Bronchoscopy and bronchoalveolar lavage in dogs and cats

Iva Šmit and Mirta Vučković*

Abstract

Diseases of the respiratory tract are widespread in small animal practice. Bronchoscopy and bronchoalveolar lavage are valuable diagnostic techniques that allow visualisation of the lumen and mucosa of a large part of the airway, and enable sampling. The decision on the sampling method depends on the patient's assessment and imaging. Bronchoalveolar lavage is a safe and simple procedure indicated for diffuse diseases of the bronchi, pulmonary interstitium or alveoli. It is performed by infusion of 0.9% saline solution into the selected regional bronchus through the working channel of a bronchoscope or as a "blind technique" and removed as quickly as possible. The samples obtained by bronchoalveolar lavage are suitable for cytology, bacteriology and fungal cultures or other diagnostic tests such as PCR or specific antigen tests. Bronchoscopy requires appropriate and expensive equipment, in addition to specialised training and experience of the physician performing the procedure. This review article provides an overview of the technique, equipment, most common indications, complications and interpretation of the results of bronchoscopy and bronchoalveolar lavage in dogs and cats.

Key words: *bronchoscopy; bronchoalveolar lavage; dog; cat*

Introduction

Diseases of the respiratory tract are common in small animal practice. Various diagnostic procedures are used in dogs and cats with signs of respiratory disease. Endoscopy is an essential part of small animal practice and is becoming increasingly available in primary care. Although it requires additional equipment and training, airway endoscopy has evolved and is one of the most important techniques for evaluating patients with signs of airway disease. It usually includes rhinoscopy, tracheoscopy and bronchoscopy (Concoran, 2004).

Bronchoscopy is an extremely valuable, minimally invasive diagnostic procedure and a useful diagnostic tool that allows direct visualisation of the lumen and mucosa of a large portion of the airway, sampling by bronchial brush, biopsy and bronchoalveolar lavage (BAL), and a therapeutic procedure: identification and removal of foreign bodies. It is a procedure that includes tracheoscopy, which is why it is often referred to as tracheobronchoscopy (Hawkins et al., 1990). Determining a definitive diagnosis requires interpretation of the bronchoscopy

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findings, cytology and culture results, and clinical and imaging findings.

Like similar procedures, bronchoscopy requires specialised training, including training on models or simulators and experience of the physician performing the procedure (McCool et al., 2020).

Equipment and preparation of the patient

The tracheobronchial tree can be examined with a flexible or rigid endoscope (Figure 1a). Rigid endoscopes consist of a rigid insertion tube, are more affordable, but do not provide the necessary ability for smooth movement of the instrument through the lower airways. Cytologic or culture samples cannot be obtained with a rigid endoscope (Johnson, 2001).

A flexible video bronchoscope is preferred. It consists of an insertion tube and a hand-operated control unit and is connected to a light source and an image processor that displays the images on a monitor. The control unit is used to bend the distal tip of the insertion tube and allow access to the operating channel. Bronchoscopes usually only have a two-way deflection. The insertion tube can be 40 to 85 cm long with an outer diameter of 3-6 mm. For large or giant dogs, a gastroscope (7-9 mm diameter and 120 cm long) can also be used (McKiernan, 2021).

Smaller diameter bronchoscopes are used in cats and small dogs, but the small diameter of the bronchoscope limits the size of the operating channel. An operating channel offers the possibility of using an instrument that can be very useful. Several flexible instruments are available for use with a bronchoscope – grasping forceps, baskets (Figure 1b), cytology brushes (Figure 1c) or biopsy forceps. When using additional instruments, it is important to ensure the compatibility of the instrument with the bronchoscope. Video bronchoscopes allow better visualization, provide better quality images and are more suitable for student training as they allow visualization on the screen (Chamness, 1999; Levitan and Kimmel, 2008).

