DIAGNOSIS OF AERODIGESTIVE DISORDERS IN DOGS UTLIZING VIDEOFLUOROSCOPIC SWALLLOW STUDY

A Thesis presented to the Faculty of the Graduate School
University of Missouri-Columbia

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

By
JENNIFER HOWARD
Dr. Carol Reinero, Thesis Supervisor
DECEMBER 2022

The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

DIAGNOSIS OF AERODIGESTIVE DISORDERS IN DOGS UTLIZING VIDEOFLUOROSCOPIC SWALLLOW STUDY

Presented by Jennifer Howard,

A candidate for the degree of Master of Science,

And hereby certify that, in their opinion, it is worthy of acceptance.

Dr. Carol Reinero

Dr. Teresa Lever

Dr. Laura Nafe

DEDICATION

l dedicate this thesis to my four wonderful feline companions: Jet, Silver, Pierre, Francois and all the cats I have loved before (Tenderheart, Kingsley, Felix – and many more). You keep me passionate for my career and continue to love me despite the many times I am home late from work, delaying your dinner.

ACKNOWLEDGEMENTS

The author would like to thank the following individuals:

Dr. Carol Reinero, for your support and willingness to help with my clinical research, training, and every-day life. Without you, this thesis, my career, and likely the continuation of my life would not have been possible. I am forever indebted to you for all you have done for me and the kindness you have shown me.

Dr. Teresa Lever, for your assistance in training and assistance in interpretation of data for my study. You have been a tremendous attribute to have during my graduate training.

Dr. Laura Nafe, for your mentorship during my clinical training. Your support has allowed me to gain the confidence needed to move forward with the next stage in my career.

My "SAIM Fam": Julia, Charlotte, Ashley, Iliana, Matt & Natalie, for your daily support both personally and professionally. You always make me smile and I would not be able to finish my post-graduate training without you all.

Supported by grant #02699 from the American Kennel Club Canine Health Foundation.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Tables Page

LIST OF ABBREVIATIONS

1. Aerodigestive Disorder(s) (AeroD(s))
2. Brachycephalic Obstructive Airway Syndrome (BOAS)
3. Bronchoalveolar Lavage Fluid (BALF)
4. Chronic Obstructive Pulmonary Disease (COPD)
5. Control Dogs (CON)
6. Computed Tomography (CT)
7. Gastroesophageal Reflux (GER)
8. Geriatric Onset Laryngeal Paralysis and Polyneuropathy (GOLPP)
9. Interquartile Range (IQR)
10. Lower Esophageal Sphincter (LES)
11. Penetration-Aspiration Score (PAS)
12. Pharyngeal Constriction Ratio (PCR)
13. Region of Interest (ROI)
14. Respiratory Dogs (RESP)
15. Total Nucleated Cell Count (TNCC)
16. Tricuspid Regurgitation Velocity (TRV)
17. Upper Esophageal Sphincter (UES)
18. Videofluroscopic Swallow Study (VFSS)

ABSTRACT

An aerodigestive disease (AeroD) is a disorder pathologically linking the respiratory and alimentary tracts. Dogs having respiratory signs without dysphagia, vomiting, or regurgitation typically lack diagnostics identifying comorbid alimentary disease. Videofluoroscopic swallow study (VFSS) identifies defects in swallowing, pathologic reflux, and aspiration. We hypothesized dogs with respiratory/no alimentary disease (RESP) would have significantly more abnormal VFSS metrics versus healthy controls (CON). We hypothesized RESP dogs with parenchymal disease would have more reflux and higher penetration-aspiration score (PAS) than those with airway disease.

