THESIS

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Bronchoalveolar lavage in dogs

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Table of Contents

List of abbreviations

BAL	bronchioalveolar lavage
BALF	bronchioalveolar lavage fluid
ELF	epithelial lining fluid
HRCT	high resolution computer tomography
RPMI	Rosswell Park Memorial Institute Medium
MEM	minimal essential medium
TNCC	total nucleated cell count
UVMB	University of Veterinary Medicine, Budapest
EGC	eosinophile granulocytes
NGC	neutrophile granulocytes
Lymph	lymphocytes
Macro	macrophages
FSC	forward scatter
SSC	side scatter

1. Introduction

1.1 Background and Relevance of Research

Bronchoalveolar lavage is a well-known technique for collecting samples from the deep airway system. The bronchioalveolar lavage (BAL) is widely used in research facilities as well as in everyday practise. This technique enables the analysis of the epithelia-lining-fluids (ELF). This fluid coats the alveoli and small airways. The ELF contains a various amount of cellular and non-cellular components, which gives valuable information about the condition and health status of the airways. (1)

In general, the BAL-technique describes the installation of a defined amount of sterile sodium chloride solution into the lungs with subsequent aspiration. The aspirated fluid is referred as bronchioalveolar fluid (BALF). The BAL-fluid contains an excessive number of cells, proteins and other components from the deeper airways. This can be used for research, examination and diagnosis of respiratory diseases, because it is a direct analysis of the ELF.

The bronchioalveolar lavage in dogs enables the detailed examination of the deeper airways in dogs, which helps in diagnosing and managing various airway related diseases. Even though the BAL-analysis is widely used in everyday practice, there are no uniform standards in performing the BAL, which lead to different results and difficulties of the comparability of data. (2)

An essential part of BAL-research in dogs, is the examination and effects of BAL-synthesis on lung tissue. Studies showed that recurrent BAL-procedures had no major impact and no permanent changes on the lung tissue, that had been used for the BAL. (1)However, in terms of sample analysis it has been shown that neutrophil cell counts in BLAF increase and peak following sampling in 24 hours. Therefore, repeat sampling is recommended after 48 hours if it is necessary (citation).

Furthermore, studies shown, that if the amount of lavage fluid that is instilled into the body is adapted to the dog´s bodyweight, more reliable and repeatable data will be obtained from the patient’s ELF. This is crucial for the better comparison of data between studies and laboratories. (3)

Therefore, it is important to standardize the methods to get more reliable and comparable results, which would lead to optimized diagnosis, treatment and research of airway related diseases.

2. Bronchioalveolar lavage in dogs

2.1 Definition

The bronchioalveolar lavage (BAL) is a diagnostic and research technique to gather samples from the lower airways. This procedure enables the examination of the epithelia-lining-fluid (ELF), which coats the alveoli and lower airways. The ELF contains an excessive amount of cellular and non-cellular components, which are crucial for the assessment of the lower respiratory tract. (4)

The BAL-technique means the instillation of sterile sodium chloride solution into the lung. Subsequently the examiner must aspirate the fluid, that is now called bronchioalveolar lavage fluid (BALF). The BALF contains cells, proteins and other components of the lower respiratory tracts, which benefits the treatment and diagnostics of airway borne diseases by direct analysis of the ELF.(5)

2.2 History and development

The history of the BAL goes back till the early 1920´s. The first documented and performed procedure was 1927. In this year it was known as the bronchial lavage with sterile sodium chloride solution. The solution was instilled via a catheter through a rigid bronchial endoscope into the desired area of the lower airways. The term “bronchial lavage” was coined by Stitt in the 1930´s. (6)

This technique originated from the treatment for septic lung diseases and alveolar proteinosis. In the early 1960´s the lavage was then used to examine the immunity of the lower airways in animals. In the late 1960´s till the 1970´s, first experiments and examinations were performed in humans. A groundbreaking article was released in 1974 using an flexible bronchoscope to reach different and even deeper parts of the lung, to examine the different secretions by installation of sterile sodium chloride solution.(4)

In the 1980´s and 90´s this procedure got more and more common, and it was widely used by many facilities. Concerns grew because till then no uniform protocol was established on how a BAL-lavage should be performed. As a result there could be a high possibility to gain different results and data, sometimes with significant differences, when using different

techniques, which would lead to different interpretation and outcome of the BAL-analysis.(1)(4)

To target this problem, different groups of scientists gathered to develop a uniform protocol for the BAL-procedure. The European Respiratory Society developed a protocol on how to perform a bronchoalveolar lavage for interstitial lung diseases and how to interpret the results. Furthermore, instructions and guidelines for technical aspects and standardisation were created. (6)

A multi organisation conglomerate was formed, sponsored by the National Institute of Health (NIH) and lead by the American Thoracic Society, to examine and compare BAL-profiles in patients with ILD (interstitial lung disease) and healthy patients.(3)

2.3 Usage of BAL-synthesis in modern medicine

Over the time the BAL established as a very useful tool for diagnosing and researching of airway borne diseases. The BAL offers possibilities to collect secretions from the apical surfaces of the bronchial and alveolar epithelia.

It is necessary to mention that the collected secretions are significantly diluted, caused by the instilled sodium chloride solution. This enables the analysis of the cellular- and non-cellular components. (7)

Despite the diagnostic potential of BALF, the application in everyday clinical practise is reduced due to its relative lack of specify and is often used as an additional diagnostic tool. Even though the BALF-nucleated immune cells have correlated well with certain forms of ILD such as sarcoidosis, the suboptimal specificity has limited their impact as a diagnostic tool in ILD. Nevertheless, BALF analysis has contributed immensely to our understanding of the immunopathogenesis of various diseases and continues to be considered as a valuable diagnostic and research tool.

2.4 Techniques and procedures of BAL sampling

2.4.1 BALF sampling

The BAL is a well-established procedure to gather samples from the lower airways. This procedure contains several steps, that must be performed very carefully to ensure the safety of the patient and the reliability of results.

The animal/ patient must be sedated and intubated before the actual procedure. Preoxygenation may be necessary and should be available for patients.

Patients suffering or being suspected of ILD (interstitial lung disease) should undergo the routine clinical evaluation. This includes clinical inquiry and a checkup for bleeding tendencies to keep the procedure-related complications as low as possible, by ruling out potential risk factors. If no clinically relevant findings are present, the patient is suitable for the BAL procedure.

Patients suffering from ILD should receive a HRCT before the BAL is performed. This helps to choose the target site based on the HRCT results, instead of the traditional BAL sites (i.e. right/ middle lobe or lingua) (5)

Furthermore, it is recommended to use the HRCT (high-resolution computer tomography) for the identification of target lung areas to acquire samples with high diagnostic value with the BAL method. The BAL procedure should be performed shortly after the HRCT because target areas or characteristic abnormalities in the parenchymal tissue may change over time Despite the lack of controlled clinical trials comparing the information gained by the BAL sites identified by the HRCT versus the standard BAL sites (these are sites with usually good accessibility and an acceptable returned volume, which could be for example the right middle lobe), some studies suggest using the HRCT to choose the site of lavage.

Gravity could possibly influence the lavage process. Due to this the position of the patient should be chosen by the practitioner based on the clinical situation regarding the patient and must be written in the procedure report. While using a flexible bronchoscopy, the bronchoscope is placed in a wedged position inside the chosen bronchopulmonary section. Through the bronchoscope, three to five aliquots of room temperature sterile saline are administered in total volume of between 15-25ml per wash or 1-2ml/kg in dogs. Instilled saline is typically collected using a negative suction pressure of less than 100 mm Hg after each aliquot has been instilled. It is necessary to modify the negative suction pressure to prevent possible airway collapse.(8) It may be useful to follow the instilled saline with 3-5

ml of air to make sure it reaches the targeted lung regions. A minimum of five percent of the injected volume should be recovered in total (optimal sampling recovers greater than thirty percent). Airway collapse will decrease fluid retrieval.

The procedure should be stopped, if less than 5% of each injected aliquot volume is recovered during the process, because most of the fluid is still retained in the lung. This is to protect the patient from further harm. Recoveries of 42-48 % and 59-75 % were reported for dogs and cats respectively.(8)

BALF obtained from healthy patients is transparent, with low cellularity. A stable foam is frequently present on top of the samples indicating the presence of surfactant from the alveoli in the sample. An increased cellularity may lead to increased turbidity.(8)

Cloudy BAL fluid (i.e., milky or light brown-beige colour) with flocculent material that settles by gravity to the bottom of the container within 15 to 20 minutes after collection could be indicative of pulmonary alveolar proteinosis (PAP). Similarly, grossly bloody BAL fluid with increasing intensity in sequential aliquots could indicate acute diffuse alveolar haemorrhage.

