was based on vomiting and/or diarrhea for more than 1-week duration. A previous diagnosis of autoimmune disease was based on historic immune-mediated hemolytic anemia, immune mediated thrombocytopenia, atopic dermatitis, inflammatory bowel disease or immune mediated glomerulonephritis. The diagnosis of immune mediated glomerulonephritis was based either on biopsy results or alternately positive response to immunosuppressive therapy following absence of response to standard therapy for proteinuria. For the remaining conditions, medical records were reviewed to confirm agreement of historical diagnosis of immune mediated disease. A previous diagnosis of neoplasia required either cytologic or histopathologic confirmation of a neoplastic process, and a previous diagnosis of osteoarthritis required appropriate radiographic classification of joint disease.

Patients were categorized into four groups based on results of BAL +/- FNA: pneumonia, pulmonary inflammation, pulmonary neoplasia or no pulmonary disease (Table 4.1). A diagnosis
of pneumonia was based on presence of suppurative inflammation with positive bacterial (including *Mycoplasma spp.*), or fungal culture, or negative culture with suppurative inflammation, presence of degenerative changes in neutrophils and positive response to antibiotics. Pulmonary inflammation was diagnosed by increased proportion of neutrophils and/or eosinophils in differential counts of cytocentrifuged BAL preparations, absence of degenerative neutrophil changes, negative culture results and negative heartworm and pulmonary parasite testing. A diagnosis of pulmonary neoplasia was derived from cytologic or histopathologic findings (ante- or post-mortem) in conjunction with appropriate radiographic changes. A diagnosis of no pulmonary disease was based upon cytologically normal BAL fluid and absence of radiographic lung changes.
<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Subtype</th>
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<td></td>
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</tr>
<tr>
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<td></td>
<td>Cytology - dog (dog)</td>
<td>&gt;5% eosinophils</td>
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<td></td>
<td></td>
<td>Cytology - cat (cat)</td>
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<td>Diagnosis of cancer</td>
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<tr>
<td></td>
<td></td>
<td>Radiography</td>
<td>No abnormalities</td>
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</table>
BAL Technique

Following evaluation by a board-certified veterinary anesthesiologist, cats and dogs underwent general anesthesia using a protocol tailored to animals with potential respiratory impairment, including pre-oxygenation prior to induction of general anesthesia, and in cats administration of bronchodilator medication upon induction of general anesthesia. In patients intubated during bronchoalveolar lavage a sterile endotracheal tube was placed. Bronchoalveolar lavage was performed using a bronchoscope. The attending clinician chose the type of bronchoscope, volume of fluid instilled, and technique of fluid retrieval. BAL samples were placed in sterile tubes and delivered to the laboratory for processing within 60 minutes of collection.

Cytologic Evaluation

A 200 µL aliquot of each sample was cytocentrifuged (Shandon Cytospin 4, Thermo Fisher Scientific Inc, Waltham, MA) for 6 minutes at 180 g, and two additional feather-edge slides were prepared from fluid sedimented for 5 minutes at 500 g. Slides were stained with Wright stain and differential cell counts of a minimum of 500 nucleated cells were performed at 400 × magnification of cytocentrifuge preparations by a board certified veterinary clinical pathologist (DB). Inflammation was classified as neutrophilic (dogs >5% neutrophils, <5% eosinophils; cats >5% neutrophils, <10% eosinophils), eosinophilic (dogs >5% eosinophils, <5% neutrophils; cats >10% eosinophils, <5% neutrophils) or mixed (dogs >5% neutrophils and >5% eosinophils; cats >5% neutrophils and >10% eosinophils). Infection was diagnosed according to positive bacterial or fungal culture or appropriate response to antibiotics in the face of degenerate neutrophilic
inflammation on cytologic evaluation. The degree of pulmonary hemosiderosis was assessed with a semi-quantitative scoring system of the proportion of macrophages containing pigment, the number of pigment granules per cell, and relative pigment staining intensity (Appendix 3 & Figure 4.1).

Figure 4.1 Different scores of BAL hemosiderosis.

A - grade 1
B - grade 3
C - grade 4
D - Prussian blue stain.
Statistical analysis

For statistical analyses, the commercial software SAS 9.3 (SAS Institute Inc., Cary, NC, USA) was used. Data distributions were assessed for normality with a Shapiro-Wilk test. Exact logistic regression was used to investigate the presence of pulmonary hemosiderosis with different variables. The variables analyzed included age, species, body weight, history of prior disease, clinical signs and vital parameters prior to BAL (heart rate, respiratory rate, temperature, coughing, nasal discharge), changes identified on thoracic radiographs, fine needle aspiration prior to BAL, duration of BAL and cytologic interpretation. P <0.05 was considered significant.