Forty-five client owned dogs with respiratory disease (RESP) and 15 hospital staff owned dogs (CON) were evaluated. Prospectively, all dogs underwent VFSS. RESP dogs had advanced respiratory diagnostics. Eight subjective and three objective VFSS metrics (pharyngeal constriction ratio, PCR; PAS; and esophageal transit time, ETT) were assessed. Fishers Exact test compared differences between groups (presence or absence of VFSS abnormalities). PCR and PAS were compared via Mann-Whitney Rank Sum test ($p<0.05$ significant). Results revealed subjective VFSS abnormalities in 34/45 (75%) RESP and 2/15 (13%) CON dogs, with RESP dogs significantly more likely to have VFSS abnormalities ($p = 0.006$). There was no significant difference in PCR between groups. RESP dogs were more likely to have pathologic PAS than CON ($p = 0.027$). RESP dogs with airway disease had higher PAS than CON ($p = 0.011$) but not RESP dogs with parenchymal disease ($p = 0.246$). Overall, seventy-five percent of RESP dogs had VFSS abnormalities, underscoring that AeroDs are common.

CHAPTER 1

INTRODUCTION & LITERATURE REVIEW

Aerodigestive disorders (AeroDs) are a complex group of diseases that highlight the close relationship between the alimentary and respiratory tracts.[1] The aerodigestive tract consists mainly of the upper airways (nasopharynx, larynx, and proximal trachea) and upper digestive tract (oropharynx and esophagus) but can also include the lower airways (bronchi, bronchioles) and the pulmonary parenchyma.[2] In both humans and dogs, the entrance to the upper digestive tract and respiratory tract are intimately associated with centimeters separating the two.[1,2] As breathing, eating, and swallowing are all vital functions for survival and have overlap in their anatomic pathways, pathology of one system can easily affect the other. Therefore, both diagnostic investigation and treatment strategies must be aimed at both systems to control signs and manage these conditions.

AeroDs can arise from swallowing disorders (dysphagia), abnormal airway defenses, reflux, or a combination of these.[1,3-5] AeroDs have been associated with significant morbidity and mortality in humans and are linked to chronic cough, inflammatory airway diseases, and even severe parenchymal changes such as pulmonary fibrosis.[5,6] Understanding of shared disorders between the alimentary and respiratory tract in dogs is in its infancy and therefore remains understudied.

Evaluation of the upper alimentary and respiratory tract is key to diagnosis. There are numerous strategies for individual evaluation of the alimentary and respiratory tracts in clinical practice. For the alimentary tract, physical examination of the oropharynx and endoscopy are commonly employed.[7] The respiratory tract can be evaluated with

examinations such as functional laryngeal assessments and bronchoscopy.[8] Additionally, imaging such as thoracic radiography or computed tomography (CT) is often utilized.[8] Novel diagnostics have been described in a research setting, notably the use of nuclear scintigraphy to detect silent reflux events or the detection of biomarkers (such as pepsin A) in airway lavage or oropharyngeal swab samples, in both dogs and humans.[9-11] These are valuable diagnostic tools, but tend to only investigate one system at a time or are not clinically available. Therefore, the criterion standard for evaluation of the upper alimentary tract disease is the videofluoroscopic swallow study (VFSS).[12] This is a radiographic procedure in which contrast added to liquid and different food consistencies is eaten by a patient, which allows the clinician to view all physiologic processes with swallowing including mastication, bolus formation, swallow, esophageal motility, and entrance into the stomach, as well as reflux and hiatal herniation after eating.[12] Therefore, VFSS is the most comprehensive method for detecting dysphagia of any kind.[13] Additionally, the upper respiratory tract can be viewed and issues such as reflux or aspiration of liquid or food into the airway can be identified.[12] While other diagnostic tools including CT and endoscopy are good ancillary tools for evaluating the separate body systems in a "static" state, VFSS is the vital diagnostic used to evaluate for AeroDs, especially in a dynamic state.

