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Interventional Endoscopy: Diagnostic and Therapeutic Applications

TITOLO IN ITALIANO

Endoscopia interventistica: applicazioni diagnostiche e terapeutiche

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1. Introduction

Endoscopy is a medical procedure that involves inserting a rigid optics or a long, flexible tube equipped with a light and camera (an endoscope) into the animal body to examine internal cavities of the organism. This minimally invasive technique allows diagnostic interventions across various organ, including gastrointestinal tract, airway, and urinary tract (Tams, 2010). The diagnostic potential of endoscopy is rooted in its ability to provide direct visualization of internal organs and mucosal surfaces, and to facilitate the collection of tissue samples. This can be subjected to cytological and histopathological examination, allowing for a definitive diagnosis of various conditions, including inflammatory diseases and neoplasms. In addition, biological samples collected during endoscopy can be subjected to microbiological tests, such as bacteriological and mycological cultures, to identify disease-causing agents. This is particularly valuable in diagnosing infectious diseases, where the identification of specific pathogens can guide targeted antimicrobial or antifungal therapy (Tams, 2010). Consequently, the therapeutic potential of endoscopy has been evident since its inception. The combination of real-time observation and tissue sampling makes endoscopy a powerful tool in both the diagnosis and management of many pathological conditions. The association of an advanced optical system with ancillary tools, either inserted through the working channel of the endoscope or used co-axially alongside it, offering a less traumatic alternative to traditional tissue sampling and surgical procedures. Interventional endoscopy represents a form of minimally invasive surgery that reduces recovery time and discomfort for the patient while also decreasing the risk of complications associated with more invasive surgeries (De Lorenzi, 2012).

The initial applications of interventional endoscopy in veterinary medicine were focused on the sampling of biological material and the retrieval of foreign bodies from various regions of the body. Today, the use and applications of these techniques have evolved significantly, offering more advanced diagnostic and therapeutic possibilities. In the gastrointestinal tract, it is possible to perform dilation of strictures under endoscopic visualization using rigid dilators inserted coaxially at the instrument or balloon dilators inserted through the working channel of the endoscope. Stenoses are more common in the esophagus and are frequently secondary to traumatic injury (e.g., foreign bodies, gastroesophageal reflux) to the wall, leading to ingrowth of fibrotic tissue. Annular stenoses can also be localized in the anorectal region, where they typically have a congenital origin. Dilatation of fibrotic stenoses can similarly be applied in the airway system, typically in the nasopharynx but also in the trachea, as well as in the urinary tract when the stricture is located in the urethra. Another application of interventional endoscopy in the gastrointestinal tract is the removal of neoplasms (benign or malignant) with pedunculated bases using diathermic snares, a technique known as polypectomy. This procedure is commonly employed for lesions located in the stomach or colon. Malignant neoplasms located in various parts of the gastrointestinal tract (e.g., esophagus, stomach, duodenum, and colon) can also be partially removed through palliative excision using diode or argon lasers in an endoscopic-assisted procedure. These lasers are employed to ablate or reduce tumor masses that cannot be completely resected, alleviating symptoms such as obstruction or bleeding. Finally, endoscopy can be used for the placement of feeding tubes in animals requiring long-term nutritional support, offering a minimally invasive solution for maintaining proper nutrition. Many applications of interventional endoscopy in the respiratory and urinary systems mirror those in the gastrointestinal tract, including the removal of foreign bodies, dilation of stenosis, and excision of neoplasms. However, another use of interventional endoscopy is stent placement, which, although possible in the gastrointestinal and urinary tracts, is more commonly utilized in the airway lumen, particularly in the trachea. This procedure helps maintain airway patency in cases of tracheal collapse, stenosis, or tumors, facilitating better airflow and reducing respiratory distress. Moreover, numerous laser-assisted and endoscopic surgical procedures can be performed on dogs and cats affected by brachycephalic syndrome. Finally, the application of interventional endoscopy in the urinary tract includes also the removal or destruction of uroliths using laser lithotripsy and the treatment of ectopic ureters, particularly when they follow an intramural course (Bottero, 2022).

The objective of this thesis is to explore in depth several endoscopic-guided procedures in airway system, providing detailed information on their clinical indications, surgical instruments and techniques, along with an analysis of their associated complications and criticisms. In particular, we propose to study the following techniques: endoscopic-guided sampling methods for biological material in the lower airway tract, stent placement for tracheal collapse in dogs, and diode-laser epiglottidectomy in dogs.

1. A prospective comparison of fiberoptic endobronchial needle aspiration, bronchial brushing and forceps biopsy for the diagnosis of broncoscopically visible canine lower airways masses

Abstract

Objectives: To compare the diagnostic yield of Endobronchial Wang Needle Aspiration (EBNA) with those of Bronchial Brushing (BB) and Forceps Biopsy (FB) in canine tracheal and endobronchial masses or submucosal infiltrations examined by fiberoptic bronchoscopy.

Materials and Methods: Flexible fiberoptic bronchoscope-guided BB, FB, and EBNA were performed consecutively in dogs with airway exophytic mass or submucosal infiltrations. The three techniques were compared for their diagnostic sensitivity, specificity, positive predictive value, negative predictive value, accuracy, and 95% confidence intervals (CI).

Results: Twenty-one dogs were included in the study. EBNA confirmed the malignancy identified by histopathological diagnosis in 90.48% of cases, FB in 80.95%, and BB in 52.38%. Agreement in the final morphologic tumor type was found in 19 of 21 cases (90.48%) by EBNA, in 15 of 21 cases (71.43%) by FB, and in 8 of 21 cases (38.10%) by BB. The technique with the highest sensitivity and accuracy (0.94 and 0.90; IC 95% + 0.99/-0.89) was EBNA, whether used alone or combined with other methods.

Clinical significance: EBNA should be used alone or in combination with other techniques for routine bronchoscopy to obtain the highest diagnostic yield.

Keywords: endobronchial submucosal lesion, exophytic mass lesion, endobronchial Wang needle aspiration, endoscopy, dogs

1.1. Introduction

Primary lung and bronchial tumors are infrequent in companion animals compared to their high incidence in humans. The occurrence is approximately 0.5% in dogs and cats but has increased at least two-fold in recent years (Moulton, 1981). In contrast, metastatic lung tumors are more common in small animals (Sharkey, 2020). Although rare, airway masses in dogs may originate from inflammatory, infective, and parasitic causes (Hill, 2008; De Lorenzi, 2009; Kliewer, 2023; Papaioannou, 2004). Lower airway masses are usually suspected based on an abnormal radiographic imaging study, often in conjunction with symptoms caused by the tumor's local or systemic effects. Historically, clinicians have used a combination of established techniques, such as forceps bronchial biopsy, bronchial brushing, bronchial washing, and trans-thoracic fine-needle aspiration (FNA) cytology or biopsy to diagnose airways and lung malignancy (Penninc, 2008; McMillian, 1988; Wood, 1998; Zekas, 2005; Teske, 1991; Roundebush, 1981). Transthoracic ultrasound, computed tomography (CT), and recently fluoroscopic-guided FNA are some of the most common techniques used to sample pulmonary lesions in veterinary medicine (Jacob, 2023). FNA is a minimally invasive technique associated with a lower rate of complications, but sampling is often limited to peripheral pulmonary lesions (Jacob, 2023).

The modality selected to diagnose a suspected lower airway mass is based on the size and location of the primary tumor in the lung. The main goals in selecting a specific diagnostic modality are (1) to maximize the yield of the selected procedure for both diagnosis and staging and (2) to avoid unnecessary invasive and dangerous tests for the patient. Although not routinely performed in the presence of endotracheal and endobronchial tumors in veterinary medicine, endoscopic-guided fine-needle aspiration is considered effective in diagnosing mucosal exophytic and submucosal (erythema, loss of bronchial markings, or thickening of the mucosa) lesions in human medicine (Horsley, 1884; Lundgren, 1983).

In a prospective study, we compared three diagnostic techniques to obtain specimens with the flexible fiberoptic bronchoscope in lower airway masses. This study aimed to compare the diagnostic yield and complications of Endobronchial Wang Needle Aspiration (EBNA) with those of Bronchial Brushing (BB) and Forceps Biopsy (FB) in canine tracheal and endobronchial masses (EBMs) or submucosal infiltrations (SI) examined by fiberoptic bronchoscopy, with particular reference to making: 1) a distinction between benign and malignant growths and 2) a correct cytologic diagnosis.

1.2.Materials and Methods

1.2.1. Animals

All dogs with a radiographic and/or tomographic diagnosis of tracheal or bronchopulmonary mass in the period January 2023 – December 2023 at the Anicura Hospital I Portoni Rossi of Zola Predosa (Bologna, Italy) were eligible for the study. Inclusion in the study required that dogs had a visible endotracheal or endobronchial abnormality during bronchoscopy. These abnormalities included exophytic masses (EM) or submucosal infiltrations (SI), defined as thickening or loss of mucosal marking with bronchial narrowing. We did not include cases with normal mucosa but apparent narrowing of airways due to extrinsic or extramural compression. We studied only those cases in which EBNA, BB e FB, and a definitive histopathological diagnosis from a surgical specimen or necroscopy had all been performed. Age, breed, sex, neutering status, and endoscopic findings were recorded before entry into the study.

All dog owners gave informed consent; they were informed that a combination of sampling techniques, including needle aspiration, brushing, and forceps biopsy, would be used.

1.2.2. Intervention

All bronchoscopies were performed by experienced operators (DDL and DB), and all cytological samples were interpreted by a board-certified clinical pathologist (DDL).

The bronchoscopic examination was performed in a standardized fashion with a flexible fiberoptic bronchoscope (Fibroscope 60003VB, KARL STORZ, Tuttlingen, Germany) under general anesthesia in all dogs: premedication with 2 μg/kg dexmedetomidine and 0.2 mg/kg methadone intramuscularly, induction with 4 mg/kg of propofol administered intravenously. A 5-minute preoxygenation period was used. A stable plane of anesthesia was maintained with a constant rate infusion of propofol at 0.1–0.4 mg/kg/min or intermittent boluses. Oxygen was delivered through the bronchoscope's working channel or by use of jet ventilation. Electrocardiogram (ECG), blood pressure, and pulse oximetry were constantly monitored. During recovery, supplemental oxygen was provided as needed. The sequence of samples was BB, EBNA, and FB.

Bronchial Brush (BB) cytology was taken using a sheathed disposable cytology brush (Code n. 9939, Aorta S.r.l., Milano, Italy). After each brushing, the brush was sheathed and then withdrawn from the bronchoscope. Brushing was smeared by gently rolling the brush on a glass slide. The procedure was then repeated as we aimed to take two separate brush specimens from each lesion. This system was used to minimize the number of cases in which the brush samples were inadequate for cytological interpretation. The slides were air-dried and stained with May-Grünwald-Giemsa (MGG) in an automatic slide stainer (Aerospray Slide Stainer 7000, WESCOR, Logan, UT, USA).

All Endobronchial Wang Needle Biopsies (EBNA) were obtained with a 22-gauge transbronchial needle catheter (WANG® Transbronchial Cytology Needle, CONMED Corporation, Utica, NY, USA). The Wang needle has an outer flexible plastic catheter, a distal 15 mm in length retractable sharp beveled needle, a middle flexible catheter, a stylet, a proximal control device that manipulates the movement of the needle, and a side port through which suction can be applied. The outer plastic catheter has a metallic hub at the distal end to protect the endoscope's working channel from the biopsy needle accidentally perforating. The middle flexible catheter transmits a negative pressure from the proximal end to the distal needle while the stylet provides rigidity to the needle for successful insertion through hard masses; it prevents clogging of the needle with bronchial epithelial cells with submucosal masses. Due to the nature of the procedure, both catheters are flexible enough to be maneuvered into more peripheral locations yet stiff enough to exert force to penetrate both soft and hard masses. A specimen was obtained by using a Wang needle inserted into the fibroscope working channel. To prevent damage to the working channel of the fibroscope by the needle, the fibroscope was kept as straight as possible, with the distal tip in the neutral position during catheter insertion. The beveled end of the needle was secured within the metal hub during its passage through the working channel and advanced and locked into place only after the metallic hub was visible beyond the tip of the fibroscope. Once the tip of the needle had penetrated the mass, the internal sheath was removed, and rapid negative pressure was applied by aspirating with a 20-mL syringe several times. After the needle was moved back and forth inside the mass, it was retrieved, and the internal sheath was removed. Positive pressure was applied using an air-filled 20-mL syringe to expel the aspirated material in the needle onto a glass slide, which was then smeared against another glass slide by using the squash preparation technique to yield cytological smears on two glass slides, as described elsewhere (De Lorenzi, 2008). The slides were air-dried and stained with MGG in an automatic slide stainer (Aerospray Slide Stainer 7000, WESCOR, Logan, UT, USA). We aimed to take two aspiration samples from each lesion.

All endobronchial Forceps Biopsies (FB) were obtained using flexible biopsy forceps (MicroTech BF – 18 – 12 AC – 1, Nanjing, China) introduced into the bronchoscope's working channel. We aimed to take three forceps biopsies from each lesion unless the collection of samples was limited by complications such as bleeding. All histological specimens obtained were placed on biopsy sponges, fixed in 10% neutral-buffered formalin, processed, and embedded in paraffin wax; 4-mm sections were stained with hematoxylin and eosin (H&E), and then evaluated by a pathologist who was unaware of the cytological diagnosis.

Cytopathologic specimens were classified, based on cytomorphologic criteria well described in the veterinary literature (Penninc, 2008; McMillian, 1988; Wood, 1998), into four groups: 1) carcinoma, 2) neuroendocrine tumor, 3) sarcoma, and 4) other neoplastic and non-neoplastic lesions.

1.2.3. *Outcome measure*

We compared the sensitivity, specificity, positive predictive value (PPV), negative predictive value (PNV), accuracy, and 95% confidence intervals (CI) of a PPV of isolated EBNA for the correct distinction between benign and malignant growth and to make a correct cytological diagnosis with that of BB sampling and FB. We also compared the sensitivity specificity, PPV, PNV, accuracy, and 95% CI of a PPV of all possible combinations of sampling (EBNA and BB; EBNA and FB; BB and FB; EBNA, FB, and BB) for the correct distinction between benign and malignant growth.

1.2.4. Statistical methods

Only descriptive statistics were provided. The results were expressed as mean and median (range) for continuous variables and number (%) for qualitative and semi-quantitative variables.

1.3.Results

Twenty-one of 26 eligible dogs were enrolled in the study. Three dogs were excluded due to concern about possible bleeding complications after EBNA and before FB. Two dogs were precluded for lack of a definitive histopathological diagnosis from a surgical specimen or necroscopy. The most common breeds included were Mixed breed (n = 8, 38.1%), English Setter (n = 2, 9.52%), and Labrador Retriever (n = 2, 9.52%). Additionally, several other breeds were represented by one case each (Boxer, Dalmatian, Doberman, German Shepherd, Golden Retriever, Fox Terrier, Schnauzer, Springer Spaniel, Rottweiler). There were 4 (19.05%) spayed females, 3 (14.29%) intact females, 12 (57.14%) intact males, and 2 (9.52%) neutered males. The mean age was 10.7 years (median 11 years, 1-16 years).