To ensure adequate diagnostics, besides choosing the right equipment, certain endoscope cleaning and disinfection procedures should be practiced. After each use, bronchoscopes should be cleaned according to the manufacturer's recommendations and then disinfected

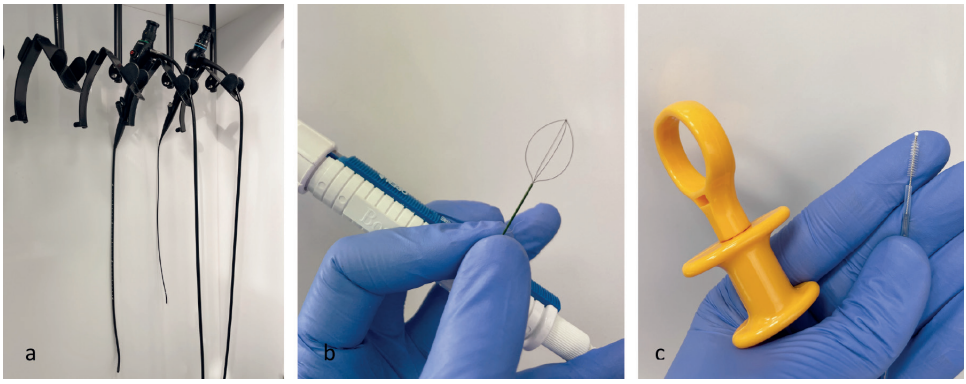

Figure 1. Bronchoscopy equipment (a) flexibile bronchoscope; (b) grasping basket; (c) cytology brush

with the recommended solution (Levitan and Kimmel, 2008). Today, there are disposable endoscopes on the market that minimise the possibility of contamination.

Bronchoscopy in dogs and cats requires general anaesthesia or deep sedation. Most clinicians prefer inhalational anaesthesia, though some authors describe injectable anaesthesia without intubation (Norris et al., 2002; Johnson and Drazenovich, 2007).

It is necessary to prevent the patient from moving and to ensure that it does not cough due to the movement of the bronchoscope. The patient is usually placed in sternal recumbency, and a soft object is placed under the chin to prevent movement of the head. A mouth gag is applied to prevent damage to the endoscope. Preoxygenation of the patient is recommended. In larger animals where visualisation of the trachea is not required, bronchoscopy can be performed via an endotracheal tube using a special T-adapter. In smaller patients, the bronchoscope may obstruct the lumen of an endotracheal tube if the procedure is performed through the endotracheal tube. In such cases, adequate delivery of oxygen and gas anaesthesia is prevented, and the examination and diagnostic sampling must be performed in several short segments or the patient can be supplied with oxygen through the working channel of the bronchoscope (Hawkins et al., 1990; McKiernan, 2005).

Indications and most common respiratory diseases in cats and dogs

The most common indications for bronchoscopy in small animal practice are cough (acute or chronic), foreign bodies in the airways, tracheal or tracheobronchial collapse, recurrent pneumonia, chronic bronchitis, asthma, tracheal or bronchial trauma, pulmonary infiltrates, haemoptysis, neoplasia, placement and evaluation of tracheal stents, ciliary dyskinesia and strictures. Tracheobronchoscopy is less useful in dogs or cats with focal pulmonary lesions, diffuse interstitial lesions, or vascular lesions (Rha and Mahony, 1999).

One of the most common clinical diagnoses in dogs with acute or chronic respiratory disease is bacterial pneumonia, and bronchoscopy and bronchoalveolar lavage are very useful in these patients, especially in patients not responding to therapeutic attempts. Some authors suggest a relationship between viral respiratory diseases and the development of bacterial pneumonia, though there are many risk factors such as age or concurrent diseases (Leroy et al., 1997, Tart et al., 2010). Eosinophilic bronchopneumopathy is a disease considered to be a manifestation of immunological hypersensitivity and is characterised by eosinophilic infiltration of the lungs and bronchial mucosa with eosinophils. It is also known as pulmonary infiltrates with eosinophils (PIE) and has been described in humans and dogs (Clercex et al., 2000). Chronic bronchitis in dogs and cats is a chronic respiratory disease caused by airway injury (irritants). It is characterised by increased BAL fluid neutrophils in the absence of detectable intracellular bacteria and growth of pathogens in culture and is a common cause of chronic cough in dogs and cats (Padrid et al., 1990, Venema and Petterson, 2010).