2.4.2 Handling of BALF samples

It is recommended to gather the BALF in containers that inhibit cell adhesion to the surface (such as silicone-coated glass, polypropylene, or other polymers used for tissue culture in suspension). The method of transport depends on the duration it takes to get the sample to the laboratory. Optimally BALF samples are analysed immediately to avoid cell deterioration. If more time is needed the sample should be kept and transported at 4° C which could be achieved by transporting the sample on ice in a Styrofoam container. The sample should be centrifuged at a speed that keeps the cell integrity (250-300 rpm for 10 minutes) and the cells can be added to a nutrient-supplemented medium if transportation and storage takes more than 60 min. This sample can be stored up to 24 hours at 4°C. If it is not possible to centrifuge the sample solution, it is recommended to add MEM (minimal essential medium) or RPMI (Rosswell Park Memorial Institute medium) to the lavage solution. The sample should be transported as quickly as possible to the laboratory, or could be stored up to 12 hours at 4°C.

A prolonged transport to the laboratory could bring false results and the BALF must not be transported frozen or transported on dry ice.

MEM and RPMI are both cell culture media that are used in laboratory settings for the growth and maintenance of cells outside their natural environment. These media provide the cells with various nutrients, essential minerals, vitamins and buffering agents.

To achieve the best results possible, the BALF samples must be processed immediately by the laboratory. Container materials should not be adherent to avoid loss of cells and alteration of the sample. The BALF can be strained through loose gauze, if the fluid is rich in mucus. Small amounts of mucus can be dissolved with dithiothreitol. Following this step, centrifuge the sample at an optimal speed (mentioned earlier) then resuspended and analysed. Concentrated samples (cytocentrifuged) may be used for cytological analysis to increase cell density. Samples that are older than 24 hours are not suitable for a proper analysis.

3. Analysis of BALF

3.1 Methods for cell counting and differentiation: manual cell counting, automated cell counting

The reference method to determine the total count of nucleated cell (TNCC) in body fluids is by using a microscope and a counting chamber (hemacytometer). Nevertheless, nowadays the vets favour rise the automatic cell count via the haematology analyser.

The counting with the hemacytometer can be very precise, but it is time consuming and prone to errors, either due to dilution, cell differentiation/identification or examiner differences. Automated haematology analysers are widely used for human probe analyses. There are some veterinary medicine versions of these devices like the Advia 2120i haematology analyser. This device is capable of producing validated results regarding the analysis of the TNCC in BALF, by using a multi species software.(4) Reported TNCC from healthy dogs is less than 500 cells/microliter.(8)

Differential cell counts can be performed. The automated cell differentiation becomes more and more popular, but the microscopic assessment of cell morphology and differentiations is still widely performed and considered gold standard. Typically, a cytospin slide preparation is frequently used, although direct smears are occasionally prepared. Cytospin preparations offer the advantage of more concentrated and evenly distributed samples. However, it has been reported that these samples underestimate lymphocytes and

overestimate neutrophil cells. The samples are then dried and stained with a Romanowsky type staining method (for example May-Grünwald-Giemsa or DiffQuick stains). During a microscopic BALF-analysis around 100 – 200 cells should be differentiated. According to de Lorenzi et al a 500 cell differential is recommended to reach optimal reproducibility.(9)

The presence of erythrocytes usually indicates the blood contamination with limited diagnostic value. However, the presence of haemoglobin breakdown products can indicate previous haemorrhage.

Epithelial cells are typically not counted in cytospin preparations, because these cells are accumulated in clusters and their numbers are in correlation with the method use for probe extraction. In general, the counting of epithelial cells is very uncommon in the published literature and without any interest, because there is very rarely a correlation between lung lesions and the total number of epithelia cells. However, if they represent upper airway contamination it is necessary to note their presence due to the differential interpretation (upper airway pathologies may be represented in the sample along with the desired target area confounding the conclusion).

Regarding the automatic counting method via the Advia 2120i haematology analyser, it is remarkable that the device is capable of precise measurements of the leukocytes, represented in the total nucleated cell count (TNCC), just like the regular blood analyses. The common problem that occurs is that the Advia in not capable of custom gating permitting successful differentiation of cells indifferent species. Nevertheless, there are some optional software changes to distinguish at least three different cells like neutrophilic, eosinophilic and mononucleated cells for rats and mice. Due to that modifying process, more and more profiles are produced to enhance the precision of these analysis. (10)

Some other devices, like various Sysmex haematology systems (xT and XN series) even have special operating protocols for BALF-analysis. These protocols can successfully generate the TNCC in various animal species. Even though the automatic counting with the various haematology analysers is very time saving, it needs to be pointed out that the devices can only detect a deviation in the TNCC. For the evaluation of morphological cellular changes, specific macrophagic vacuoles or other important and by the haematology analysers not recognized cell types (for example mast cells or neoplastic cells) the microscopic evaluation, performed by a skilled operator, is irreplaceable. (11)

3.2 Application of flow cytometry

Flow cytometry is a very useful tool that's becoming more important in veterinary practise. It's especially useful for analysing body fluids in dogs, like BALF. This technique helps veterinarians quickly and accurately count and study the cells in a sample. It works by sending the cells through a fluid stream and past a laser beam to measure their physical and chemical traits, giving vets detailed insights into the health of the animal.(7)

3.2.1 Principle of flow cytometry

The basic principle of flow cytometry is measuring light scattering and fluorescence caused by cells as they pass through a focused laser beam. Light scattering is divided into two main components: forward scatter (FSC), which provides information about cell size, and side scatter (SSC), which gives insights into the granularity or internal complexity of the cells. Additionally, fluorescent dyes can be used to label specific cell components or markers, which are then measured by fluorescence detectors.

In veterinary medicine, flow cytometry is often used to analyse BALF to determine cell composition and the presence of inflammatory cells. This is particularly useful for diagnosing and monitoring airway related diseases in dogs. A typical example of a flow cytometer used in veterinary medicine is the ADVIA 2120i haematology analyser. This device uses laser optics to measure light scattering and absorption, enabling the identification and counting of different cell types. Giving this the ADVIA 2120i guarantees a lot of advantages. The haematology analyser is quick and efficient. It can analyse a large amount of sample is a relatively short period of time which would make it perfect for large facilities or laboratories.

Also worth to mention that the results, given by the analyser are very precise and constant, that enables the comparability and reproducibility of data/ results. Finally, the multidimensional analysis. This describes the ability to measure several parameters simultaneously, which enables the better characterisation of cells and identification of cell population. (12)(7)

Other, more complex devices offer deeper insight into various cell subsets (such as CD4 or CD8 positive lymphocytes) and are mainly used for research purposes.(13)

3.3 Comparison of methods

3.3.1 Comparison of automated and manual methods

The manual cell counting is the traditional method, which is till today the gold standard. This method allows the direct observation and counting of cells under the microscope. The problem with this method is that it is very time consuming, and it is prone to human error. The automated cell counting methods are advanced technologies such as flow cytometry and offering a quick and precise alternative to the existing manual methods.

An example for an automated system is the ADVIA 2120i haematology-analyser. Studies shown that the ADVIA 2120i system is capable of precise cell counting and differentiation with certain limitations. The device was capable of analysing probes with moderate to intense pleocytosis (increased cell amount). Nevertheless, the accuracy decreases in probes with lower cell amounts indicates the need for careful calibrations and possibly species-specific settings.

An advantage of the ADVIA 2120i compared to the manual, human-based, cell counting is the reproducibility, higher accuracy of results and faster processing, because the ADVIA 2120i system uses highly standardized processes. These described advantages could be especially useful in clinical laboratories with a high throughput rate.(14)

3.4 Normal and pathological findings in Dogs

Table 1:Normal findings in dogs.

Normal Findings in Dogs		
Cell Type	**Dogs (Normal)**	**Method**
Macrophages	78 %	Bronchoalveolar lavage (BAL) was performed, and cell counts from BAL fluid were analysed.
Lymphocytes	7 %	Bronchoalveolar lavage (BAL) was performed, and cell counts from BAL fluid were analysed.
Neutrophils	5 %	Bronchoalveolar lavage (BAL) was performed, and cell counts from BAL fluid were analysed.
Eosinophils	6 %	Bronchoalveolar lavage (BAL) was performed, and cell counts from BAL fluid were analysed.
Mast Cells	1 %	Bronchoalveolar lavage (BAL) was performed, and cell counts from BAL fluid were analysed.
Epithelial Cells	1 %	Bronchoalveolar lavage (BAL) was performed, and cell counts from BAL fluid were analysed.
Total Nucleated Cells/µl	Less than 500	Bronchoalveolar lavage (BAL) was performed, and cell counts from BAL fluid were analysed.

Table 2: Pathological findings in dogs.