In veterinary medicine, VFSS has traditionally been performed on dogs in lateral recumbency while manually restrained and force fed contrast mixed in food and/or liquid. However, this method poses concern for risk of aspiration, especially in dysphagic dogs. Furthermore, the use of non-physiologic feeding conditions hampers interpretation as well as increases radiation exposure to staff. Therefore, a standardized protocol was

developed to allow dogs to freely feed in a standing position to improve evaluation and mimic physiologic feedings and decrease other risks mentioned previously.[12]

In humans, advanced diagnostics to identify AeroDs have been used for years and thus a number of aerodigestive diseases have been discovered. A heterogenous group of disorders, they are organized into issues of the esophagus, upper and lower airways, and pulmonary parenchyma. Disease of the esophagus can include abnormal motility including spasms, hypomotility, amotility or achalasia, gastroesophageal reflux (GER), and structural disorders such as strictures or intramural neoplasms, and inflammatory forms of esophagitis. Retention or re-entry of liquid or food into the upper digestive tract can potentially cause damage to tissues from acid or enzymes, and aspiration of this material can lead to respiratory disease.[2,14,15] In the larynx, responsible for protection of the airways, stenosis, paresis/paralysis, and clefts (abnormal openings between the esophagus and larynx) have been identified in both humans and dogs that leave the airways more vulnerable to aspiration and subsequent damage to the respiratory tract, potentially predisposing to development of AeroDs.[15-19] Diseases of the respiratory tract that have been associated with AeroDs include asthma, softening of the airways (malacia), and chronic lung diseases such as bronchitis, chronic obstructive pulmonary disease (COPD), and pulmonary fibrosis.[14,20-22]

To date, only a small number of AeroDs in dogs have been thoroughly investigated. The most common, aspiration pneumonia, occurs secondary to risk factors such as esophageal or laryngeal disease, vomiting, regurgitation, or anesthetic events, among other causes.[23-25] Inhalation of solid or liquid material into the airways and pulmonary parenchyma can result in aspiration pneumonia, defined as inhalation of

contents of the gastrointestinal tract with bacteria, or aspiration pneumonitis, where inhalation of low pH stomach contents causes chemical injury without an initial infectious component.[25] Treatment is aimed at supportive care and addressing the underlying cause of aspiration, where gastrointestinal disease is often implicated. This highlights the importance of investigating aerodigestive diseases.

Other aspiration-related respiratory diseases have been recognized in veterinary medicine besides aspiration pneumonia. Upper airway disorders leading to secondary aspiration largely include GER but can also include laryngeal dysfunction.[26-28] GER is well recognized in humans and can result in oropharyngeal, nasopharyngeal, laryngeal, and proximal trachea inflammation.[6,29] Previous literature has described laryngeal hyperemia in 54% of dogs with acute or chronic cough.[30] While non-specific, this could represent a link between macro- or micro-aspiration and cough. GER has been recognized as a clinical syndrome in dogs.[31,32] Consequences affecting the alimentary tract specifically have been recognized including esophagitis, ulceration, strictures, and epithelia metaplasia.[31] Respiratory complications in a population of dogs with GER has not been thoroughly described. In humans with select respiratory disease, GER has been estimated to effect up to 90% of patients.[5,14] Common respiratory diseases that affect dogs include inflammatory airway disease (such as chronic bronchitis), bronchomalacia, bronchiectasis, tracheal collapse, and idiopathic pulmonary fibrosis.[5] These diseases can be a cause of significant morbidity and mortality in dogs.

Aspiration-related respiratory diseases have been also shown to affect the small airways, defined as bronchioles with inner diameters of <2mm.[33]Aspiration-related small airway diseases are recognized in humans as a consequence of recurrent aspiration.[34]

Diagnosis is often made with a combination of documentation of aspiration on VFSS, CT imaging, and sometimes histologic evaluation.[35] GER has been recognized as a predisposing factor for humans with small airway disease.[35] Additionally, bronchiolectasis, defined as irreversible widening of bronchioles due to destruction of the elastic and muscular components of airway walls, can often be a consequence of congenital or acquired defects.[36] These defects can lead to chronic cycles of inflammation and further damage to the airway.[36] Aspiration plays a role in humans with small airway disease and may contribute to similar findings in dogs, but further studies are needed to investigate this potential link.