In cats, bacterial pneumonia is less common than inflammatory feline bronchial disease (Dear, 2014). Feline asthma is a chronic inflammatory disease caused by the inhalation of allergens, which over time leads to hypersensitivity and a de-

Figure 2. Foreign body from the left principal bronchus of a dog – clay ball

crease in lung function. Bronchoconstriction causes airway obstruction due to mucus and oedema (Trzil and Reinero, 2014; Garrity et al., 2019).

Inhaled foreign bodies are a less common cause of respiratory symptoms, but they are dangerous and life-threatening as they are also a source of mixed bacterial and fungal infections (Dear, 2014) (Figure 2). The most common foreign bodies in small animals are plant material such as blades of grass (Tenwolde et al., 2010), who conducted a retrospective study of 37 cases and found that bronchoscopy was successful in removing airway foreign bodies, regardless of animal size or the duration of clinical signs.

Technique

The anatomy of the canine tracheobronchial tree has been described with a detailed schematic system of nomenclature (Amis and Mckiernan, 1986). Each lung has a cranial and a caudal lobe, with the right side having both a middle and an accessory lobe. This system is accepted and used by most authors and practitioners and allows for detailed examination reports. The system uses a set of numbers and letters to identify the principal, lobar, segmental, and subsegmental bronchi by their order of origination and their dorsal or ventral orientation. Most authors and practitioners also use this system for tracheobronchoscopy in cats (Caccamo et al., 2007). The procedure begins by entering the trachea, proceeds to the carina and gently guides the bronchoscope through the respiratory tract, without using force at any time. During bronchoscopy in most dogs and cats, it is usually possible to reach both the right and left main bronchi as well as the origins of the lobar bronchi. In large dogs, a bronchoscope can be passed through lobar, segmental and some subsegmental bronchi. The respiratory tree should be

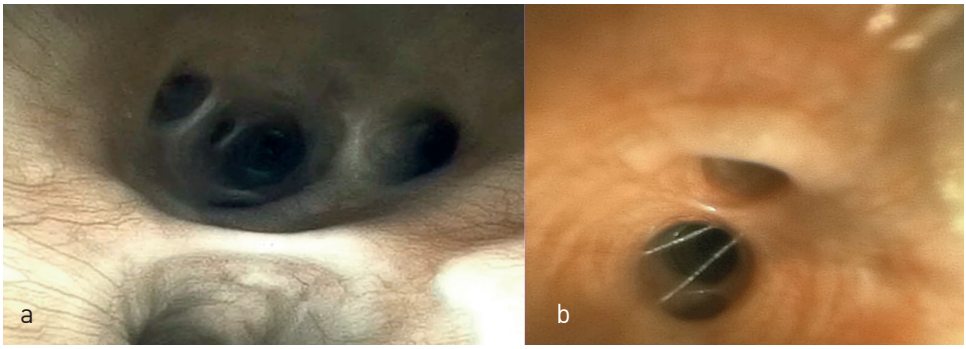

Figure 3. Bronchoscopy of a dog (a): Clearly visible submucosal vessels; (b) excess mucus in airways

examined systematically. The endoscope should be retracted to the level of carina and the procedure repeated to allow for reorientation if the clinician performing the procedure loses sight of the anatomical position of the bronchoscope (Johnson, 2001).

During the examination, it is mandatory to describe the colour and texture of the mucosa, patency, presence and nature of secretions, and presence and location of foreign bodies or masses. The normal finding in tracheobronchoscopy is a moist, pale pink mucosa with small amounts of secretions, and clearly visible submucosal vessels (Figure 3a). The carina appears as a sharp separation between the right and left principal bronchi. The normal entrances to the principal bronchi are round and smooth (Creevy, 2009).

The depth and extent to which the bronchi can be reached with the bronchoscope depends on the size of both the equipment and the patient. Each accessible airway should be evaluated and any changes in size, shape and mucosal appearance noted. In chronic bronchitis patients, a common finding is a loss of mucosal vascularity, roughening of the mucosa, sometimes with nodular thickening. The airways can be dilated and with excess mucus (Figure 3b).