4.4 Results

Signalment

Between May 2007 and May 2016, BAL samples from 205 animals (171 canine, 34 feline) with complete medical records were assessed. The 171 dogs were between 0.3 and 16.0 years of age (median = 6.0 years) and weighed 1.4 to 80.0 kg (median = 21.0 kg). There were 22 intact males (12.9%), 75 castrated males (43.9%), 16 intact females (9.4%) and 58 spayed females (33.9%). The population included 38 mixed breed dogs, 13 Labrador Retrievers, 9 German Shepherds, 8 Yorkshire Terriers, 6 English Bulldogs, 5 Pugs, 4 each of Dachshunds, Bernese Mountain Dogs, and Golden Retrievers, 3 each of Newfoundlands, Siberian Huskies, Australian Shepherds, Wheaten Terriers, Shih Tzus, Whippets and French Bulldogs, 2 each of Basset Hounds, Cocker Spaniels, Irish Wolfhounds, Border Collies, Boxers, Cattle Dogs, Great Danes, Bichon Frise, Rough Collies, Chihuahuas, Boston Terriers, West Highland White Terriers, English Springer Spaniels, Beagles and Jack Russell Terriers and 1 each of Hungarian Vizla, Kerry Blue
Terrier, Swedish Vallhund, Bullmastiff, Standard Poodle, Doberman, Shetland Sheepdog, Leonberger, Rottweiler, Havanese, Rhodesian Ridgeback, Coonhound, Redbone Hound, Belgian Teruven, Portuguese Waterdog, Korean Jindo, Polish Lowland Sheepdog, Akita, Papillion, German Shorthaired Pointer, Neapolitan Mastiff, Curly Coat Retriever, Miniature Poodle, Cane Corso, Miniature Pinscher, Bull Terrier, Maltese, Welsh Corgi and Lhasa Apso. The 34 cats were between 0.3 and 15.0 years of age (median = 4.5 years) and between 2.3 to 8.4 kg in body weight (median = 4.3 kg). There was 1 intact male (2.9%), 15 castrated males (44.1%), 1 intact female (2.9%) and 17 spayed females (50.0%). The population included 23 domestic short-hair cats, 3 domestic long-hair cats, and 1 each of domestic medium-hair cat, Burmese, Siamese, Lynx, Maine Coon, Himalayan, Savannah and Devon Rex.

Hemosiderin was reported in 13/171 (7.6%) canine samples and 18/34 (52.9%) feline samples. Cats were significantly more likely to have pulmonary hemosiderosis on BAL cytology compared to dogs (OR 13.33, confidence interval [CI] 5.18 to 35.71, p <0.001). There were no significant associations between age, sex or weight and the presence or severity of hemosiderosis in dogs (Table 4.2) or cats (Table 4.3).
## Table 4.2 Characteristics of dogs with and without hemosiderin in BAL

<table>
<thead>
<tr>
<th>Hemosiderin</th>
<th>Present</th>
<th>Absent</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of dogs</td>
<td>13</td>
<td>158</td>
<td></td>
</tr>
<tr>
<td>Age (yr), median and range</td>
<td>8.0 (1.0-14.0)</td>
<td>6.0 (0.3-16.0)</td>
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<tr>
<td>Weight (kg), median and range</td>
<td>29.7 (5.6-59.0)</td>
<td>20.9 (1.4-80.0)</td>
<td>0.32</td>
</tr>
</tbody>
</table>

### Clinical signs
- **Coughing**: 8/13 (62%) vs. 139/158 (88%) | P = 0.20
- **Nasal discharge**: 1/13 (8%) vs. 42/158 (27%) | P = 0.16
- **HR (bpm), median and range**<sup>a</sup>: 120 (64-152) vs. 120 (56-200) | P = 0.83
- **RR (bpm), median and range**<sup>b</sup>: 52 (20-120) vs. 36 (16-130) | P = 0.04
- **Temperature (C), median and range**: 38.8 (37.5-41.2) vs. 38.8 (36.0-41.9) | P = 0.32
- **O₂ requirement**<sup>c</sup>: 7/13 (54%) vs. 52/158 (33%) | P = 0.27

### Prior disease
- **Neoplasia**: 4/13 (31%) vs. 17/158 (11%) | P = 0.12
- **Autoimmune disease**: 1/13 (8%) vs. 1/158 (0.6%) | P = 0.42
- **Gastrointestinal disease**: 4/13 (31%) vs. 30/158 (19%) | P = 0.49
- **Osteoarthrosis**: 3/13 (23%) vs. 3/158 (2%) | P = 0.01

### Radiographic findings
- **Cardiomegaly**: 1/13 (8%) vs. 8/158 (5%) | P = 1.00
- **Pulmonary disease**: 12/13 (92%) vs. 97/158 (61%) | P = 0.18

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<sup>a</sup> Heart rate, beats per minute  
<sup>b</sup> Respiratory rate, breaths per minute  
<sup>c</sup> Requirement for pre-anesthetic oxygen supplementation
Table 4.3 Characteristics of cats with and without pulmonary hemosiderosis