Lastly, chronic microaspiration has been well established as a contributor to the development of interstitial lung diseases in humans, particularly idiopathic pulmonary fibrosis.[37] Interstitial lung diseases represent a heterogenous group of inflammatory or fibrotic disorders affecting the space between the pulmonary epithelium and vascular endothelium as well as alveolar filling disorders[38] The understandings of interstitial lung diseases in veterinary medicine are currently in infancy; therefore, investigation of contributions of alimentary disorders to these parenchymal diseases is warranted.

Other recognized AeroDs in veterinary medicine include concurrent esophageal and laryngeal dysfunction in dogs with geriatric onset laryngeal paralysis and polyneuropathy (GOLPP), with affected dogs having significantly more abnormalities on VFSS compared to control dogs.[39] Furthermore, dysphagia, GER, and hiatal hernia, among other alimentary disorders, have been recognized in brachycephalic dogs with brachycephalic obstructive airway syndrome (BOAS). Previous studies have reported a prevalence of up to 73% of esophageal dysmotility in brachycephalic dogs.[40,41] A recent

study described that 84% of brachycephalic dogs had evidence of reflux via proximal and distal esophageal pH measurements.[42] This study did not find an association with severity of reflux and degree of BOAS signs but did not evaluate for respiratory pathology. Multiple retrospective studies have documented anatomical abnormalities such as a sliding hiatal hernia in brachycephalic dogs, that can predispose to reflux and comorbid respiratory disease.[43,44] Additionally, a high prevalence of gastrointestinal histopathologic lesions in dogs with BOAS has been reported.[41] While multiple alimentary abnormalities have been recognized, potential sequalae or link to respiratory disease, particularly in a prospective manner, has not been investigated.

As mentioned previously, VFSS is the criterion standard for evaluation for upper alimentary tract disease and dysphagia in dogs.[12] Previous literature has looked closely at disease processes that can be identified by VFSS. Besides standardization of protocols, the literature has also described normal views of the pharynx and upper esophageal sphincter (UES) in relation to swallowing.[45] Previously, cricopharyngeal achalasia, defined as the functional abnormality involving the failure of the UES to open fully or at the appropriate time,[46] has been shown to have specific features on VFSS including delayed time to opening and closure of the UES.[47] Other causes of dysphagia have also been investigated. A retrospective study by Pollard et al in 2017 described anatomical areas responsible for dysphagia in 216 dogs that underwent VFSS. Of those, 72% of dogs had dysphagia, with 45% having esophageal dysmotility, 45% having dysfunction of the lower esophageal sphincter (LES), 13% having pharyngeal dysphagia, 9% having a focal esophageal abnormality such as a stricture, and 8% having cricopharyngeal achalasia. Notably, 38% of dogs had multiple abnormalities present contributing to dysphagia.[48]

This study also added to a previous study by the same authors evaluating pharyngeal constriction ratios (PCR),[49] a measurement of pharyngeal contractility[50,51], and found that higher PCRs were associated with both pharyngeal and other forms of dysphagia. These studies were monumental in establishing VFSS metrics for evaluating causes of dysphagia in dogs. However, these studies did not investigate sequalae of dysphagia to the neighboring respiratory tract.

Due to the link between the upper gastrointestinal tract and airways noted in humans, the literature has examined co-morbidities between the two in dogs. Poncet et al in 2005 evaluated 73 brachycephalic dogs with BOAS and determined that 97% of dogs had at least one upper gastrointestinal tract abnormality.[41] While VFSS was not used as a method of alimentary tract evaluation, a correlation was found between the severity of gastrointestinal signs and respiratory signs suggesting AeroDs were contributing to disease.