In bronchopneumonia normal or hyperaemic mucosa can be seen, with purulent exudate (Corcoran, 2004). Haemorrhage or increased mucosal friability may be associated with lung contusion, parasitic infection (e.g., *Oslerus*, *Paragonimus*), foreign bodies, mucosal trauma induced from chronic coughing, or neoplasia. Primary mucosal tumours of the airways are uncommon (McKiernan, 2021).

The training and skills of the physician performing the procedure are also important factors. Sampling is performed after complete visual examination. The most common technique for sampling is bronchoalveolar lavage. Less common techniques are biopsy and cytological brush. Biopsy is performed with a small endoscopic biopsy forceps and can be used for collection of samples from masses or nodules. However, sampling is difficult and can lead to bleeding. In addition, the samples are very small and are often damaged or crushed, making this the least commonly performed technique for diagnostic sampling during bronchoscopy. Brushing is a diagnostic procedure for collection cells and/or bacteria adherent to the mucosa (Greenway et al., 2019). This is performed with special endoscopic brushes that are passed through the

working channel of the bronchoscope, pulled out of the plastic sheath, pushed very gently over the mucous membrane and then pulled out of the working channel again covered with the plastic sheath. The samples obtained in this way can be used for cytology by carefully rolling the brush over glass slides, or for culture by swirling the brush through a culture medium (Creevy, 2009). Bronchoalveolar lavage is described further in this article.

Contraindications and limitations

There are certain limitations to bronchoscopy that need to be mentioned, and some of these are based on the characteristics of the scope. The diameter of the insertion tube is a limiting factor in how far the endoscope can be inserted, taking into account the diameter of the airway. In addition, a smaller diameter of the insertion tube may prevent the presence of a sufficiently large working channel required for the use of additional instruments such as grasping forceps (Johnson, 2001).

As patients undergoing bronchoscopy and the associated sampling procedures are typically animals with respiratory disease, extreme caution is required as there is concern that the suppression of respiration by anaesthesia could exacerbate collapse of the diseased airways (Roudebush, 1990). Complications associated with bronchoscopy are rare and include worsening respiratory symptoms, induction of bronchospasm, pneumothorax, bleeding or complications related to anaesthesia and oxygenation. Cats are particularly susceptible to bronchospasm due to their hyperreactive airways (Roudebush, 1990; Bianco et al., 2020).

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) is a minimally invasive diagnostic procedure used to evaluate cats and dogs with lower airway disease, particularly when diffuse disease of the bronchi, pulmonary interstitium or alveoli is present and confirmed by radiography or CT. Bronchoalveolar lavage is a less successful and recommended method for sampling local lesions. It allows samples to be taken from the lower airways. These samples can be examined cytologically and microbiologically for evidence of inflammation, neoplastic cells and infectious agents (Andreassen, 2003). In the study by Kajin et al. (2018), 30 dogs and 7 cats were examined and BAL, along with other diagnostic methods, was found to be useful in making a diagnosis in 63% of dogs and 28% of cats.

Before starting bronchoalveolar lavage it is necessary to define adequate amount of sterile saline to be used. Most authors agree that at least two bolus infusions per site should be performed. The dose of saline is determined by the size of the patient. In a retrospective study of 68 cats by Johonson and Drazenovich (2007), the mean volume of fluid infused was between 2.62 and 5.05 mL/kg. Hawkins (2004) recommends at least four boluses of 10 mL per affected area for dogs under 8 kg and for all cats, and a report by Hawkins et al. (2006) used a total volume of 15 to 75 mL per dog, divided into two or more aliquots. In dogs and cats, recovery of 40% to 75% of the infusion material was reported (Hawkins et al., 2006; Johonson and Drazenovich, 2007).

BAL is always performed after completion of visual examination but before other more invasive sampling procedures. More invasive sampling procedures such as biopsy or brushing can

cause bleeding, which can alter the results of bronchoalveolar lavage (Creevy, 2009).