Endotracheal or endobronchial endoscopic abnormalities detected were EM in 14 out of 21 cases (66.67%) and SI in 7 out of 21 dogs (33.33%). The distribution of endoscopic findings was focal in all cases except for one animal, which had a multifocal localization of submucosal bronchial infiltrations. In 6/21 dogs (28.57%), the abnormalities were localized in the trachea; four were EM, and two were SI. The remaining 15 endoscopic lesions (71.43%) were in the segmental or subsegmental bronchi. According to the classification of Amis & McKiernan (1986), bronchial abnormalities were detected in LPB, LB1, LB2, LB2D2, LB2V2, RPB, RB1, RB2, RB3, RB4, and RB4V1 (Amis, 1986).

A definitive histopathological diagnosis was established for all dogs using necropsy or surgical specimens. Malignancy was found in 17 cases (80.95%), while non-malignant lesions were found in 4 (19.05%). The most common tumor type was carcinoma, followed by sarcoma, carcinoid, melanoma, and mast cell tumors (MCT). The most prevalent benign lesions were abscesses, followed by granulomas and chondromas. The results of the BB, EBNA, FB, and histopathological definitive diagnosis are given in **Table 1**.

Table 1 - BB, EBNA, and FB cytologic or histologic diagnosis, and definitive diagnosis of 21 dogs included in the study.

	Brush cytology (BB) (n = 21)	**Endobronchial Wang Needle Biopsies (EBNA) (n = 21)**	**Forceps biopsies (FB) (n = 21)**	**Histologic definitive diagnosis (n = 21)**
Malignancy	7 (33.33%)	17 (80.95%)	13 (61.9%)	17 (80.95%)
1. Carcinoma	5 (23.81%)	9 (42.86%)	8 (38.1%)	10 (47.62%)
2. Sarcoma	1 (4.76%)	4 (19.05%)	2 (9.52%)	3 (14.29%)
3. Neuroendocrine tumor	-	2 (9.52%)	-	-
Carcinoid	-	-	-	2 (9.52%)
4. Other neoplastic lesions				
Mast cell tumors	1 (4.76%)	1 (4.76%)	1 (4.76%)	1 (4.76%)
Melanoma	-	1 (4.76%)	1 (4.76%)	1 (4.76%)
Malignant tumor unspecified	-		1 (4.76%)	-
Non-neoplastic lesions	14 (66.67%)	4 (19.05%)	8 (38.1%)	4 (19.05%)
Normal mucosa	1 (4.76%)	-	-	-
Necrosis and/or flogosis and/or dysplasia	13 (61.9%)	4 (19.05%)	7 (33.33%)	-
Abscess	-	-	-	2 (9.52%)
Granuloma	-	-	-	1 (4.76%)
Chondroma	-	-	1 (4.76%)	1 (4.76%)

BB: Brush Cytology; EBNA: Endobronchial Wang Needle Biopsies; FB: Forceps Biopsies

The EBNA yielded the highest number of positive results for malignancy, followed by FB and then BB. EBNA confirmed the malignancy or benignity identified by histopathological definitive diagnosis in 19 out of 21 cases (90.48%). FB confirmed the malignancy or benignity in 17 out of 21 cases (80.95%). Finally, BB confirmed the malignancy or benignity in only 11 out of 21 cases (52.38%). Similar results were obtained for the accuracy of the diagnosis; there was a difference in results between the three methods regarding tumor types or diagnosis. In 19 out of 21 cases (90.48%), EBNA agreed with the final morphologic tumor type or diagnosis. FB confirmed the final morphologic tumor type or diagnosis in 15 out of 21 cases (71.43%), while BB confirmed it in only 8 out of 21 cytology samples (38.10%). **Table 2** shows the results of sensitivity, specificity, accuracy, PPV, PNV, and IC 95% together with the various techniques and combinations used.

Table 2 - Sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (PNV) for various techniques (BB, ENBA e FB) and combinations of these.

	Sens.	Spec.	Acc.	PPV	PNV	IC 95%
BB	0.29	1	0.42	1	0.25	± 1
ENBA	0.94	0.75	0.90	0.94	0.75	+ 0.99 / - 0.89
FB	0.76	1	0.80	1	0.5	± 1
BB + ENBA	1	0.75	0.95	0.94	1	+ 0.99 / - 0.89
BB + FB	0.82	1	0.85	1	0.57	± 1
FB + ENBA	1	0.66	0.95	0.94	1	+ 0.99 / - 0.89
ENBA + BB + FB	0.82	1	0.85	1	0.57	± 1

Acc.: Accuracy; BB: Brush Cytology; ENBA: Endobronchial Wang Needle Biopsies; FB: Forceps Biopsies; IC 95%: 95% Interval of Confidence; PPV: Positive Predictive Value; PNV: Negative Predictive Value; Sens.: Sensitivity; Spec.: Specificity

The technique with the highest sensitivity for detecting malignancy was EBNA, whether used alone or in combination with other methods. Diagnostic accuracy was highest when the methods were used jointly, but particularly when EBNA was used in combination with FB or BB.

1.4.Discussion

Flexible fiberoptic bronchoscopy is the most beneficial, non-invasive technique for investigating endobronchial abnormalities (Dobler, 2009). Common non-malignant causes of mucosal airway abnormalities in dogs include inflammatory diseases such as chronic bronchitis and eosinophilic bronchopneumopathy (Zhu, 2015; Clercx, 2000). Primary or metastatic lung tumors less commonly cause mucosal changes in dogs. They typically develop in the periphery and impinge on the airway without evidence of exophytic masses or mucosal invasion, which is more typical of human lung cancer. Exophytic masses within the lumen of the airways in dogs may originate from neoplastic or inflammatory causes (Hill, 2008; Brownlie, 1990). In human medicine, bronchoscopy is essential for definitively diagnosing lung tumors. However, it has marginal importance in veterinary medicine because endobronchial neoplasms are rarely observed (De Lorenzi, 2012). Although rare in small animals, airway granulomas were previously reported as a consequence of mycobacterial infection or parasitic infestation (Kliewer, 2023; De Lorenzi, 2009; Pechman Jr, 1980). Although bronchial epithelium appears to respond to irritation in limited ways, grossly visible changes may not be pathognomonic for any specific disease (Kirk, 1986). Therefore, samples from the airways are used to establish an etiologic or specific diagnosis. For these reasons, we need to find reliable diagnostic methods that yield a high number of positive results and accurately identify the type of tumor or lesion.

Bronchial brush cytology (BB) is a complementary airway diagnostic method that allows direct cytological evaluation of visible endobronchial lesions. BB should be performed by gently rotating and moving the brush back and forth against the airway mucosa to collect cells. Organic material collected from the BB should be immediately smeared onto glass slides (Zhu, 2015). The use of BB for the detection of bronchial inflammatory disease is widely validated in veterinary medicine, while few data are available regarding its use for malignancy detection (Zhu, 2015). In a study by Zhu et al. (2015) comparing BB and bronchial alveolar lavage (BAL) in dogs with chronic cough, BB failed to detect malignancy in a case of carcinoma, instead revealing neutrophilic inflammation along with hyperplasia and dysplasia (Zhu, 2015). In our study, we did not use BAL as a diagnostic method because the site of the lesion was not alveolar.

Moreover, retrospective studies revealed that BAL is specific but insensitive for the diagnosis of pulmonary neoplasia in small animals (Jacobs, 2023). However, our results align with the data from this previous study. In 10 out of 17 cases, BB identified necrosis, inflammation, and dysplasia in the presence of neoplasia. In human medicine, BB has a sensitivity of up to 71% for the diagnosis of malignancy, while in veterinary medicine, the sensitivity of BB in neoplasm detection has not been reported (Zhu, 2015). In this study, we observed a sensitivity of 0.29, specificity of 1, and accuracy of 0.42 for BB. BB was the sampling method with the most minor sensitivity and accuracy compared to other techniques used. However, the diagnostic capacity of BB improved when used jointly with FB and EBNA. No complications were experienced in obtaining BB samples in this study.

Endobronchial biopsy, although less common in veterinary medicine compared to human medicine, can be a valuable diagnostic tool. It offers detailed insights into the morphological changes occurring in the bronchial tissues, which can be crucial for diagnosing and understanding chronic bronchial inflammation. Additionally, endobronchial biopsy may occasionally help identify neoplastic lesions (such as tumors) and inflammatory neoformation (Buechner-Maxwell, 1996; Tams, 2010). Mucosal biopsy samples (FB) could be obtained when definite changes are seen or when a mass lesion is visualized. Samples acquired via endoscopic forceps are typically small (less than 2 mm), and interpretation might be difficult (McCarthy, 2005; Tams, 2010). The retrieved mucosa from FB is usually 1-1.5 mm in size and is frequently crushed. The presence of crush artifacts and small size makes histologic interpretation of the sample difficult (Tams, 2010). When a biopsy is performed, multiple specimens are obtained, if possible, to give the pathologist a greater chance to make a correct diagnosis. In human medicine, FB has a sensitivity of up to 74% for the diagnosis of cancer, while in veterinary medicine, this data is lacking (Govert, 1999). In this study, we observed a sensitivity of 0.76, a specificity of 1, and an accuracy of 0.80 for FB. According to previous reports in humans, the sensitivity of FB is higher when this sampling technique is used in combination with other methods, particularly EBNA. As reported in the literature, FB procedures are safe diagnostic techniques, and no complications were experienced in obtaining FB samples in this study.

Endo-bronchial biopsy using a Wang biopsy needle (EBNA) is an important bronchoscopy sampling technique commonly used in human medicine for the diagnosis and staging of malignancy. EBNA is routinely performed for sampling submucosal endobronchial lesions (e.g., thickening of the mucosa) or masses that compress the bronchial lumen extrinsically and is occasionally used for sampling exophytic endobronchial tumors (Lundgren, 1983). The routine use of EBNA has not yet been established in veterinary medicine, and to the best of the authors' knowledge, this is the first study to evaluate the use of EBNA during airway abnormalities. For these reasons, this is also the first report on the sensitivity, specificity, and accuracy of using EBNA for the diagnosis of airway diseases in animals. We observed a sensitivity of 0.94 for EBNA, a specificity of 0.75, and an accuracy of 0.90. EBNA was the sampling method with greater sensitivity and accuracy compared to other techniques. This appears to be true whether the sensitivity for tumor only and for all lesions is considered. In only one case, EBNA collected necrotic tissue, while the definitive diagnosis was carcinoma. We suspect that EBNA is more effective than BB or FB in diagnosing airway abnormalities due to the needle's ability to penetrate the mucosa and submucosa of a lesion easily, thereby avoiding sampling inflammation and necrosis that may surround neoplastic tissue. One potential criticism of EBNA for diagnosing lung tumors is the potential for misclassifying cell types. In one case, it identified a sarcoma when the definitive diagnosis was a benign tumor (chondroma).

However, EBNA was the only technique that showed greater accuracy. According to previous reports in humans, our study found that using a combination of EBNA, FB, and BB is highly sensitive and accurate for diagnosing airway diseases and cancer (Govert, 1999). EBNA appears to provide cytological samples of diagnostic quality in our series of dogs. This is an additional advantage of EBNA over FB, as it allows for obtaining a diagnostic sample through a single sampling. A complication was experienced during EBNA, primarily moderate bleeding that prevented performing FB. However, this complication did not result in clinical issues for the animals. EBNA is sometimes associated with persistent bleeding and pneumothorax (Tams, 2010). For these reasons, the flexible bronchoscope should be maintained at the sampled sites to monitor for bleeding, and the vital parameters of animals should be monitored to identify signs of iatrogenic pneumothorax rapidly.

Our study has some limitations. The main one is the absence of a comparison between the endoscopy-guided techniques analyzed (BB, FB, and EBNA) and the methods commonly used to evaluate pulmonary lesions in veterinary medicine, such as trans-thoracic FNA. However, the intraluminal localization of the airway lesions described in our study made the use of FNA, which is suitable for sampling pulmonary areas near the chest, less appropriate. Another limitation is the small number of animals included in the study due to the low incidence of this type of injury in dogs.

This study demonstrates that EBNA detects upper airway malignancy in many cases where histopathological neoplasms are found at necropsy or surgery. It also shows that EBNA has the highest sensitivity for detecting malignancy compared to BB and FB. The diagnostic power was enhanced when EBNA was used in conjunction with BB and FB. Similar results were found for benign lesions. In veterinary medicine, EBNA is not routinely used. To the best of the authors' knowledge, this is the first study that explores the use of EBNA in airway disease in small animals. No complications were found when using EBNA in combination with other complementary sampling methods; only in two dogs did EBNA generate content bleeding that prevented performing FB. Although specific instruments and the expertise of a skilled medical practitioner are required to use EBNA, it provides the highest diagnostic accuracy with minimal risk to the animals. Even though this technique in our series of dogs appears to be safe, drawing a more definitive conclusion about the safety of EBNA would require future studies to include a larger number of animals. Moreover, future studies could compare EBNA with other sampling methods for collecting cells in lung tumors, such as FNA cytology and biopsy.

2. Dumon silicone stents can improve respiratory function in dogs with grade IV tracheal collapse: 12 cases (2019–2023)

Abstract

Objective: To evaluate the efficacy, complications, and outcome of Dumon silicone stent placement for dogs with grade IV tracheal collapse (TC).

Animals: 12 client-owned dogs.

Clinical presentation: Each dog was diagnosed with grade IV TC unresponsive to medical therapy and had severe obstructive respiratory failure.

Results: 12 dogs were included in the study. By the end of the study, 5 of 12 (41.7%) remained alive, while 7 of 12 (58.3%) dogs died. Survival times after stent placement ranged from 97 to 1,310 days (mean, 822.43 days; median, 810 days). Three of the 12 (25%) dogs died spontaneously, while 4 of 12 (33.3%) were euthanized. The cause of death was determined for 6 of 7 (85.7%) dogs and was TC related for 3 of 7 (50%). Causes of death related to TC were progressive airway collapse (2/3 [66.6%]) and incoercible cough (1/3 [33.4%]). Complications occurred in 9 of 12 (75%) cases and included granulation tissue growth (3/12 [25%]), incoercible cough (2/12 [16.7%]), progressive airway collapse (2/12 [16.7%]), stent migration (1/12 [8.3%]), and stent deformation (1/12 [8.3%]). Reduction of obstructive dyspnea and episodes of asphyxiation was achieved after Dumon silicone stent placement.

Clinical relevance: The placement of an intraluminal Dumon silicone stent was a successful salvage treatment for TC in dogs that did not respond to medical management. Disease progression is inevitable, but substantial improvement of respiratory function may be achieved for months to years.