Pathological Findings in Dogs		
Pathological Findings	**Correlated Changes in cell counts**	**Methods**
Eosinophilic pneumonitis secondary to canine heartworm disease	Increased total nucleated cell count, slightly increased eosinophils, increased reactive lymphocytes	Bronchoalveolar lavage (BAL) was performed, and cell counts from BAL fluid were analysed.
Blastomycosis	High numbers of mature, non-degenerate neutrophils, reactive lymphocytes, plasma cells, and macrophages	BAL was performed, and cytospin preparation of BAL fluid was analysed.
Suppurative inflammation	Neutrophile are primary cells, nondegenerate neutrophiles, occasionally contain intracellular cocci	BAL was performed, and cytospin preparation of BAL fluid was analysed.
Macrophagic/ mixed inflammation	Alveolar macrophages in acute/ chronic forms, Predominant cell-type	BAL was performed, and cytospin preparation of BAL fluid was analysed.
Granulomatous inflammation	Presence of epithelioid macrophages, multinucleated giant cells	BAL was performed, and cytospin preparation of BAL fluid was analysed.
Hypersensitivity airway disease	Eosinophilic and neutrophilic cells, lower TCC	BAL was performed, and cytospin preparation of BAL fluid was analysed.
Viral pneumonia	Neutrophilic inflammation, degenerated neutrophilic cells	BAL was performed, and cytospin preparation of BAL fluid was analysed.
Bacterial pneumonia	mucus, high numbers of macrophages, degenerated neutrophiles with intracellular bacteria	BAL was performed, and cytospin preparation of BAL fluid was analysed.

3.5 Presence of epithelial cells and how it influences the diagnosis

Epithelial cells in BAL can appear in various forms and can influence the diagnosis of airway diseases in dog and cats. They play a major role in identifying neoplastic or inflammatory processes. There is a wide range of detectable epithelial cells, from normal cells to highly pleomorphic cells. The morphologic properties of these atypical cells are anisokaryosis, variations in nucleocytoplasmic ratio, prominent and/or multiple, often abnormally placed or shaped nucleoli and uneven chromatin patterns. These properties are crucial for diagnosing malignant diseases like carcinomas.

In cases of chronic-active inflammation, moderate amounts of epithelial cells accruing in cohesive clusters can be detected. These cells are often morphologically normal and support the diagnosis of epithelial hyperplasia. The differentiation between inflammatory and neoplastic epithelial cells can be impended by the presence of inflammation. Studies shown that intense inflammatory reactions can mask the morphological properties of cells, so it is important to gather additional diagnostic information, like medical history, known diseases and radiological results for an successful analysis of epithelial cells.(15)

3.6 Nonrepresentative sample

The bronchioalveolar lavage relies on the analysis of cellular and noncellular components. Without these components the BALF sample has no diagnostic value in evaluating airway diseases. A BALF sample can be seen as non-representative, if the cellular composition of the alveolar space is not adequately reflected. This can happen under various causes and are crucial for both clinical- and research purposes.

A main indicator that a BALF sample is not representative is the absence of nucleated cells. This may occur if the sample was improperly handled or processed and could lead to cell-lysis or degeneration. (16)

The viability of nucleated cells is a critical parameter with roundabout 90% viability a sample can be seen as representative. Is the viability under 80% can be seen as a significant impairment and is suboptimal for analysis.

Another important indicator for a nonrepresentative sample is the presence of cylindrical epithelia cells in larger amounts compared to the alveolar macrophages. In a representative BAL sample, the alveolar macrophages are the primary immune cells in the alveolar area.

The sample is contaminated from the proximal airways and not from the alveolar space if more than 5% of cylindrical epithelial cells are present. This is a common error in lavage procedures which leads to a different cellular profile and low analytical value, because it doesn't represent the lower airways. The cylindrical epithelia could also block the clear view in the alveolar macrophages and other important immune cells which are crucial for a correct diagnosis. This could lead to misdiagnosing of serious interstitial lung diseases or other pulmonary diseases. (15)

To minimize the risk of non-representative samples, several procedural recommendations were created. Some recommendations are standardized voluminal and aliquots during the lavage procedure, to ensure a sufficient sampling of the alveolar space. Furthermore, a careful handling and processing of the sample material is essential to maintain the cell integrity and viability. Suitable staining- and counting techniques should be used to ensure a meaningful result. (17)

4. Aims

The aim of this thesis is to provide an overview of the BAL cytological analysis performed et the UVMB and to identify preanalytical and analytical variables which might increase the diagnostic value of BALF samples.

5. Materials & methods

5.1 BALF collection Procedure

Dogs underwent BALF sampling procedure under standardized circumstances performed at the Diagnostic Imaging Unit of the University Teaching Hospital of the University of Veterinary Medicine, Budapest (UVMB).

5.2 BALF cell counting and cytological evaluation

BAL fluid was submitted to the Clinical Laboratory of the Department of Clinical Pathology and Oncology on the day of collection in plain and EDTA collection tubes. Samples were measured for total nucleated cell count (TNCC) and red blood cell count (RBC) on the Advia

2120i haematology analyser (Siemens Healthineers, Erlangen, Germany) on standard canine peripheral blood measurement settings. Some samples were excluded because of excessive mucus clot formation. In these cases, TNCC and RBC counts were not available.

Concentrated smears and direct smears were created according to the clinical pathologist's preference. Concentrated smears were prepared using 100 microliter aliquots spun in a cytocentrifuge. Cytological analysis was performed by either a board certified veterinary clinical pathologist (dr. Péter Vajdovich) or a resident (dr Márton Márialigeti).

5.3 Data collection and processing

Clinical records and laboratory results of dogs undergoing BAL sampling procedure from 2 January 2023 to 30 October 2024 at the teaching hospital of UVMB were reviewed retrospectively. Signalment, history as well as aerobic and fungal culture and parasitology results were collected from the Doki for Vets Connect practice management software (Alpha-vet Kft, Székesfehérvár, Hungary, v 10.00.1942). BALF cytology results were collected from tha LabSoftLims software (NetCare.hu Kft, Kalocsa, Hungary, rev 15236).

Collected data included the following: signalment (breed, age, sex and body weight), clinical observations (clinical diagnosis: tracheal collapse with grade, bronchomalacia, presence and character of airway inflammation, oedema, haemorrhage, stenosis, fibrosis, suspicion of neoplasia, signs of reflux and/or aspiration, upper respiratory tract disorders, concurrent diseases, concurrent medication), aerobic and fungal culture results, parasitology results, results of imaging studies and histopathological examination. The laboratory data included total nucleated cell count, red blood cell count (measured on the Advia 2120i system), total cells counted on microscopy and differential cell counts.

Cases included for differential cell counts were ones where at least 50 nonepithelial cells were counted.

Descriptive statistics and statistical methods were used to assess the samples in patients with different respiratory disorders. R software (R Foundation for Statistical Computing, Vienna, Austria) was used to do the appropriate statistical tests.

6. Results

The analysis is focused on the cytological profile and various respiratory diseases in dogs. Especially the total number of nucleated cells (TNCC) and different cytological properties of each disease was crucial for the analysis. These features were analysed in the context of the dominant concurrent diseases and compared across the different disease groups to highlight their special pathophysiologic mechanisms.

6.1 Patient Population

Samples from 236 patients were collected and analysed. 60 breeds and mixed breed dogs were represented (figure 1). 111 females (76 neutered, 35 intact) and 122 males (55 neutered and 67 intact) were recorded. Sex was not recorded for 3 dogs (figure 2)

Age was available for 233 dogs. Mean age was 8,7 years (SD 4,1, range <1-17 years). Weight data was available for 221 dogs. Mean weight was 11,4 kg (SD 10,7, range 1,6-70 kg,

figure 3)

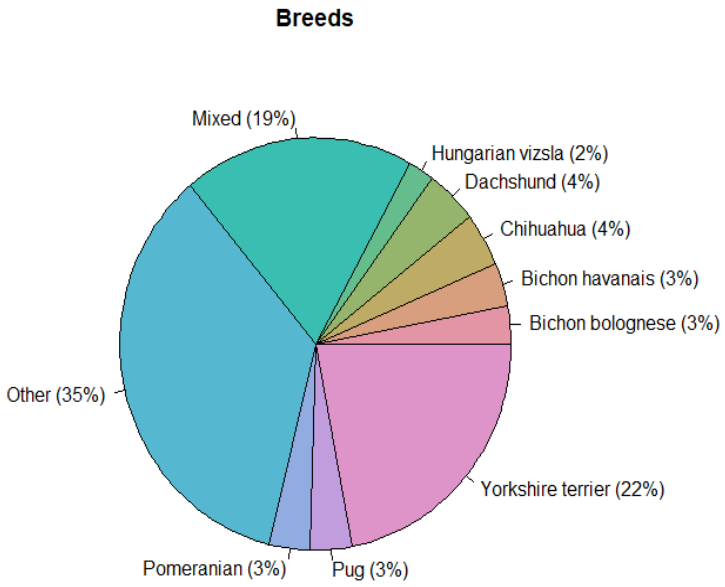

Figure 1: breed distribution. Represented breeds are those which gave at least 2 % (5 individuals) of the whole population.