BAL can be performed endoscopically or using the sterile endotracheal tube technique, called "blind BAL". In endoscopic BAL, the endoscope should be passed through the trachea into the desired region. This is normally performed on both the right and left sides. The bronchoscope is inserted into the trachea until it wedges into the smallest bronchus it can fit into, depending on the length and diameter of the bronchoscope. The procedure is then repeated on the other side (Figure 4). Once the endoscope has been placed in the desired position, sterile 0.9% saline solution is infused through the working channel into the regional bronchus and retrieved as quickly as possible. The saline solution is pushed as a bolus from the syringe. The same syringe is used to aspirate the saline solution through the working channel. Sometimes aspiration results in negative pressure and no fluid is collected (Andreassen, 2003).

This is a consequence of airway collapse in response to aspiration, and the bronchoscope should be retracted a few millimetres and aspiration repeated. Instead of the procedure with syringes, a special collection container can be used so that the vacuum suction is connected to the designated connection of the container and the liquid is collected through the working channel immediately after the bolus injection. Vacuum pump aspiration improves fluid collection compared to syringe aspiration but has no significant effect on the rate of diagnostic success (Woods et al., 2014). The sample should be divided and placed in an EDTA tube to preserve the cells, and at least one sample should be placed in a sterile tube without anticoagulant for culture or other tests such as PCR (Andreassen, 2003). BAL without the guidance of an endoscope is usually performed via

Figure 4. „Blind" bronchoalveolar lavage in a cat

Figure 5. Bronchoalveolar fluid sample

an endotracheal tube, with a small diameter, soft, open-ended catheter placed as far as possible, without force, due to risk for iatrogenic pneumothorax, then the saline is infused and withdrawn while the patient is in the lateral position, and the procedure is repeated on the other side (Figure 4). The cellularity of the bronchoalveolar lavage fluid obtained by "blind" BAL technique is sufficient (McCauley et al., 1998).

The samples obtained by BAL are suitable for cytology, bacteriology and fungal culture or other diagnostic tests such as PCR or specific antigen tests (Andreassen, 2003) (Figure 5).

Cytology samples obtained by BAL are from the lower branches of the respiratory tree. Samples are collected in EDTA tubes and processed according to the chosen laboratory procedure using cytospin techniques. Cytospin preparations are considered to be of higher quality and are recommended when assessing BAL fluid with low total cell counts (Dehard et al., 2008), though slide smear preparation is a simple and accurate method for quantifying the cytology of bronchoalveolar lavage fluid (Thompson et al., 1996). Storage leads to a change in the cytological diagnosis in up to 57% of stored specimens, and therefore cytological analysis of BAL fluid should be performed promptly (Nafe et al., 2011). Some clinicians prefer to determine the total number of nucleated cells from BAL samples, but the interpretation of these results is controversial as it depends on the amount of saline infused (Rebar et al., 1980; Mordelet-Dambrine et al., 1984).

BAL fluid usually contains macrophages (> 70%) as the most abundant cells, epithelial cells, which include ciliated respiratory epithelial cells and goblet cells, and surfactant, which is responsible for foam formation (Andreasen, 2003; McKiernan, 2005).

Bronchoalveolar lavage fluid values

for healthy dogs are reported as 200±86 total cells per microlitre with 70±11% macrophages, 7±5% lymphocytes, 5±5% neutrophils, 6±5% eosinophils, 1±1% mast cells, and 1±1% epithelial cells (Vail et al., 1995). Bronchoalveolar lavage fluid values for healthy cats are reported as 241±101 total cells per microlitre with 70.6±0.8% macrophages, 4.6±3.2% lymphocytes, 6.7±4.0% neutrophils, and 16.1±6.8% eosinophils (Hawkings et al., 1994).

Different percentages and morphologies of cells are expected depending on the disease causing the respiratory signs, such as a relative increase in white blood cells in infection and inflammation (Hawkins et al., 1995).

Findings of degenerated neutrophils in BAL cytology and neutrophils containing bacteria are usually indicative of pneumonia (Johnson and Vernau, 2019).