Keywords: stents, dogs, tracheal collapse, endoscopy, minimally invasive procedures

2.1.Introduction

TC is a common cause of respiratory difficulty and cough in older small and toy-breed dogs (Chisnell, 2015; Weisse, 2019). Endoscopy is considered the gold standard for diagnosis of TC, detecting concurrent diseases, and identifying the location, grade, and severity of TC (Della Maggiore, 2020; De Lorenzi, 2012). According to the classification by Tangner and Hobson (Tangner, 1982), 4 degrees of gravity are distinguished by the reduction of the tracheal luminal diameter. The treatment of TC varies with the location and grade of collapse. The treatment of choice is conservative medical therapy that includes weight control, avoidance of neck leads, management of comorbidities, and use of various medications (antitussive agents, glucocorticoids, bronchodilators, antibiotics) (Weisse, 2019; Della Maggiore, 2020; Bonagura, 2014; Buback, 1996). In patients refractory/unresponsive to medical treatment or with severe (grade IV) TC, surgical treatment or placement of an intraluminal stent should be attempted (Weisse, 2019; Della Maggiore, 2020; Suematsu, 2019). Common surgical options include using extraluminal ring prosthetics or endoluminal stents to reestablish airway patency (Weisse, 2019; Suematsu, 2019). Intratracheal stents can be distinguished into silicone and self-expanding metal stents (Sun, 2008). Metal stents are associated with several side effects. Potential complications include migration, stent fracture, stent collapse, stent deformation, tracheal perforation, development of obstructive granulation tissue, and inflammatory and bacterial tracheitis (Weisse, 2019; Della Maggiore, 2020; Sun, 2008). In human medicine, the use of metal stents for benign airway disease is not recommended and managing airway obstruction involves the use of Dumon silicone stents (Folch, 2018; Serio, 2014; Semaan, 2015). However, Dumon silicone stents have complications, including migration, obstruction from accumulated secretions, and granulation tissue growth at the proximal or distal ends (Folch, 2018). Numerous studies have evaluated the efficacy and complications of tracheal metallic stents in treating TC in dogs (Weisse, 2019; Raske, 2018; Durant, 2012; Kim, 2008). To the authors' knowledge, there are no similar studies in reference to Dumon silicone stents and only 1 study16 concerning biocompatibility and applicability in normal canine trachea (Xavier, 2008).

This study aimed to investigate the efficacy of Dumon silicone stent placement in dogs with grade IV TC. Additional objectives were the characterization of complications in a 6-month follow-up from stent placement.

2.2.Methods

Medical records were retrospectively reviewed of dogs diagnosed with grade IV TC and subjected to Dumon silicone stent placement by the Anicura Hospital I Portoni Rossi of Zola Predosa (Bologna, Italy) between January 2019 and January 2023. Inclusion criteria for stent placement consisted of a diagnosis of grade IV TC unresponsive to medical therapy and severe obstructive respiratory failure. Exclusion criteria for stent placement were severe laryngeal disease (grade III collapse, paralysis, epiglottic retroversion), severe cardiopathy (class B2 of Consensus American College of Veterinary Internal Medicine degenerative mitral disease), and organ failure (renal, hepatic). All cases included were subjected to clinical examination, hematology and serum biochemistry, radiographic study including a lateral projection of the neck and 2 orthogonal projections of the thorax, and endoscopic study of the upper airway tract.

Data reviewed from electronic medical records included patient signalment, clinical findings, endoscopic and radiographic findings, stent diameter, length and thickness, complications, and follow-up procedures. Patients were excluded from this study if their digital files were incomplete or lacked follow-up (absence of telephone contacts by owners or clinical checks) in the 6 months following stent placement. Complications were categorized as perioperative when they occurred during the stent application and postoperative when they occurred during the postoperative period.

2.2.1. *Dumon stent*

The Dumon silicone stent (Novatech) is a dedicated tracheobronchial stent used to treat various tracheal and bronchial obstructive diseases (Dumon, 1990). This prosthesis is made of polydimethylsiloxane (biocompatible silicone) and presents a serrated external surface with teeth protruding for intercalation and fixation in the lumen of the airways **(Figure 1)**.

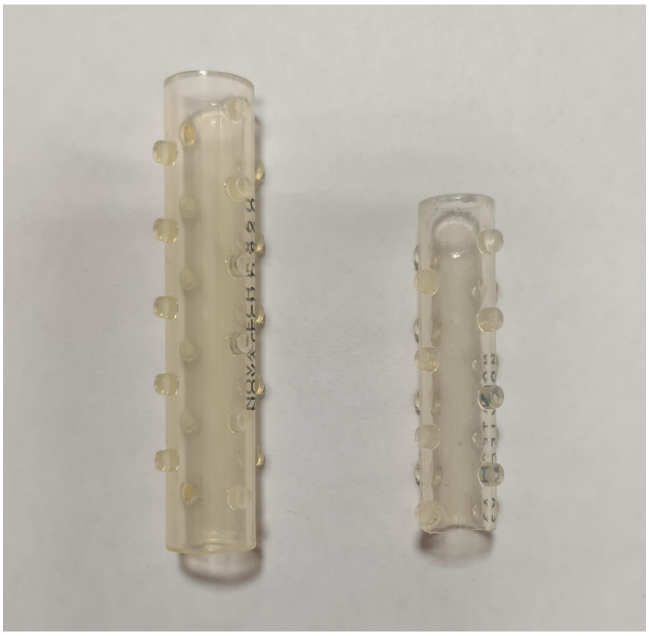

Figure 1 - The Dumon silicone stent presents a serrated external surface with teeth protruding for intercalation and fixation in the lumen of the airways.

The stents have a highly polished inner surface that prevents adhesion of dense mucus, blood, or other materials from the respiratory tree and smooth extremities to prevent friction-related damage (Tangner, 1982; Dumon, 1990). A complete prosthesis set includes several diameters and lengths.

2.2.2. *Endoscopy, stent sizing, and placement*

All endoscopic procedures were performed by the same investigator (DDL). An endoscopy was performed before stent placement to confirm the diagnosis of TC, assess its extent, and identify abnormalities of the upper airway and bronchi. The patients were subjected to endoscopic study under general total IV anesthesia technique. The dogs were premedicated with 2 μg/kg dexmedetomidine and 0.2 mg/kg methadone IM, and anesthesia was induced with 4 mg/kg of propofol administered IV. Anesthesia was maintained with propofol administered in bolus, fentanyl administered in bolus of 2 μg/kg, or fentanyl in constant rate infusion at a dosage of 5 μg/kg/h. Patients were placed in sternal recumbency with the neck extended and support under the chin to ensure the trachea was as straight as possible. The examination of the larynx and cervical trachea was performed with a 2.7 mm, 18 cm, 30° oblique rigid endoscope (64018 BS; Karl Storz SE and Co KG). The intrathoracic trachea and bronchi examination was performed with a 5.2 mm, 85 cm flexible fiberscope (60001 VL2 Bronco-Fiberscope; Karl Storz SE and Co KG).

Stent sizing (length and diameter) was based on endoscopic findings. The appropriate length of stent was determined by measuring the extent of the TC from its beginning to its end. To achieve this, a graduated scale on the operating tube of the flexible bronchoscope was utilized. For the diameter measurement, a comparative analysis was conducted between the diameter of a healthy tracheal segment and a known measuring element, such as a biopsy clamp with a predetermined width of 3 mm inserted into the working channel of the endoscope and kept in an open position. A stent 1 cm longer than the extent of the TC was chosen to include normal trachea 0.5 cm cranially and caudally as previously reported in humans (Dutau, 2013).

The placement was made after direct bronchoscopic visualization of the selected site. The stent had to be placed 0.5 cm away from the cricoid cartilage and bronchial bifurcation to prevent interfering with laryngeal function and air passage under the airway tract. The Dumon stent was introduced into the dedicated pusher (Tonn applicator; Novatech). When the size of the dogs did not allow for use of the Tonn applicator, the stent was grasped and placed with a long and thin bayonet clamp through the laryngeal adytum and under direct bronchoscopic visualization. Correct placement was verified with endoscopic visualization of the site. A transtracheal suture was placed through surgical cervical ventral access to ensure fixation of the stent on the tracheal surface. Transtracheal suture was placed in the cranial cervical tract of the trachea with a suture thread of Dafilon 3/0. After the stent's placement, the animals were evaluated with a radiographic study of the cervical-thoracic region of the trachea for evaluation of stent positioning **(Figure 2)**. This image was used as a reference for radiographic revisions performed during follow-up.

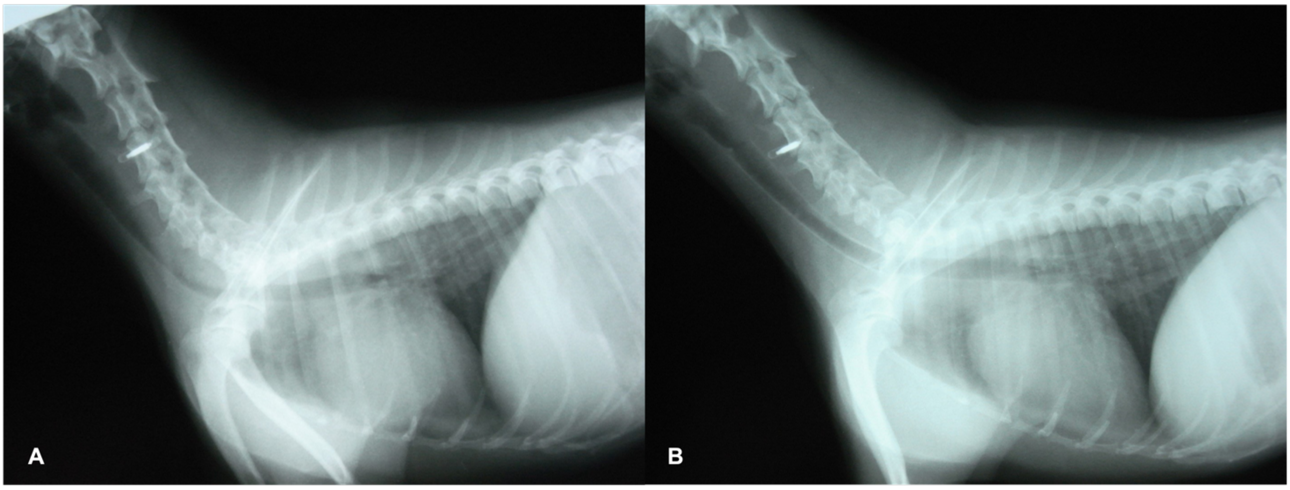

Figure 2 - Lateral thoracic radiographic images of a dog with tracheal collapse (TC) before (A) and immediately after (B) endoluminal placement of a Dumon silicone stent. A) Lateral radiographic view showing cervical TC. B) Appropriate stent placement is confirmed.

After the placement, medical therapy was performed to reduce inflammation, promote mucusciliary clearance, and suppress cough. Postoperative medications included anti-inflammatories like budesonide 0.5 mg (Aircort) via aerosol 3 times a day and prednicortone (Deltacortene) 0.5 mg/kg twice a day until the minimum effective dose was obtained, and an antitussive like butorphanol (Dolorex) PO 0.2 mg/kg twice a day if needed.

2.2.3. *Follow-up*

Follow-up information was collected from the owner for each dog by means of phone calls. Pet owners were contacted every 2 weeks for the first 3 months and then once a month for 6 months. On the basis of the clinical condition described by the owner, endoscopic and radiographic controls were decided. Collected information included the presence and severity of clinical signs, medications administered, complications, survival time, and cause of death when applicable.

2.2.4. *Statistical analysis*

Only descriptive statistics were provided. The results were expressed as mean and median for continuous variables, and number (percentage) for other variables.

2.3.Results

A review of the medical records revealed 12 dogs with grade IV TC that were subjected to the placement of intraluminal Dumon stent of the operative unit of Interventional Pulmonology of the Anicura Hospital I Portoni Rossi during the study period (**Table 1**).

Table 1 - Characteristics (breed, sex, age, localization of collapse, stent characteristics, follow-up, and complications) of 12 dogs included in the study.

Patient	Breed	Sex	Age (months)	Localization of collapse	Diameter (ø, mm) - Length (cm) - Thickness (mm)	Complications	Follow-up (days)
1	Yorkshire Terrier	M	72	C	10 - 8 - 1,5	Stent migration	1275 (†)
2	Yorkshire Terrier	M	48	C + T	10 - 8 - 1,5	Stent deformation	745 († [e])
3	Poodle	F	120	C	9 - 10 - 1,5	Development of thoracic TC and bronchial collapse	1110 days (†)
4	Yorkshire Terrier	F	96	C + T + B	10 - 8 - 1,5	Development of laryngeal collapse and bronchial collapse	420 days (†)

5	Pomeranian	M	84	C + T	11 - 8 - 1,5	Obstruction from granulation tissue growth	1310 days († [e])
6	Yorkshire Terrier	M	48	C + B	10 - 8 - 1,5	Paroxysmal cough	97 days († [e])
7	Maltese	F	100	T	10 - 8 - 1,5	Paroxysmal cough	810 days († [e])
8	Poodle	F	108	C + T + B	9 - 8 - 1,5	Obstruction from granulation tissue growth	650 days
9	Yorkshire Terrier	M	78	C + T	10 - 10 - 1,5		360 days
10	Pomeranian	M	100	C	11 - 8 - 1,5	Obstruction from granulation tissue growth	350 days
11	Pomeranian	M	36	C	10 - 8 - 1,5		280 days
12	Poodle	F	70	C + T	9 - 10 - 1,5		240 days

M = male; F = female; C = cervical tracheal collapse (TC); T = thoracic TC; B = bronchial collapse; (ø) = diameter; † = spontaneous death; † e = euthanized.

Four dog breeds were represented. The most common was Yorkshire Terrier (41.7% [5/12]), Pomeranian (25% [3/12]), Poodle (25% [3/12]), and Maltese (8.3% [1/12]). Males (41.7% [5/12]) and females (58.3% [7/12]) ranged in age from 36 to 120 months (mean, 81 months; median, 80 months). Three (25% [3/12]) dogs had cervical TC, 1 (8.3% [1/12]) had thoracic TC, 5 (41.7% [5/12]) had cervical-thoracic TC, 1 (8.3% [1/12]) had cervical TC and bronchial collapse, and 2 (16.7% [2/12]) had cervical-thoracic TC and bronchial collapse.

2.3.1. *Stent*

In 4 (33.3% [4/12]) patients, stents were placed only in the cervical trachea; of these, 3 (25% [3/12]) dogs had cervical TC, while 1 (8.3% [1/12]) had cervical TC and a bronchial collapse. In 1 (8.3% [1/12]) patient, a Dumon silicone stent was placed only in the thoracic trachea. In 7 (58.4% [7/12]) dogs, Dumon silicone stents were placed in the entire trachea; of these, 5 (41.7% [5/12]) patients had cervical and thoracic TC, while 2 (16.7% [2/12]) had cervical and thoracic TC and bronchial collapse. Dumon silicone stents had a thickness of 1.5 mm, diameter ranging from 9 to 11 mm, and length ranging from 8 to 10 cm (**Table 1**). The most used stents were those with a diameter and length of 10 mm and 8 cm (50% [6/12]). Stents with a diameter and length of 11 mm and 8 cm (16.7% [2/12]), 9 mm and 10 cm (16% [2/12]), 10 mm and 10 cm (8.3% [1/12]), and 9 mm and 8 cm (83% [1/12]) were also used.