<table>
<thead>
<tr>
<th></th>
<th>Hemosiderin</th>
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<tbody>
<tr>
<td></td>
<td>Present</td>
<td>Absent</td>
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<tr>
<td>Number of cats</td>
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<tr>
<td>Age (yr), median and range</td>
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<td>3.0 (0.3-15.0)</td>
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<td>Weight (kg), median and range</td>
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<td>4.6 (2.3-8.4)</td>
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<tr>
<td>Clinical signs</td>
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<tr>
<td>Coughing</td>
<td>9/18 (50%)</td>
<td>6/16 (38%)</td>
<td>0.98</td>
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<tr>
<td>Nasal discharge</td>
<td>2/18 (11%)</td>
<td>3/16 (19%)</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>HR (bpm), median and range&lt;sup&gt;a&lt;/sup&gt;</td>
<td>195 (160-240)</td>
<td>200 (100-260)</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>RR (bpm), median and range&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48 (16-80)</td>
<td>41 (24-80)</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Temperature (C), median and range</td>
<td>38.4 (35.5-40.7)</td>
<td>38.9 (37.5-40.6)</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>O₂ requirement&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7/18 (39%)</td>
<td>11/16 (69%)</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>Prior disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neoplasia</td>
<td>1/18 (6%)</td>
<td>0/16 (0%)</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Autoimmune disease</td>
<td>1/18 (6%)</td>
<td>0/16 (0%)</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal disease</td>
<td>4/18 (22%)</td>
<td>2/16 (13%)</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>Radiographic findings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiomegaly</td>
<td>1/18 (6%)</td>
<td>0/16 (0%)</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Pulmonary disease</td>
<td>12/18 (67%)</td>
<td>10/16 (63%)</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Heart rate, beats per minute  
<sup>b</sup> Respiratory rate, breaths per minute  
<sup>c</sup> Requirement for pre-anesthetic oxygen supplementation

Clinical signs

Prior to BAL, coughing and nasal discharge was noted for 146/171 (85.4%) and 46/171 (26.9%) dogs, respectively. Coughing or sneezing prior to BAL was not significantly associated with the presence of hemosiderin on BAL cytology in dogs (Table 4.2). Prior to BAL median respiratory rate (RR) was 36 breaths per minute (range 16 to 130), median heart rate was 120 beats per minute (range 56 to 200), and median body temperature was 38.8°C (range 36.0°C to 41.9°C). An increased RR prior to BAL was associated with a significantly increased risk of pulmonary
hemosiderosis (median RR in dogs with hemosiderosis was 52, range 20 to 120; median RR in dogs without hemosiderosis was 36, range 16 to 130; p = 0.04). There was no significant association of heart rate or body temperature with pulmonary hemosiderosis in dogs (Table 4.2).

Prior to BAL, 59/171 (34.5%) dogs received oxygen therapy. Administration of oxygen therapy prior to BAL was not significantly associated with pulmonary hemosiderosis (Table 5.2).

In 15/34 (44.1%) and 5/34 (14.7%) cats coughing and nasal discharge, respectively, was recorded prior to BAL. Neither coughing nor nasal discharge were significantly associated with pulmonary hemosiderosis (Table 4.3).

Prior to BAL, median RR was 44 breaths per minute (range 16 to 80), median heart rate was 200 beats per minute (range 100 to 260) and median temperature was 38.5°C (range 35.5°C to 40.5°C). There was no significant association of heart rate, respiratory rate or body temperature with hemosiderosis (Table 4.3).

**Prior disease conditions**

Of 171 dogs undergoing BAL, prior illnesses included neoplasia (21, 12.3%), osteoarthritis (6, 3.5%), autoimmune disease (3, 1.8%) and gastrointestinal disease (34, 19.9%). Osteoarthritis was associated with a 14.9x (CI 1.8 to 125, p = 0.01) increased risk of hemosiderosis in BAL. None of the other conditions were significantly associated with hemosiderosis (Table 4.2).
Among 34 cats that underwent BAL, one each (2.9%) had a prior history of neoplasia and autoimmune disease, and 6 (17.6%) had a prior history of gastrointestinal disease. No cats had a prior history of osteoarthritis. None of these conditions were significantly associated with hemosiderosis (Table 4.3).

**Radiographic findings**

Two- or three-view survey radiographs were performed in all dogs prior to BAL. Cardiomegaly was detected in 9/171 (5.3%) dogs, and pulmonary parenchymal disease in 109/171 (63.7%) of dogs. Presence of cardiomegaly or pulmonary parenchymal changes on survey thoracic radiographs was not significantly associated with hemosiderosis (Table 4.2).

Survey radiographs as above were also performed in all cats prior to BAL. Cardiomegaly was detected in 1/34 (2.9%) and pulmonary parenchymal disease in 26/34 (76.5%) cases. Neither cardiomegaly nor pulmonary parenchymal changes on survey radiographs were significantly associated with pulmonary hemosiderosis (Table 4.3).