An increased number of neutrophils (Figure 6) may be associated with bacterial, fungal, mycoplasmal or allergic diseases, such as chronic bronchitis, obstructive lung disease or foreign bodies (Rakich and Latimer, 1989).

In dogs testing negative for heartworms, lungworms, or a failed fenbendazole trial, bronchoscopy findings of yellow mucus, airway collapse or hyperaemia, and a high percentage of eosinophils in the BAL are usually indicative of eosinophilic bronchopneumopathy (Clercex et al., 2000). Johonson et al. (2019), in a study on 75 dogs with eosinophilic lung disease, concluded that dogs can be categorised as having eosinophilic bronchitis, eosinophilic bronchopneumopathy and eosinophilic granuloma based on imaging, bronchoscopy and cytology findings of BAL fluid. In a study of 104 dogs conducted by Johnson and Vernau

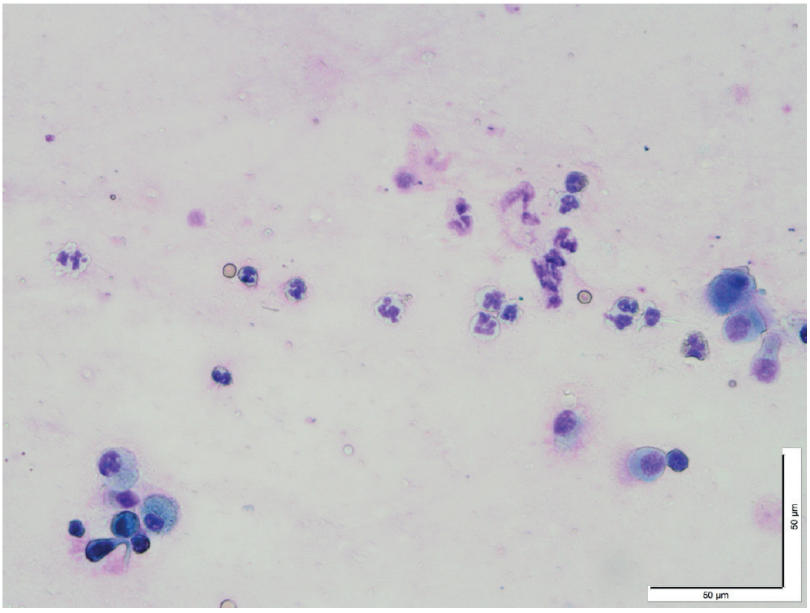

Figure 6. Bronchoalveolar fluid smear (sedimented, MGG stain, magnification 40 x): mixed inflammatory cells with a prevalence of neutrophils (courtesy of V. Đurić)

(2019), lymphocytosis was found to be common in BAL fluid and is likely to be a common response to airway injury.

Depending on the predominant type of inflammatory cells in the airways, a distinction is made between feline asthma and chronic bronchitis, as asthma is primarily characterised by eosinophilic and chronic bronchitis by neutrophilic airway inflammation (Moise et al., 1989; Johnson and Vernau, 2011; Allerton et al., 2013; Gareis et al., 2022).

Sometimes even neoplastic cells can be found, but in several studies the correlation between BAL and histopathology was incomplete as not all neoplasms exfoliate (Norris et al., 2002; Conti et al., 2010). In a study of dogs with multicentric lymphoma involving the lung, bronchoalveolar lavage fluid contained neoplastic lymphocytes in 66% of cases (Hawkins et al., 1993). Kim et al. (2022) found suspected pulmonary mesothelioma in BAL fluid in a dog.

Analysis of BAL fluid can be helpful in the diagnosis of some parasitic diseases. For example, Kavarnos et al. (2022) found that cytology of BAL fluid revealed histiocytic inflammation in 14/31 (45.2%) dogs with *Leishmania infantum* where the parasite was identified in one dog (3.2%). The immunofluorescence antibody test in the BAL fluid was positive in 15/31 (48.4%) of dogs examined.

