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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).

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

1. A prospective comparison of fiberoptic endobronchial needle aspiration, bronchial brushing and forceps biopsy for the diagnosis of bronoscopically 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

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

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

105 In a prospective study, we compared three diagnostic techniques to obtain specimens with the flexible
106 fiberoptic bronchoscope in lower airway masses. This study aimed to compare the diagnostic yield
107 and complications of Endobronchial Wang Needle Aspiration (EBNA) with those of Bronchial
108 Brushing (BB) and Forceps Biopsy (FB) in canine tracheal and endobronchial masses (EBMs) or

109 submucosal infiltrations (SI) examined by fiberoptic bronchoscopy, with particular reference to
110 making: 1) a distinction between benign and malignant growths and 2) a correct cytologic diagnosis.

111

112 ***1.2.Materials and Methods***

113 ***1.2.1. Animals***

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

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

126 ***1.2.2. Intervention***

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

129 The bronchoscopic examination was performed in a standardized fashion with a flexible fiberoptic
130 bronchoscope (Fibroscope 60003VB, KARL STORZ, Tuttlingen, Germany) under general anesthesia
131 in all dogs: premedication with 2 µg/kg dexmedetomidine and 0.2 mg/kg methadone intramuscularly,
132 induction with 4 mg/kg of propofol administered intravenously. A 5-minute preoxygenation period
133 was used. A stable plane of anesthesia was maintained with a constant rate infusion of propofol at
134 0.1–0.4 mg/kg/min or intermittent boluses. Oxygen was delivered through the bronchoscope's

135 working channel or by use of jet ventilation. Electrocardiogram (ECG), blood pressure, and pulse
136 oximetry were constantly monitored. During recovery, supplemental oxygen was provided as needed.
137 The sequence of samples was BB, EBNA, and FB.

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

145 All Endobronchial Wang Needle Biopsies (EBNA) were obtained with a 22-gauge transbronchial
146 needle catheter (WANG® Transbronchial Cytology Needle, CONMED Corporation, Utica, NY,
147 USA). The Wang needle has an outer flexible plastic catheter, a distal 15 mm in length retractable
148 sharp beveled needle, a middle flexible catheter, a stylet, a proximal control device that manipulates
149 the movement of the needle, and a side port through which suction can be applied. The outer plastic
150 catheter has a metallic hub at the distal end to protect the endoscope's working channel from the
151 biopsy needle accidentally perforating. The middle flexible catheter transmits a negative pressure
152 from the proximal end to the distal needle while the stylet provides rigidity to the needle for successful
153 insertion through hard masses; it prevents clogging of the needle with bronchial epithelial cells with
154 submucosal masses. Due to the nature of the procedure, both catheters are flexible enough to be
155 maneuvered into more peripheral locations yet stiff enough to exert force to penetrate both soft and
156 hard masses. A specimen was obtained by using a Wang needle inserted into the fibroscope working
157 channel. To prevent damage to the working channel of the fibroscope by the needle, the fibroscope
158 was kept as straight as possible, with the distal tip in the neutral position during catheter insertion.
159 The beveled end of the needle was secured within the metal hub during its passage through the
160 working channel and advanced and locked into place only after the metallic hub was visible beyond

161 the tip of the fibroscope. Once the tip of the needle had penetrated the mass, the internal sheath was
162 removed, and rapid negative pressure was applied by aspirating with a 20-mL syringe several times.
163 After the needle was moved back and forth inside the mass, it was retrieved, and the internal sheath
164 was removed. Positive pressure was applied using an air-filled 20-mL syringe to expel the aspirated
165 material in the needle onto a glass slide, which was then smeared against another glass slide by using
166 the squash preparation technique to yield cytological smears on two glass slides, as described
167 elsewhere (De Lorenzi, 2008). The slides were air-dried and stained with MGG in an automatic slide
168 stainer (Aerospray Slide Stainer 7000, WESCOR, Logan, UT, USA). We aimed to take two aspiration
169 samples from each lesion.

170 All endobronchial Forceps Biopsies (FB) were obtained using flexible biopsy forceps (MicroTech BF
171 – 18 – 12 AC – 1, Nanjing, China) introduced into the bronchoscope's working channel. We aimed to
172 take three forceps biopsies from each lesion unless the collection of samples was limited by
173 complications such as bleeding. All histological specimens obtained were placed on biopsy sponges,
174 fixed in 10% neutral-buffered formalin, processed, and embedded in paraffin wax; 4-mm sections
175 were stained with hematoxylin and eosin (H&E), and then evaluated by a pathologist who was
176 unaware of the cytological diagnosis.

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

180 *1.2.3. Outcome measure*

181 We compared the sensitivity, specificity, positive predictive value (PPV), negative predictive value
182 (PNV), accuracy, and 95% confidence intervals (CI) of a PPV of isolated EBNA for the correct
183 distinction between benign and malignant growth and to make a correct cytological diagnosis with
184 that of BB sampling and FB. We also compared the sensitivity specificity, PPV, PNV, accuracy, and
185 95% CI of a PPV of all possible combinations of sampling (EBNA and BB; EBNA and FB; BB and
186 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 necropsy. 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

213 by granulomas and chondromas. The results of the BB, EBNA, FB, and histopathological definitive
 214 diagnosis are given in **Table 1**.

215

216 **Table 1** - BB, EBNA, and FB cytologic or histologic diagnosis, and definitive diagnosis of 21 dogs included in the
 217 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%)

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

219

220 The EBNA yielded the highest number of positive results for malignancy, followed by FB and then
 221 BB. EBNA confirmed the malignancy or benignity identified by histopathological definitive
 222 diagnosis in 19 out of 21 cases (90.48%). FB confirmed the malignancy or benignity in 17 out of 21
 223 cases (80.95%). Finally, BB confirmed the malignancy or benignity in only 11 out of 21 cases
 224 (52.38%). Similar results were obtained for the accuracy of the diagnosis; there was a difference in
 225 results between the three methods regarding tumor types or diagnosis. In 19 out of 21 cases (90.48%),
 226 EBNA agreed with the final morphologic tumor type or diagnosis. FB confirmed the final
 227 morphologic tumor type or diagnosis in 15 out of 21 cases (71.43%), while BB confirmed it in only
 228 8 out of 21 cytology samples (38.10%). **Table 2** shows the results of sensitivity, specificity, accuracy,
 229 PPV, PNV, and IC 95% together with the various techniques and combinations used.

230

231 **Table 2** - Sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (PNV) for
 232 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

233 Acc.: Accuracy; BB: Brush Cytology; ENBA: Endobronchial Wang Needle Biopsies; FB: Forceps Biopsies; IC 95%:
 234 95% Interval of Confidence; PPV: Positive Predictive Value; PNV: Negative Predictive Value; Sens.: Sensitivity;
 235 Spec.: Specificity

236

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

240

241 ***1.4.Discussion***

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

259 Bronchial brush cytology (BB) is a complementary airway diagnostic method that allows direct
260 cytological evaluation of visible endobronchial lesions. BB should be performed by gently rotating
261 and moving the brush back and forth against the airway mucosa to collect cells. Organic material
262 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

289 diagnosis. In human medicine, FB has a sensitivity of up to 74% for the diagnosis of cancer, while in
290 veterinary medicine, this data is lacking (Govert, 1999). In this study, we observed a sensitivity of
291 0.76, a specificity of 1, and an accuracy of 0.80 for FB. According to previous reports in humans, the
292 sensitivity of FB is higher when this sampling technique is used in combination with other methods,
293 particularly EBNA. As reported in the literature, FB procedures are safe diagnostic techniques, and
294 no complications were experienced in obtaining FB samples in this study.

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

312 However, EBNA was the only technique that showed greater accuracy. According to previous reports
313 in humans, our study found that using a combination of EBNA, FB, and BB is highly sensitive and
314 accurate for diagnosing airway diseases and cancer (Govert, 1999). EBNA appears to provide

315 cytological samples of diagnostic quality in our series of dogs. This is an additional advantage of
316 EBNA over FB, as it allows for obtaining a diagnostic sample through a single sampling. A
317 complication was experienced during EBNA, primarily moderate bleeding that prevented performing
318 FB. However, this complication did not result in clinical issues for the animals. EBNA is sometimes
319 associated with persistent bleeding and pneumothorax (Tams, 2010). For these reasons, the flexible
320 bronchoscope should be maintained at the sampled sites to monitor for bleeding, and the vital
321 parameters of animals should be monitored to identify signs of iatrogenic pneumothorax rapidly.

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

328 This study demonstrates that EBNA detects upper airway malignancy in many cases where
329 histopathological neoplasms are found at necropsy or surgery. It also shows that EBNA has the highest
330 sensitivity for detecting malignancy compared to BB and FB. The diagnostic power was enhanced
331 when EBNA was used in conjunction with BB and FB. Similar results were found for benign lesions.

332 In veterinary medicine, EBNA is not routinely used. To the best of the authors' knowledge, this is the
333 first study that explores the use of EBNA in airway disease in small animals. No complications were
334 found when using EBNA in combination with other complementary sampling methods; only in two
335 dogs did EBNA generate content bleeding that prevented performing FB. Although specific
336 instruments and the expertise of a skilled medical practitioner are required to use EBNA, it provides
337 the highest diagnostic accuracy with minimal risk to the animals. Even though this technique in our
338 series of dogs appears to be safe, drawing a more definitive conclusion about the safety of EBNA
339 would require future studies to include a larger number of animals. Moreover, future studies could

340 compare EBNA with other sampling methods for collecting cells in lung tumors, such as FNA
341 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

368 TC is a common cause of respiratory difficulty and cough in older small and toy-breed dogs (Chisnell,
369 2015; Weisse, 2019). Endoscopy is considered the gold standard for diagnosis of TC, detecting
370 concurrent diseases, and identifying the location, grade, and severity of TC (Della Maggiore, 2020;
371 De Lorenzi, 2012). According to the classification by Tangner and Hobson (Tangner, 1982), 4 degrees
372 of gravity are distinguished by the reduction of the tracheal luminal diameter. The treatment of TC
373 varies with the location and grade of collapse. The treatment of choice is conservative medical therapy
374 that includes weight control, avoidance of neck leads, management of comorbidities, and use of
375 various medications (antitussive agents, glucocorticoids, bronchodilators, antibiotics) (Weisse, 2019;
376 Della Maggiore, 2020; Bonagura, 2014; Buback, 1996). In patients refractory/unresponsive to
377 medical treatment or with severe (grade IV) TC, surgical treatment or placement of an intraluminal
378 stent should be attempted (Weisse, 2019; Della Maggiore, 2020; Suematsu, 2019). Common surgical
379 options include using extraluminal ring prosthetics or endoluminal stents to reestablish airway
380 patency (Weisse, 2019; Suematsu, 2019). Intratracheal stents can be distinguished into silicone and
381 self-expanding metal stents (Sun, 2008). Metal stents are associated with several side effects.
382 Potential complications include migration, stent fracture, stent collapse, stent deformation, tracheal
383 perforation, development of obstructive granulation tissue, and inflammatory and bacterial tracheitis
384 (Weisse, 2019; Della Maggiore, 2020; Sun, 2008). In human medicine, the use of metal stents for
385 benign airway disease is not recommended and managing airway obstruction involves the use of
386 Dumon silicone stents (Folch, 2018; Serio, 2014; Semaan, 2015). However, Dumon silicone stents
387 have complications, including migration, obstruction from accumulated secretions, and granulation
388 tissue growth at the proximal or distal ends (Folch, 2018). Numerous studies have evaluated the
389 efficacy and complications of tracheal metallic stents in treating TC in dogs (Weisse, 2019; Raske,
390 2018; Durant, 2012; Kim, 2008). To the authors' knowledge, there are no similar studies in reference
391 to Dumon silicone stents and only 1 study¹⁶ concerning biocompatibility and applicability in normal
392 canine trachea (Xavier, 2008).