**BAL procedure and transthoracic fine needle aspiration**

For dogs, median BAL procedure time was 15 minutes (range 10 to 70). Longer BAL times were associated with increased risk of hemosiderosis (median BAL time in dogs with hemosiderosis 20 minutes, range 15 to 60; median BAL time in dogs without hemosiderosis 15 minutes, range 10 to 70; p = 0.04). For every 1-minute increase in BAL time, there was a 1.05x (CI 1.00 to 1.11, p = 0.04) increased risk of hemosiderosis in BAL cytology. Eleven of 171 (6.4%)
of dogs had transthoracic fine needle aspiration performed in conjunction with BAL. Six of the 11 dogs (54.5%) had FNA performed prior to BAL and the remainder had FNA performed following BAL. Dogs that underwent transthoracic FNA during the same general anesthetic as for BAL had a 5.52x (CI 0.82 to 28.57, p = 0.02) increased risk hemosiderosis in BAL (Table 4.4).

Table 4.4 BAL procedural aspects for dogs with and without pulmonary hemosiderosis

<table>
<thead>
<tr>
<th>Hemosiderin</th>
<th>Present</th>
<th>Absent</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of dogs</td>
<td>13</td>
<td>158</td>
<td></td>
</tr>
<tr>
<td>BAL duration(a), median (range)</td>
<td>20 (15-60)</td>
<td>15 (10-70)</td>
<td>0.04</td>
</tr>
<tr>
<td>Transthoracic FNA(b)</td>
<td>3/13 (23%)</td>
<td>8/171 (5%)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

\(a\) Minutes  
\(b\) Transthoracic fine needle aspiration and BAL under same general anesthetic

The median BAL procedure time in cats was 15 minutes (range 10 to 45). There was no significant association between BAL duration and hemosiderosis. Three of 34 (8.8%) cats underwent transthoracic FNA in conjunction with BAL, and this was not associated with a significantly higher risk of hemosiderosis on BALF cytology in cats (Table 4.5).
Table 4.5 BAL procedural aspects for cats with and without pulmonary hemosiderosis

<table>
<thead>
<tr>
<th></th>
<th>Hemosiderin</th>
<th></th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAL duration⁵, median (range)</td>
<td>18</td>
<td>15 (10-30)</td>
<td>15 (10-45)</td>
<td>0.37</td>
</tr>
<tr>
<td>Transthoracic FNA‡</td>
<td></td>
<td>2/18 (11%)</td>
<td>1/16 (6%)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

⁵ Minutes
‡ Transthoracic fine needle aspiration and BAL under same general anesthetic

BAL cytologic findings

Pneumonia was diagnosed in 65/171 (38.0%) dogs and 8/34 (23.5%) cats, pulmonary inflammation in 82/171 (48.0%) dogs and 23/34 (67.6%) cats, pulmonary neoplasia in 5/171 (2.9%) dogs and 2/34 (5.9%) cats, and no pulmonary disease in 19/171 (11.1%) dogs and 1/34 (2.9%) cats (Table 4.6 and 4.7). The type of cytologic interpretation and therefore category of pulmonary disease was significantly associated with hemosiderosis in dogs (Table 4.6), but not in cats (Table 4.7). Dogs with a cytologic diagnosis of neoplasia had an 11.97x (CI 0.74 to 191.55, p = 0.02) increased risk of hemosiderosis compared to dogs with a cytologic diagnosis of inflammatory disease, and a 14.13x (CI 0.83 to 245.64, p = 0.01) increased risk of hemosiderosis compared to dogs with a cytologic diagnosis of pneumonia (Table 4.6).

None of the assessments of different degrees of hemosiderosis had a significant effect on factors associated with an increased risk of pulmonary hemosiderosis.
Table 4.6 Cytologic classification of 171 BAL samples from dogs

<table>
<thead>
<tr>
<th></th>
<th>Present</th>
<th>Absent</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of dogs</td>
<td>13</td>
<td>158</td>
<td></td>
</tr>
<tr>
<td>Cytologic diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary neoplasia</td>
<td>3/13 (23%)</td>
<td>2/158 (2%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Pulmonary inflammation</td>
<td>6/13 (46%)</td>
<td>76/158 (48%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>3/13 (23%)</td>
<td>62/158 (39%)</td>
<td>0.01</td>
</tr>
<tr>
<td>No pulmonary disease</td>
<td>1/13 (8%)</td>
<td>18/158 (11%)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

<sup>a</sup> Risk of hemosiderosis for pulmonary neoplasia was compared to risk of hemosiderosis for pulmonary inflammation, pneumonia and no pulmonary disease combined. P <0.05 indicates significant difference in risk of hemosiderosis for other diagnostic categories compared to pulmonary neoplasia.