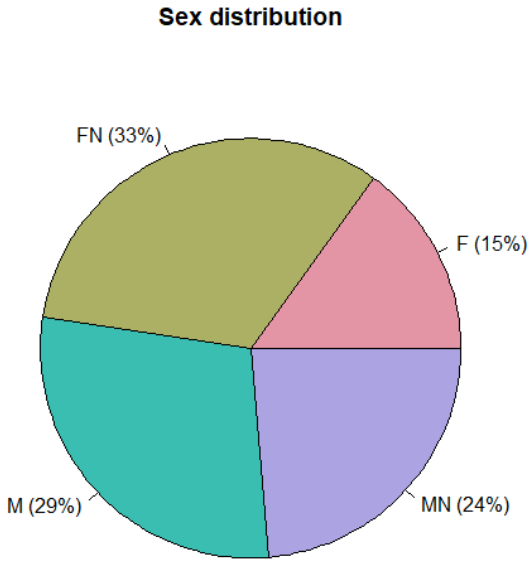

Figure 2: sex distribution of the sampled dogs.

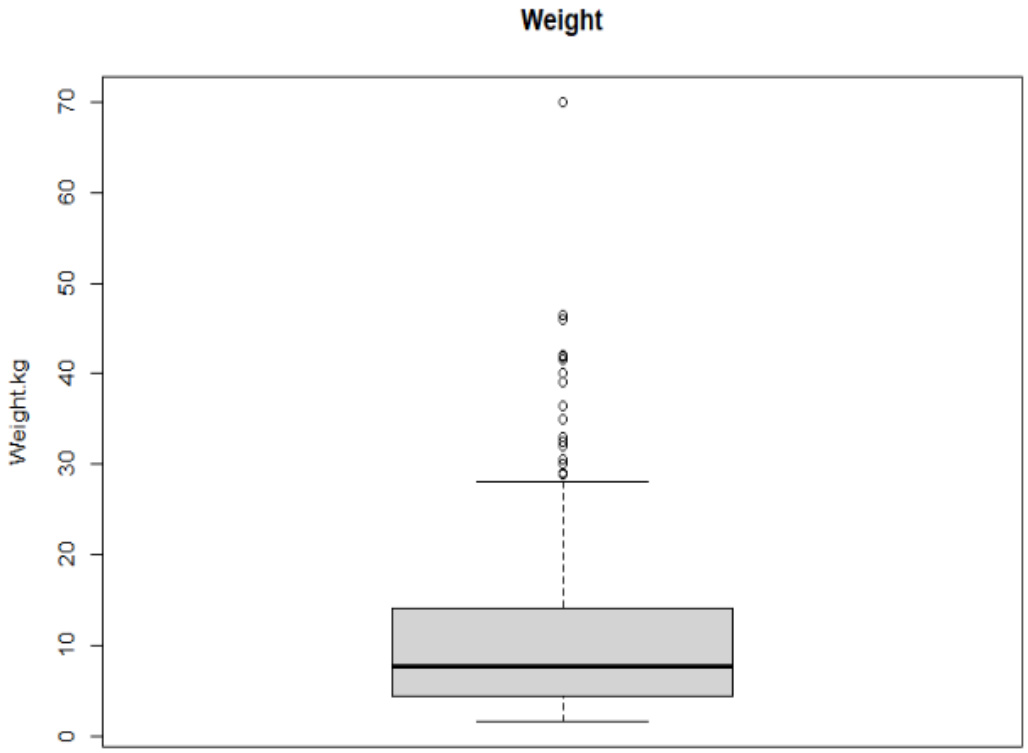

Figure 3: weight distribution of dogs undergoing BAL sampling. Small dogs are overrepresented.

6.2 Clinical characteristics of the patients

Inflammatory diseases were frequently observed (173/236 cases). The most frequent clinical diagnosis was chronic bronchitis (77/173) followed by serous bronchitis (18/173) and catarrhal bronchitis (9/173). Tracheal collapse was diagnosed in 99 cases (21, 30, 29 and 19 cases for grade 1, 2, 3 and 4 respectively). Bronchomalacia was observed in 71 cases.

6.3 Results of the laboratory analyses

6.3.1 Differences between small and large dogs

From the 236 cases 186 had at least 50 nonepithelial cells counted. 89 samples had less than 10 % epithelial cells counted. Total nucleated cell counts measured on the Advia 2120i system were available in 151 cases (mean $1{,}14 \times 10^9$/L, SD 2,68, range 0-18). Nonepithelial cell count mean was $0{,}59 \times 10^9$/L (SD1,93, range 0-17,7). Red blood cells were generally absent (none counted in 132 cases, mean $0{,}01 \times 10^{12}$/L, SD 0,03, range 0-0,2)

Total nucleated cell counts (figure 4), and epithelial percentages (figure 5) were compared amongst small (<= 5 kg) and large breed (> 5 kg) dogs (figure 4, figure 5). Significant difference was found in in total nucleated cell count (p=0,004) with small breed dogs having lower cell counts (small dogs mean: $0{,}48 \times 10^9$/L, SD 0,87, range 0-5,8; large breeds: mean $1{,}55 \times 10^9$/L, SD 3,3, range 0-18,0). At the same time difference between epithelial cell ratio was not significant (p=0,27).

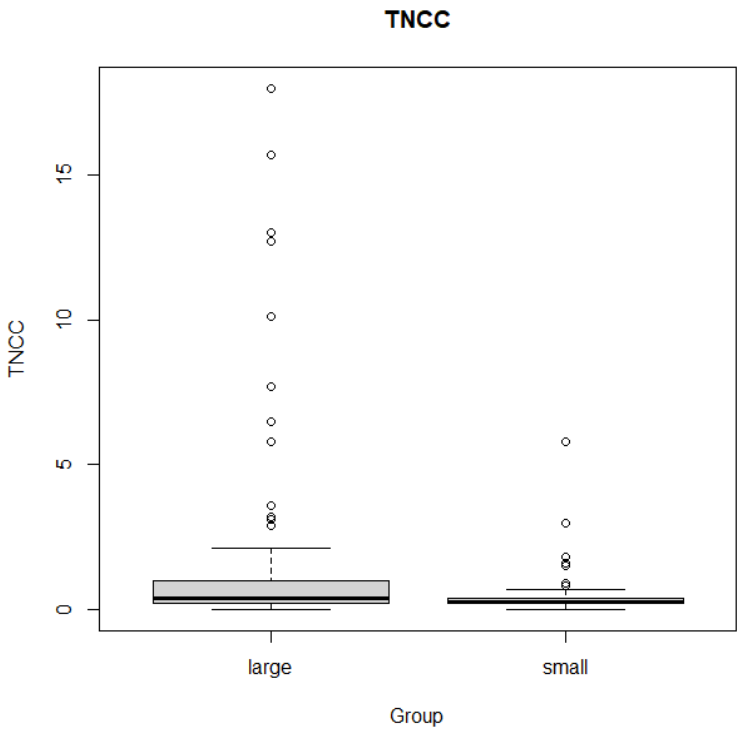

Figure 4: TNCC in small and large dogs.

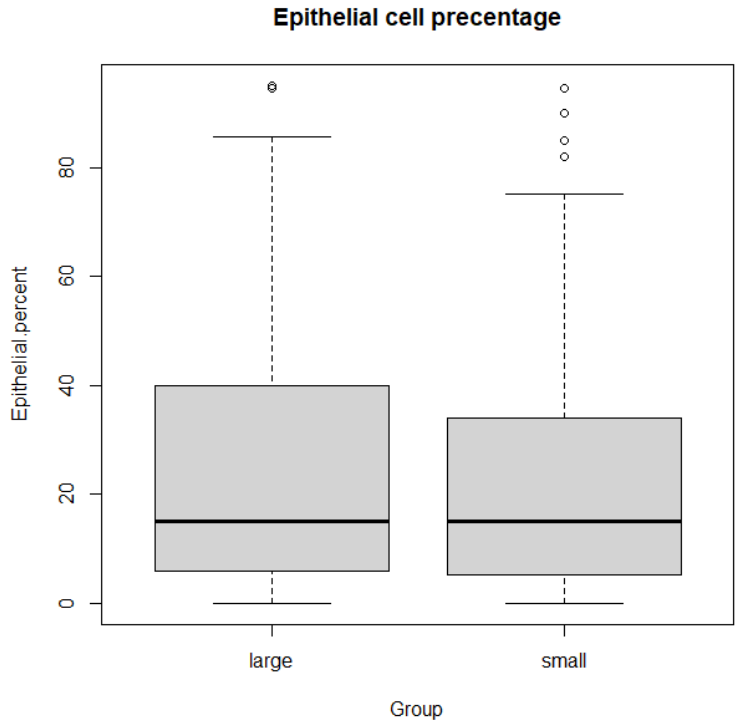

Figure 5: epithelial cell percentages in small and large breed dogs.