2.3.2. *Complications*

No perioperative complications occurred in patients (0% [0/12]). Postoperative complications occurred in 75% (9/12) of dogs (Table 1). In 58.3% (7/12) of cases, complications were associated with the procedure and included the development of obstructive granulation tissue (25% [3/12]), stent migration (8.3% [1/12]), stent deformation (8.3% [1/12]), and paroxysmal cough (16.7% [2/12]). Tracheal contact granulomas occurred at 350, 650, and 1,310 days after placement (mean, 770 days; median, 650 days) and were treated with laser surgery. Stent migration occurred 10 days after stent placement, and the issue was successfully resolved by repositioning the stent and applying an additional transtracheal suture 1 cm behind the original stitch. The deformed stent was replaced by a new one 400 days after the first stent placement. Paroxysmal cough occurred at 97 and 810 days after stent placement (mean, 453 days; median, 453 days). The mean time for complications associated to stent placement was 518 days (median, 400 days). Two (25%) dogs had complications related to the progression of the disease and not to the presence of the stent; these complications included the development of thoracic TC and bronchomalacia (8.3% [1/12]) that occurred 1,110 days after stent placement and development of laryngeal collapse and bronchomalacia (8.3% [1/12]) that occurred 410 days after stent placement. The mean time for complications not related to the presence of the stent was 760 days (median, 760 days). The remaining patients (25% [3/12]) did not have post-operative complications.

2.3.3. *Follow-up*

Follow-up times after stent placement for all 12 dogs included in the study ranged from 97 days (recorded minimum survival time) to 180 days (6 months). By the end of the study, 5 of 12 (41.7%) dogs were alive, while 7 of 12 (58.3%) were dead. The mean survival time for the 7 dogs that died was 822.43 days (median, 810 days). The minimum survival time recorded was 97 days, while the maximum survival time was 1,310 days (**Table 1**). Cause of death was determined for 85.7% (6/7) of dogs, and of these, 50% (3/6) were related to TC and 50% (3/6) were unrelated to TC. Four (33.3% [4/12]) dogs were euthanized after 97, 745, 810, and 1,310 days after stent placement. Euthanasia was performed due to incoercible cough (25% [1/4]), worsening of bronchial collapse (50% [2/4]), and hepatic carcinoma (25% [1/4]). Spontaneous deaths occurred in 25% (3/12) of cases; for these patients, death occurred at 410, 1,100, and 1,275 days after stent placement. The causes of death were, respectively, unknown, kidney failure, and bite trauma to the neck and chest. Patients alive (5/12) had a good follow-up with an improvement in respiratory function perceived by owners. This improvement was observed by owners for 240, 280, 350, 360, and 650 days from stent placement.

2.4.Discussion

To our knowledge, this was the first tracheal stenting report investigating endoluminal silicone Dumon stent placement in dogs with grade IV TC. During the 3 years of the study, 12 dogs with grade IV TC were subjected to the placement of endoluminal Dumon silicone stents and included in the study.

Four breeds were represented in the included dogs, and Yorkshire Terrier accounted for 47.1% (5/12) and was the prevalent breed; these data were according to that reported in the literature (Weisse, 2019; Becker, 2012). Age at the time of stent placement ranged between 2 and 10 years, supporting previous evidence that this condition may manifest at any time with a variable rate of progression (Tangner, 1982). Involvement of the bronchial wall is termed bronchomalacia and is reported in 45% to 83% of dogs with TC (Della Maggiore, 2020). Cervical TC presented alone (33.3% [4/12]) and in association to collapse of the thoracic trachea (33.33% [4/12]) and bronchi (8.3% [1/12]). Two (16.7% [2/12]) dogs were affected by diffuse malacia, cervical-thoracic TC, and bronchial collapse. One dog was affected by thoracic TC (8.3% [1/12]). Our information supports previous evidence that malacia can affect the trachea, bronchi, or both (Della Maggiore, 2020). Cervical TC was present in 91.7% (11/12) of patients included in the study, supporting the authors' opinion that endoluminal stent placement is necessary when patients manifest obstructive dyspnea.

Endoluminal stents are medical devices for maintaining the patency of tubular organs (Stehlik, 2015). Tracheal stenting can be performed using fluoroscopy, endoscopy, or digital radiography (Weisse, 2015). Self-expanding nitinol stents are often preferred because these devices can be placed quickly and noninvasively (Weisse, 2019; Della Maggiore, 2020; Sun, 2008). Previous and numerous studies described intraluminal stenting with metal stents under fluoroscopic guidance in dogs with terminal TC with evidence of numerous side effects and success rates ranging from 61% to 89% (De Lorenzi, 2012; Raske, 2018). In human medicine, it is currently recommended to use Dumon silicone stents to manage malignant and benign airway obstruction in adults and children (Serio, 2014; Semaan, 2015). Indeed, metallic stents are associated with more side effects compared to silicone Dumon stents, including issues such as stent fracture, stent collapse, and tracheal perforation. Additionally, the metal meshes tend to become incorporated into the mucosa, making their removal impossible once they are positioned (Weisse, 2019; Della Maggiore, 2020; Serio, 2014; Semaan, 2015). In humans, Dumon silicone stent placement is made after direct bronchoscopic visualization of the selected site; the insertion is usually achieved by pushing the stent off from a loader using a prosthesis pusher (Tonn applicator; Novatech) (Dumon, 1990). In our study, the stent was placed under bronchoscopic guidance as previously described for humans, and rigid bronchoscopy and general an-esthesia were needed (Dumon, 1990). When the small size of the dogs did not allow the use of the Tonn applicator, the stent was folded and placed with a long and thin bayonet clamp through the laryngeal adytum and under direct bronchoscopic visualization. Our work suggests that Dumon silicone stenting is a possible minimally invasive and easy technique in dogs with TC, as already evidenced in human medicine for airway stenosis (Chen, 2021). Perioperative complications were not observed in any patients.

In the use of self-expanding metallic stents, an accurate measurement of the tracheal diameter and length is essential for selecting an appropriate stent size (Raske, 2018). These stents are made of metal mesh that is reconstrainable and undergoes foreshortening. Foreshortening refers to the decrease in the length of the stent, which occurs as it expands to its maximum diameter (Monaco, 2014). The Dumon silicone stent is made of polydimethylsiloxane (biocompatible silicone) and does not present the characteristics described above (Dumon, 1990). Therefore, measurements for stent selection as previously described are not necessary (Weisse, 2015). In our work, measurements were estimated by endoscopic findings. A complete set of Dumon silicone stents includes several diameters and lengths (Dumon, 1990). This is important given the considerable variability of breeds in veterinary practice. Stent sizes for the 12 dogs included in our study ranged from 9 to 11 mm (diameter) and 8 to 10 cm (length), while all stents had a thickness of 1.5 mm. Reduced variability in the size of the stents is due to the predisposition of small and toy breeds to TC (Della Maggiore, 2020).

Postoperative complications have been seen with the use of Dumon silicone stents for the treatment of malignant and benign airway obstruction in human medicine. Similar postoperative complications were seen in our series of dogs including stent migration, stent deformation, granulation tissue formation around the stent, and poor patient tolerance (Durant, 2012; Zakaluzny, 2003; Sura, 2008). Migration is caused by inappropriate sizing or misplacement of the stent and can be life-threatening (Monaco, 2014). For the placement of a nitinol stent, the usual practice involves obtaining the maximal tracheal diameter from positive-pressure thoracic radiographs. It is crucial to make an accurate measurement of the tracheal lumen to determine the appropriate size for the pros- thesis (Durant, 2012; Kim, 2008). As previously reported, stent migration for self-expanding metallic stents in dogs occurred up to 37% (Weisse, 2015). Despite a less precise measurement in our study, the data showed that this complication occurred in 8.3% (only 1/12) of patients. This may be due to the reduced number of patients included in the study or the different characteristics of Dumon silicone stents compared to metallic ones. However, it is possible that a more accurate measurement of tracheal length and diameter could have resulted in a decreased rate of stent migration. Stent migration was resolved by repositioning the stent and applying a transtracheal suture. The significant advantage of Dumon silicone stents compared to metallic stents is the ability to easily reposition and remove using rigid grasping forceps (Folch, 2018). Stent deformation occurred in 8.3% (1/12) of cases. The deformed stent was replaced with another Dumon silicone stent. This complication was not observed with metallic stents, which are more rigid and difficult to deform but can break because of the continuous mechanical stimulation caused by coughing (Moritz, 2004). In our study, the development of exuberant granulation tissue occurred in 25% (3/12) of cases, predominantly at the stent ends (**Figure 3**).

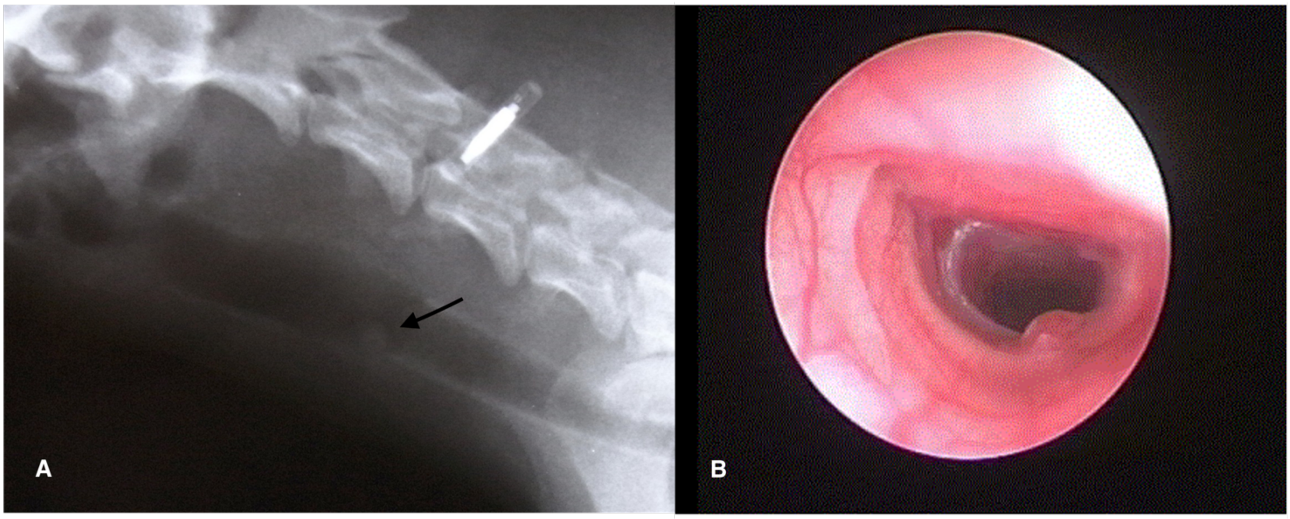

Figure 3 - Development of exuberant granulation tissue. A) Lateral thoracic radiographic view of a dog with TC after placement of an endoluminal Dumon silicone stent. Notice the granulation tissue ingrowth at the stent ends. B) Bronchoscopic view of the granulation tissue ingrowth in a trachea of a dog after placement of an intraluminal Dumon silicone stent.

Localization to the stent ends may have been in response to the edges of the stent in this area or to increased motion and friction between the mucosa and implant on-site. In similar studies, the prevalence of granulation tissue ingrowth was 50% and granulation tracheal tissue responded well to corticosteroid treatment (Sun, 2008; Moritz, 2004). In our case, we preferred to remove the newly formed tissue through endoscopy-guided laser surgery. Indeed, overgrowth tissue was obstructive and severely hampered respiratory function. The use of laser allowed for an immediate intervention at the time of diagnosis, whereas steroid therapy entails longer healing times. Progressive TC beyond the stent was a critical complication and the reason that dogs in the present study required euthanasia. A further cause for euthanasia was incoercible cough. Cough was an expected complication given the continued presence of a foreign object (stent) in the trachea. The cough was controlled by medical therapy. Additional complications have been noted with metallic stents in humans and dogs, including problems with placement and removal and stent fracture (Durant, 2012; Zakaluzny, 2003). In our study and that of Xavier et al (Xavier, 2008), these complications were not registered with the use of silicone stents. Three of 12 (25%) dogs did not have postoperative complications. On the basis of these data, we can conclude that the Dumon silicone stent is associated with a lower rate of complications, which can be managed by medical therapy, repositioning, or laser surgery.

Survival rates in the study were like those previously reported for metallic stents (Weisse, 2015; Moritz, 2004). Mean survival time in our series was 822.43 days. Euthanasia was performed in 4 (33.3% [4/12]) dogs. Only 1 dog subjected to euthanasia was associated with stent placement (incoercible cough). Euthanasia was performed in a mean of 740.5 days from stent placement. Spontaneous death was registered in 25% (3/12) of cases and associated with causes not correlated with disease or stent placement. Death occurred in a mean of 931.66 days from stent placement. The remaining 41.7% (5/12) of dogs were considered by owners to have a good outcome at follow-up 2 years from stent placement. As reported by the owners during phone calls, surviving dogs had an improved quality of life as indicated by an improvement in respiratory function, which was subsequently confirmed by the veterinarian during clinical checkup.

Our study had several limitations, including its retrospective nature. The dogs were not randomized, and there was a small dataset to examine. Six dogs were excluded for inadequate follow-up; for this reason, the cases included in the study were reduced. The data and measured outcomes were collected before the study started and, therefore, were not standardized. Finally, another potential confounding factor was that the results were compared with those of other studies, which differed in the characteristics of the population examined.

Nevertheless, we conclude that the Dumon silicone stent can be successfully used as a palliative treatment for dogs with terminal cervical TC and should be considered in patients not responsive to medical treatment. Dumon silicone stents, unlike stents in nitinol, seem to be appropriate since removal or replacement is always possible. Despite the presence of a stent, there will invariably be disease progression, but substantial improvement in respiratory function (reduction of obstructive dyspnea and episodes of asphyxiation) may be achieved for a medium-long period. Dumon silicone stents have efficacy for treating grade IV TC with approximately 50% incidence of complication. Regular bronchoscopy follow-up should be conducted.

Our results suggest that using a Dumon silicone stent for grade IV TC is possible, effective, well tolerated by the patient, and associated with minimal and acceptable complications.

3. **Management and outcomes of 35 dogs treated with Diode Laser Epiglottidectomy (DLE)**

Abstract

Objective: To describe the management and outcomes of dogs with epiglottic conditions treated with diode laser epiglottidectomy (DLE).

Study Design: Single-institutional observational prospective study.

Animals: Thirty-five dogs diagnosed with epiglottic disease undergoing DLE.

Methods: In all cases, an epiglottic disease was documented with a laryngoscopy combined, when needed, with fluoroscopy, computed tomographic (CT) scan, and biopsy. Sub-total (SDLE) and total (TDLE) diode laser epiglottidectomy were performed under endoscopic guidance according to the diagnosis. Follow-up was obtained by a reexamination visit and endoscopy, and telephone follow-up with the owner.

Results: The most common epiglottic disorder was epiglottic retroversion (ER) (57.1%). SDLE was performed in 32/35 (91.4%) dogs, while 3/35 (8.6%) dogs underwent TDLE. Intraoperative complications occurred during 11.4% surgeries, and were represented by significant bleeding. Postsurgical complications were reported in 8.5% cases post-SDLE and were represented by transient airway obstruction caused by local oedema. Follow-up (median 18 months, minimum 3 months – maximum 21 months) consultations revealed prolonged resolution of upper airway obstruction without signs of respiratory tract compromise or dysphagia.