Table 4.7 Cytologic classification of 34 BAL samples from cats

<table>
<thead>
<tr>
<th></th>
<th>Present</th>
<th>Absent</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of dogs</td>
<td>13</td>
<td>158</td>
<td></td>
</tr>
<tr>
<td>Cytologic diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary neoplasia</td>
<td>2/18 (11%)</td>
<td>0/16 (0%)</td>
<td>0.06</td>
</tr>
<tr>
<td>Pulmonary inflammation</td>
<td>14/18 (78%)</td>
<td>9/16 (56%)</td>
<td>0.40</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>2/18 (11%)</td>
<td>6/16 (38%)</td>
<td>0.13</td>
</tr>
<tr>
<td>No pulmonary disease</td>
<td>0/18 (0%)</td>
<td>1/16 (6%)</td>
<td>0.33</td>
</tr>
</tbody>
</table>

<sup>a</sup> Risk of hemosiderosis for pulmonary neoplasia was compared to risk of hemosiderosis for pulmonary inflammation, pneumonia and no pulmonary disease combined. P <0.05 indicates significant difference in risk of hemosiderosis for other diagnostic categories compared to pulmonary neoplasia.
4.5 Discussion

Pulmonary hemosiderosis was a more common feature of BAL cytology of cats than dogs. The frequency of pulmonary hemosiderosis in feline patients in this study was similar to that previously reported for feline tracheal wash samples. Cats may be more prone to pulmonary hemosiderosis than dogs due to increased susceptibility to alveolar hemorrhage or a reduced rate of hemosiderin degradation by alveolar macrophages. Factors that might contribute to increased permeability of the blood gas barrier, leading to alveolar hemorrhage, include increased capillary transmural pressures, high pulmonary vascular pressure or vascular congestion.

In species with a thinner blood gas barrier, a lower capillary transmural pressure is required to cause stress failure and alveolar hemorrhage. The thickness of the blood gas barrier has not been investigated in cats; however, considering that larger animals tend to have thicker blood gas barriers and an increased threshold for stress failure, it would be anticipated that cats, compared to dogs, might have a thinner blood gas barrier and lower threshold for stress failure.

Increased pulmonary vascular pressure can arise as a result of systemic or pulmonary hypertension. Blood pressure was inconsistently recorded; therefore, evaluation of an association between systemic hypertension and frequency of pulmonary hemosiderosis could not be determined. Pulmonary hypertension was not documented in any patient in this study; however, all patients undergoing BAL had respiratory abnormalities, which are risk factors for pulmonary hypertension. A positive correlation between the degree of hemosiderosis, mean pulmonary arterial pressure, and pulmonary vascular resistance, has been reported in humans with idiopathic pulmonary fibrosis. Similar studies have not been performed in veterinary medicine.
Aside from differences in the threshold for stress failure of the blood gas barrier, cats may also have a slower rate of clearance of hemosiderin-laden macrophages compared to dogs. Hemosiderin-laden macrophages have been reported to persist in humans and mice for up to 3 months. Slower clearance of hemosiderin-laden macrophages may result in cumulative hemosiderosis, translating to an increased likelihood of detecting pulmonary hemosiderosis in feline BAL samples.

Only an increased respiratory rate prior to BAL was significantly associated with the frequency of pulmonary hemosiderosis in dogs. Increased respiratory rate can be an indicator of both chronicity and severity of respiratory disease. An associated increased frequency of pulmonary hemosiderosis could be related to vascular micro-trauma from increased negative airway pressure in chronic respiratory disease, or erythrocyte diapedesis subsequent to vasodilation, reduced endothelial integrity and inflammatory cytokine release.

A prior history of osteoarthritis was associated with increased risk of pulmonary hemosiderosis in dogs. A causal relationship between osteoarthritis and development of pulmonary hemosiderosis is not readily apparent. Primary hemostatic disorders could manifest with hemarthrosis and pulmonary bleeding, or non-steroidal anti-inflammatory drug therapy could induce hemorrhage into joints and lung. Alternatively, increased circulating inflammatory cytokines secondary to osteoarthritis could affect pulmonary vascular function. Complete history of non-steroidal anti-inflammatory use and temporal association with the time of BAL was inconsistently recorded, and patients receiving this medication did not undergo hemostatic testing. Therefore, a potential association between non-steroidal anti-inflammatory drug use and
pulmonary hemosiderosis remains undetermined. Finally, an increased risk of pulmonary 
hemosiderosis from osteoarthrosis may represent a type I error, or false positive finding.