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

396

397 **2.2.Methods**

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

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

414 **2.2.1. Dumon stent**

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



420

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

423

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

427 **2.2.2. Endoscopy, stent sizing, and placement**

428 All endoscopic procedures were performed by the same investigator (DDL). An endoscopy was per-
429 formed before stent placement to confirm the diagnosis of TC, assess its extent, and identify
430 abnormalities of the upper airway and bronchi. The patients were subjected to endoscopic study under
431 general total IV anesthesia technique. The dogs were premedicated with 2 µg/kg dexmedetomidine
432 and 0.2 mg/kg methadone IM, and anesthesia was induced with 4 mg/kg of propofol administered IV.
433 Anesthesia was maintained with propofol administered in bolus, fentanyl administered in bolus of 2
434 µg/kg, or fentanyl in constant rate infusion at a dosage of 5 µg/kg/h. Patients were placed in sternal
435 recumbency with the neck extended and support under the chin to ensure the trachea was as straight
436 as possible. The examination of the larynx and cervical trachea was performed with a 2.7 mm, 18 cm,
437 30° oblique rigid endoscope (64018 BS; Karl Storz SE and Co KG). The intrathoracic trachea and

438 bronchi examination was performed with a 5.2 mm, 85 cm flexible fiberscope (60001 VL2 Bronco-
439 Fiberscope; Karl Storz SE and Co KG).

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

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

460

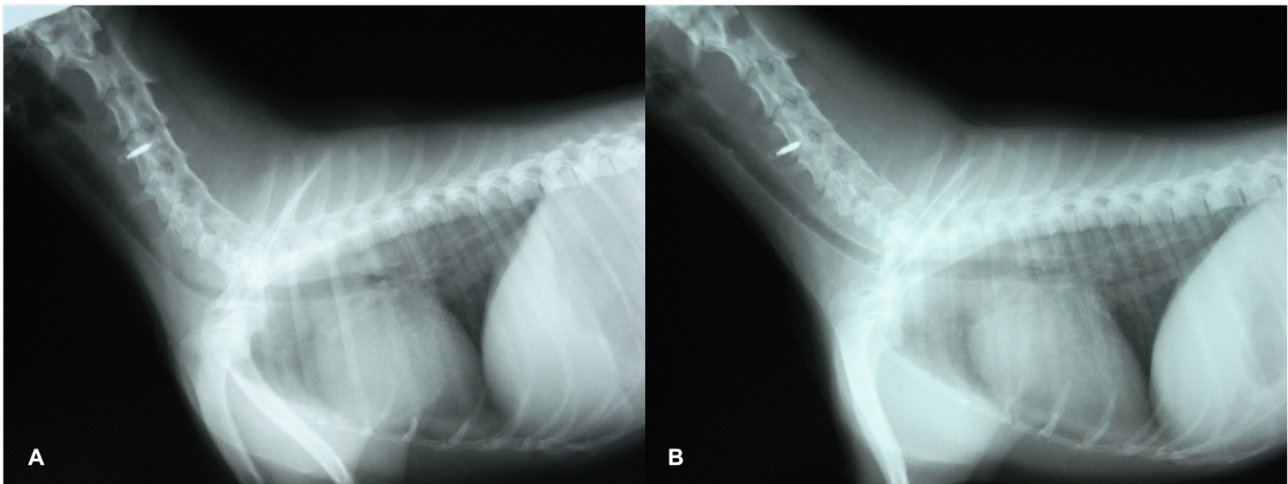


Figure 2 - Lateral thoracic radiographic images of a dog with tracheal collapse (TC) before (A) and immediately after (B) endoluminal placement of a Dumon silicone stent. A) Lateral radiographic view showing cervical TC. B) Appropriate stent placement is confirmed.

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

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

485

486 **Table 1** - Characteristics (breed, sex, age, localization of collapse, stent characteristics, follow-up, and
 487 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 († °)
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

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

490

491 Four dog breeds were represented. The most common was Yorkshire Terrier (41.7% [5/12]),
492 Pomeranian (25% [3/12]), Poodle (25% [3/12]), and Maltese (8.3% [1/12]). Males (41.7% [5/12])
493 and females (58.3% [7/12]) ranged in age from 36 to 120 months (mean, 81 months; median, 80
494 months). Three (25% [3/12]) dogs had cervical TC, 1 (8.3% [1/12]) had thoracic TC, 5 (41.7% [5/12])
495 had cervical-thoracic TC, 1 (8.3% [1/12]) had cervical TC and bronchial collapse, and 2 (16.7%
496 [2/12]) had cervical-thoracic TC and bronchial collapse.

497 **2.3.1. Stent**

498 In 4 (33.3% [4/12]) patients, stents were placed only in the cervical trachea; of these, 3 (25% [3/12])
499 dogs had cervical TC, while 1 (8.3% [1/12]) had cervical TC and a bronchial collapse. In 1 (8.3%
500 [1/12]) patient, a Dumon silicone stent was placed only in the thoracic trachea. In 7 (58.4% [7/12])
501 dogs, Dumon silicone stents were placed in the entire trachea; of these, 5 (41.7% [5/12]) patients had
502 cervical and thoracic TC, while 2 (16.7% [2/12]) had cervical and thoracic TC and bronchial collapse.
503 Dumon silicone stents had a thickness of 1.5 mm, diameter ranging from 9 to 11 mm, and length
504 ranging from 8 to 10 cm (**Table 1**). The most used stents were those with a diameter and length of 10
505 mm and 8 cm (50% [6/12]). Stents with a diameter and length of 11 mm and 8 cm (16.7% [2/12]), 9
506 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
507 also used.

508 **2.3.2. Complications**

509 No perioperative complications occurred in patients (0% [0/12]). Postoperative complications
510 occurred in 75% (9/12) of dogs (Table 1). In 58.3% (7/12) of cases, complications were associated
511 with the procedure and included the development of obstructive granulation tissue (25% [3/12]), stent
512 migration (8.3% [1/12]), stent deformation (8.3% [1/12]), and paroxysmal cough (16.7% [2/12]).
513 Tracheal contact granulomas occurred at 350, 650, and 1,310 days after placement (mean, 770 days;
514 median, 650 days) and were treated with laser surgery. Stent migration occurred 10 days after stent
515 placement, and the issue was successfully resolved by repositioning the stent and applying an
516 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

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

546 Four breeds were represented in the included dogs, and Yorkshire Terrier accounted for 47.1% (5/12)
547 and was the prevalent breed; these data were according to that reported in the literature (Weisse, 2019;
548 Becker, 2012). Age at the time of stent placement ranged between 2 and 10 years, supporting previous
549 evidence that this condition may manifest at any time with a variable rate of progression (Tangner,
550 1982). Involvement of the bronchial wall is termed bronchomalacia and is reported in 45% to 83% of
551 dogs with TC (Della Maggiore, 2020). Cervical TC presented alone (33.3% [4/12]) and in association
552 to collapse of the thoracic trachea (33.33% [4/12]) and bronchi (8.3% [1/12]). Two (16.7% [2/12])
553 dogs were affected by diffuse malacia, cervical-thoracic TC, and bronchial collapse. One dog was
554 affected by thoracic TC (8.3% [1/12]). Our information supports previous evidence that malacia can
555 affect the trachea, bronchi, or both (Della Maggiore, 2020). Cervical TC was present in 91.7% (11/12)
556 of patients included in the study, supporting the authors' opinion that endoluminal stent placement is
557 necessary when patients manifest obstructive dyspnea.

558 Endoluminal stents are medical devices for maintaining the patency of tubular organs (Stehlik, 2015).
559 Tracheal stenting can be performed using fluoroscopy, endoscopy, or digital radiography (Weisse,
560 2015). Self-expanding nitinol stents are often preferred because these devices can be placed quickly
561 and noninvasively (Weisse, 2019; Della Maggiore, 2020; Sun, 2008). Previous and numerous studies
562 described intraluminal stenting with metal stents under fluoroscopic guidance in dogs with terminal
563 TC with evidence of numerous side effects and success rates ranging from 61% to 89% (De Lorenzi,
564 2012; Raske, 2018). In human medicine, it is currently recommended to use Dumon silicone stents
565 to manage malignant and benign airway obstruction in adults and children (Serio, 2014; Semaan,
566 2015). Indeed, metallic stents are associated with more side effects compared to silicone Dumon
567 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 anesthesia 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 prosthesis (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).