Conclusions: Laser epiglottidectomy can be performed via a transoral approach with minimal morbidity by using a 980 μm diode laser under endoscopic guidance. Due to proper dogs selection and surgical technique, no significant complications regarding respiration or swallowing were reported in this study.

Clinical Significance: The surgical techniques described in this study have proven effective and minimally invasive for treating epiglottic-related airway obstruction.

3.1.Introduction

Despite its prominent location at the entrance of the laryngeal airway, little is known about the epiglottis' function and conditions. During the swallowing reflex, the epiglottis protects the lower airways from aspiration of liquids and solids as it covers the laryngeal inlet by acting as a hinged lid. The role of the epiglottis in the mechanism of breathing is not completely understood (De Lorenzi, 2015; Amis, 1998).

Veterinary literature regarding epiglottis disorders and medical treatment in dogs is minimal (De Lorenzi, 2015; Flanders, 2009; Skerrett, 2015; Mullins, 2014; Mullins, 2019; Shoieb, 2014). Only a few studies have been published concerning the incidence of epiglottic diseases and their treatment was limited to 79 cases in total, 77 of which were affected by the same disorder (epiglottic retroversion) (Flanders, 2009; Skerrett, 2015; Mullins, 2014; Mullins, 2019). Only two studies describe the surgical treatment of a primary epiglottic malignant tumour (De Lorenzi, 2015; Shoieb, 2014). Although epiglottic retroversion (ER) is increasingly recognized as a cause of continuous or intermittent upper airway obstruction in dogs, its aetiology remains unknown. Hypothesis about the aetiology in dogs include hypothyroidism-associated peripheral neuropathy, epiglottic fracture, epiglottic malacia, and failure of the hyoepiglottic muscles to drag the epiglottis rostrally and ventrally to oppose the negative pressure generated during inspiration (Skerrett, 2015; Mullins, 2019). However, in the current literature, only 3 cases diagnosed with ER underwent a histopathological examination of the epiglottis, although no attempt was made to assess the histological features of the cartilage (Dallman, 1988).

Therapeutic management of epiglottic retroversion (ER) can be medical or surgical; in dogs, epiglottopexy and subtotal or total epiglottidectomy are described as surgical treatment of ER (Skerrett, 2015; Mullins, 2014; Mullins, 2019). Total epiglottidectomy is also used for surgical resolution of primary epiglottic malignant tumours in dogs (De Lorenzi, 2015; Amis, 1998; Flanders, 2009; Skerrett, 2015; Mullins, 2014; Mullins, 2019; Shoieb, 2014). Epiglottidectomy is well tolerated and associated with a lower rate of postsurgical complications compared to a more invasive procedure such as incisional or non-incisional epiglottopexy (Mullins, 2019). A high incidence of surgical complications and failures was reported. In one paper, approximately one-third of a cohort of 19 dogs were euthanized after surgery because of respiratory complications (Skerrett, 2015). Another study regarding 50 surgically treated dogs reports that 48.7% had major postsurgical complications, and 24% of dogs died as a consequence of clinical conditions or were euthanized (mean 301.5 days, range 3-1212 days) (Mullins, 2019). In human medicine, video-assisted laser epiglottidectomy is the golden standard surgical procedure for treating epiglottic-related airway obstruction, as in neoplastic or chronic inflammatory diseases, and for sleep apnea. The surgery is usually well tolerated by patients, with no significant postoperative complications or worsening in swallowing, breathing, or phonation (Zeitels, 1990; Golz, 2000; Kanemaru, 2007).

The purpose of this study is: 1) to characterize the signalment, history, clinical signs, endoscopic features, comorbidities, complications, and outcomes of a cohort of 35 dogs with an epiglottic disease treated with DLE; 2) to describe a standardized surgical technique; 3) to describe the pathological features of the epiglottic cartilage related to the different diagnosed diseases.

3.2.Materials and methods

From January 2022 to December 2023, dogs with a history of respiratory distress and a final diagnosis of epiglottic disease treated with sub-total (SDLE) or total diode laser epiglottectomy (TDLE) were included in this study. When respiratory comorbidities were present, dogs were excluded from the study if they concurrently underwent surgical management for their other airway disorders and epiglottis surgery.

For each dog, the recorded information included signalment, history, clinical signs, cervical and thoracic radiographs (and, when available, computed tomographic scan and fluoroscopy of the affected area) findings, upper and lower airway endoscopic findings, cytological and histological findings, surgical procedures, and outcomes.

3.2.1. *Endoscopic procedure*

All dogs underwent a complete endoscopic examination, including pharyngoscopy, laryngoscopy, and tracheobronchoscopy. All endoscopic and surgical procedures were performed by the same clinician (DDL) and were performed with the dogs in sternal recumbency, the jaw held open with a gag and with gentle traction of the tongue (**Figure 1**).

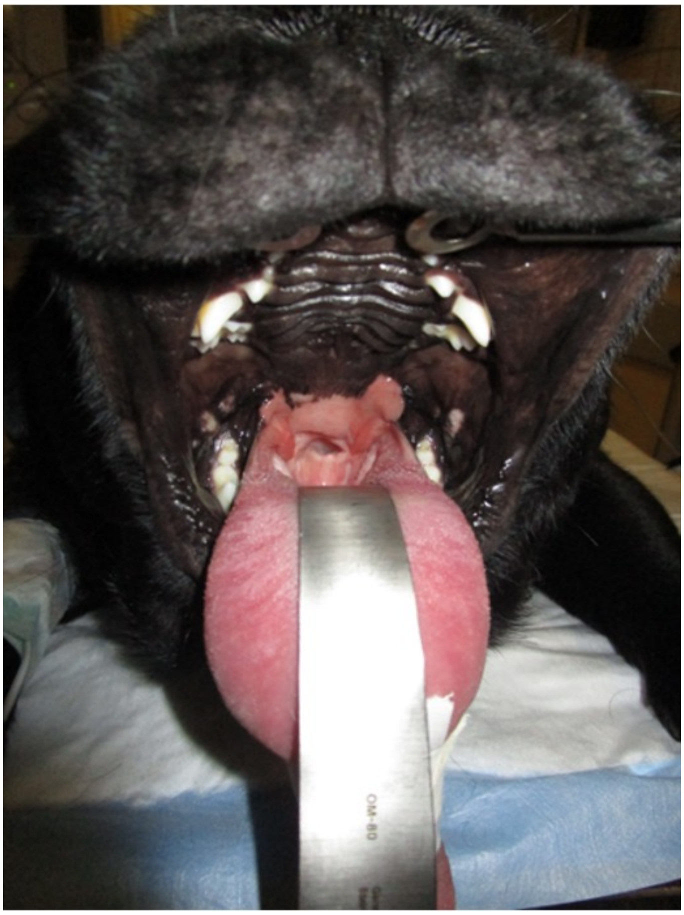

Figure 1 - Patient positioning during the endoscopy and diode laser epiglottidectomy (DLE).

Laryngoscopy, pharyngoscopy, and cervical tracheoscopy were performed under sedation or light injective anaesthesia via a 2.7 mm x 18 cm rigid telescope (Telescope K Storz 64018BS, Karl-Storz-Endoscopy, Tuttlingen, Germany) with a halogen light supply. Bronchoscopy was performed under inhalation anaesthesia passing a 2.7 mm x 67 cm flexible endoscope (Fiberscope K Storz 11278AK, Karl-Storz-Endoscopy) through a T-adapter inserted between the tracheal tube and the tubing of the anaesthetic machine, using the same halogen light supply.

In relation to endoscopic findings, disorders of the epiglottis were classified into 4 major categories: epiglottis verticalization (EV), characterized by a normal-shaped epiglottis permanently posteriorly displaced, partially, or hiding/impeding the inspection of the glottis (**Figure 2**); ER characterized by a normal shaped epiglottis posteriorly displaced during inspiration, partially or obstructing the glottic space; epiglottis deformation (ED) characterized by an abnormally shaped epiglottis which partially obstructs the glottis; epiglottis neoplasia (EN) characterized by an epiglottic mass that partially obstructs the glottis that was cytologically or histologically diagnosed as a neoplasm.

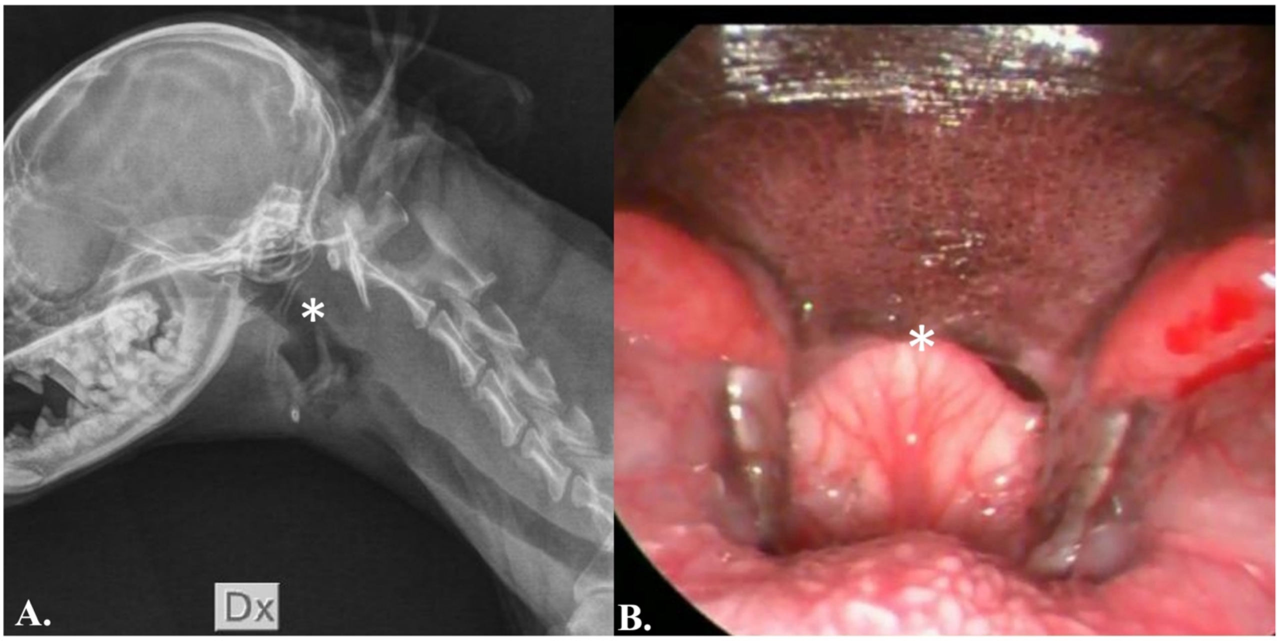

Figure 2 - Epiglottic verticalization (EV). A) Lateral neck radiographic view reveals the caudal shift of the epiglottis, indicated by the asterisk; B) Endoscopic view of EV, the epiglottis is indicated by the asterisk.

Laryngeal collapse was graded as proposed by Leonard (Leonard, 1960), tracheal collapse was graded as proposed by Tagner and Hobson (Tagner, 1982), while bronchial collapse was graded based on the bronchial diameter reduction, as proposed by De Lorenzi et al (De Lorenzi, 2009).

3.2.2. *Surgical Procedure*

SDLE was the preferred procedure for all dogs with epiglottic benign conditions such as epiglottic retroversion, epiglottic verticalization, and epiglottic deformation; TDLE was the surgery of choice for the treatment of neoplastic diseases of the epiglottis.

The dogs were subjected to surgical procedures under the general total intravenous anaesthesia technique (TIVA). The dogs were premedicated with 2 μg/kg dexmedetomidine and 0.2 mg/kg methadone intramuscularly, and the anesthesia was induced with 4 mg/kg of propofol administered intravenously. Anesthesia was maintained with propofol administered in bolus, fentanyl administered in bolus of 2 μg/kg, or fentanyl in constant rate infusion (CRI) at a dosage of 5 μg/kg/h. Although displaced posteriorly, the endotracheal tube could impede surgical manoeuvres; its bulk impedes the manipulation of the epiglottis, and the traction and exposure of the epiglottis are less than ideal. Furthermore, the endotracheal tube is at constant risk of being struck by the laser beam. For these reasons, all the surgical procedures described in our study were performed in an extubated dog with oxygen supplementation administrated through a nasotracheal cannula.

All the dogs were placed in sternal recumbency with the neck extended, the head suspended from a frame, and the jaw held wide open with a zinc oxide tape placed from around the tongue to the surgical table. A better visualization of the surgical field was obtained by placing a malleable retractor (BT752R, B Braun Aesculap, Milan, Italy) with the tip positioned anterior to the epiglottis to expose the vallecula and the base of the tongue, thus allowing optimal exposure (**Figure 1**). Once positioned, the retractor was held in place by fixing it to the table with zinc oxide tape.

Epiglottectomy was performed using a 270 nm or a 350 nm diameter fibre, 4W power, 980 μm wavelength diode laser (Quanta System, Milan, Italy) in contact, continuous wave mode. For both SDLE and TDLE, a 2.7 mm x 18 cm, 30° oblique rigid endoscope (HOPKINS II Forward-Oblique Telescope 30°, 64018 BA, Karl Storz) and a sheath 14.5 Fr x 15 cm with 3 different ports (67065 C, Karl Storz) were used. The central port was used to insert the laser fibre, and the 2 lateral ports were used for continuous smoke and fluid aspiration.

The procedure was performed by starting from the left side of the epiglottis. The first incision was performed in contact mode. The excision was accomplished after applying a traction force in the mid-lateral portion of the epiglottis using a cup laryngeal forceps (Dufner 27970-01, Dufner instruments GmbH, Tuttlingen, Germany). Maintaining the epiglottis in maximum traction, a U-shaped wedge was incised at the level of the suprahyoid portion of the epiglottis if SDLE was performed. When TDLE was performed, the whole cartilage, including the epiglottic stalk (petiolus epiglottidis), was removed by resecting it from the dorso-caudal surface of the thyroid cartilage. The diode laser was used to cut through both aryepiglottic folds and to incise the lateral edge of the epiglottis. These incisions were then united across the vallecular surface of the epiglottis, resulting in an *en bloc* excision of the cartilage. Careful dissection along the lingual surface of the epiglottis is necessary to avoid significant bleeding.

Immediately after the surgical procedure, the dogs were intubated, and a refrigerated saline-soaked sponge was placed on the surgical area and left in place for 3-5 minutes. The tissue defect heals by secondary re-epithelization without primary closure. A double bolus of 0,5 mg/kg dexamethasone sodium phosphate (Dexadreson 2 mg/ml, MSD Segrate, Milan, Italy) was administered intravenously before induction of anesthesia and 12 hours post-surgery to inhibit traumatic oedema of the operative field. Perioperative antibiotics (amoxicillin + acid clavulanic, 12,5 mg/kg twice daily subcutaneously) were also administered to all the dogs. Ranitidine chlorhydrate (Zantadine 3 gr/100 ml, CEVA S.p.A, Milan, Italy) at a dose of 2 mg/kg twice daily orally was employed as an antiacid to prevent delayed healing in any patient suspected of gastroesophageal reflux. Usually, postoperative alimentation was given ad libitum, with soft food, starting 12 hours after surgery.