Iatrogenic trauma induced by procedures such as transthoracic needle aspiration can lead 
to hemorrhage into airways or alveoli, and hence contribute to pulmonary hemosiderosis. Only a 
small number of dogs and cats underwent transthoracic needle aspiration prior to BAL, which 
occurred in each case during the same general anesthetic. In mice and humans hemosiderin-laden 
macrophages arise between 50 hours and 3 days after acute alveolar hemorrhage,\(^9,10\) therefore, it 
seems unlikely that transthoracic needle aspiration in dogs would contribute to pulmonary 
hemosiderosis within less than an hour. Therefore, association of transthoracic needle aspiration 
and hemosiderosis is more likely a reflection of the severity of pulmonary disease rather than the 
procedure itself. Similarly, although dogs necessitating longer times for BAL had significantly 
increased risk of pulmonary hemosiderosis, this is also more likely related to the severity of 
pulmonary disease rather than the procedure itself.

There was no relationship between hemosiderophages and cardiac disease in this cohort of 
cats and dogs; however, only few dogs and cats had cardiomegaly, and none had congestive heart 
failure. BAL is not usually indicated in patients with congestive heart failure, therefore the 
presence of hemosiderophages in such patients could not be evaluated.

Dogs with a cytologic diagnosis of pulmonary neoplasia had an increased frequency of 
hemosiderosis. Neoplasia could result in airway hemorrhage from tissue disruption, vascular 
leakage and necrosis. The correlation between pulmonary hemosiderosis and a cytologic diagnosis 
of inflammatory disease compared to other disease categories was close to significant. Non-septic 
lung inflammation has been considered to convey an increased risk of tracheal hemosiderosis due
to increased negative airway pressure causing vascular micro-trauma and increased erythrocyte
diapedesis secondary to cytokine release and vasodilation.\textsuperscript{1,12}

Interestingly, hemosiderosis was uncommon in cats with BAL eosinophilic inflammation
but relatively common in cats with neutrophilic or mixed inflammation. This differs from previous
reports of high frequency of hemosiderosis in cats with asthma diagnosed by tracheal wash.\textsuperscript{1} Of
the cats diagnosed with pulmonary inflammation on cytology, only 2/23 had eosinophilic
inflammation. Therefore, a lack of association between hemosiderosis and eosinophilic
inflammation may have been related to small sample size.

Varied degrees of hemosiderosis were found with each of the different cytologic diagnoses
in both dogs and cats but no significant association was found between any of the risk factors and
presence of hemosiderosis. This was similar to previously reported findings in feline tracheal wash
samples. Degree of hemosiderosis was not related to cytologic diagnosis. It is likely that factors
such as location, extent, severity and chronicity of disease are more important determinants of the
degree of hemorrhage, phagocytosis and hemosiderin breakdown.

Overall, the frequency of hemosiderosis was much higher in cats than in dogs. However,
the number of feline cases was relatively small, limiting conclusions to be drawn regarding the
association of hemosiderosis with particular conditions, types of pulmonary disease and absence
of pulmonary disease. A cause for pulmonary hemosiderosis is not always identified in humans
and various environmental factors such as inhalation of mold spores have been implicated.\textsuperscript{6,8,16,17}
Although chronic exposure to toxic mold has been associated with severe pulmonary hemorrhage
under anesthesia in two cats,\textsuperscript{18} a potential relationship between environmental agents and
frequency of hemosiderosis in cats and dogs remains to be determined.
There are limitations inherent to retrospective studies. Neither the BAL procedure nor investigation of pulmonary disease was standardized. Information such as systemic blood pressure, specific duration of clinical signs, hemostatic testing, and long-term outcomes of patients was unavailable. Cats compared to dogs can have a longer duration of clinical illness prior to investigative procedures such as BAL, which may inherently have increased the frequency of pulmonary hemosiderosis.

In conclusion, this study is the first relatively large-scale assessment of factors associated with pulmonary hemosiderosis as diagnosed by BAL in dogs and cats. Findings indicate that cats with respiratory disease more often than dogs have pulmonary hemosiderosis. The degree of respiratory impairment, as indirectly implied by increased respiratory rate and prolonged BAL procedure, and a diagnosis of historical osteoarthritis, were significantly associated with hemosiderosis in dogs. A prospective study incorporating standardized investigative and outcome assessment would be helpful to correlate pulmonary hemosiderosis with prognosis.
4.6 Reference


5 Summary and Conclusions

5.1 Summary

The diagnostic process to determine the underlying etiology of lower respiratory tract clinical signs in cats presents multiple challenges for veterinarians.

Radiography is the most commonly utilized and most widely available imaging modality to examine the thoracic structures of cats; however, CT has provided more accurate characterization of severity, pattern of change and localization of disease, and allowed for more detailed diagnostic information.\textsuperscript{1-9} Computed tomography is becoming increasingly accessible to veterinarians. Requirement of general anesthesia was previously a major limitation; however, with the use of plexiglass chambers thoracic CT can now be performed in awake or minimally sedated patients.\textsuperscript{10,11} Due to the increasing availability of this imaging modality, it is important for veterinarians to gain an understanding of how thoracic CT can optimize the diagnostic evaluation of cats with lower respiratory tract disease.