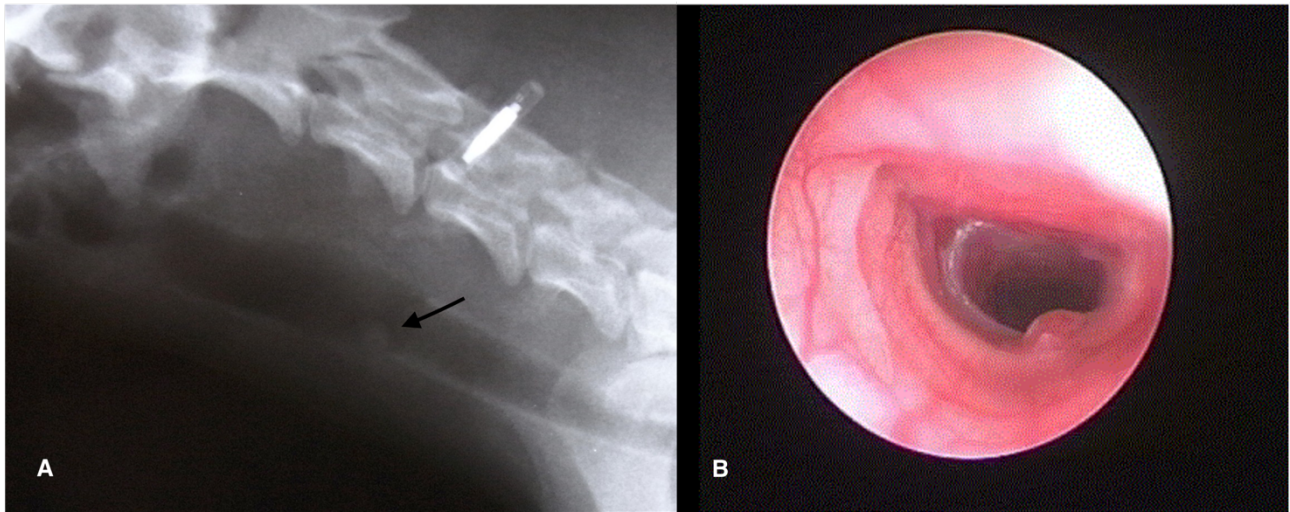


Figure 3 - Development of exuberant granulation tissue. A) Lateral thoracic radiographic view of a dog with TC after placement of an endoluminal Dumon silicone stent. Notice the granulation tissue ingrowth at the stent ends. B) Bronchoscopic view of the granulation tissue ingrowth in a trachea of a dog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

660 Our results suggest that using a Dumon silicone stent for grade IV TC is possible, effective, well
661 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

713 such as incisional or non-incisional epiglottopexy (Mullins, 2019). A high incidence of surgical
714 complications and failures was reported. In one paper, approximately one-third of a cohort of 19 dogs
715 were euthanized after surgery because of respiratory complications (Skerrett, 2015). Another study
716 regarding 50 surgically treated dogs reports that 48.7% had major postsurgical complications, and
717 24% of dogs died as a consequence of clinical conditions or were euthanized (mean 301.5 days, range
718 3-1212 days) (Mullins, 2019). In human medicine, video-assisted laser epiglottidectomy is the golden
719 standard surgical procedure for treating epiglottic-related airway obstruction, as in neoplastic or
720 chronic inflammatory diseases, and for sleep apnea. The surgery is usually well tolerated by patients,
721 with no significant postoperative complications or worsening in swallowing, breathing, or phonation
722 (Zeitels, 1990; Golz, 2000; Kanemaru, 2007).

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

727

728 ***3.2. Materials and methods***

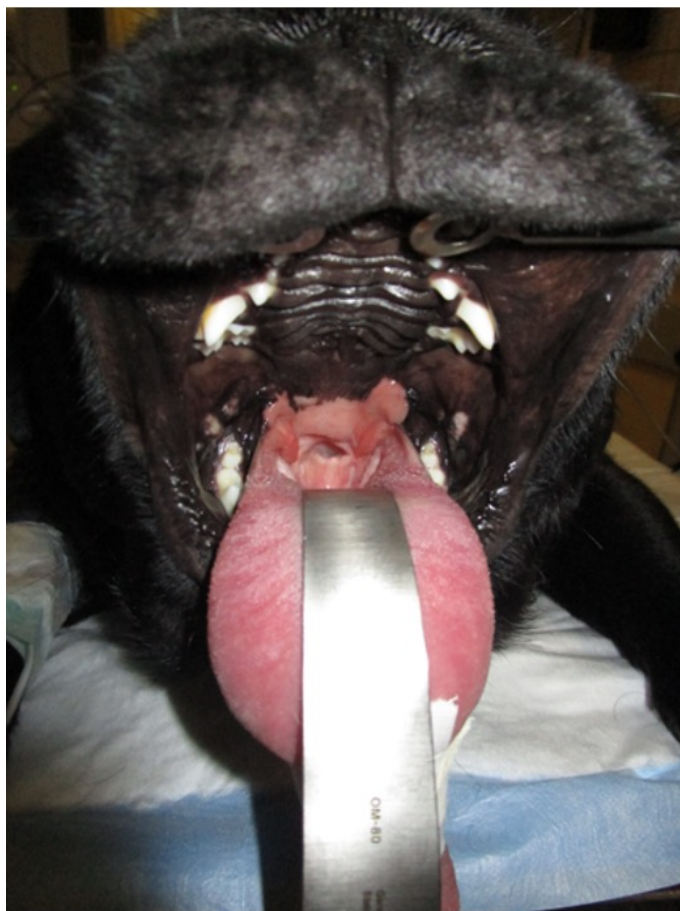
729 From January 2022 to December 2023, dogs with a history of respiratory distress and a final diagnosis
730 of epiglottic disease treated with sub-total (SDLE) or total diode laser epiglottectomy (TDLE) were
731 included in this study. When respiratory comorbidities were present, dogs were excluded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

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

738 ***3.2.1. Endoscopic procedure***

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

743



744

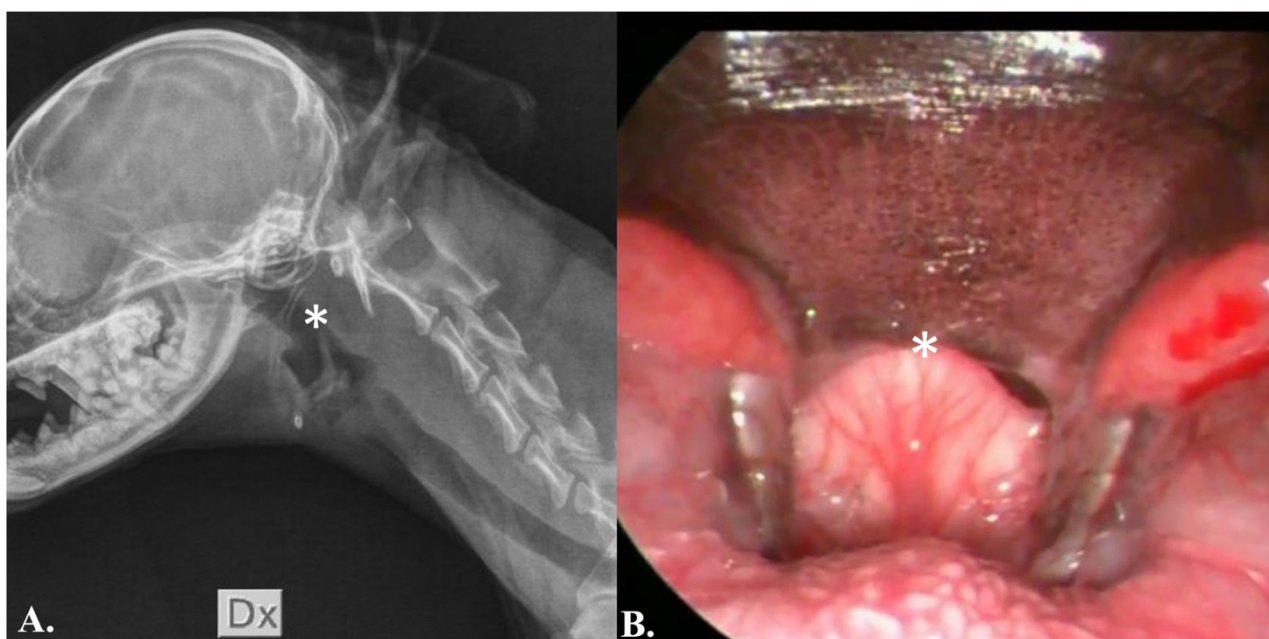
745 **Figure 1** - Patient positioning during the endoscopy and diode laser epiglottidectomy (DLE).

746

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

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

760



761

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

764

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

768 3.2.2. Surgical Procedure

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

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

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

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

794 The procedure was performed by starting from the left side of the epiglottis. The first incision was
795 performed in contact mode. The excision was accomplished after applying a traction force in the mid-
796 lateral portion of the epiglottis using a cup laryngeal forceps (Dufner 27970-01, Dufner instruments
797 GmbH, Tuttlingen, Germany). Maintaining the epiglottis in maximum traction, a U-shaped wedge

798 was incised at the level of the suprahypoid portion of the epiglottis if SDLE was performed. When
799 TDLE was performed, the whole cartilage, including the epiglottic stalk (petiolus epiglottidis), was
800 removed by resecting it from the dorso-caudal surface of the thyroid cartilage. The diode laser was
801 used to cut through both aryepiglottic folds and to incise the lateral edge of the epiglottis. These
802 incisions were then united across the vallecular surface of the epiglottis, resulting in an *en bloc*
803 excision of the cartilage. Careful dissection along the lingual surface of the epiglottis is necessary to
804 avoid significant bleeding.

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

815 3.2.3. *Histological findings*

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

822 3.2.4. *Follow-up*

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

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

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

842 3.2.5. *Statistical analysis*

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

845

846 3.3. *Results*

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

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

851

852 **Table 1** - Signalment (breed, sex, age), pre-operative clinical signs, and diagnostic (endoscopic and radiographic)
 853 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

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

859

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

863 Mongrel (n = 5, 14.2%) and German Shepherd (n = 1, 2.8%). There were 16 (45.7%) spayed females,
864 1 (2.8%) intact female, 15 (42.8%) intact males and 3 (8.5%) neutered males. The mean age was 66
865 months (12-120 months, median 61.4 months).

866 The most frequent pre-operative clinical signs were dysphagia (n = 33, 94.2%), exercise intolerance
867 (n = 33, 94.2%), dyspnea (n = 33, 94.2%) and stridor (n = 30, 85.7%). Other clinical signs reported
868 were hypersomnolence (n = 11, 33.4%), discomfort during sleeping (n = 11, 33.4%), and cough (n =
869 6, 17.14%). Individual pre-operative clinical signs are listed in **Table 1**.

870 **3.3.1. Diagnostic findings**

871 Epiglottic abnormalities seen during the laryngoscopy were 20 ER (57.1 %), 6 EV (17.1%), 6 ED
872 (17.1%), and 3 EN (8.6%). Nineteen dogs (54.2%) were affected by additional respiratory
873 abnormalities observed endoscopically: 5 dogs (26.3%) had 1 concomitant respiratory tract
874 abnormality, 10 dogs (52.6%) had 2 concomitant respiratory tract abnormalities, and 4 dogs (21.0%)
875 had 3 or more concomitant respiratory tract abnormalities. Additional upper and lower respiratory
876 tract abnormalities (**Table 1**) included: tracheal collapse (n = 10, 28.6%), bronchial collapse (n = 9,
877 25.7%), arytenoid collapse (n = 7, 20%), soft palate hyperplasia (n = 5, 14.3%), and arytenoid
878 immobility (n = 2, 5.7%).