3.2.3. *Histological findings*

All histological specimens of epiglottis, obtained sampling both normal and abnormal appearing tissue, were fixed in 10% neutral-buffered formalin, processed, and embedded in paraffin wax; 4-µm sections were stained with hematoxylin and eosin (H&E), and then evaluated by a board certificate pathologist. The histological specimens were classified into 3 groups: 1) nonspecific findings (when only mixed inflammation was present), 2) necrosis (when severe degenerative cartilage changes were evident), and 3) neoplasia (when neoplastic cells were present).

3.2.4. *Follow-up*

Follow-up consultations were conduced 1 month post-surgery (short-term follow-up) through clinical visit and laryngoscopic examination, and at 3 months post-surgery (long-term follow-up) solely through clinical check. No specific rubric for the evaluation of outcomes was provided to the owners. Instead, during these visits, owners were administered a questionnaire to assess the outcome of the surgery. They were asked whether the dog showed an improvement or worsening of respiratory function, whether there was persistence of pre-operative clinical signs, and whether the dog exhibited new clinical signs. Based on the information collected by the owners during clinical checks (short- and long-term follow-up), the outcome was classified as follows:

- Poor: when the dog presented unchanged or worsened clinical signs;
- Moderate: when the dog showed good improvement in respiratory function with the disappearance of some clinical signs but persistent limitations in physical activity;
- Adequate: when the dog showed complete improvement in respiratory function and the disappearance of clinical signs.

If owners did not authorize a laryngoscopy examination, follow-up was conducted only by clinical visit, and endoscopy was performed during intubation for another procedure (if necessary, in a period between 1 to 6 months after STLS or TDLS). In addition, throughout the study period, information on the outcomes (clinical aggravation, death, etc…) was obtained through telephone contacts with dog owners. Further visits or endoscopic checks were scheduled based on the outcomes discussed during these telephone conversations.

3.2.5. *Statistical analysis*

Only descriptive statistics were provided. The results were expressed as median (range) for continuous variables and number (%) for qualitative and semi-quantitative variables.

3.3.Results

Thirty-five dogs diagnosed with an epiglottic disease and undergoing DLE at the Operative Unit (OU) of the Interventional Pulmonology of the Anicura Hospital *I Portoni Rossi* during the study period

(January 2022-December 2023) were included in the study. Thirty-two out of 35 dogs underwent SDLE (91.4%) and 3 TDLE (8.6%). Individual signalments are listed in **Table 1**.

Table 1 - Signalment (breed, sex, age), pre-operative clinical signs, and diagnostic (endoscopic and radiographic) findings of 35 dogs included in the study.

Case number	**Signalment (breed, sex, age in months)**	**Pre-operative clinical signs**	**Endoscopic findings**	**Radiographic findings**
1	Pomeranian, FS, 18	IS; Dy; EI;	ER	/
2	Chihuahua, M, 48	IS; Dy; EI;	ER	CDE
3	Yorkshire Terrier, M, 60	IS; Dy; EI;	ER, TC (II°)	/
4	Chihuahua, FS, 86	IS; Dy; EI; DDS; H; Co;	ER, TC (III°), BC (III°)	BIP
5	Pomeranian, MC, 74	IS; Dy; EI;	ER, TC (III°), BC (II°)	TC, BIP, GD
6	Poodle, FS, 48	IS; Dy; EI;	EV	/
7	Pug, FS, 36	Dy; EI; DDS; H; Ds;	EV, SPH, LC (II°), BC (III°)	SPH
8	French Bulldog, FS, 96	IS; Dy; EI;	ED, SPH, LC (II°)	AES, SPH
9	Chihuahua, M, 84	IS; Dy; EI; DDS; H;	ER, TC (III°), BC (III°), LC (II°)	TC, BIP
10	Mongrel, M, 120	Co;	EN	/
11	Boxer, M, 24	IS; Dy; EI;	ER	/
12	Chihuahua, MC, 72	IS; Dy; EI; DDS; H; Co;	ER, TC (II°), BC (II°)	TC, GD
13	Pomeranian, M, 36	IS; Dy; EI; DDS; H;	ER	CDE, GD
14	Pomeranian, FS, 54	IS; Dy; EI; DDS; H;	ER	/
15	Mongrel, FS, 74	Dy; EI;	ED	AES
16	Pug, M, 48	IS; Dy; EI; DDS; H; Ds;	EV, BC (III°), LC (II°)	SPH, BIP, GD
17	Pomeranian, M, 12	IS; Dy; EI;	ER, LC (II°), AI	/

18	Mongrel, FS, 60	IS; Dy; EI;	EV	/
19	Poodle, F, 84	IS; Dy; EI;	ER, TC (III°)	CDE
20	Yorkshire Terrier, FS, 78	IS; Dy; EI; Co;	EV, TC (III°), BC (III°)	TC, BIP, GD
21	Pomeranian, M, 54	IS; Dy; EI;	ER	/
22	German Sheperd, FS, 108	Co;	EN	/
23	Pug, MC, 42	IS; Dy; EI; DDS; H;	ER, BC (II°), LC (II°)	SPH
24	Yorkshire Terrier, FS, 12	IS; Dy; EI;	ER, TC (IV°), LC (II°), AI	TC
25	Pomeranian, FS, 72	IS; Dy; EI;	ER, TC (II°)	CDE
26	Yorkshire Terrier, M, 24	IS; Dy; EI; Co;	ER, TC (III°)	TC, GD
27	Chihuahua, M, 42	IS; Dy; EI;	EV	CDE
28	CKCS, M, 66	IS; Dy; EI; DDS; H;	ED, SPH, LC (II°)	AES, SPH, GD
29	Chihuahua, FS, 80	IS; Dy; EI;	ER, TC (III°), BC (II°)	TC, BIP
30	French Bulldog, M, 72	IS; Dy; EI; DDS; H;	ED, LC (III°), AI	AES, GD
31	Poodle, FS, 42	IS; Dy; EI;	ED	/
32	CKCS, M, 72	IS; Dy; EI; DDS; H; Ds;	ED, SPH	AES, SPH
33	Chihuahua, M, 24	IS; Dy; EI;	ER	/
34	Mongrel, FS, 84	Dy; EI;	ER	CDE, GD
35	Mongrel, FS, 120	IS; Dy; EI;	EN	AES

AEI: Abnormal Epiglottic Shape; AI: Aritenoid Immobility; CKCS: Cavalier King Charles Sphaniel; CDE: Caudally Displaced Epiglottis; Co: Cough; DDS: Discomfort During Sleeping; BC: bronchial collapse; BIP: Bronco-interstitial pattern;; Dy: Dyspnea; Ds: Dysphagia; EI: Exercise Intolerance; ED: Epiglottic deformation; EN: Epiglottic neoplasia; ER: Epiglottic retroversion; EV: Epiglotic verticalization; GD: Gastric dilatation; H: Hypersomnolence; IS: Inspiratory stridor; LC (grade): laryngeal collapse; SPH: Soft Palate Hyperplasia; TC (grade): tracheal collapse (grade).

The most common breeds included were Pomeranian (n = 7, 20%), Chihuahua (n = 7, 20%), and Yorkshire Terrier (n = 4, 11,4%). Also, there were eight brachycephalic breed dogs: Pug (n = 3, 8.5%), CKCS (n = 2, 5.7%), French Bulldog (n = 2, 5.7%), Boxer (n = 1, 2.8%). Other breeds present were

Mongrel (n = 5, 14.2%) and German Shepherd (n = 1, 2.8%). There were 16 (45.7%) spayed females, 1 (2.8%) intact female, 15 (42.8%) intact males and 3 (8.5%) neutered males. The mean age was 66 months (12-120 months, median 61.4 months).

The most frequent pre-operative clinical signs were dysphagia (n = 33, 94.2%), exercise intolerance (n = 33, 94.2%), dyspnea (n = 33, 94.2%) and stridor (n = 30, 85,7%). Other clinical signs reported were hypersomnolence (n = 11, 33.4%), discomfort during sleeping (n = 11, 33.4%), and cough (n = 6, 17.14%). Individual pre-operative clinical signs are listed in **Table 1**.

3.3.1. *Diagnostic findings*

Epiglottic abnormalities seen during the laryngoscopy were 20 ER (57.1 %), 6 EV (17.1%), 6 ED (17.1%), and 3 EN (8.6%). Nineteen dogs (54.2%) were affected by additional respiratory abnormalities observed endoscopically: 5 dogs (26.3%) had 1 concomitant respiratory tract abnormality, 10 dogs (52.6%) had 2 concomitant respiratory tract abnormalities, and 4 dogs (21.0%) had 3 or more concomitant respiratory tract abnormalities. Additional upper and lower respiratory tract abnormalities (**Table 1**) included: tracheal collapse (n = 10, 28.6%), bronchial collapse (n = 9, 25.7%), arytenoid collapse (n = 7, 20%), soft palate hyperplasia (n = 5, 14.3%), and arytenoid immobility (n = 2, 5.7%).

Radiographic abnormal findings were recorded in 23 dogs (67.7%). These included gastric dilation (n = 8, 22.8%), tracheal collapse (n = 7, 20%), abnormal epiglottic shape (n = 6, 17.1%), caudally displaced epiglottis (n = 6, 17.1%) (**Figure 2**), broncho-interstitial pattern (n = 6, 17.1%), and soft palate hyperplasia (n = 5, 14.2%). Fluoroscopy was performed in 7 (20%) alert and awake dogs to confirm a clinical and endoscopic diagnosis of ER. In all cases, fluoroscopy was used to confirm the abnormal caudal movement of the epiglottis during inspiration. Computed tomography (CT) was obtained in 5 (14.2%) dogs with an endoscopic suspect of epiglottic neoplasia. Abnormal findings included moderately to severely deformed epiglottis in all cases (5/5, 100%), epiglottic mineralization in 4 (80%) cases, and sub-mandibular lymph node enlargement in 2 (40%) cases.

3.3.2. *Complications*

In this study, intraoperative complications occurred during 4 out of 35 (11.4%) surgeries, 2 (7.2%) during SDLE, and 2 (2.7%) during TDLE. The only intraoperative reported complication was significant bleeding. In all cases, the bleeding was controlled by grasping the vessel with an alligator forceps and cauterizing it with a bipolar electrosurgery device.

Postsurgical complications were reported in 3 (8.5%) cases post-SDLE. In this study, the only reported complication was local oedema that caused transient airway obstruction and required temporary re-intubation; all 3 dogs were regularly extubated within 2 hours after surgery. This study did not report aspiration pneumonia and temporary tracheostomy as postsurgical complications.

3.3.3. *Histological findings*

All 35 resected epiglottic specimens underwent pathological evaluation. Nonspecific findings were found in 27 (77.1%) cases (20 ER, 6 EV, 1 ED) and included subepithelial oedema (27/27, 100%), dilated lymphatics (22/27, 81.4%), mixed inflammatory infiltrate (15/27, 55.5%) and cartilage mineralization (5/27, 18.5%). Epiglottic necrosis was found in 5 (14.2%) cases of ED (**Figure 3**).

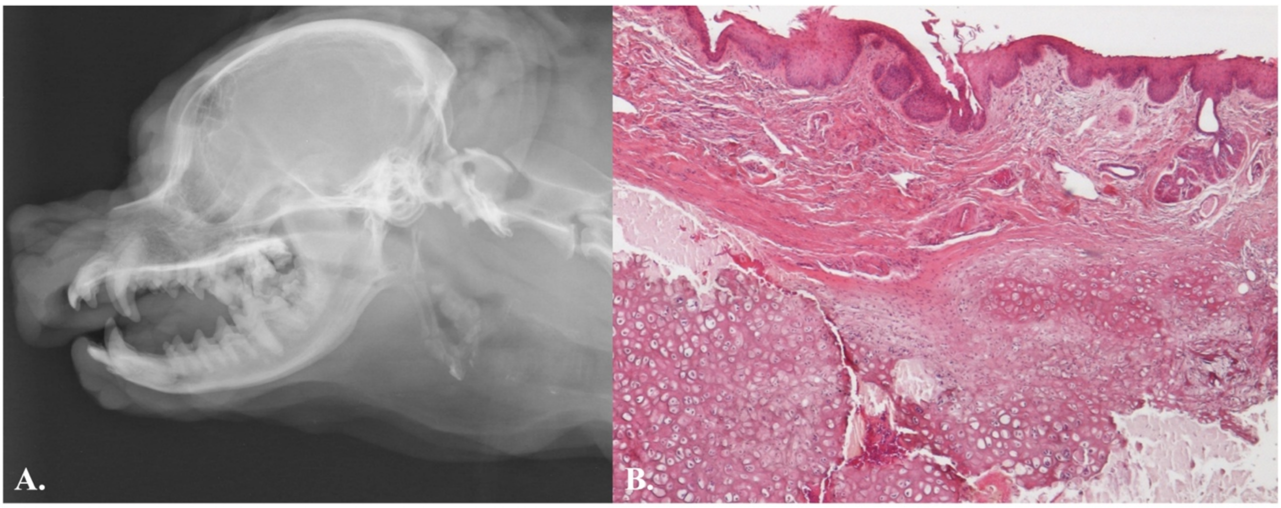

Figure 3 - Epiglottic necrosis. A) Lateral neck radiographic view reveal alterations in the shape and radiodensity of the epiglottis; B) Histological findings in hematoxylin and eosin (H&E) coloration during epiglottic necrosis.

The diagnosis of neoplasia was established in all 3 (8.5%) cases of EN; 2 were diagnosed as chondrosarcomas, and 1 identified as an undifferentiated sarcoma.

3.3.4. *Follow-up*

The median duration of follow-up is 18 months (min 3 months – max 21 months). At the time of drafting, 29 (82.8%) dogs were alive. Four dogs died of non-respiratory related conditions (2 of severe cardiac insufficiency, 1 of a road accident, 1 of adrenal gland adenocarcinoma). Two dogs died of respiratory-related conditions (severe generalized bronchial collapse and III° grade laryngeal collapse).

Of the 35 dogs that underwent DLE surgery and included in the study, 30 (85%) had an adequate outcome, 3 (8.6%) had a moderate outcome, and 2 (5.8%) had a poor outcome. No difference was observed in the short-term (1 month) and long-term (3 months) outcomes recorded during interviews with the owners at clinical examination. No differences in outcomes were reported by the owners during the telephone contacts following the examination visit performed at 3 months.

A follow-up laryngoscopy was performed in 17 (48.5%) dogs either electively (1 months after surgery) or at the time of intubation for another procedure (1 to 6 months after STLS or TDLS). In 15 cases, the larynx displayed no scar retraction, granulation tissue, or twisting, and there was no distortion other than the defect in the treated area (**Figure 4**). In 2 cases, a small area of residual epiglottic cartilage was exposed and not covered by the mucosal layer, apparently with no functional consequences.