6.3.2 Infectious background

Altogether 227 aerobic and fungal cultures were performed. No cultures were performed in 9 cases. A positive result was seen in 47 cases, of which 2 were fungal, the rest bacterial. Sterile results were achieved in 180 cases. Microorganisms were seen in 43 cases and found absent in 193 cases on cytological examination. Sensitivity of cytology for the detection of microorganism was 39,5 %, specificity 87,1 % and diagnostic accuracy was 78,4 %. There were differences between the two cytopathologists, the largest of which was observed in sensitivity (cytopathologist 1: sensitivity 25 %, specificity 92 %, accuracy 81,2%, cytopathologist 2: sensitivity 61,1 %, specificity 80,3 %, accuracy 76,2 %);. Culture results are demonstrated in table 3.

Table 3: Culture Results

Culture Result		
Culture Result	Number of Samples	%
sterile	180	79,3
Pharyngeal flora	9	4,0
Bordetella bronchiseptica	8	3,5
Mycoplasma sp	5	2,2
Mixed colonising flora	5	2,2
Pasteurella multocida	4	1,8
Pseudomonas aeruginosa	4	1,8
Staphilococcus pseudointermedius	3	1,3
Streptococcus sp	3	1,3
Intestinal flora	2	0,9

Escherichia coli	2	0,9
Mixed Gram + flora	1	0,4
Aspergillus fumigatus	1	0,4
Klebsiella pneumoniae	1	0,4
Acinetobacter calcoaceticus	1	0,4
Malassezia pachydermatis	1	0,4
Enterobacter sp	1	0,4

Faecal examination by flotation and larva isolation was performed in 21 cases. Two cases of Angiostrongylus vasorum infestation were found, both of which associated with larvae observed on cytopathology. Other, non-respiratory parasites were occasionally found.

6.3.3 Neoplasia

Neoplastic causes were suspected on the cytopathological examination in 14 cases. 3 confirmed neoplastic cases (proven by aspiration of the lung or histopathology after surgical excision) were found. Additionally, 2 other cases were labelled as highly suspicious for malignancy but definitive diagnosis in these cases is lacking. Excluding these cases the sensitivity of cytopathology for neoplasia was 100 %; specificity is very low with a diagnostic accuracy of 18 %.

6.3.4 Effect of medication at the time of sampling

Concurrent medication administered at the time of sampling was characterised. Apart of medication taken for concurrent illnesses and antibiotics frequently administered agents were theophylline and prednisolone.

With theophylline treatment a significant difference was found in eosinophil cell ratios (men for treated dogs 0,64 %, mean for untreated dogs 4,4 %, p=0,0006) whereas other cell ratios

were unaffected. This was in tandem with a significant difference in sample cellularity (TNCC 0,46x10^9/L vs 1,38x10^9/L for treated and untreated dogs respectively, p=0,004).

In the case of systemic corticosteroid application a significant difference was found for cellularity (TNCC 0,56x10^9/L vs 1,41x10^9/L for the treated and untreated populations respectively, p=0,01) with significantly different eosinophil and neutrophil cell percentages (eosinophils 0,68 vs 4,48 % for treated vs untreated dogs respectively, p=0,0006 and neutrophils 20,4 vs 34,7 % for treated vs untreated dogs respectively, p=0,02).

7. Discussion

7.1 Patient population

The analysis is focused on the cytological profile and various respiratory diseases in dogs. Especially the total number of nucleated cells (TNCC) and different cytological properties of each disease was crucial for the analysis. These features were analyzed in the context of the dominant concurrent diseases and compared across the different disease groups to highlight their special pathophysiologic mechanisms.

Cases included for cytological comparisons were those where at least 50 nonepithelial cells were counted. This threshold was set to avoid mistakes due to the inherent uncertainty of microscopic cell counts (the lower the cell ratio, the higher the uncertainty of the cell count).

It is notable that epithelial cells are found in abundance in several samples. This feature is somewhat discordant with the literature, in which epithelial cells are usually found in lower proportion (<5%). Many of these samples contained high numbers of columnar epithelial cells indicating proximal airway contact. Possible explanations include epithelial cell hyperplasia due to inflammation or sampling methods yielding higher ratios of these cells. Samples with high epithelial cell ratios may not be comparable. An epithelial cell ratio of 10 % was set in this study. The reason for the higher epithelial cell ratio then the published data (5%) was that a higher number of cases could be analyzed. Only 89 of the 236 cases had lower than 10 % epithelial cells. Statistical probes were conducted amongst these samples to see whether there are differences; no such differences were found.

7.1.1 Chronic Bronchitis

77 cases with the clinical diagnosis of chronic bronchitis were found. In 9 of these cases bacteria were cultured from the BALF, cultures from the other cases were sterile. Statistically significant differences were only found for eosinophil granulocyte percentages (lower in septic cases, mean nonseptic 3,24 %, mean septic 0,28 %, p=0,03). The most frequent comorbidity apart of respiratory disorders such as bronchomalacia was cardiac diseases (25 cases). Adequate (>50 nonepithelial cells counted) differential cell counts were found in 51 cases. In cases where less than 10 % epithelial cells were present lymphocytes were seen in higher proportion at the expense of other cell types; however, this difference was statistically not significant. Cell composition of nonseptic chronic bronchitis cases are illustrated in figure 6. Cell counts are summarised in table 4.

Table 4: Cell counts for cases of chronic nonseptic bronchitis. Nonepithelial cell % is derived excluding epithelial cells from the calculation.

	Epithelial cell %	**Macrophages %**	**Neutrophils %**	**Eosinophils %**	**Lymphocytes %**	**Mast cells %**	**Other %**
min	0,0	2,6	0,0	0,0	0,0	0,0	0,0
max	50,8	100,0	89,6	53,0	38,8	0,2	43,9
mean	16,5	60,2	26,6	3,0	8,4	0,0	0,9
SD	13,8	28,4	24,7	9,2	9,6	0,0	5,8

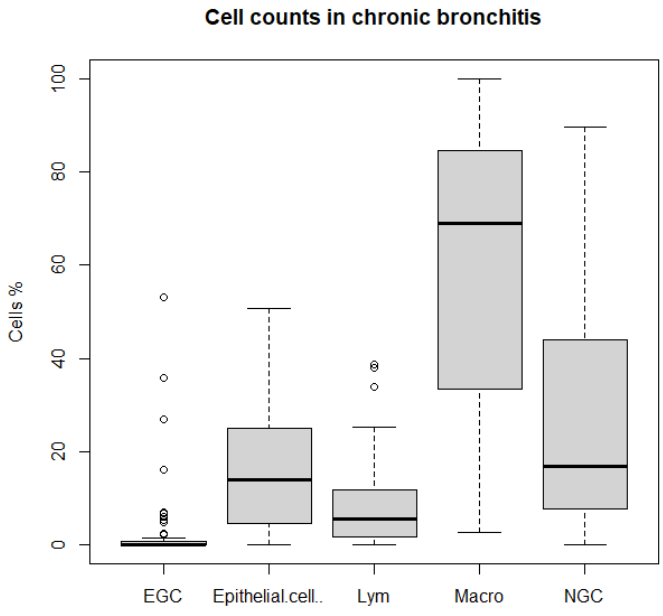

Figure 6. Cell ranges for chronic bronchitis cases.

Breeds Commonly Affected: Poodles, Cocker Spaniels, and Terriers.

Why These Breeds Are Affected: These breeds are frequently affected due to their genetic predisposition to airway hypersensitivity and chronic inflammation. Their smaller body size and airway structure can lead to increased susceptibility to irritants and allergens that trigger chronic bronchitis.

Prominent Cytological Features:

Neutrophils are indicative of chronic inflammation, suggesting ongoing irritation and immune response. Compared to mucopurulent bronchopneumonia, the neutrophil count is lower, reflecting a less acute but persistent inflammatory process.

The presence of epithelial cells indicates hyperplasia due to chronic irritation. This is more pronounced than in serous bronchitis, where fluid predominates over cellular changes.

Macrophages are involved in phagocytosis and tissue repair, highlighting the chronic nature of the disease. This is like catarrhal bronchitis, where macrophages also play a significant role in managing mucus and debris.

TNCC Changes: Elevated TNCC (up to 20,000 cells/µL or higher) reflects sustained immune cell recruitment, distinguishing it from serous bronchitis, which has a lower TNCC due to less cellular infiltration

Connection with Concurrent Illnesses:

Tracheal Collapse: This condition exacerbates airway obstruction and inflammation. The mechanical stress from tracheal collapse can cause further irritation and mucus production, complicating chronic bronchitis management. The collapse leads to increased turbulence and pressure changes in the airways, promoting chronic irritation and inflammation.