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

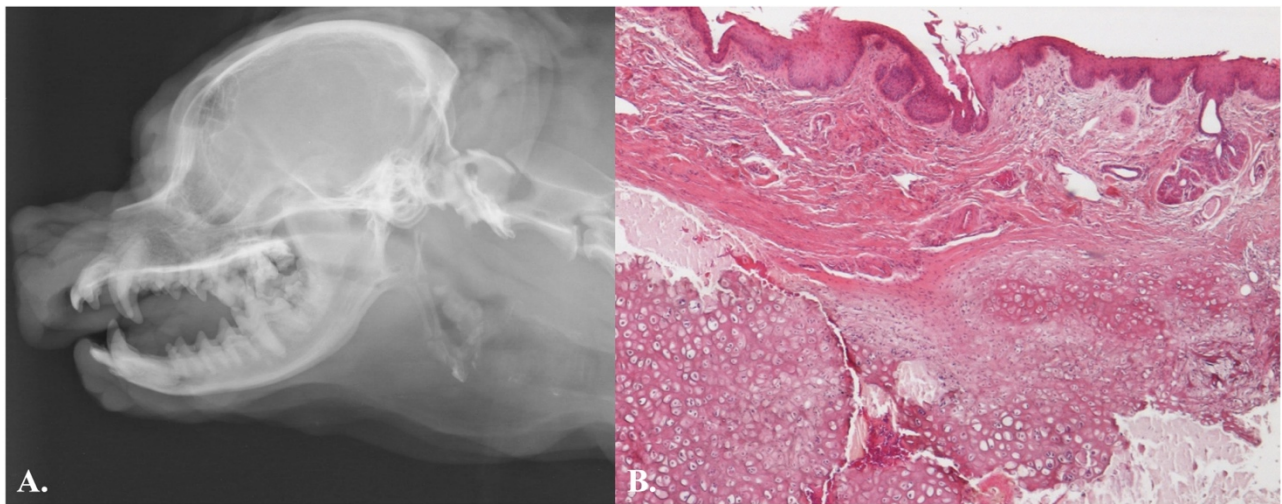
888 **3.3.2. Complications**

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

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

897 **3.3.3. Histological findings**

898 All 35 resected epiglottic specimens underwent pathological evaluation. Nonspecific findings were
899 found in 27 (77.1%) cases (20 ER, 6 EV, 1 ED) and included subepithelial oedema (27/27, 100%),
900 dilated lymphatics (22/27, 81.4%), mixed inflammatory infiltrate (15/27, 55.5%) and cartilage
901 mineralization (5/27, 18.5%). Epiglottic necrosis was found in 5 (14.2%) cases of ED (**Figure 3**).
902



903
904 **Figure 3 - Epiglottic necrosis.** A) Lateral neck radiographic view reveal alterations in the shape and radiodensity of the epiglottis; B)
905 Histological findings in hematoxylin and eosin (H&E) coloration during epiglottic necrosis.

906

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

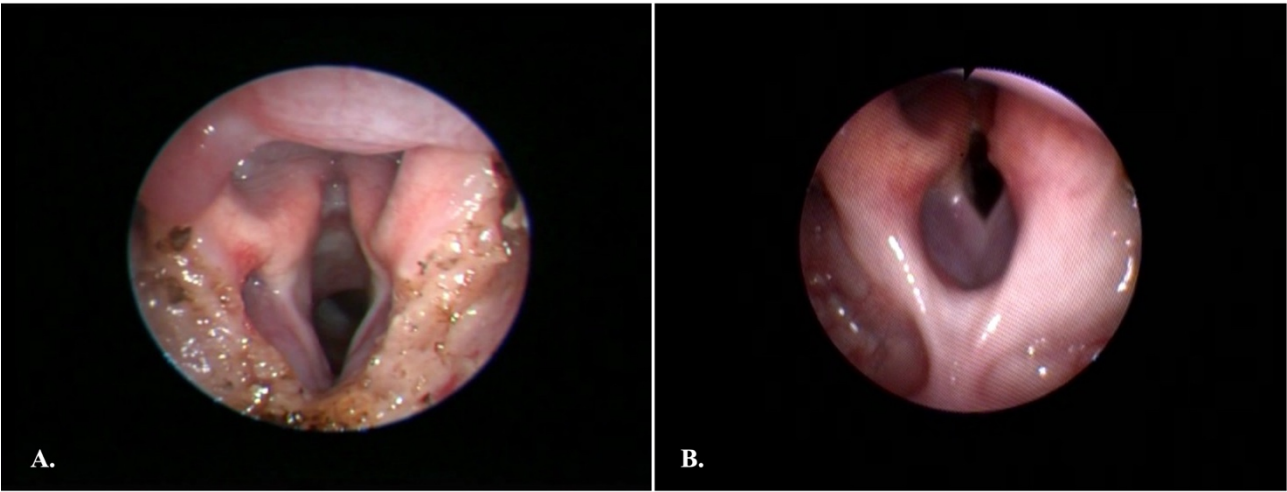
909 **3.3.4. Follow-up**

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

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

920 A follow-up laryngoscopy was performed in 17 (48.5%) dogs either electively (1 months after
921 surgery) or at the time of intubation for another procedure (1 to 6 months after STLS or TDLS). In
922 15 cases, the larynx displayed no scar retraction, granulation tissue, or twisting, and there was no
923 distortion other than the defect in the treated area (**Figure 4**). In 2 cases, a small area of residual
924 epiglottic cartilage was exposed and not covered by the mucosal layer, apparently with no functional
925 consequences.

926



927

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

930

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

957 endoscopic inspection. In some dogs, an aberrant craniocaudal movement was only detected at a light
958 anesthesia plane, possibly due to the increased air volume and speed during inhalation if compared
959 with a deeper anesthesia plane, as previously described in humans (Kanemaru, 2007). This could
960 explain the intermittent clinical signs in dogs clinically normal between obstructive respiratory
961 episodes precipitated by stress or exercise. Indeed, the most frequently recorded clinical sign in this
962 study was exercise intolerance (33/35 cases, 94.2%). Due to intermittent symptomatology, the
963 diagnosis of ER may be significantly underestimated. Moreover, veterinary clinicians should be
964 aware that a missed diagnosis of ER when performing an upper airway examination could result not
965 only from excessive tongue traction or downward pressure of the inspecting tool on the epiglottis but
966 could be secondary to a deep anesthesia plane (Skerrett, 2015; Mullins, 2014). Fluoroscopy should
967 be performed, in doubt, in awake dogs to confirm a clinical and endoscopic suspect of ER. In this
968 study, fluoroscopy was performed in 7 (20%) awake dogs, and in all cases, it was possible to confirm
969 the abnormal caudal movement of the epiglottis during the inspiratory act.

970 EV can be defined as the posterior displacement of the epiglottis against the posterior pharyngeal
971 wall. This displacement is independent of the respiratory phase, and the epiglottis appears
972 macroscopically normal. EV and ER have been considered different disorders in human medicine,
973 and we prefer to differentiate between these two conditions (Salamanca, 2019). During the endoscopic
974 investigation, no substantial changes in epiglottic position were noticed in correlation to the
975 anesthesia stage in the presence of EV. Moreover, no anatomical abnormality of the epiglottis was
976 observed in all EV cases. The causes of EV in these dogs may be challenging to determine as several
977 possibilities exist, such as congenital abnormalities or laryngeal trauma. We hypothesize that, in some
978 cases, the hypertrophic tongue may play a role in epiglottis verticalization. In our theory, the base of
979 a hypertrophic tongue may exert a positive pressure on the lingual aspect of the epiglottis, displacing
980 it posteriorly and causing epiglottic back position and subsequent verticalization, but further
981 investigation is warranted. In our study, 2 out of the 6 cases of EV involved brachycephalic dogs,
982 supporting our thesis but highlighting the presence of many pathogenetic causes that are still

983 unknown. Additional hypotheses regarding the etiology of EV in dogs could include epiglottic
984 fracture or malacia, hypothyroidism-associated peripheral neuropathy, and denervation of the
985 hypoglossal nerve, the glossopharyngeal nerve, or both, as already suspected for ER (Mullins, 2019).
986 The possibility that a generalized malacia is a predisposing factor for epiglottis pathologies such as
987 EV and ER is supported by a prevalence of small breeds (Pomeranian, Chihuahua, and Yorkshire
988 Terrier), known to be predisposed to conditions such as tracheal and bronchial collapse (Della
989 Maggiore, 2020).

990 ED could be defined as a congenital or acquired anatomical alteration of the epiglottis that can
991 obstruct the glottis. This study found 6 cases of severe epiglottic deformation, 4 of which were in
992 brachycephalic breed dogs (CKCS and French Bulldog). The epiglottis demonstrated a markedly
993 distorted shape in these dogs, partially obstructing the laryngeal inlet. Two cases demonstrated both
994 left and right borders folded dorsally and axially, approximately mid-body, with the rostral portion
995 deviated ventrally and the cartilage thickened with the micro-nodular aspect of the mucosa of the
996 laryngeal surface of the epiglottis (**Figure 5**). In three cases, the endoscopic exam revealed a
997 transverse kink in the mid-body of the epiglottis with the rostral portion deviated caudo-ventrally and
998 to the left in 2 dogs and to the right in 1 dog (**Figure 5**). In the last case, the epiglottis curled on itself
999 and was omega-shaped, probably secondary to severely shortened aryepiglottic folds (**Figure 5**). In
1000 all these cases, the apex was nodular and thickened.



1001
1002 **Figure 5** - Endoscopic view of epiglottic deformation (ED). A) Epiglottis exhibits folding along its borders in both dorsal and axial
1003 directions. The rostral portion of the epiglottis is bent or deviated ventrally. The mucosa appears to have a micro-nodular aspect; B)
1004 Epiglottic had a transverse kink in the middle portion, and the rostral portion deviated caudo-ventrally; C) Epiglottic is folded on the
1005 same "omega"-shaped.

1006

1007 Histopathological examination of specimens collected from the ED of these dogs revealed tissue
1008 necrosis in 5 out of 6 cases. Epiglottic necrosis is an unusual condition rarely described in human
1009 medicine that may occur as a potential complication of epiglottitis in immunocompromised patients
1010 (Sengör, 2018). The causes of epiglottic deformity in these dogs may be difficult to determine; our
1011 theory is that the epiglottic deformation could be congenital or acquired secondary to a chronic
1012 inflammatory condition. Our 6 dogs have no medical history of immunodeficiency disease, laryngeal
1013 infection, or chronic inflammation to corroborate our theory.

1014 Concomitant upper or lower airway disorders were diagnosed in 54.2% of cases, suggesting that
1015 epiglottic conditions are either a component of these disorders or occur secondary to chronic
1016 increased inspiratory airway pressures that may occur with these processes. Furthermore, ER, EV,
1017 and ED may represent an unrecognized component of brachycephalic airway obstructive syndrome
1018 (BAOS), or this population may be at risk for epiglottis conditions because of abnormal inspiratory
1019 pressures. However, brachycephalic breeds represented only 22.8% of this study's population;
1020 therefore, no statistical conclusions can be drawn.