Bronchoalveolar lavage is one of the most widely used diagnostic techniques for obtaining samples from the lower respiratory tract. Two major BAL techniques have been described – bronchoscopic BAL (B-BAL) and non-bronchoscopic (NB-BAL).\textsuperscript{12-15} Each technique has advantages and disadvantages. Bronchoscopic-BAL provides visually directed sampling from specific lung lobes, which adds diagnostic information regarding localization of lower respiratory tract disease and macroscopic airway changes.\textsuperscript{16} However, bronchosopes are not available to every practitioner and macroscopic mucosal changes may not provide additional diagnostic information in cats with lower respiratory tract disease.\textsuperscript{17} Non-bronchoscopic BAL is obviously performed without a bronchoscope and hence without direct visualization of specific lobes and
In order to compare the utility of these two different techniques, the first objective of this dissertation was their assessment in healthy cats without signs of lower respiratory tract disease. Results of this study showed that there were no significant differences in cytologic quality or differential cell counts between the two techniques, that both techniques provided samples of excellent cytologic quality, and that the retrieved volume of infused saline was higher with the B-BAL technique.

In this group of healthy cats, defined as lacking clinical signs of lower respiratory tract disease and having normal thoracic radiographic results, cytologic findings of BALF were abnormal relative to published guidelines. In most cats (75%), subsequent thoracic CT showed pulmonary parenchymal changes that were considered to be unrelated to the BAL procedure. It was reported previously up to 23% of cats with signs of lower respiratory tract disease had normal thoracic radiographs. Conversely, bronchial disease can be detected on thoracic CT in up to 24% of cats without respiratory signs and is more common in older cats. Whether such changes represent pre-clinical or clinically insignificant inflammatory lower airway disease is unknown. The proportion of cats with clinical signs of lower respiratory tract disease normal thoracic radiographs, abnormal thoracic CT findings, and abnormal BALF cytology is also unknown. Although with CT pulmonary abnormalities that were undetectable on with radiography may identified, there may be a proportion of cats that only have functional disease (bronchospasm or airway collapse). In people with asthma or chronic bronchitis, functional change may precede structural change, and therefore a small proportion of people with clinical signs and diagnosis of asthma will have normal thoracic CT findings. Hence, it may be possible that a proportion of cats with asthma or chronic bronchitis has functional disease without structural change, and therefore normal thoracic CT and radiography results despite chronic lower respiratory tract signs.
During comparison of B-BAL and NB-BAL in healthy cats, it was realized that fluoroscopy could be utilized for direct sampling of specific lung lobes using the NB-BAL technique. A bronchoscope might therefore not be required to reach specific lung sites while the advantage of multi-segment lavage would be maintained. It was hypothesized that using this technique, it would be feasible to obtain BAL samples from specific lung lobes in cats with signs of lower respiratory tract disease. Therefore, in 2 affected cats, BAL of multiple lung lobes was performed without an endoscope under fluoroscopic guidance. The proportion of infusate retrieved was comparable to that of NB-BAL and B-BAL techniques in healthy cats and cats with lower respiratory tract disease. The samples had excellent cytologic quality, cellular preservation and cellularity. Fluoroscopic guided BAL could be considered an acceptable alternative to B-BAL to sample multiple, specific lung lobes. Although fluoroscopy can be used to identify airway collapse and is commonly used to characterize this condition in dogs with tracheal collapse, bronchospasm or airway collapse was not identified in any of the cats undergoing F-BAL. The resolution of fluoroscopy is less than that of radiography, precluding visualization of small diameter airways. Bronchospasm in cats typically occurs in the bronchioles or distal bronchi, which are likely to be too small to be visualized with fluoroscopy. Therefore, bronchoscopy, particularly using a small diameter bronchoscope that allows endoscopic examination of the distal bronchi, might improve detection of bronchospasm or airway collapse.

During comparison of B-BAL and NB-BAL in healthy cats, in conjunction with abnormal BALF cytologic findings, hemosiderophages were detected in all BAL samples obtained. Hemosiderin is a common finding in BAL samples, but the frequency and risk factors associated
with BALF hemosiderosis have not been determined. In the retrospective study in chapter 4 in this thesis, hemosiderosis was more common in feline samples than in canine BALF samples. A longer course of subclinical illness and differences in the blood-gas barrier have been proposed to account for the difference in frequency of BALF hemosiderosis between cats and dogs.\textsuperscript{22,23} Specific risk factors were not associated with BALF hemosiderosis in cats; however, increased respiratory rate, prolonged BAL time, concurrent transthoracic lesion aspiration and the type of cytologic diagnosis were associated with BALF hemosiderosis in dogs. In both dogs and cats, the degree of hemosiderosis was not associated with a specific cytologic diagnosis, but because of the retrospective nature of the study and lack of follow up, the effect of hemosiderosis on long-term prognosis is undetermined. Prospective studies are required to characterize the effect of hemosiderosis, its development and long-term consequences in the lung to provide an improved understanding of the significance of this finding.