Except for cytology, BAL fluid is always sent for culture. The airways are not sterile, so some bacteria are likely to be of unknown clinical significance (McKiernan et al., 1984; Peeters et al., 2008). In dogs, the lung microbiota has only recently been studied and little data is available (Ericsson et al., 2016; Fastrès et al., 2019, 2020a). Samples of BAL fluid can be stored at 4°C for 24 hours prior to culture without altering the culture results (Curran et al., 2020). In order to objectify the significance of the bacteria found, it is assumed that more than 10^4 colony forming units (CFU)/mL represent a true infection, while less than 10^3 CFU/mL represent contamination (Padrid, 2000). A study by Peeters et al. (2008) found that greater than 1.7 x 10^3 CFU/mL or more indicates a sensitivity of 86% and a specificity of 100% for the diagnosis of a respiratory infection. However, this threshold may not accurately predict whether antibiotics are required (Lebastard et al., 2022). Bronchoalveolar lavage fluid analysis can be useful in identifying fungal infections with lung involvement (Hawkins and DeNicola, 1990). Commonly isolated organisms from BAL fluid in dogs and cats are *Mycoplasma* spp., *Pasteurella* spp., *Bordetella bronhiseptica, Escherichia coli, Klebsiella pneumoniae, Salmonella typhimurium, Pseudomonas* sp. *and Cryptococcus neoformans* (Foster et al., 2004a, 2004b; Peeters et al., 2008)

In addition to the above tests, BAL fluid can be tested by PCR for various pathogens such as *Angiostrongylus vasorum* (Canonne et al., 2015), *Bordetella bronchiseptica* (Cannone, 2016), and *Mycoplasma spp* (Chan et al., 2013).

In the last decade, several other markers from bronchoalveolar fluid have been investigated that can be used to detect various diseases. Tasaka et al. (2012) found that elevated CXC chemokine levels in airspaces obtained by BAL in human patients can be associated with emphysematous lung changes in patients with pulmonary fibrosis.

Fastrès et al. (2020b) used single-cell RNA sequencing to characterise disease-related heterogeneity within macrophage/monocyte cell populations in the BAL fluid of five dogs with idiopathic canine pulmonary fibrosis compared to three healthy dogs.

Conclusion

Bronchoscopy and bronchoalveolar lavage are valuable diagnostic techniques that allow direct visualisation and sampling from the respiratory tree and should be used frequently, especially in patients not responding to therapeutic attempts. Since the samples obtained by bronchoalveolar lavage are suitable for cytology, bacteriology and fungal cultures or other diagnostic tests such as PCR or specific antigen tests, this makes BAL very useful in practice and research. Efforts should be made in the training of physicians and equipment to ensure the safety and efficacy of the procedure.

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Bronhoskopija i bronhoalveolarna lavaža u pasa i mačaka

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Bolesti dišnih prohoda vrlo su česte u pasa i mačaka. Bronhoskopija i bronhoalveolarna lavaža korisne su dijagnostičke metode koje omogućuju vizualizaciju lumena i sluznice velikog dijela dišnog prohoda te uzimanje uzorka. Odluka o metodi uzorkovanja ovisi o procjeni stanja pacijenta i provedenoj slikovnoj dijagnostici. Bronhoalveolarna lavaža je siguran i jednostavan postupak indiciran u dijagnostici difuznih bolesti bronha, plućnog intersticija ili alveola; izvodi se aplikacijom 0,9 % fiziološke otopine u odabrani regionalni bronh kroz radni kanal bronhoskopa ili kao „slijepa tehnika“, potom se tekućina što je moguće brže uzorkuje. Uzorci dobiveni bronhoalveolarnom lavažom prikladni su za citološku pretragu, bakteriološku i mikološku pretragu ili druge dijagnostičke testove kao što su PCR ili specifični antigenski testovi. Bronhoskopija zahtijeva odgovarajuću opremu te specijaliziranu obuku i iskustvo veterinara koji izvodi zahvat. U ovom preglednom članku iznesen je prikaz osnovne tehnike, opreme, najčešćih indikacija, komplikacija i tumačenja rezultata bronhoskopije i bronhoalveolarne lavaže u pasa i mačaka.

Ključne riječi: *bronhoskopija, bronhoalveolarna lavaža, pas, mačka*