Obesity: Excess weight can exacerbate respiratory issues by increasing the workload on the respiratory system, leading to more severe symptoms. Obesity can also contribute to systemic inflammation, further complicating chronic bronchitis

Therapeutic Approach: Anti-inflammatory medications, bronchodilators, and lifestyle modifications to minimize airway stress are crucial strategies. Weight management is also essential to reduce respiratory strain.

Interestingly the only significant difference between cases with positive culture result vs sterile samples was a slightly lower eosinophil percentage in the culture positive group.

However, eosinophil percentages in both cases are comparable to the statistical uncertainty of cell counts in this case and therefore this finding may not be relevant in clinical scenarios.

7.1.2 Serous Bronchitis

11 cases with the clinical diagnosis of serous bronchitis were identified. All contained adequate cells for differential cell counts. Epithelial cell ratios were occasionally higher than 10 %. Infectious agents were not cultured from these samples. Differential cell counts are represented in table 5 and figure 7.

Table 5: Cell counts for cases of serous bronchitis. Nonepithelial cell % is derived excluding epithelial cells from the calculation.

	Epithelial cells %	**Macrophages %**	**Neutrophils %**	**Eosinophils %**	**Lymphocytes %**	**Mast cells %**	**Other cells %**
min	2,0	7,2	0,0	0,0	0,0	0,0	0,0
max	40,0	100,0	92,8	7,7	42,7	0,0	0,0
mean	15,2	62,6	26,0	1,1	10,3	0,0	0,0
SD	10,9	31,0	30,3	2,4	16,0	0,0	0,0

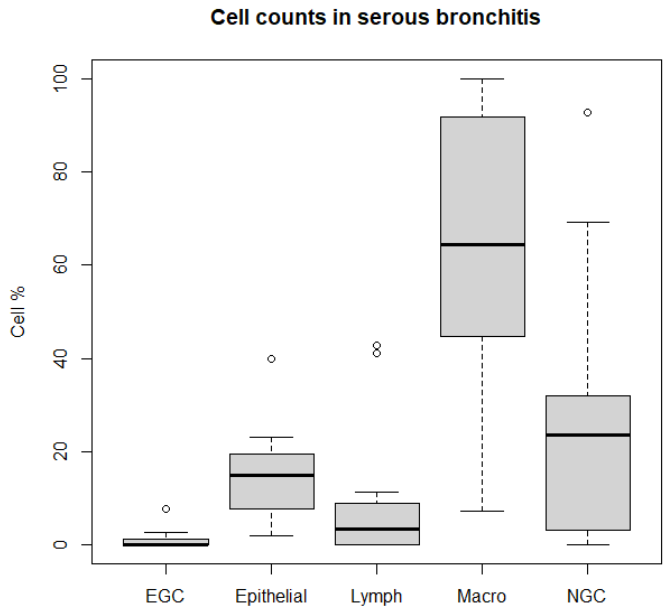

Figure 7: Differential cell counts of serous bronchitis cases.

Breeds Commonly Affected: Yorkshire Terriers and Chihuahuas

Why These Breeds Are Affected: These small breeds are particularly susceptible to serous bronchitis due to their sensitive airways and tendency for allergic reactions. Their compact anatomy can amplify the effects of allergens and irritants, leading to increased mucus production. Also, the Tracheal collapse can lead to increased airway resistance and chronic coughing, which irritates the airways and promotes inflammation. Furthermore, it will lead to increased mucus production and fluid accumulation, characteristic of serous bronchitis.

Prominent Cytological Features:

Epithelial Cells: These cells indicate glandular activity and fluid production, which is more prominent than in chronic bronchitis, where cellular infiltration is higher.

Eosinophils: Eosinophils suggest an allergic component, which is less common in mucopurulent bronchopneumonia, where neutrophils dominate.

TNCC Changes: Moderately increased TNCC (10,000-15,000 cells/μL) due to fluid presence rather than cellular infiltration, contrasting with the high TNCC in mucopurulent bronchopneumonia

Connection with Concurrent Illnesses:

Atopic Dermatitis: Often coexists with serous bronchitis, reflecting a systemic hypersensitivity that can exacerbate respiratory symptoms. The skin and respiratory tract share similar immune responses, and allergens affecting the skin can also trigger respiratory symptoms.

Asthma: Allergic reactions can trigger asthma attacks, leading to increased mucus production and airway constriction. Asthma can exacerbate serous bronchitis by increasing airway reactivity and inflammation

Therapeutic Approach: Involves managing environmental allergens, antihistamines, and possibly corticosteroids to reduce secretion. Asthma management may include bronchodilators and inhaled corticosteroids.

7.1.3 Mucopurulent/Purulent Bronchopneumonia

8 cases were identified. One case was contained very high proportion of epithelial cells but adequate numbers of nonepithelial cells were counted to allow inclusion for descriptive

statistics. Pathogens were cultured from 5 of the 8 cases. A notable feature in these cases is the lack of eosinophil cells. Differential cell counts are shown in table 6 and figure 8.

Table 6: Cell counts for cases of purulent airway disorders. Nonepithelial cell % is derived excluding epithelial cells from the calculation.

	Epithelial cells %	Macrophages %	Neutrophils %	Eosinophils %	Lymphocytes %	Mast cells %	Other cells %
min	8	0	6,3	0	0	0	0
max	76,3	82,1	96,4	0	11,6	0	10,9
mean	29,64286	28,72857	64,54286	0	3,885714	0	2,857143
SD	27,54737	33,1884	36,34153	0	4,339585	0	5,195254

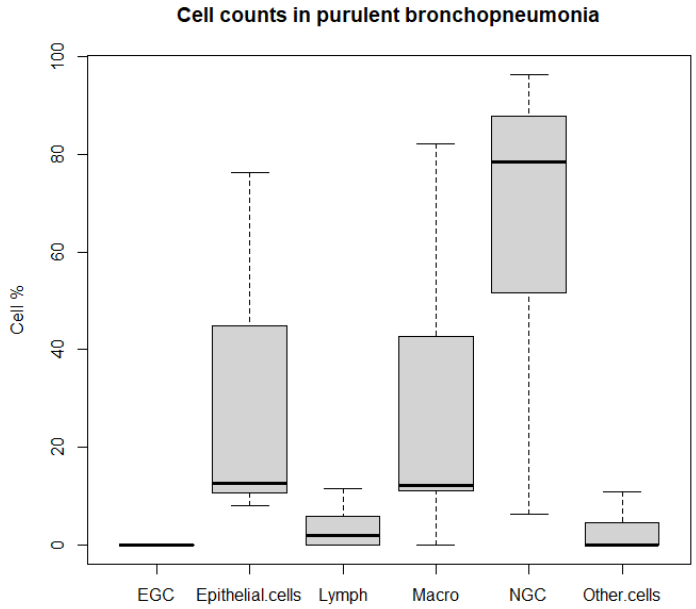

Figure 8: Differential cell counts in purulent airway disorders

Breeds Commonly Affected: Bulldogs and Boxers.

Why These Breeds Are Affected: These brachycephalic breeds are particularly susceptible to mucopurulent bronchopneumonia due to their anatomical features that can lead to airway obstructions. Their short noses and narrow airways favor the accumulation of secretions and the development of infections.

Prominent Cytological Features:

Neutrophils: Range: 50-85%; Mean: ~75%. High neutrophil counts indicate acute inflammation and infection, distinguishing it from chronic bronchitis, where neutrophil levels are lower.

Macrophages: Range: 10-25%; Mean: ~18%. Macrophages are involved in debris clearance, like catarrhal bronchitis but in a more acute setting.

TNCC Changes: Drastically increased TNCC (often >25,000 cells/µL), reflecting significant infiltration due to acute infection, unlike the moderate TNCC in catarrhal bronchitis

Connection with Concurrent Illnesses:

Viral Infections: Such as canine influenza or parainfluenza can predispose dogs to secondary bacterial infections, worsening bronchopneumonia. Viral infections can damage the respiratory epithelium, making it easier for bacteria to colonize and cause infection.

Immunosuppression: Conditions like Cushing's disease or prolonged steroid use can impair immune response, increasing susceptibility to infections. Immunosuppression reduces the body's ability to fight off infections, leading to more severe and prolonged disease

Therapeutic Approach: Administering broad-spectrum antibiotics alongside supportive care like IV fluids is essential. Addressing underlying immunosuppressive conditions is also crucial.

7.1.4 Catarrhal Bronchitis

12 cases were identified. An interesting feature here is a generally high observed epithelial cell proportion, possibly reflecting epithelial cell hyperplasia. Differential cell count results are presented in table 7 and figure 9.

Table 7: Cell counts for cases of catarrhal airway disorders. Nonepithelial cell % is derived excluding epithelial cells from the calculation.