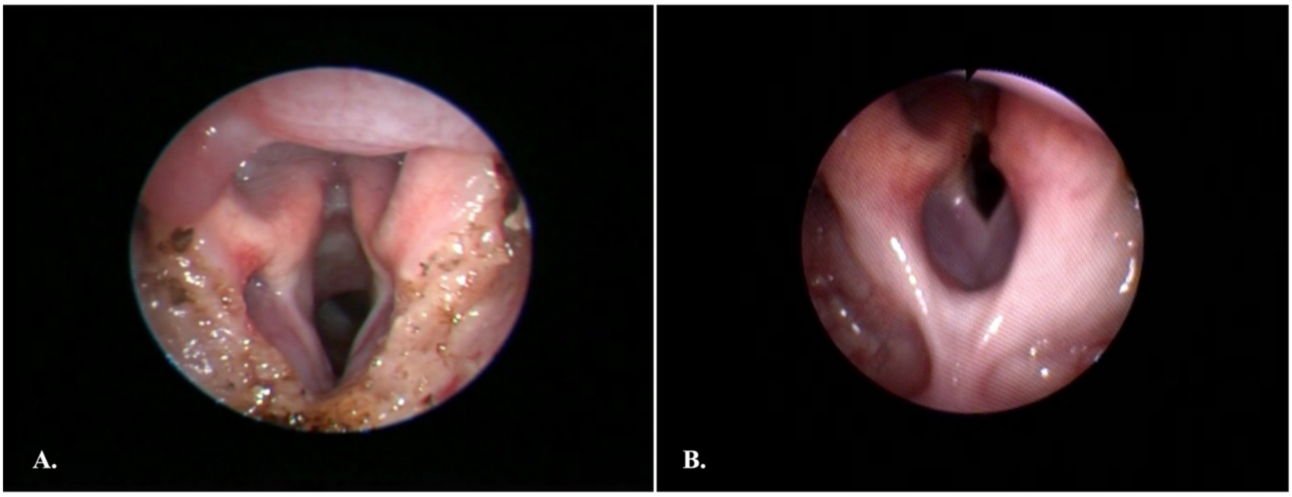

Figure 4 – Post-operatory videoendoscopic images of surgical site. A) Videoendoscopic image taken immediately after the surgery; B) Videoendoscopic image taken 1 month after surgery reveal the larynx displaying no defects in the treated area.

3.4. Discussion

To the best of our knowledge, few studies in veterinary medicine investigated video-assisted diode laser epiglottidectomy in dogs with obstructive epiglottic disease. During the 3 years of the study, 35 dogs with a diagnosis of epiglottic disease were treated with sub-total (SDLE) or total epiglottectomy (TDLE).

The exact identification of the respiratory tract obstruction site is essential in selecting the best therapeutic strategy. The epiglottis is a thin lamella of elastic cartilage shaped like a leaf. It projects obliquely behind the root of the tongue and ventral into the entrance of the larynx. The epiglottis protects the lower respiratory tract by acting as a valve during the swallowing reflex. The concurrent adduction of the arytenoid cartilages and the vocal folds assists its function. The active movement of the epiglottis while breathing is induced by the contraction of the hyoepiglottic muscle. This muscle originates from the ceratohyoid bone and inserts on the ventral aspect of the epiglottis; thus, its contraction moves the epiglottis ventrally, providing a mechanism for active control of the epiglottic position during breathing (Amis, 1998; Amis, 1996). Early research on upper airway obstructive breathing disorders largely ignored the epiglottis. However, more recent studies have shown, both in human and veterinary medicine, that the epiglottis plays a vital role on its own or in combination with other respiratory structures (Flanders, 2009; Skerrett, 2015; Mullins, 2014; Mullins, 2019).

Epiglottic retroversion (ER) was this study's most common epiglottic disorder (20/35 cases, 57.1%). The same condition, both in pediatric and in adult human patients with obstructive sleep apnea, could be the cause of severe and life-threatening complications due to associated respiratory difficulties (Zeitels, 1990). Both in dogs and humans, an unusually flaccid epiglottis (so-called "floppy epiglottis") is displaced posteriorly against the posterior pharyngeal wall during inspiration. In extreme cases, the negative pressure developed within the laryngeal inlet may cause the epiglottis to prolapse downward and inward, resulting in sub-total or total airway obstruction (Mullins, 2019; Zeitels, 1990). Despite all the cases of ER reported in this study, an abnormally flaccid epiglottis was perceived to cause respiratory symptoms, and epiglottis prolapse was not always evident during the endoscopic inspection. In some dogs, an aberrant craniocaudal movement was only detected at a light anesthesia plane, possibly due to the increased air volume and speed during inhalation if compared with a deeper anesthesia plane, as previously described in humans (Kanemaru, 2007). This could explain the intermittent clinical signs in dogs clinically normal between obstructive respiratory episodes precipitated by stress or exercise. Indeed, the most frequently recorded clinical sign in this study was exercise intolerance (33/35 cases, 94.2%). Due to intermittent symptomatology, the diagnosis of ER may be significantly underestimated. Moreover, veterinary clinicians should be aware that a missed diagnosis of ER when performing an upper airway examination could result not only from excessive tongue traction or downward pressure of the inspecting tool on the epiglottis but could be secondary to a deep anesthesia plane (Skerrett, 2015; Mullins, 2014). Fluoroscopy should be performed, in doubt, in awake dogs to confirm a clinical and endoscopic suspect of ER. In this study, fluoroscopy was performed in 7 (20%) awake dogs, and in all cases, it was possible to confirm the abnormal caudal movement of the epiglottis during the inspiratory act.

EV can be defined as the posterior displacement of the epiglottis against the posterior pharyngeal wall. This displacement is independent of the respiratory phase, and the epiglottis appears macroscopically normal. EV and ER have been considered different disorders in human medicine, and we prefer to differentiate between these two conditions (Salamanca, 2019). During the endoscopic investigation, no substantial changes in epiglottic position were noticed in correlation to the anesthesia stage in the presence of EV. Moreover, no anatomical abnormality of the epiglottis was observed in all EV cases. The causes of EV in these dogs may be challenging to determine as several possibilities exist, such as congenital abnormalities or laryngeal trauma. We hypothesize that, in some cases, the hypertrophic tongue may play a role in epiglottis verticalization. In our theory, the base of a hypertrophic tongue may exert a positive pressure on the lingual aspect of the epiglottis, displacing it posteriorly and causing epiglottic back position and subsequent verticalization, but further investigation is warranted. In our study, 2 out of the 6 cases of EV involved brachycephalic dogs, supporting our thesis but highlighting the presence of many pathogenetic causes that are still unknown. Additional hypotheses regarding the etiology of EV in dogs could include epiglottic fracture or malacia, hypothyroidism-associated peripheral neuropathy, and denervation of the hypoglossal nerve, the glossopharyngeal nerve, or both, as already suspected for ER (Mullins, 2019). The possibility that a generalized malacia is a predisposing factor for epiglottis pathologies such as EV and ER is supported by a prevalence of small breeds (Pomeranian, Chihuahua, and Yorkshire Terrier), known to be predisposed to conditions such as tracheal and bronchial collapse (Della Maggiore, 2020).

ED could be defined as a congenital or acquired anatomical alteration of the epiglottis that can obstruct the glottis. This study found 6 cases of severe epiglottic deformation, 4 of which were in brachycephalic breed dogs (CKCS and French Bulldog). The epiglottis demonstrated a markedly distorted shape in these dogs, partially obstructing the laryngeal inlet. Two cases demonstrated both left and right borders folded dorsally and axially, approximately mid-body, with the rostral portion deviated ventrally and the cartilage thickened with the micro-nodular aspect of the mucosa of the laryngeal surface of the epiglottis (**Figure 5**). In three cases, the endoscopic exam revealed a transverse kink in the mid-body of the epiglottis with the rostral portion deviated caudo-ventrally and to the left in 2 dogs and to the right in 1 dog (**Figure 5**). In the last case, the epiglottis curled on itself and was omega-shaped, probably secondary to severely shortened aryepiglottic folds (**Figure 5**). In all these cases, the apex was nodular and thickened.

Figure 5 - Endoscopic view of epiglottic deformation (ED). A) Epiglottis exhibits folding along its borders in both dorsal and axial directions. The rostral portion of the epiglottis is bent or deviated ventrally. The mucosa appears to have a micro-nodular aspect; B) Epiglottic had a transverse kink in the middle portion, and the rostral portion deviated caudo-ventrally; C) Epiglottic is folded on the same "omega"-shaped.

Histopathological examination of specimens collected from the ED of these dogs revealed tissue necrosis in 5 out of 6 cases. Epiglottic necrosis is an unusual condition rarely described in human medicine that may occur as a potential complication of epiglottitis in immunocompromised patients (Sengör, 2018). The causes of epiglottic deformity in these dogs may be difficult to determine; our theory is that the epiglottic deformation could be congenital or acquired secondary to a chronic inflammatory condition. Our 6 dogs have no medical history of immunodeficiency disease, laryngeal infection, or chronic inflammation to corroborate our theory.

Concomitant upper or lower airway disorders were diagnosed in 54.2% of cases, suggesting that epiglottic conditions are either a component of these disorders or occur secondary to chronic increased inspiratory airway pressures that may occur with these processes. Furthermore, ER, EV, and ED may represent an unrecognized component of brachycephalic airway obstructive syndrome (BAOS), or this population may be at risk for epiglottis conditions because of abnormal inspiratory pressures. However, brachycephalic breeds represented only 22.8% of this study's population; therefore, no statistical conclusions can be drawn.

In veterinary medicine, laryngeal neoplasms are rarely reported in small animals; they include epithelial and mesenchymal types, with the epiglottis as the most unusual primary neoplastic subsite (Wilson, 2002; Salk, 1986; Muraro, 2013). To the authors' knowledge, only two cases of primary malignant epiglottis neoplasms have been reported (De Lorenzi, 2015; Shoieb, 2014); in both cases, the conclusive diagnosis was low-grade chondrosarcoma microscopically characterized by well-developed chondroid lobules, which primarily originated from the elastic cartilage of the epiglottis. The inadequate number of reports regarding cartilaginous tumours of the epiglottis in veterinary medical literature imposes some diagnostic and therapeutic challenges. In human medicine, both conventional surgery and video-assisted laser epiglottidectomy are used to treat supraglottic airway obstruction secondary to neoplastic diseases (Zeitels, 1990). Following the successful outcome of previously published cases and cases included in our case series, it is concluded that with careful selection and adequate knowledge of laser surgery, laser supraglottic laryngectomy is a suitable treatment for epiglottic cancer. However, further investigation is necessary to assess the outcome in a more significant number of cases (De Lorenzi, 2015).

Various surgical techniques have been described in humans for treating benign epiglottic obstructive diseases such as ER, EV, and ED. These treatments include epiglottopexy, laser epiglottoplasty, epiglottis stiffening operation, and sub-total laser epiglottidectomy (Salamanca, 2019). In veterinary medicine, epiglottectomy and epiglottopexy have been described for treating benign epiglottic obstructive conditions (Mullins, 2014; Mullins, 2019). Total epiglottectomy, traditional and laser guidance, has also been described for the treatment of epiglottic neoplasms (De Lorenzi, 2015; Shoieb, 2014). To the best of the authors' knowledge, no surgical treatments are described for epiglottis deformation in dogs. In this study, we propose video-assisted DLE as a treatment for all obstructive diseases of the epiglottis. TDLE or SDLE was the procedure of choice for all our dogs: diode laser is easy to manipulate, shortens surgical time, and provides a high degree of precision, maintaining hemostasis and minimizing postoperative oedema. We performed the same procedure described in humans by Catalfumo et al. by resecting the suprahyoid portion of the epiglottis (Catalfumo, 1998). This procedure was safe, effective, and tolerated by all our dogs. No severe intra- or postsurgical complications have been observed. The only intraoperative reported complication was significant bleeding. The diode laser provides a dry operative field during most of the procedure. However, branches of superior laryngeal vascularization may be encountered, and to avoid significant bleeding, careful dissection along the lingual surface of the epiglottis is necessary. In 4 cases, bleeding of the superior laryngeal vessel branches was controlled by grasping them with alligator forceps and applying a coagulating electric current, with no further complications. In a retrospective study aiming to report intraoperative and major postoperative complications in dogs treated surgically for ER by comparing the incidence of major postoperative complications between procedures, authors found that although intraoperative complications were uncommon, major postoperative complications were common, especially after epiglottopexy procedures (Mullins, 2019). More specifically, major postoperative complications were documented in 22 dogs after 36 of 74 (48.7%) procedures.

Postoperative complications occurred after 7 of 12 (58.3%) non-incisional epiglottopexy, 23 of 43 (53.5%) incisional epiglottopexy, 2 of 4 (50%) partial epiglottectomy, 2 of 12 (16.7%) subtotal epiglottectomy, and 2 of 3 (66.7%) other surgical procedures. The authors concluded that surgical treatment of ER is associated with a high rate of major postoperative complications. This statement is in contrast with the results of the present study: postsurgical complications were reported in 3 cases post-SDLE, with the only reported complication being local oedema that caused transient airway obstruction and required temporary re-intubation; all 3 dogs were regularly extubated within 2 hours after surgery. The latter could be associated with a lower rate of complications from the laser technique, as proposed by the authors in the present study.

Epiglottectomy in infants and adult patients with epiglottic obstructive disease results in relief of symptoms of airway obstruction in 85-90% of cases (Holinger, 1989). This is consistent with what we observed in our study, where there was a substantial improvement in both short and long-term respiratory function without developing a swallowing disorder. To prevent misinterpretation of the dog's clinical status by the owner, improvement of respiratory function was evaluated through regular clinical visits and questionnaires administered by clinicians at each follow-up. Laryngoscopy enables a direct examination of the anatomy and dynamic movement of the epiglottis. This diagnostic technique and the histopathological evaluation of the collected specimens permitted us to obtain a better awareness of the diseases of the epiglottis. The surgical techniques described in this study have been proven effective for treating epiglottic-related airway obstruction. SDLE or TDLE with the 980 μm diode has been proven to be a safe and relatively simple procedure by a well-trained surgeon and provides a high degree of precision and hemostasis, minimizing postoperative oedema. An accurate clinical evaluation, medical history collection, and adequate diagnostic endoscopy are fundamental for properly selecting dogs for surgical procedures. The results obtained in this study are promising: 85% of the dogs had an adequate outcome. No significant respiratory discomfort or swallowing disorder was reported during the postoperative evaluation.

6. Discussion

Interventional endoscopy has emerged as a pivotal tool in both the diagnosis and treatment of various gastrointestinal, urinary and airway disorders. This advanced technique allows for minimally invasive procedures that can provide real-time diagnosis and, in many cases, immediate therapeutic interventions. This doctoral thesis reviews some diagnostic and therapeutic applications of interventional endoscopy in airway tract, highlighting its role in managing complex cases that would otherwise require more invasive surgical procedures.