Laryngeal paralysis has been a well described clinical syndrome in which almost all of the intrinsic muscles of the larynx become paralyzed, causing significant morbidity, with affected dogs developing upper airway obstruction.[19,52] It is often a disease of older, large breed dogs but congenital forms have been seen in Bouviers des Flandres and Siberian Huskies.[53,54] Though it can be secondary to traumatic, iatrogenic, or neoplastic processes, it is often idiopathic in nature[55] and is thought to be in part due to neurogenic atrophy of the laryngeal muscles[56] because of progressive degeneration of the recurrent laryngeal nerves and possibly secondary to a generalized polyneuropathy.[52,57] As the treatment for life-threatening laryngeal paralysis (unilateral arytenoid lateralization) leaves great risk for aspiration, investigation for concurrent esophageal dysmotility is of

interest.[58] Previous research has investigated esophageal dysfunction in dogs with idiopathic laryngeal paralysis compared to control dogs and found that esophageal dysmotility was more severe in dogs with laryngeal paralysis in comparison to controls and more severe esophageal dysmotility was associated with aspiration pneumonia post-surgery.[39] Additionally, another study in dogs with laryngeal paralysis revealed that they were more likely to have GER compared to clinically normal dogs.[59] These studies highlight a link between dysmotility and reflux of the upper alimentary and respiratory tracts though no respiratory diseases besides aspiration pneumonia were investigated.

In canines, any potential link between the alimentary and respiratory tracts remain poorly understood due to diagnostic limitations and poor clinical recognition.[1] To date, there has been little investigation into possible AeroDs in dogs. Recognition of these diseases are important as it can allow the clinician to target therapy and enables investigation into novel treatments. Two previous retrospective studies investigated AeroDs specifically in dogs. A retrospective study by Grobman et al documented that in dogs presenting for cough in the absence of reported gastrointestinal signs, swallowing abnormalities were identified in 81% of dogs.[60] Additionally, a primary respiratory disorder was found in only 55% of the population. Another retrospective study by Luciani et al, described signs of AeroDs in dogs with sliding hiatal hernias. Dogs diagnosed with a sliding hiatal hernia presented for both alimentary and respiratory signs. Interestingly, brachycephalic dogs were more likely to present with severe respiratory signs such as overt distress highlighting the likely interplay between the respiratory and alimentary tracts.[44] These studies suggests that a population of dogs with respiratory signs or disease may have an AeroD and may benefit from treatment aimed at the

alimentary tract. Identifying more aerodigestive diseases in dogs is warranted, particularly in a prospective manner, as it can allow for better understanding of these diseases and lead to development of specific therapies to limit severity of disease and improve patient outcomes. Therefore, the aim of this thesis was to prospectively identify the prevalence of VFSS abnormalities on dogs presenting with respiratory clinical signs compared to healthy controls. With the addition of advanced respiratory diagnostics, we sought to further establish a link between the lung and the gut and describe the potential sequalae of alimentary pathology on the neighboring respiratory system.

CHAPTER 2

DOCUMENTING THE PATHOLOGIC LINK BETWEEN THE LUNG AND GUT: VIDEOFLUOROSCOPIC SWALLOW STUDY DIAGNOSIS OF AERODIGESTIVE DISORDERS IN DOGS

Introduction

Aerodigestive disorders (AeroD) are a complex group of diseases that highlight the close relationship and shared pathology between the alimentary and respiratory tracts.[1] Impaired airway protection, dysphagia, GER, regurgitation, vomiting, or combinations thereof can cause or exacerbate respiratory disease. In dogs, aspiration pneumonia is the most recognized AeroD but a wide spectrum of other disorders are described.[23,25] In humans, AeroD have been linked to chronic sinusitis, laryngeal disorders, chronic cough, inflammatory airway and parenchymal diseases, and others.[5,14] Notably, improvement or resolution of respiratory signs largely depends on treatment of the alimentary disorder.[14] This is mirrored by a study showing improved outcomes in brachycephalic dogs with upper airway obstruction receiving tandem medical therapy for alimentary tract disease with corrective upper airway surgery[61] as brachycephalic dogs are known to have a high prevalence (97%) of concurrent digestive disorders[41]. Diagnostic investigations linking respiratory and alimentary tract disorders in dogs are limited by poor clinical recognition and few specific testing modalities.[1,60] Recognition of AeroD are important as they can allow the clinician to provide a comprehensive therapeutic plan and may affect prognosis. Additionally, idiopathic cough (i.e., cough in absence of identifiable primary respiratory abnormalities) is thought to arise secondary to digestive disorders which must be directly addressed for clinical benefit.[62]