	Epithelial cells %	Macrophages %	Neutrophils %	Eosinophils %	Lymphocytes %	Mast cells %	Other cells %
min	1,4	2,2	0,0	0,0	0,0	0,0	0,0
max	75,5	81,8	97,1	30,6	34,4	0,0	0,0
mean	26,5	53,6	33,8	4,8	7,9	0,0	0,0
SD	23,2	29,7	28,9	9,5	9,0	0,0	0,0

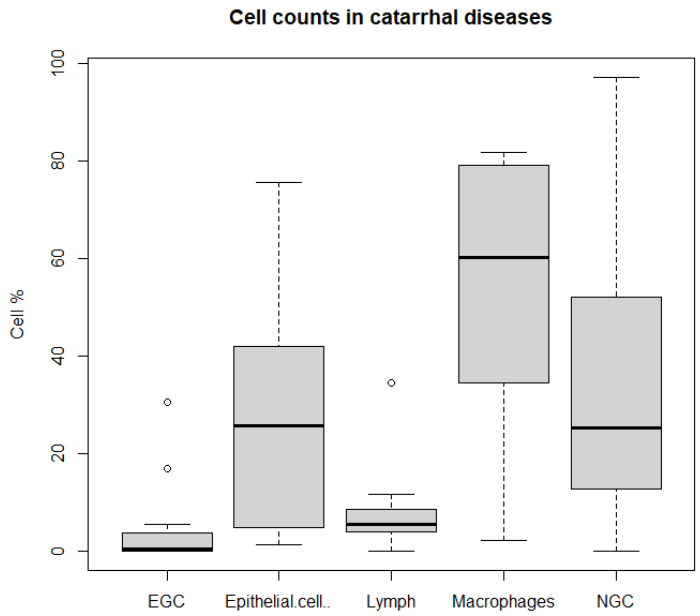

Figure 9: Differential cell counts in catarrhal airway disorders

Breeds Commonly Affected: Dachshunds and Basset Hounds.

Why These Breeds Are Affected: These breeds are particularly susceptible to catarrhal bronchitis due to their anatomical features that can lead to increased mucus production. Their long noses and narrow airways favor the accumulation of secretions and the development of inflammation.

Prominent Cytological Features:

Mucus: Range: 30-60%; Mean: ~45%. High mucus content is a defining feature, more so than in chronic bronchitis, where cellular infiltration is more prominent.

Epithelial Cells: Range: 5-15%; Mean: ~10%. Indicates mucosal turnover, like chronic bronchitis but with more mucus involvement.

Lymphocytes: Range: 5-20%; Mean: ~12%. Lymphocytes indicate chronic inflammation, less acute than in mucopurulent bronchopneumonia.

TNCC Changes: Moderate TNCC elevation (10,000-18,000 cells/μL), indicating mucus-related cellular presence, unlike the high TNCC in mucopurulent bronchopneumonia

Connection with Concurrent Illnesses:

Chronic Sinusitis: Can exacerbate catarrhal discharge, leading to increased mucus production and airway obstruction. Sinusitis can cause post-nasal drip, contributing to mucus accumulation in the airways.

Allergic Rhinitis: Allergies can trigger excessive mucus production, complicating catarrhal bronchitis. Allergic rhinitis can cause inflammation and mucus production in the nasal passages, which can extend to the lower airways

Therapeutic Approach: Use of expectorants and anti-inflammatories to alleviate mucus buildup. Managing underlying sinusitis or allergies is also important.

7.1.5 Bronchomalacia

55 cases were identified. Differential cell counts are provided in table 8 and figure 10.

Table 8: Cell counts for cases of bronchomalacia. Nonepithelial cell % is derived excluding epithelial cells from the calculation.

	Epithelial cell %	Macrophages %	Neutrophils %	Eosinophils %	Lymphocytes %	Mast cells %	Other cells %
min	0,0	1,8	0,0	0,0	0,0	0,0	0,0
max	67,6	100,0	95,8	16,0	42,1	52,9	3,0
mean	15,9	60,7	29,1	0,8	8,3	1,0	0,1
SD	14,7	29,7	29,5	2,5	10,0	7,1	0,5

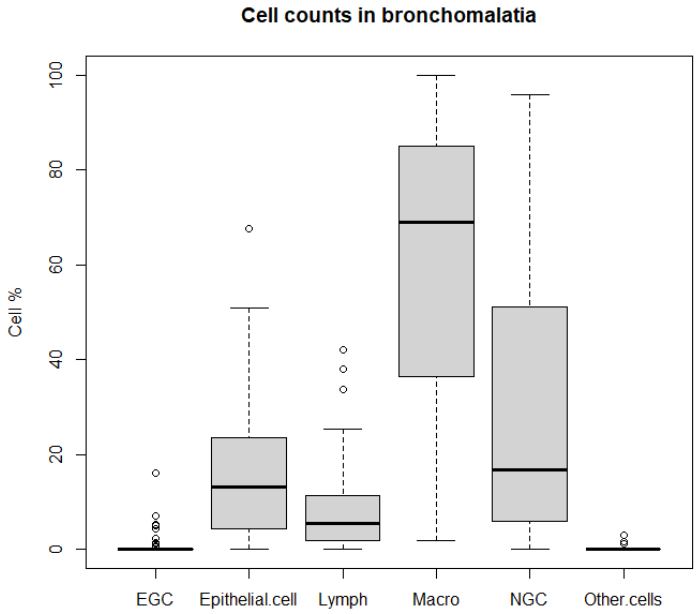

Figure 10: Differential cell counts in bronchomalacia.

Breeds commonly affected: Yorkshire Terriers are frequently affected by bronchomalacia. This breed, along with others like the Bichon Bolognese and West Highland White Terrier, are often predisposed due to their anatomical and genetic predispositions which may include structural airway weaknesses

Prominent cytological features:

Columnar Epithelial Cells: These cells are often present in high percentages, indicating epithelial hyperplasia and chronic irritation. For instance, in one case, columnar epithelial cells constituted 80% of the cell population.

Cuboidal Epithelial Cells: These cells are less frequently observed but can be present in smaller percentages, such as 5% in some samples.

Macrophages: A significant presence of macrophages is noted, often comprising a substantial portion of the cell population, such as 15% in some cases. This indicates an ongoing inflammatory response as macrophages are involved in phagocytosis and tissue repair.

Neutrophil Granulocytes (NGC): These cells can also be present, indicating an acute inflammatory component. For example, in one sample, NGCs made up 5% of the cells.

Lymphocytes and Other Cells: Occasionally, lymphocytes and other cell types like mast cells may be present, reflecting a mixed inflammatory response.

Connection with concurrent illnesses:

Bronchomalacia is often associated with chronic bronchitis and can be linked with cardiac conditions such as mitral endocarditis. These concurrent diseases suggest a multifactorial aetiology where respiratory and cardiac problems may exacerbate each other.

Therapeutic approach: Treatment typically involves bronchodilators and anti-inflammatory medications. Aminophylline and prednisolone are commonly used to manage symptoms by reducing inflammation and improving airway patency.

7.2 Differences amongst small/large dogs

A cutoff of 5 kg was selected because this is defined as a standard threshold for endoscope diameter change. It appears that samples from large breed dogs yield samples with higher cellularity and therefore the diagnostic yield may be increased due to less samples with low cell counts. At the same time a significant difference in epithelial cell count was not observed. However, considering the diverse range of diseases for which BAL sampling was performed these results should be interpreted with caution.

7.3 Infectious background

It appears that cytology shows an acceptable concordance with culture results. Sensitivity of cytology for the presence of infectious agents is relatively low but specificity is high compared to culture results. Interobserver differences were seen in sensitivity; this may be due to different sample preferences between the two investigators. A lower sensitivity found in the case of one of the cytopathologists can be explained by his preference of direct smears where the material is spread out on a larger surface. Cytospin preparations may be better suited for the detection of bacteria. However proper estimation of interobserver differences would have required simultaneous sample analysis which hasn’t been performed in this case and therefore the results should be interpreted with this knowledge.

Mycoplasma was removed from sensitivity/specificity and accuracy calculations due to low observability on cytology preparations.

Bacterial background was associated with an increased TNCC (mean 1,67x10^9/L, range 0-12,7, SD 2,75) as well as neutrophil cell percentage. Characteristic results are presented in table 9 and figure 11.

Table 9: Cell counts for cases with a positive bacterial culture. Nonepithelial cell % is derived excluding epithelial cells from the calculation.