1021 In veterinary medicine, laryngeal neoplasms are rarely reported in small animals; they include
1022 epithelial and mesenchymal types, with the epiglottis as the most unusual primary neoplastic subsite
1023 (Wilson, 2002; Salk, 1986; Muraro, 2013). To the authors' knowledge, only two cases of primary
1024 malignant epiglottis neoplasms have been reported (De Lorenzi, 2015; Shoieb, 2014); in both cases,
1025 the conclusive diagnosis was low-grade chondrosarcoma microscopically characterized by well-
1026 developed chondroid lobules, which primarily originated from the elastic cartilage of the epiglottis.

1027 The inadequate number of reports regarding cartilaginous tumours of the epiglottis in veterinary
1028 medical literature imposes some diagnostic and therapeutic challenges. In human medicine, both
1029 conventional surgery and video-assisted laser epiglottidectomy are used to treat supraglottic airway
1030 obstruction secondary to neoplastic diseases (Zeitels, 1990). Following the successful outcome of
1031 previously published cases and cases included in our case series, it is concluded that with careful
1032 selection and adequate knowledge of laser surgery, laser supraglottic laryngectomy is a suitable

1033 treatment for epiglottic cancer. However, further investigation is necessary to assess the outcome in
1034 a more significant number of cases (De Lorenzi, 2015).

1035 Various surgical techniques have been described in humans for treating benign epiglottic obstructive
1036 diseases such as ER, EV, and ED. These treatments include epiglottopexy, laser epiglottoplasty,
1037 epiglottis stiffening operation, and sub-total laser epiglottidectomy (Salamanca, 2019). In veterinary
1038 medicine, epiglottectomy and epiglottopexy have been described for treating benign epiglottic
1039 obstructive conditions (Mullins, 2014; Mullins, 2019). Total epiglottectomy, traditional and laser
1040 guidance, has also been described for the treatment of epiglottic neoplasms (De Lorenzi, 2015;
1041 Shoieb, 2014). To the best of the authors' knowledge, no surgical treatments are described for
1042 epiglottis deformation in dogs. In this study, we propose video-assisted DLE as a treatment for all
1043 obstructive diseases of the epiglottis. TDLE or SDLE was the procedure of choice for all our dogs:
1044 diode laser is easy to manipulate, shortens surgical time, and provides a high degree of precision,
1045 maintaining hemostasis and minimizing postoperative oedema. We performed the same procedure
1046 described in humans by Catalfumo et al. by resecting the suprahyoid portion of the epiglottis
1047 (Catalfumo, 1998). This procedure was safe, effective, and tolerated by all our dogs. No severe intra-
1048 or postsurgical complications have been observed. The only intraoperative reported complication was
1049 significant bleeding. The diode laser provides a dry operative field during most of the procedure.
1050 However, branches of superior laryngeal vascularization may be encountered, and to avoid significant
1051 bleeding, careful dissection along the lingual surface of the epiglottis is necessary. In 4 cases, bleeding
1052 of the superior laryngeal vessel branches was controlled by grasping them with alligator forceps and
1053 applying a coagulating electric current, with no further complications. In a retrospective study aiming
1054 to report intraoperative and major postoperative complications in dogs treated surgically for ER by
1055 comparing the incidence of major postoperative complications between procedures, authors found
1056 that although intraoperative complications were uncommon, major postoperative complications were
1057 common, especially after epiglottopexy procedures (Mullins, 2019). More specifically, major
1058 postoperative complications were documented in 22 dogs after 36 of 74 (48.7%) procedures.

1059 Postoperative complications occurred after 7 of 12 (58.3%) non-incisional epiglottopexy, 23 of 43
1060 (53.5%) incisional epiglottopexy, 2 of 4 (50%) partial epiglottectomy, 2 of 12 (16.7%) subtotal
1061 epiglottectomy, and 2 of 3 (66.7%) other surgical procedures. The authors concluded that surgical
1062 treatment of ER is associated with a high rate of major postoperative complications. This statement
1063 is in contrast with the results of the present study: postsurgical complications were reported in 3 cases
1064 post-SDLE, with the only reported complication being local oedema that caused transient airway
1065 obstruction and required temporary re-intubation; all 3 dogs were regularly extubated within 2 hours
1066 after surgery. The latter could be associated with a lower rate of complications from the laser
1067 technique, as proposed by the authors in the present study.

1068 Epiglottectomy in infants and adult patients with epiglottic obstructive disease results in relief of
1069 symptoms of airway obstruction in 85-90% of cases (Holinger, 1989). This is consistent with what
1070 we observed in our study, where there was a substantial improvement in both short and long-term
1071 respiratory function without developing a swallowing disorder. To prevent misinterpretation of the
1072 dog's clinical status by the owner, improvement of respiratory function was evaluated through regular
1073 clinical visits and questionnaires administered by clinicians at each follow-up. Laryngoscopy enables
1074 a direct examination of the anatomy and dynamic movement of the epiglottis. This diagnostic
1075 technique and the histopathological evaluation of the collected specimens permitted us to obtain a
1076 better awareness of the diseases of the epiglottis. The surgical techniques described in this study have
1077 been proven effective for treating epiglottic-related airway obstruction. SDLE or TDLE with the 980
1078 μm diode has been proven to be a safe and relatively simple procedure by a well-trained surgeon and
1079 provides a high degree of precision and hemostasis, minimizing postoperative oedema. An accurate
1080 clinical evaluation, medical history collection, and adequate diagnostic endoscopy are fundamental
1081 for properly selecting dogs for surgical procedures. The results obtained in this study are promising:
1082 85% of the dogs had an adequate outcome. No significant respiratory discomfort or swallowing
1083 disorder was reported during the postoperative evaluation.

1084 **6. Discussion**

1085

1086 Interventional endoscopy has emerged as a pivotal tool in both the diagnosis and treatment of various
1087 gastrointestinal, urinary and airway disorders. This advanced technique allows for minimally invasive
1088 procedures that can provide real-time diagnosis and, in many cases, immediate therapeutic
1089 interventions. This doctoral thesis reviews some diagnostic and therapeutic applications of
1090 interventional endoscopy in airway tract, highlighting its role in managing complex cases that would
1091 otherwise require more invasive surgical procedures.

1092 Endoscopy of the airway system has widespread applications because the majority of the respiratory
1093 tract can be explored using rigid and flexible endoscopes (De Lorenzi, 2012). Endoscopy with airway
1094 sampling techniques, including forceps biopsies, trans-tracheal lavage (TTL), bronchial brushing
1095 (BB), and bronchoalveolar lavage (BAL), is frequently employed for the diagnosis of respiratory
1096 diseases in veterinary practice (Tams, 2010). Common causes of airway clinical signs include
1097 anatomical defects, infections, inflammatory conditions, and malignant airway diseases. While
1098 anatomical defects can be diagnosed visually during endoscopy, confirming whether the cause is
1099 infectious, inflammatory, or neoplastic requires cytologic, histologic and microbiologic analyses.
1100 These analyses can be performed on fluid samples that have contacted the epithelial lining (es. TTL,
1101 BAL), brush samples, fine needle aspirates (FNA), or biopsy specimens (Zhu, 2015). The primary
1102 objective of a sampling technique is to be both sensitive and specific, with minimal collateral effects
1103 for the animal. For these reasons, one of the first applications of interventional endoscopy was the
1104 sampling of biological material through minimally invasive techniques. Endobronchial biopsy using
1105 a Wang biopsy needle is an important bronchoscopy sampling technique commonly used in human
1106 medicine (Lundgren, 1983). In our prospective study, we have compared the diagnostic yield of
1107 EBNA with those of BB and Forceps Biopsy (FB) in canine tracheal and endobronchial masses or
1108 submucosal infiltrations examined by fiberoptic bronchoscopy. This is the first study to evaluate the
1109 use of EBNA during airway abnormalities in medicine veterinary. In humans, bronchoscopy is an

1110 essential procedure for definitively diagnosing lung tumors that develop in both peripheral and central
1111 locations of the bronchus and represent one of the most common fatal malignancies in adults (Govert,
1112 1999; Jones, 2001). It is important to obtain the maximum amount of diagnostic information during
1113 each bronchoscopy procedure, as a negative result may necessitate further investigations, leading to
1114 time delays and potential discomfort for the patient. In contrast to the situation in humans, primary
1115 lung and bronchial tumors are infrequent in companion animals, with an occurrence of approximately
1116 0.5%, which has increased at least two-fold in recent years. Metastatic lung tumors, which typically
1117 develop in the periphery of the organ as exophytic masses or, more rarely, as mucosal invasions, are
1118 more common in animals (Moulton, 1981). Although rare, exophytic masses within the airway lumen
1119 can also arise secondary to inflammation, parasitic infestation, or mycobacterium infection (De
1120 Lorenzi, 2012). In addition to the reasons listed above, in veterinary medicine, reaching a diagnosis
1121 quickly is crucial, as the ability to perform diagnostic examinations often depends on the owner's
1122 financial resources. Therefore, it is essential to use a sampling method that offers the highest levels
1123 of accuracy, sensitivity, and specificity, both in human and veterinary medicine. In our study, none of
1124 the sampling methods examined achieved 100% sensitivity, specificity, and accuracy. However,
1125 EBNA demonstrated a higher diagnostic yield compared to BB and FB. EBNA proved to be the
1126 sampling method with the greatest sensitivity and accuracy for detecting both malignant and
1127 inflammatory lesions. Additionally, the combination of EBNA with BB and FB further enhanced
1128 accuracy and sensitivity, reducing the possibility of diagnostic errors. All techniques were found to
1129 be safe for the animals, both when used individually and in combination, demonstrating the potential
1130 to maximize diagnostic power without significant adverse effects. In human medicine, some authors
1131 consider the complementary use of EBNA, BB and FB because increase the sensitive and accuracy
1132 for the diagnosis of airway diseases and cancer (Govert, 1999; Jones, 2001). Our study lays the
1133 foundation for the routine use of EBNA, either alone or in combination with other sampling methods,
1134 in the diagnosis of airway diseases in animals.