There are several limitations to the studies reported here. Ideally, thoracic CT, and heartworm antibody, coagulation testing and NT-proBNP, would have been performed in all cats prior to comparing BAL procedures. Obtaining samples from the affected lung lobes would have been interesting to further characterize the cytologic changes that are associated with the structural changes appreciable on thoracic CT. The sample size was relatively small in each study, and therefore cases were limited to those with inflammatory airway disease or pneumonia. To determine whether specific CT or radiographic findings are associated with certain cytologic interpretations, a larger group of cats would need to be evaluated. Furthermore, to better evaluate the diagnostic utility of CT relative to radiography, comparison of cytologic findings in BALF from the most severely affected lung lobes based on radiography and CT would be required. This could not be accomplished without excessive infused lavage fluid volume and would have been
unsafe for cats. The proportion of fluid retrieval did not affect the cytologic quality; however, a universal definition of parameters that constitute acceptable cytologic quality is not currently available. The grading of samples in these studies was based on expert opinion rather than evidence-based recommendations.

Aspects of feline BAL that warrant further investigation include evaluation of suction pump aspiration on sample quality and retrieval, and studies to determine the optimal infusate volume. Fluoroscopic BAL could be further developed since it incorporates some of the advantages afforded by B-BAL without need for an actual endoscope. Further investigation of the utility of thoracic CT compared to thoracic radiographs to select the optimal BAL site is also warranted.

5.2 Conclusions

In healthy cats, there was no significant difference in cytologic quality, differential cell count or complications between B-BAL and NB-BAL. Fluoroscopic guided BAL provided BALF samples of excellent cytologic quality and can be utilized for directed sampling of the right middle, accessory, right caudal and left caudal lung lobes.

Cats have an increased risk of BALF hemosiderosis compared to dogs; however, no specific risk factors for BALF hemosiderosis were identified in cats. Several risk factors for BALF hemosiderosis were identified in dogs. Prospective study is required to determine the development, effect and consequence of hemosiderosis in cats and dogs.
5.3 Reference


APPENDICES

Appendix 1: Description of scoring system used to assess anesthetic recovery of cats after BAL

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Very calm, smooth, no excitement and no oxygen supplementation required after 10 min post-extubation to maintain SpO₂ ≥95%</td>
</tr>
<tr>
<td>2</td>
<td>Mild excitement; no additional sedation required and oxygen supplementation needed for 10 - 30 min post-extubation to maintain SpO₂ ≥95%</td>
</tr>
<tr>
<td>3</td>
<td>Severe excitement; additional sedation required and oxygen supplementation needed for 30 - 60 min post-extubation to maintain SpO₂ ≥95%</td>
</tr>
<tr>
<td>4</td>
<td>Airway complication requiring re-intubation, cardiac arrest followed by successful resuscitation, or oxygen supplementation needed for more than 60 min post-extubation to maintain SpO₂ ≥95%, cat transferred to ICU</td>
</tr>
<tr>
<td>5</td>
<td>Permanent arrest/death/euthanasia</td>
</tr>
<tr>
<td>Variable</td>
<td>Score</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>Cellularity (leukocytes per slide)</td>
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</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
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<tr>
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<tr>
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<tr>
<td>cells per slide)</td>
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<td>Epithelial cells (number per slide)</td>
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<td>3</td>
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<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>RBC (percent per slide)</td>
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<tr>
<td></td>
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</tr>
<tr>
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<td>3</td>
</tr>
<tr>
<td>Bacteria (organisms/slide)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
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<tr>
<td></td>
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<tr>
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<tr>
<td>Hemosiderophages (cells/slide)</td>
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<tr>
<td>Mucus</td>
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<tr>
<td></td>
<td>3</td>
</tr>
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</table>

Scoring system was created by use of criteria described elsewhere.²,¹⁰
Appendix 3: Semi-quantitative scale for assessing the degree of pulmonary hemosiderosis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Score</th>
<th>Criterion</th>
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<td>Percent macrophages containing</td>
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<td>pigment</td>
<td>1</td>
<td>1-10%</td>
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<tr>
<td></td>
<td>2</td>
<td>11-30%</td>
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<tr>
<td></td>
<td>3</td>
<td>31-50%</td>
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<tr>
<td></td>
<td>4</td>
<td>&gt;50%</td>
</tr>
<tr>
<td>Number of pigment granules</td>
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<td>Absent</td>
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<tr>
<td>per cell</td>
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<td>1-2</td>
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<tr>
<td></td>
<td>2</td>
<td>3-5</td>
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<tr>
<td></td>
<td>3</td>
<td>6-10</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Pigment stain intensity</td>
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<td>Absent</td>
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<tr>
<td></td>
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<td>Faint</td>
</tr>
<tr>
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<td>2</td>
<td>Faint to moderate</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Faint to intense</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Intense</td>
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</tbody>
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