Endoscopy of the airway system has widespread applications because the majority of the respiratory tract can be explored using rigid and flexible endoscopes (De Lorenzi, 2012). Endoscopy with airway sampling techniques, including forceps biopsies, trans-tracheal lavage (TTL), bronchial brushing (BB), and bronchoalveolar lavage (BAL), is frequently employed for the diagnosis of respiratory diseases in veterinary practice (Tams, 2010). Common causes of airway clinical signs include anatomical defects, infections, inflammatory conditions, and malignant airway diseases. While anatomical defects can be diagnosed visually during endoscopy, confirming whether the cause is infectious, inflammatory, or neoplastic requires cytologic, histologic and microbiologic analyses. These analyses can be performed on fluid samples that have contacted the epithelial lining (es. TTL, BAL), brush samples, fine needle aspirates (FNA), or biopsy specimens (Zhu, 2015). The primary objective of a sampling technique is to be both sensitive and specific, with minimal collateral effects for the animal. For these reasons, one of the first applications of interventional endoscopy was the sampling of biological material through minimally invasive techniques. Endobronchial biopsy using a Wang biopsy needle is an important bronchoscopy sampling technique commonly used in human medicine (Lundgren, 1983). In our prospective study, we have compared the diagnostic yield of EBNA with those of BB and Forceps Biopsy (FB) in canine tracheal and endobronchial masses or submucosal infiltrations examined by fiberoptic bronchoscopy. This is the first study to evaluate the use of EBNA during airway abnormalities in medicine veterinary. In humans, bronchoscopy is an essential procedure for definitively diagnosing lung tumors that develop in both peripheral and central locations of the bronchus and represent one of the most common fatal malignancies in adults (Govert, 1999; Jones, 2001). It is important to obtain the maximum amount of diagnostic information during each bronchoscopy procedure, as a negative result may necessitate further investigations, leading to time delays and potential discomfort for the patient. In contrast to the situation in humans, primary lung and bronchial tumors are infrequent in companion animals, with an occurrence of approximately 0.5%, which has increased at least two-fold in recent years. Metastatic lung tumors, which typically develop in the periphery of the organ as exophytic masses or, more rarely, as mucosal invasions, are more common in animals (Moulton, 1981). Although rare, exophytic masses within the airway lumen can also arise secondary to inflammation, parasitic infestation, or mycobacterium infection (De Lorenzi, 2012). In addition to the reasons listed above, in veterinary medicine, reaching a diagnosis quickly is crucial, as the ability to perform diagnostic examinations often depends on the owner's financial resources. Therefore, it is essential to use a sampling method that offers the highest levels of accuracy, sensitivity, and specificity, both in human and veterinary medicine. In our study, none of the sampling methods examined achieved 100% sensitivity, specificity, and accuracy. However, EBNA demonstrated a higher diagnostic yield compared to BB and FB. EBNA proved to be the sampling method with the greatest sensitivity and accuracy for detecting both malignant and inflammatory lesions. Additionally, the combination of EBNA with BB and FB further enhanced accuracy and sensitivity, reducing the possibility of diagnostic errors. All techniques were found to be safe for the animals, both when used individually and in combination, demonstrating the potential to maximize diagnostic power without significant adverse effects. In human medicine, some authors consider the complementary use of EBNA, BB and FB because increase the sensitive and accuracy for the diagnosis of airway diseases and cancer (Govert, 1999; Jones, 2001). Our study lays the foundation for the routine use of EBNA, either alone or in combination with other sampling methods, in the diagnosis of airway diseases in animals.

The application of stents is one of the most influential developments in interventional endoscopy, both in humans and animals. In veterinary medicine, stenting is usually performed in the respiratory and urinary tracts, though there are also cases of stenting in blood vessels or gastrointestinal structures. Stents are tubular devices made of biocompatible materials, designed to maintain the patency of tubular organs. They have become an attractive alternative to several surgical procedures due to their easy application and the reduction of side effects (Graczyk, 2023). Endoscopic-guided stent placement can be used in the gastrointestinal tract for the palliative treatment of esophageal stenosis, particularly in cases where strictures fail to respond to repeated dilation procedures. Esophageal stent can be BioDegradable Stent (BDS), Self-Expanding Metallic Stent (SEMS) and Self-Expanding Plastic Stent (SEPS). Endoscopic stent placement can be attempted to reduce recurrence rates in Refractory Benign Esophageal Strictures (RBES) and to lower the risk of perforation and laceration. Esophageal stent placement was performed under endoscopic and/or fluoroscopic guidance and left at the site of the stricture for 3-5 weeks, as previously reported in human practice. Before placement, the stricture must be dilated to the same diameter than the stent. (Bottero, 2022). Stent placement is associated with numerous short-term and long-term complications, including dissolution of the BDS, stent migration, infection, hyperplastic tissue ingrowth within the stent, and dilation of the esophagus caudal to the stent (Lam, 2013). To reduce migration rates, it is essential to know the exact dimensions (length and diameter) of the esophageal stenosis and/or to use stents with anti-migration mechanisms, and/or to suture the stent to the esophageal wall. To prevent infection due to the persistence of food between the stent and the esophageal wall, the use of a feeding tube is recommended. The complication rate with esophageal stents is high, while clinical improvement is infrequent (Bottero, 2022). The use of a removable silicone stent has been described in cats with recurrent acquired nasopharyngeal stenosis (ANS). ANS is an obstruction caused by scar tissue formation in the nasopharynx. Various treatment methods have been reported, including surgical excision of the stenotic membrane, mucosal advancement flaps, and balloon dilation. However, all of these techniques have been associated with high recurrence rates of stenosis. In cases of refractory ANS, silicone stent placement can be attempted after dilating the strictures with Kelly forceps or balloon dilation. In the latter case the procedure is monitored via the endoscope retroflexed over the soft palate. Moreover, the correct stent placement is ascertained by radiography and nasopharyngoscopy. As with treatment of RBES, the stent application is temporary and is typically removed after 3 weeks (De Lorenzi, 2015). Stent placement has also been applied as a palliative treatment, particularly in cases where an organ, such as the colon or urethra, is affected by mucosal cancer. In these cases, it can significantly alleviate clinical symptoms, such as difficulty with defecation or urination. While was reported colonoscopy placement of stent, to the best known of the author urethra stent placement in veterinary practice was made with fluoroscopic guide (Graczyk, 2023; Culp, 2011), even if their placement under endoscopic vision is possible. The use of intraluminal stents in the respiratory tract is common in dogs affected by tracheal collapse that fail to respond to medical management (Robin, 2024). Tracheal collapse in dogs is a condition primarily affecting toy and small breeds, is a common cause of cough, and in severe cases, it can lead to significant respiratory failure due to airway obstruction. The therapeutic management of affected dogs is predominantly medical, with a focus on reducing inflammation, managing cough, addressing comorbidities, and preventing trauma to the neck (Weisse, 2015; Della Maggiore, 2020). However, in cases where pharmacological treatments fail, airway patency can be restored by applying prostheses, either extra-tracheal or intra-tracheal (Weisse, 2015). Tracheal stenting can be performed using various imaging techniques such as fluoroscopy, endoscopy, or digital radiography. The types of stents that can be utilized in both human and veterinary medicine in the respiratory tract include include BDS, SEMS and silicone Dumon Stent (Weisse, 2015; Serio, 2014). In veterinary practice, the most commonly used and described type of stent in the literature is the SEMS. Our work is the first to explore the use of the Dumon silicone stent in dogs with tracheal collapse. Our results align with current literature, as the complications observed with the Dumon silicone stent, such as migration, granulation tissue formation, and deformation, are similar to those reported with SEMS in the respiratory tract and other organs. The key advantage of the Dumon stent over SEMS is that complications such as migration or deformation can be easily resolved through removal and repositioning. This makes the Dumon stent a more favorable option not only in cases of tracheal collapse in dogs but also for broader use within the respiratory tract. Our opinion reflects the position in human medicine, where the use of metal stents for benign airway diseases is not recommended. Instead, managing airway obstruction typically involves the use of Dumon silicone stents (Folch, 2018). It's crucial to highlight that the primary indication for stent placement in tracheal collapse of dog is obstructive dyspnea. Therefore, stenting is most frequently indicated for tracheomalacia of the cervical tract. In our study, most of the dogs presented with cervical tracheal collapse, either alone or in combination with thoracic or bronchial collapse. The majority of animals showed significant improvement in respiratory function following the procedure. However, medical therapy was still necessary to control airway inflammation and manage potential coughing, which could arise as a side effect of the stent or as a result of the underlying tracheomalacia. In conclusion, the stenting procedure, whether applied to the airway tracts or other organs, is a minimally invasive technique that can be highly effective for the palliative management of stenoses that do not respond to medical treatment and cause organ obstruction. However, stenting is not without potential complications, some of which can be life-threatening. Therefore, frequent clinical, endoscopic, and radiographic monitoring is essential to manage these risks and ensure the well-being of the animals.

Light Amplification by Stimulated Emission of Radiation (LASER) is a form of electromagnetic radiation produced by an emission system. This system consists of a solid or gaseous "active medium" that, when stimulated by electrical or optical energy, emits photons. These photons are then amplified, producing a concentrated and powerful beam of light. LASER emits light at a single wavelength (λ) and is composed of parallel waves that travel in synchrony through space and time. These characteristics allow the laser to concentrate a significant amount of power onto a specific point on a surface, resulting in high intensity at that precise location. The tissue alteration caused by LASER is due to the transformation of electromagnetic energy into thermal energy. The effects on tissue depend on the temperature: at 65°C, denaturation of protein components and collagen contraction occur; at

90°C, fluid evaporation leads to tissue retraction and coagulation; and when the temperature exceeds 100°C, tissue carbonization takes place. Tissue effects are also influenced by the wavelength of the LASER, the distance between the laser and the tissue, the exposition time, and the tissue's water content and pigmentation. In veterinary practice, the most commonly used LASER is the diode laser, which is transmitted via flexible quartz fiber (Sullins, 2002). This flexibility allows the laser to be used with the majority of endoscopic instruments, whether rigid or flexible, as the fiber can be inserted through the working channel of the endoscope or used coaxially with the optical system. The use of laser through an endoscope offers many advantages, including illumination and magnification of the surgical site, which enhances precision during procedures. Additionally, the endoscope's aspiration system can effectively remove laser-generated smoke, ensuring a clearer field of vision. These features contribute to safer and more efficient procedures (Sullins, 2002; Bottero, 2022). In veterinary medicine, there are numerous applications of endoscopy-guided laser technology. Many pathologies affecting the gastrointestinal, respiratory, and urinary tracts can be treated, and in some cases resolved, through laser ablation. Video-endoscopic guided laser technology is utilized in the gastrointestinal system for the treatment of neoformations in the esophagus, stomach, and colon. While the use of laser ablation in these cases is often palliative, it can lead to significant clinical improvements in animals, especially when there is an obstruction in the organ. This can help alleviate conditions such as secondary megaesophagus or the impossibility on defecation, allowing for better quality of life. Similar applications of video-assisted laser technology are also found in the urinary tract, particularly for treating obstructive neoformation. Laser therapy can be beneficial in addressing congenital anatomical defects such as ectopic ureters and ureteroceles. However, one of the significant complications associated with laser use in these conditions is the potential for organ rupture. This risk is especially pronounced when working with neoplastic tissues, where it can be challenging to predict the laser's effects on the pathological tissue (Bottero, 2022). Finally, there are numerous applications of laser endoscopy in the respiratory system, particularly in dogs affected by brachycephalic obstructive airway syndrome (BOAS). In these cases, laser applications include: staphylectomy, laryngectomy (performed in cases of grade III laryngeal collapse), turbinectomy, destruction of nasopharyngeal sialocele. Once a time, video-assisted laser techniques are utilized for the ablation of benign and malignant neoplasms within the airway tract (nasal, laryngeal and tracheal), and it also provide solutions for certain congenital anatomical defects, such as choanal atresia. The most concerning complication associated with the use of lasers in airway procedures is tissue edema, which can result from the heat generated during laser application (Bottero, 2020). To mitigate this adverse effect, it is crucial to employ several strategies:

- Cooling the tissue: applying ice water to the affected area immediately after laser surgery can help cool down the tissue and reduce edema.
- Anti-inflammatory medications: administering anti-inflammatory drugs post-surgery can further decrease inflammation and tissue swelling.
- Monitoring: close monitoring of the animal during the recovery phase is essential. This includes being vigilant for signs of obstructive edema, which may manifest as stridor and inspiratory dyspnea.
- Intubation: in cases where significant obstructive edema develops, it may become necessary to intubate the animal to ensure adequate airway patency (Bottero, 2022).

In our study, we propose diode laser epiglottidectomy (DLE), following the same procedure described by Catalfumo et al. (Catalfumo, 1998) in human medicine, which involves resecting the suprahyoid portion of the epiglottis. This approach was used to treat four obstructive epiglottic conditions in dogs: epiglottic retroversion (ER), epiglottic verticalization (EV), epiglottic deformation (ED), and epiglottic neoplasm (EN). In human medicine, video-assisted laser epiglottidectomy is considered the gold standard for addressing epiglottic airway obstructions, with an 85-90% success rate in relieving symptoms of benign obstruction (Zeitels, 1990; Golz, 2000; Kanemaru, 2007; Holinger, 1989). In veterinary medicine, subtotal epiglottidectomy and epiglottopexy have been employed to manage ER and EV, while total epiglottidectomy (both traditional and laser-assisted) has been used to treat EN (De Lorenzi, 2015; Mullins, 2014; Mullins, 2019; Shoieb, 2014). However, no surgical interventions for epiglottic deformation in dogs have been previously reported. Conventional surgical approaches for epiglottic conditions often result in significant postoperative complications, a finding that contrasts with the outcomes of our study (Mullins, 2014; Mullins, 2019). In our series, the only intraoperative complication encountered was notable bleeding from the branches of the superior laryngeal vessels, which was effectively controlled by grasping them with alligator forceps and applying electrocautery. The only postoperative complication was localized edema, which caused temporary airway obstruction, necessitating re-intubation. Preoperatively, common clinical signs included dysphagia, exercise intolerance, dyspnea, and stridor. Following DLE, we observed substantial improvements in both short- and long-term respiratory function in 85% of cases, with no instances of swallowing disorders developing. Both total DLE (TDLE) and subtotal DLE (SDLE) were effective for treating epiglottic-related airway obstruction in dogs. The diode laser is advantageous for its ease of use, reduced surgical time, precision, and the ability to maintain hemostasis while minimizing postoperative edema. These techniques have proven to be straightforward and efficient for surgeons experienced in laser and endoscopic procedures, with significantly fewer complications compared to conventional surgical treatments for epiglottic pathology.

In conclusion, the use of interventional endoscopy in the airway system offers numerous diagnostic and therapeutic applications. A clear understanding of the clinical indications for applying these techniques, along with specific training and expertise of clinicians or surgeons, is essential for performing endoscopic procedures with precision and efficiency, reducing the risk of complications. Although infrequent, complications associated with assisted endoscopic procedures are still possible. Therefore, it is crucial to be aware of the potential adverse effects associated with these procedures to enable prompt intervention should they occur. Additionally, monitoring animals during the postoperative period and conducting regular follow-ups after discharge are essential for ensuring their overall health and well-being. As the availability of endoscopic instruments for veterinarians expands, the routine use of interventional endoscopy is expected to grow. However, further studies are necessary to fully understand the potential benefits and limitations of these techniques, ensuring they are applied effectively and safely in veterinary practice.

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