1135 The application of stents is one of the most influential developments in interventional endoscopy,
1136 both in humans and animals. In veterinary medicine, stenting is usually performed in the respiratory
1137 and urinary tracts, though there are also cases of stenting in blood vessels or gastrointestinal
1138 structures. Stents are tubular devices made of biocompatible materials, designed to maintain the
1139 patency of tubular organs. They have become an attractive alternative to several surgical procedures
1140 due to their easy application and the reduction of side effects (Graczyk, 2023). Endoscopic-guided
1141 stent placement can be used in the gastrointestinal tract for the palliative treatment of esophageal
1142 stenosis, particularly in cases where strictures fail to respond to repeated dilation procedures.
1143 Esophageal stent can be BioDegradable Stent (BDS), Self-Expanding Metallic Stent (SEMS) and
1144 Self-Expanding Plastic Stent (SEPS). Endoscopic stent placement can be attempted to reduce
1145 recurrence rates in Refractory Benign Esophageal Strictures (RBES) and to lower the risk of
1146 perforation and laceration. Esophageal stent placement was performed under endoscopic and/or
1147 fluoroscopic guidance and left at the site of the stricture for 3-5 weeks, as previously reported in
1148 human practice. Before placement, the stricture must be dilated to the same diameter than the stent.
1149 (Bottero, 2022). Stent placement is associated with numerous short-term and long-term
1150 complications, including dissolution of the BDS, stent migration, infection, hyperplastic tissue
1151 ingrowth within the stent, and dilation of the esophagus caudal to the stent (Lam, 2013). To reduce
1152 migration rates, it is essential to know the exact dimensions (length and diameter) of the esophageal
1153 stenosis and/or to use stents with anti-migration mechanisms, and/or to suture the stent to the
1154 esophageal wall. To prevent infection due to the persistence of food between the stent and the
1155 esophageal wall, the use of a feeding tube is recommended. The complication rate with esophageal
1156 stents is high, while clinical improvement is infrequent (Bottero, 2022). The use of a removable
1157 silicone stent has been described in cats with recurrent acquired nasopharyngeal stenosis (ANS). ANS
1158 is an obstruction caused by scar tissue formation in the nasopharynx. Various treatment methods have
1159 been reported, including surgical excision of the stenotic membrane, mucosal advancement flaps, and
1160 balloon dilation. However, all of these techniques have been associated with high recurrence rates of

1161 stenosis. In cases of refractory ANS, silicone stent placement can be attempted after dilating the
1162 strictures with Kelly forceps or balloon dilation. In the latter case the procedure is monitored via the
1163 endoscope retroflexed over the soft palate. Moreover, the correct stent placement is ascertained by
1164 radiography and nasopharyngoscopy. As with treatment of RBES, the stent application is temporary
1165 and is typically removed after 3 weeks (De Lorenzi, 2015). Stent placement has also been applied as
1166 a palliative treatment, particularly in cases where an organ, such as the colon or urethra, is affected
1167 by mucosal cancer. In these cases, it can significantly alleviate clinical symptoms, such as difficulty
1168 with defecation or urination. While was reported colonoscopy placement of stent, to the best known
1169 of the author urethra stent placement in veterinary practice was made with fluoroscopic guide
1170 (Graczyk, 2023; Culp, 2011), even if their placement under endoscopic vision is possible. The use of
1171 intraluminal stents in the respiratory tract is common in dogs affected by tracheal collapse that fail to
1172 respond to medical management (Robin, 2024). Tracheal collapse in dogs is a condition primarily
1173 affecting toy and small breeds, is a common cause of cough, and in severe cases, it can lead to
1174 significant respiratory failure due to airway obstruction. The therapeutic management of affected dogs
1175 is predominantly medical, with a focus on reducing inflammation, managing cough, addressing
1176 comorbidities, and preventing trauma to the neck (Weisse, 2015; Della Maggiore, 2020). However,
1177 in cases where pharmacological treatments fail, airway patency can be restored by applying
1178 prostheses, either extra-tracheal or intra-tracheal (Weisse, 2015). Tracheal stenting can be performed
1179 using various imaging techniques such as fluoroscopy, endoscopy, or digital radiography. The types
1180 of stents that can be utilized in both human and veterinary medicine in the respiratory tract include
1181 include BDS, SEMS and silicone Dumon Stent (Weisse, 2015; Serio, 2014). In veterinary practice,
1182 the most commonly used and described type of stent in the literature is the SEMS. Our work is the
1183 first to explore the use of the Dumon silicone stent in dogs with tracheal collapse. Our results align
1184 with current literature, as the complications observed with the Dumon silicone stent, such as
1185 migration, granulation tissue formation, and deformation, are similar to those reported with SEMS in
1186 the respiratory tract and other organs. The key advantage of the Dumon stent over SEMS is that

1187 complications such as migration or deformation can be easily resolved through removal and
1188 repositioning. This makes the Dumon stent a more favorable option not only in cases of tracheal
1189 collapse in dogs but also for broader use within the respiratory tract. Our opinion reflects the position
1190 in human medicine, where the use of metal stents for benign airway diseases is not recommended.
1191 Instead, managing airway obstruction typically involves the use of Dumon silicone stents (Folch,
1192 2018). It's crucial to highlight that the primary indication for stent placement in tracheal collapse of
1193 dog is obstructive dyspnea. Therefore, stenting is most frequently indicated for tracheomalacia of the
1194 cervical tract. In our study, most of the dogs presented with cervical tracheal collapse, either alone or
1195 in combination with thoracic or bronchial collapse. The majority of animals showed significant
1196 improvement in respiratory function following the procedure. However, medical therapy was still
1197 necessary to control airway inflammation and manage potential coughing, which could arise as a side
1198 effect of the stent or as a result of the underlying tracheomalacia. In conclusion, the stenting
1199 procedure, whether applied to the airway tracts or other organs, is a minimally invasive technique
1200 that can be highly effective for the palliative management of stenoses that do not respond to medical
1201 treatment and cause organ obstruction. However, stenting is not without potential complications,
1202 some of which can be life-threatening. Therefore, frequent clinical, endoscopic, and radiographic
1203 monitoring is essential to manage these risks and ensure the well-being of the animals.

1204 *Light Amplification by Stimulated Emission of Radiation* (LASER) is a form of electromagnetic
1205 radiation produced by an emission system. This system consists of a solid or gaseous "active medium"
1206 that, when stimulated by electrical or optical energy, emits photons. These photons are then amplified,
1207 producing a concentrated and powerful beam of light. LASER emits light at a single wavelength (λ)
1208 and is composed of parallel waves that travel in synchrony through space and time. These
1209 characteristics allow the laser to concentrate a significant amount of power onto a specific point on a
1210 surface, resulting in high intensity at that precise location. The tissue alteration caused by LASER is
1211 due to the transformation of electromagnetic energy into thermal energy. The effects on tissue depend
1212 on the temperature: at 65°C, denaturation of protein components and collagen contraction occur; at

1213 90°C, fluid evaporation leads to tissue retraction and coagulation; and when the temperature exceeds
1214 100°C, tissue carbonization takes place. Tissue effects are also influenced by the wavelength of the
1215 LASER, the distance between the laser and the tissue, the exposition time, and the tissue's water
1216 content and pigmentation. In veterinary practice, the most commonly used LASER is the diode laser,
1217 which is transmitted via flexible quartz fiber (Sullins, 2002). This flexibility allows the laser to be
1218 used with the majority of endoscopic instruments, whether rigid or flexible, as the fiber can be inserted
1219 through the working channel of the endoscope or used coaxially with the optical system. The use of
1220 laser through an endoscope offers many advantages, including illumination and magnification of the
1221 surgical site, which enhances precision during procedures. Additionally, the endoscope's aspiration
1222 system can effectively remove laser-generated smoke, ensuring a clearer field of vision. These
1223 features contribute to safer and more efficient procedures (Sullins, 2002; Bottero, 2022). In veterinary
1224 medicine, there are numerous applications of endoscopy-guided laser technology. Many pathologies
1225 affecting the gastrointestinal, respiratory, and urinary tracts can be treated, and in some cases resolved,
1226 through laser ablation. Video-endoscopic guided laser technology is utilized in the gastrointestinal
1227 system for the treatment of neoformations in the esophagus, stomach, and colon. While the use of
1228 laser ablation in these cases is often palliative, it can lead to significant clinical improvements in
1229 animals, especially when there is an obstruction in the organ. This can help alleviate conditions such
1230 as secondary megaesophagus or the impossibility on defecation, allowing for better quality of life.
1231 Similar applications of video-assisted laser technology are also found in the urinary tract, particularly
1232 for treating obstructive neoformation. Laser therapy can be beneficial in addressing congenital
1233 anatomical defects such as ectopic ureters and ureteroceles. However, one of the significant
1234 complications associated with laser use in these conditions is the potential for organ rupture. This risk
1235 is especially pronounced when working with neoplastic tissues, where it can be challenging to predict
1236 the laser's effects on the pathological tissue (Bottero, 2022). Finally, there are numerous applications
1237 of laser endoscopy in the respiratory system, particularly in dogs affected by brachycephalic
1238 obstructive airway syndrome (BOAS). In these cases, laser applications include: staphelectomy,

1239 laryngectomy (performed in cases of grade III laryngeal collapse), turbinectomy, destruction of
1240 nasopharyngeal sialoceles. Once a time, video-assisted laser techniques are utilized for the ablation of
1241 benign and malignant neoplasms within the airway tract (nasal, laryngeal and tracheal), and it also
1242 provide solutions for certain congenital anatomical defects, such as choanal atresia. The most
1243 concerning complication associated with the use of lasers in airway procedures is tissue edema, which
1244 can result from the heat generated during laser application (Bottero, 2020). To mitigate this adverse
1245 effect, it is crucial to employ several strategies:

- 1246 - Cooling the tissue: applying ice water to the affected area immediately after laser surgery can
1247 help cool down the tissue and reduce edema.
- 1248 - Anti-inflammatory medications: administering anti-inflammatory drugs post-surgery can
1249 further decrease inflammation and tissue swelling.
- 1250 - Monitoring: close monitoring of the animal during the recovery phase is essential. This
1251 includes being vigilant for signs of obstructive edema, which may manifest as stridor and
1252 inspiratory dyspnea.
- 1253 - Intubation: in cases where significant obstructive edema develops, it may become necessary
1254 to intubate the animal to ensure adequate airway patency (Bottero, 2022).

1255 In our study, we propose diode laser epiglottidectomy (DLE), following the same procedure described
1256 by Catalfumo et al. (Catalfumo, 1998) in human medicine, which involves resecting the suprahyoid
1257 portion of the epiglottis. This approach was used to treat four obstructive epiglottic conditions in
1258 dogs: epiglottic retroversion (ER), epiglottic verticalization (EV), epiglottic deformation (ED), and
1259 epiglottic neoplasm (EN). In human medicine, video-assisted laser epiglottidectomy is considered the
1260 gold standard for addressing epiglottic airway obstructions, with an 85-90% success rate in relieving
1261 symptoms of benign obstruction (Zeitels, 1990; Golz, 2000; Kanemaru, 2007; Holinger, 1989). In
1262 veterinary medicine, subtotal epiglottidectomy and epiglottopexy have been employed to manage ER
1263 and EV, while total epiglottidectomy (both traditional and laser-assisted) has been used to treat EN
1264 (De Lorenzi, 2015; Mullins, 2014; Mullins, 2019; Shoieb, 2014). However, no surgical interventions

1265 for epiglottic deformation in dogs have been previously reported. Conventional surgical approaches
1266 for epiglottic conditions often result in significant postoperative complications, a finding that
1267 contrasts with the outcomes of our study (Mullins, 2014; Mullins, 2019). In our series, the only
1268 intraoperative complication encountered was notable bleeding from the branches of the superior
1269 laryngeal vessels, which was effectively controlled by grasping them with alligator forceps and
1270 applying electrocautery. The only postoperative complication was localized edema, which caused
1271 temporary airway obstruction, necessitating re-intubation. Preoperatively, common clinical signs
1272 included dysphagia, exercise intolerance, dyspnea, and stridor. Following DLE, we observed
1273 substantial improvements in both short- and long-term respiratory function in 85% of cases, with no
1274 instances of swallowing disorders developing. Both total DLE (TDLE) and subtotal DLE (SDLE)
1275 were effective for treating epiglottic-related airway obstruction in dogs. The diode laser is
1276 advantageous for its ease of use, reduced surgical time, precision, and the ability to maintain
1277 hemostasis while minimizing postoperative edema. These techniques have proven to be
1278 straightforward and efficient for surgeons experienced in laser and endoscopic procedures, with
1279 significantly fewer complications compared to conventional surgical treatments for epiglottic
1280 pathology.