	Epithelial cell %	Macrophages %	Neutrophil granulocytes %	Eosinophil granulocytes %	Lymphocytes %	Mast cells %	Other cells %
min	0,0	2,1	0,0	0,0	0,0	0,0	0,0
max	80,0	100,0	97,1	10,5	45,3	1,5	3,9
mean	21,2	44,3	45,6	1,2	8,6	0,0	0,2
SD	22,4	33,3	38,2	2,4	11,9	0,2	0,8

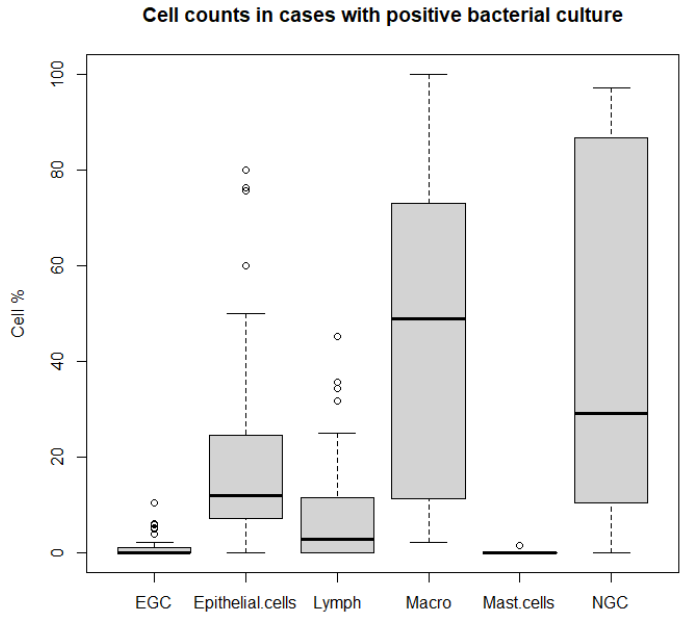

Figure 11: Differential cell counts in cases with a positive bacterial culture.

20 patients received antibiotic treatment at the time of sampling; 7 had positive culture result and 3 had positive cytology; it is possible that the antibiotics applied may have eradicated many bacteria and therefore they weren't observed on the smears.

Of note, only 1 of the 8 cases of Bordetella bronchiseptica infection was caught by cytopathology. This is in contrast with the available literature where cytopathology is noted as a sensitive method for Bordetella bronchiseptica infection. (13)

Improvements of the detection could be possibly achieved by counting more cells thereby increasing sensitivity; improved specificity can be possibly achieved by rigorous sample handling technique. Oropharyngeal contamination does not seem to play a role as eliminating samples with cytological indication of oropharyngeal contamination did not lead to major improvement in diagnostic accuracy (78,4 % for samples including oropharyngeal contamination, 79,4 % for samples excluding oropharyngeal contamination).(8)

Only two cases of positive fungal culture were found. In one of these Malasseza sp. was observed which probably represents contamination. Aspergillosis was found with culture in one case where fungal elements hasn't been observed on the smears. This finding is consistent with some of the available literature. (18) In this case a higher eosinophil cell percentage (18,9 %) was observed which is occasionally found in dogs with pulmonary aspergillosis. A human disease, allergic bronchopulmonary aspergillosis shares the cytological characteristics of this case but its existence in dogs is not proven.

Two cases of respiratory parasitosis were found, both of which were confirmed either with parasitological or cytopathological methods, indicating that cytopathology is an asset in the detection of pulmonary parasitosis.

7.4 Neoplasia

Although the sensitivity of cytopathology for neoplasia in the present study was excellent, the specificity was virtually zero and the diagnostic accuracy was poor. One possible explanation for this feature may be the high proportion of epithelial cells in the samples and a high proportion of distorted cells in the samples. Hyperplastic epithelial cells in inflammatory disorders share cytologic features with low grade malignancies which may explain the results found in the present study. The cytopathologic diagnosis of neoplasia should be interpreted with caution and other diagnostic methods such as imaging and histopathology should be used to confirm the diagnosis.

7.5 Effect of medication at the time of sampling

Some differences were found in total nucleated cell counts and cell percentages under the influence of concurrent theophylline and corticosteroid treatments. In general cell counts were decreased if treatments were administered at the time of sampling. A potential

explanation is offered by the anti-inflammatory effect of corticosteroids. In the case of theophylline an indirect anti-inflammatory effect may be suspected due to its broncho dilating action. The effect of these substances on the differential cell percentages may confound the interpretation of the BALF analyses and should be considered. Notably eosinophils were decreased in both cases which may influence the interpretation of BALF samples from patients suffering from diseases which otherwise induce an eosinophilic infiltration (such as eosinophilic bronchopneumopathy, parasitosis or hypersensitivity disorders). A decreased eosinophilic inflammatory response was described in humans undergoing theophylline treatment. (19) To the author's knowledge such a change has not been described in veterinary medicine.

8. Conclusion

In the present study a retrospective analysis of canine BALF samples from dogs undergoing endoscopy in the Veterinary Teaching Hospital of UVMB was presented, with a highlight on select conditions. The insight gained may help to characterise various diseases where BALF analysis may contribute to the diagnosis.

Observations of importance include the high ratio of epithelial cells in the obtained samples; differences in cell counts amongst small and large dogs; and insights into the diagnostic accuracy of cytological analyses as well es changes in cell counts observed under the effect of the bronchodilator theophylline and anti-inflammatory systemic corticosteroids.

Some recommendations can be made especially regarding the cytological analysis. It appears that a concentrated smear offers a higher sensitivity for the detection of bacterial colonisation/infection of the respiratory tract with a minimal decrease in specificity and diagnostic accuracy which may be advantageous. The cytopathologic diagnosis of neoplasia should be interpreted with caution.

9. Abstract

This thesis describes the application and optimization of bronchoalveolar lavage (BAL) in dogs, especially for diagnosing and management of airway diseases. The BAL technique enables the collection of samples from the deep airways which leads to a better analysis of the epithelia lining fluid (ELF). The fluids contain various amounts of cellular and non-cellular components, which provides us with valuable information about the health status of the airways.

The central goal of this thesis is the provide insight into cytopathological characteristics of BALF samples to ensure a better comparability of the different results and consequently a better diagnosis and treatment of airway related diseases.

The cellular and non-cellular components of the BAL-fluid were examined to identify possible biomarker for various airway related diseases in dogs.

Important findings include an acceptable diagnostic accuracy of cytopathological analysis for infectious diseases with high specificity and variable sensitivity amongst techniques (concentrated vs direct smears), a low diagnostic accuracy of cytology for neoplastic diseases and differences in BALF cell counts obtained from small vs large breed dogs. A decreased TNCC and decreased eosinophil cell percentages were observed in dogs undergoing theophylline or systemic corticosteroid treatment at the time of sampling. Decreased neutrophil percentage was observed in the case ofsystemic corticosteroid treatment.

In general, it can be recommended to investigate the observed high epithelial cell counts in samples because these may interfere with the interpretation and comparability of the results. Yet another recommendation can be to increase the nonepithelial cells counted on cytopathologic specimens to ensure the high reproducibility of the results obtained. Concentrated cytopathologic preparations can be recommended for the detection of bacteria in the samples.

10. Absztrakt

Jelen szakdolgozatban a BAL mintavétel körülményeinek és optimalizálási lehetőségeinek leírását tartalmazza, légúti megbetegedések vizsgálatának céljából. A BALF mintavétel segítségével légúti minták nyerhetőek, melyek elősegítik az ELF vizsgálatát. A minták sejtes és egyéb összetevőket tartalmaznak, melyek értékes információt szolgáltathatnak a légutak állapotáról.

A szakdolgozatban elsősorban a BALF minták citopatológiai elemzésére került abban a reményben, hogy a minták eredményeinek összehasonlíthatósága növekszik. Ez job diagnosztikai és terápiás kimenetelhez vezethet.

A BALF minták sejtes és egyéb össztevőinek elemzésére került sor, melyek között potenciális biomarkerek találhatóak.

A legfontosabb feltárt információk között említhető a citopatológiai vizsgálatok elfogadható pontossága (magas sepcificitással és változós szenzitivitással attól függően, hogy koncentrált vagy direct kenet vizsgálatára kerül sor); a citopatológiai vizsgálatok alacsony diagnosztikai pontossága a daganatos megbetegedések detekciójában; illetve a minták cellularitásának változása a kistestű és nagytestű egyedek esetében. Csökkent magvas sejtszám mellett csökkent eosinophil arány volt detektálható azon egyedek minátiban, melyek a mintavétel idején teofillin vagy szisztémás kortikoszteroid kezelésben részesültek. A szisztémás kortikoszteroid kezelés mellett csökkent neutrophil arány is észlelhető volt.

Javasolható a dolgozatben talált magas epithelialis sejtszám okának feltárása, ez ugyanis befolyásolhtaja a citopatológiai kép értelmezését és az irodalmi adatokkal való összehasonlíthatóságát. E mellett ajánlható a mintákban található egyéb sejtek számolásakor magasabb számú sejt differenciálása, mely az eredmények reprodukálhatóságának növeléséhez vezethet. A kórokozók detekciójára alkalmasabbnak tűnnek a koncentrált citopatológiai minták.

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