1281 In conclusion, the use of interventional endoscopy in the airway system offers numerous diagnostic
1282 and therapeutic applications. A clear understanding of the clinical indications for applying these
1283 techniques, along with specific training and expertise of clinicians or surgeons, is essential for
1284 performing endoscopic procedures with precision and efficiency, reducing the risk of complications.
1285 Clinical indications for EBNA sampling include masses or mucosal infiltrations in the airway tract.
1286 The application of Dumon tracheal stents is indicated for dogs with IV° TC in the cervical or
1287 cervicothoracic regions and clinical signs of suffocation. Finally, diode laser DLE is indicated for
1288 epiglottic diseases causing obstruction of the glottis. Although infrequent, complications associated
1289 with assisted endoscopic procedures are still possible. EBNA sampling could cause moderate
1290 bleeding, which resolved spontaneously in our cases. In humans, pneumothorax secondary to this

1291 sampling method has been reported, and theoretically, it is also possible in animals. Complications
1292 associated with Dumon tracheal stents include stent migration or deformation, growth of granulation
1293 tissue, and coughing. These complications can be managed with regular clinical and endoscopic
1294 monitoring and, when necessary, through stent removal or repositioning, medical therapy, or
1295 minimally invasive surgical interventions. Finally, complications observed during diode-laser DLE
1296 included intra-operative bleeding and post-operative local edema secondary to laser action. The
1297 bleeding was resolved through surgical cauterization of the affected vessels, while the local edema
1298 resolved with corticosteroid therapy and temporary intubation of the dogs. Therefore, it is crucial to
1299 be aware of the potential adverse effects associated with these procedures to enable prompt
1300 intervention should they occur. Additionally, monitoring animals during the postoperative period and
1301 conducting regular follow-ups after discharge are essential for ensuring their overall health and well-
1302 being. As the availability of endoscopic instruments for veterinarians expands, the routine use of
1303 interventional endoscopy is expected to grow. However, further studies are necessary to fully
1304 understand the potential benefits and limitations of these techniques, ensuring they are applied
1305 effectively and safely in veterinary practice.

1306 **References**

- 1307 1. Amis T.C., McKiernan B.C. *Systematic identification of endobronchial anatomy during*
1308 *bronchoscopy in the dog*. In “Am J Vet Res” 1986, n° 47(12), 2649-2657
- 1309 2. Amis T.C., O'Neill N., Di Somma E., Wheatley J.R. *Epiglottic movements during breathing*
1310 *in humans*. In “J Physiol” 1998, 1, 307-314
- 1311 3. Amis T.C., O'Neill N., Van der Touw T., Brancatisano A. *Control of epiglottic position in*
1312 *dogs: role of negative upper airway pressure*. In “Respir Physiol” 1996, n° 105(3), 187-94
- 1313 4. Becker W.M., Beal M., Stanley B.J., Hauptman J.G. *Survival after surgery for tracheal*
1314 *collapse and the effect of intrathoracic collapse on survival*. In “Vet Surg” 2012, n° 41(4),
1315 501-506
- 1316 5. Bonagura J.D., *Kirk's Current Veterinary Therapy* 15th Ed, Saunders Elsevier, Philadelphia
1317 2014, United States of America, pp. 630-635
- 1318 6. Bottero E., *Interventional Endoscopy in dog and cat* 1th Ed, Poletto Editore, Milano 2022,
1319 Italia
- 1320 7. Brownlie S.E. *A retrospective study of diagnosis in 109 cases of canine lower respiratory*
1321 *disease*. In “J Small Anim Pract” 1990, n ° 31, 371-376
- 1322 8. Buback J.L., Boothe H.W., Hobson H.P. *Surgical treatment of tracheal collapse in dogs: 90*
1323 *cases (1983–1993)*. In “JAVMA” 1996, n° 208(3), 380384
- 1324 9. Buechner-Maxwell V., Crisman M., Murray M., Ley W., Saunders G., Walton A.
1325 *Transendoscopic biopsy of the horse's airway mucosa*. In “Journal of Equine Veterinary
1326 Science” 1996, n° 16(9), 375-379
- 1327 10. Catalfumo F.J., Golz A., Westerman S.T., Gilbert L.M., Joachims H.Z., Goldenberg D. *The*
1328 *epiglottis and obstructive sleep apnoea syndrome*. In “J Laryngol Otol” 1998, n° 112(10),
1329 940-3

11. Chen D.F., Chen Y., Zhong C.H., Chen X.B., Li S.Y. *Long-term efficacy and safety of the Dumon stent for benign tracheal stenosis: a meta-analysis*. In “J Thorac Dis” 2021, n° 13(1), 82-91
12. Chisnell H.K., Pardo A.D. *Long-term outcome, complications and disease progression in 23 dogs after placement of tracheal ring prostheses for treatment of extrathoracic tracheal collapse*. In “Vet Surg” 2015, n° 44(1), 103-113
13. Clercx C., Peeters D., Snaps F., Hansen P., McEntee K., Detilleux J., Henroteaux M., Day M.J. *Eosinophilic Bronchopneumopathy in Dogs*. In “J Vet Intern Med” 2000, n° 14, 282-291
14. Culp W.T.N., MacPhail C.M., Perry J.A., Jensen T.D. *Use of a Nitinol Stent to Palliate a Colorectal Neoplastic Obstruction in a Dog*, In “JAVMA” 2011, n° 239, 222–227
15. Dallman M.J., RMcClure R.C., Brown E.M. *Histochemical Study of Normal and Collapsed Tracheas in Dogs*. In “Am J Vet Res” 1988, n° 49(12), 2117-25
16. De Lorenzi D., Bertoncello D., Bottero E. *Squash-preparation cytology from nasopharyngeal masses in the cat: cytological results and histological correlations in 30 cases*. In “J Feline Med Surg” 2008, n° 10(1), 55-60
17. De Lorenzi D., Bertoncello D., Comastri S., Bottero E. *Treatment of acquired nasopharyngeal stenosis using a removable silicone stent*, In “J Feline Med Surg” 2015, n. 17(2), 117-24
18. De Lorenzi D., Bertoncello D., Dentini A. *Intraoral diode laser epiglottidectomy for treatment of epiglottitis chondrosarcoma in a dog*. In “J Small Anim Pract” 2015, 56, 675-678
19. De Lorenzi D., Bertoncello D., Drigo M. *Bronchial abnormalities found in a consecutive series of 40 brachycephalic dogs*. In “JAVMA” 2009, n° 235(7), 835-40
20. De Lorenzi D., *Diseases of the Respiratory System of the Dog and Cat* 1th ed, Elsevier Masson, Amsterdam 2012, Netherlands
21. De Lorenzi D., Solano-Gallego L. *Tracheal granuloma because of infection with a novel mycobacterial species in an old FIV-positive cat*. In “J Small Anim Pract” 2009, n° 50(3), 143-6

22. Della Maggiore A. *An update on tracheal and airway collapse in dogs*. In “Vet Clin North Am Small Anim Pract” 2020, n° 50(2), 419-430
23. Dobler C.C., Crawford A.B.H. *Bronchoscopic diagnosis of endoscopically visible lung malignancies: should cytological examinations be carried out routinely?* In “Internal Medicine Journal” 2009, n° 39, 806-811
24. Dumon J.F. *A dedicated tracheobronchial stent*. In “Chest” 1990, n° 97(2), 328-332
25. Durant A.M., Sura P., Rohrbach B., Bohling M.W. *Use of nitinol stents for end-stage tracheal collapse in dogs*. In “Vet Surg” 2012, n° 41(7), 807-817
26. Dutau H. *Principles and Practice of Interventional Pulmonology* 1th Ed, Springer, Berlin 2013, Germany, pp. 311-321
27. Flanders J.A., Thompson M.S. *Dyspnea caused by epiglottic retroversion in two dogs*. In “JAVMA” 2009, n° 235, 1330-1335
28. Folch E., Keyes C. *Airway stents*. In “Ann Cardiothorac Surg” 2018, n° 7(2), 273-283
29. Golz A., Goldenberg D., Westerman S.T., Catalfumo F.J., Netzer A., Westerman L.M., Joachims H.Z. *Laser partial epiglottidectomy as a treatment for obstructive sleep apnea and laryngomalacia*. In “Ann Otol Rhinol Laryngol” 2000, n° 109, 1140-1145
30. Govert J.A., Dodd L.G., Kussin P.S., Samuelson W.M. *A prospective comparison of fiberoptic transbronchial needle aspiration and bronchial biopsy for bronchoscopically visible lung carcinoma*, in “Cancer” 1999, n° 87(3), 129-34
31. Graczyk S., Paślowski R., Grzeczka A., Litwińska L., Jagielski D., Paśławska U., *Stents in Veterinary Medicine*, In “Materials (Basel)” 2023, n° 16(4), 1480
32. Hill R.C., Ginn P.E., Thompson M.S., Seguin M.A., Miller D., Taylor D.P. *Endobronchial polyp derived from a myxosarcoma in the lung of a dog*. In “J Am Anim Hosp Assoc” 2008, n° 44(6), 327-34
33. Holinger L.D., Konior R.J. *Surgical management of severe laryngomalacia*. In “Laryngoscope” 1989, n° 99(2), 136-42

34. Horsley J.R., Miller R.E., Amy R.W., King E.G. *Bronchial submucosal needle aspiration performed through the fiberoptic bronchoscope*. In “Acta Cytol” 1984, n° 28, 211-7
35. Jacob, F. *Fluoroscopy-guided fine-needle aspiration of deep-seated pulmonary masses in dogs and cats appears safe and accurate*. In “JAVMA” 2023, n° 262(1), 1-7
36. Jones A.M., Hanson I.M., Armstrong G.R., O'Driscoll B.R. *Value and accuracy of cytology in addition to histology in the diagnosis of lung cancer at flexible bronchoscopy*, In “Respir Med” 2001, n° 95(5), 374-8
37. Kanemaru S., Kojima H., Fukushima H., Tamaki H., Tamura Y., Yamashita M., Umeda H., Ito J. *A case of Floppy Epiglottis in adult: a simple surgical remedy*. In “Auris Nasus Larynx” 2007, n° 34(3), 409-11
38. Kim J.Y., Han H.J., Yun H.Y., Lee B., Jang H.Y., Eom K.D., Park H.M., Jeong S.W. *The safety and efficacy of a new self-expandable intratracheal nitinol stent for the tracheal collapse in dogs*. In “J Vet Sci” 2008, n° 9(1), 91-93
39. Kirk R.W. *Current veterinary therapy* 9th Ed, Saunders, Philadelphia 1986, United States of America
40. Kliewer M., Gu J., Paulin M.V., Sukut S., Cosford, K. *Computed tomographic and bronchoscopic diagnosis of *Oslerus osleri* infection in a dog*. In “Vet Radiol Ultrasound” 2023, n° 64(6), E83-E87
41. Lam N., Weisse C., Berent A., Kaae J., Murphy S., Radlinsky M., Richter K., Dunn M., Gingerich K. *Esophageal stenting for treatment of refractory benign esophageal strictures in dogs*, In “J Vet Intern Med” 2013, n. 27(5), 1064-70
42. Leonard H.C. *Collapse of the larynx and adjacent structures in the dog*. In “JAVMA” 1960, n° 137, 360-363
43. Lundgren R., Bergman F., Angstrom T., *Comparison of transbronchial needle aspiration biopsy, aspiration of bronchial secretion, bronchial washing, brush biopsy and forceps biopsy in the diagnosis of lung cancer*, In “Eur J Resp Dis” 1983, n. 64, p. 378-85

44. McCarthy, T.C. *Veterinary Endoscopy for the Small Animal Practitioner* 1st Ed. Elsevier Saunders, St Louis 2005, United States of America, pp. 226
45. McMillian M.C., Kleine L.J., Carpenter J.L. *Fluoroscopically-guided percutaneous fine-needle aspiration biopsy of thoracic lesions in dogs and cats*. In “Vet Radiol” 1988, n° 29, 194-197
46. Monaco T.A., Taylor J.A., Langenbach A., Gordon S., Vance E. *Intra- and inter-observer reliability of combined segmental measurement techniques for predicting immediate post-deployment intraluminal tracheal stent length in dogs*. In “Can Vet J” 2014, n° 55(5), 435-441
47. Moritz A., Schneider M., Bauer N. *Management of advanced tracheal collapse in dogs using intraluminal self-expanding biliary wallstents*. In “J Vet Intern Med” 2004, n° 18(1), 31-42
48. Moulton J.E., Von Tscharner C., Schneide, R. *Classification of lung carcinomas in the dog and cat*, In “Vet Pathol” 1981, n° 18(4), 513-28
49. Mullins R., McAlinden A.B., Goodfellow M. *Subtotal epiglottectomy for the management of epiglottic retroversion in a dog*. In “J Small Anim Pract” 2014, n° 55, 383-385
50. Mullins R.A., Stanley B.J., Flanders J.A., López P.P., Collivignarelli F., Doyle R.S., Schuenemann R., Oechtering G., Steffey M.A., Lipscomb V.J., Hardie R.J., Kirby B.M., McAlinden A.B. *Intraoperative and major postoperative complications and survival of dogs undergoing surgical management of epiglottic retroversion: 50 dogs (2003-2017)*. In “Vet Surg” 2019, n° 48, 803-819
51. Muraro L., Aprea F., White R.A. *Successful management of an arytenoid chondrosarcoma in a dog*. In “J Small Anim Pract” 2013, n° 54, 33-5
52. Papaioannou N., Psalla D., Papadopoulos E., Adamama-Moraitou K.K., Petanidis T., Rallis T., Vlemmas I. *Obstructive, Granulomatous Tracheitis caused by Onchocerca sp. in a Dog*. In “Journal of Veterinary Medicine Series A” 2004, n° 51, 354-357

53. Pechman Jr R.D. *Pulmonary paragonimiasis in dogs and cats: a review*. In “J Small Anim Pract” 1980, n° 21, 87-95
54. Penninc, D., D’Anjou, M.A. *Atlas of small animals ultrasonography* 2nd edn. Wiley-Blackwell, Hoboken 2008, United States of America
55. Raske M., Weisse C., Berent A.C., McDougall R., Lamb K. *Immediate, short-, and long-term changes in tracheal stent diameter, length, and positioning after placement in dogs with tracheal collapse syndrome*. In “J Vet Intern Med” 2018, n° 32(2), 782-791
56. Robin T., Robin E., Le Boedec K. *A systematic review and meta-analysis of prevalence of complications after tracheal stenting in dogs*, In “J Vet Intern Med” 2024, n° 38(4), 2034-2044
57. Roundebush P., Green R.A., Digilio K.M. *Percutaneous fine needle aspiration biopsy of the lung in disseminated pulmonary disease*. In “J Am Anim Hosp Assoc” 1981, n° 17(1), 109-116
58. Salamanca F., Leone F., Bianchi A., Bellotto R.G.S., Costantini F., Salvatori P. *Surgical treatment of epiglottis collapse in obstructive sleep apnoea syndrome: epiglottis stiffening operation*. In “Acta Otorhinolaryngol Ital” 2019, n° 39(6), 404-408
59. Salk J.E., Toll S.L., Goldschmidt M.H. *Canine and feline laryngeal neoplasia a 10 year survey*. In “J Am Anim Hosp Assoc” 1986, n° 22, 359-65
60. Semaan R., Yarmus L. *Rigid bronchoscopy and silicone stents in the management of central airway obstruction*. In “J Thorac Dis” 2015, n° 7(suppl 4), S352-S362
61. Sengör A., Willke A., Aydin O., Gündes S., Almaç A. *Isolated necrotizing epiglottitis: report of a case in a neutropenic patient and review of the literature*. In “Ann Otol Rhinol Laryngol” 2004, n° 113(3 Pt 1), 225-8
62. Serio P., Fainardi V., Leone R., Baggi R., Grisotto L., Biggeri A., Mirabile L. *Tracheobronchial obstruction: follow-up study of 100 children treated with airway stenting*. In “Eur J Cardiothorac Surg” 2014, n° 45(4), e100-e109

63. Sharkey L.C., Radin M.J. & Seelig, D. *Veterinary Cytology*. 1st Ed. Wiley-Blackwell, Hoboken 2020, United States of America
64. Shoieb A.M. *Managing epiglottal chondrosarcoma of a dog: A case report*. In “J Interdiscipl Histopathol” 2014, n° 2(4), 224-227
65. Skerrett S.C., McClaran J.K., Fox P.R., Palma D. *Clinical features and outcome of dogs with epiglottic retroversion with or without surgical treatment: 24 cases*. In “J Vet Intern Med” 2015, n° 29, 1611-1618
66. Stehlik L., Hytych V., Letackova J., Kubena P., Vasakova M. *Biodegradable polydioxanone stents in the treatment of adult patients with tracheal narrowing*. In “BMC Pulm Med” 2015, n° 15(1), 164
67. Suematsu M., Suematsu H., Minamoto T., Machida N., Hirao D., Fujiki M. *Long-term outcomes of 54 dogs with tracheal collapse treated with a continuous extraluminal tracheal prosthesis*. In “Vet Surg” 2019, n° 48(5), 825-834
68. Sullins K.E. *Diode laser and endoscopic laser surgery*. In “Vet Clin North Am Small Anim Pract” 2002, n° 32(3), 639-48
69. Sun F., Usón J., Ezquerro J., Crisóstomo V., Luis L., Maynar M. *Endotracheal stenting therapy in dogs with tracheal collapse*. In “Vet J” 2008, n° 175(2), 186-193
70. Sura P.A., Krahwinkel D.J. *Self-expanding nitinol stents for the treatment of tracheal collapse in dogs: 12 cases (2001–2004)*. In “JAVMA” 2008, n° 232(2), 228-236
71. Tams, T.R., Rawlings, C.A., *Small Animals Endoscopy* 3th ed, Elsevier Saunders, St Louis 2010, United States of America, pp.
72. Tangner C.H., Hobson H.P. *A retrospective study of 20 surgically managed cases of collapsed trachea*. In “Vet Surg” 1982, n° 11(4), 146-149
73. Teske E., Stokhof A.A., Van den Ingh T.S.G.A.M., Wolvekamp W.T.C., Slappendel R.J., de Vries H.W. *Transthoracic needle aspiration biopsy of the lung in dogs with pulmonic diseases*. In “J Am Anim Hosp Assoc” 1991, n° 27(3), 289-294.

74. Weisse C., Berent A. *Veterinary Image-Guided Interventions* 1th Ed, John Wiley & Sons, New York, 2015, United States of America, pp. 73-82.
75. Weisse C., Berent A., Violette N., McDougall R., Lamb K. *Short-, intermediate-, and long-term results for endoluminal stent placement in dogs with tracheal collapse*. In “JAVMA” 2019, n° 254(3), 380-392
76. Wilson D.W., Dungworth D.L. *Tumors in Domestic Animals* 4th Ed. Iowa State Press, Iowa 2002, United States of America
77. Wood E.F., O’Brian R.T., Young K.M. *Ultrasound-guided fine-needle aspiration of focal parenchymal lesions of the lungs in dogs and cats*. In “J Vet Int Med” 1998, 12(5), 338-42
78. Xavier R.G., Sanches P.R., de Macedo Neto A.V., Kuhl G., Vearick S.B., Michelon M.D. *Development of a modified Dumon stent for tracheal applications: an experimental study in dogs*. In “J Bras Pneumol” 2008, n° 34(1), 21-26
79. Zakaluzny S.A., Lane J.D., Mair E.A. *Complications of tracheobronchial airway stents*. In “Otolaryngol Head Neck Surg” 2003, n° 128(4), 478-488
80. Zeitels S.M., Vaughan C.W., Domanowsky C.F., Fuleihan N.S., Simpson G.T. *Laser epiglottidectomy: endoscopic technique and indications*. In “Otolaryngol Head Neck Surg” 1990, 103, 337-343
81. Zekas L.J., Crawford J.T., O’Brien R.T. *Computed tomography-guided fine-needle aspirate and tissue-core biopsy of intrathoracic lesions in thirty dogs and cats*. In “Vet Radiol Ultrasound” 2005, 46(3), 200-4
82. Zhu B.Y., Johnson L.R., Vernau W., *Tracheobronchial brush cytology and bronchoalveolar lavage in dogs and cats with chronic cough: 45 cases (2012-2014)*, in “J Vet Intern Med” 2015, n. 29(2), p. 526-32