The criterion standard for evaluation of functional upper alimentary tract disease and dysphagia in dogs is the videofluoroscopic swallow study (VFSS).[12] A standardized VFSS protocol has been validated in unrestrained dogs allowed to free-feed contrast-laden liquid, slurry, and kibble to investigate dysphagia.[12] Previous studies have also utilized VFSS to identify common swallowing defects in dogs including abnormal esophageal motility, cricopharyngeal achalasia, and GER,[39,45,48] as well as identifying a higher prevalence of esophageal dysmotility in brachycephalic dogs compared to dogs with other head conformations.[40] Additionally, standardized VFSS has been used to refine the diagnosis of cases previously thought to be idiopathic megaesophagus into a known cause (a functional obstruction called LES achalasia-like syndrome).[63] While these studies[12,39,45,48,60] succeeded in characterizing abnormalities on VFSS, they failed to investigate potential consequences to the respiratory tract. A recent retrospective study using the standardized VFSS protocol documented swallowing abnormalities in 81% of dogs presenting for cough in the absence of reported alimentary tract signs.[60] This suggests that a potentially large population of dogs with respiratory signs may have AeroD and may benefit from treatment aimed at the gastrointestinal tract.

Prospective evaluation of VFSS in dogs with respiratory clinical signs but absent alimentary clinical signs compared to healthy control dogs is warranted. We hypothesized that dogs presenting for respiratory disease evaluation while lacking signs of dysphagia, vomiting, or regurgitation (i.e., RESP group) would have a proportionally greater degree of abnormal VFSS metrics compared with healthy control dogs (CON) lacking clinical signs of respiratory and alimentary disease. The objectives of our study were to (1) demonstrate that RESP dogs would have more occurrences of abnormal subjective VFSS

metrics (oral-preparatory phase defects, pharyngeal phase defects, abnormal esophageal contraction and peristalsis, megaesophagus, pathologic GER, nasopharyngeal reflux, pathologic aerophagia, and hiatal hernia) versus CON, (2) document that objective VFSS metrics in RESP dogs would be consistent with swallowing dysfunction (larger pharyngeal constriction ratio, PCR; higher penetration-aspiration score (PAS); and longer esophageal transit time (ETT)) versus CON, and (3) demonstrate RESP dogs with parenchymal disease have more severe reflux and a higher PAS than RESP dogs with airway disease.

Materials and Methods

Case selection

For this prospective, case-control study, 60 dogs presented to the University of Missouri Veterinary Health Center between July 1st 2020, and May 1st 2022. Forty-five dogs presenting for clinical signs of cough, audible respiratory disease (stertor, stridor, wheeze), or respiratory distress, without dysphagia, regurgitation or vomiting in the prior 2 months were enrolled in the RESP group. RESP dogs underwent an unrestrained, free-fed VFSS that included baseline respiratory fluoroscopy views, CT scan with paired inspiratory and expiratory breath holds (ventilator-controlled), tracheobronchoscopy, and bronchoalveolar lavage fluid (BALF) submitted for both cytology and bacterial culture. Dogs were excluded if they were refused to spontaneously drink or eat the offered liquid, slurry, and kibble consistencies during the VFSS. Fifteen dogs owned by faculty and staff of the University of Missouri Veterinary Health Center were enrolled in the CON group based on the absence of respiratory and alimentary signs in the preceding 2 months. CON

dogs only had a VFSS study performed. CON dogs were excluded if dysphagia or regurgitation was visually noted by observation of the dog in the clear plexiglass kennel during the VFSS (n=2). Written informed consent was obtained from all owners and the study was performed according to institutional guidelines for animal care and use (IACUC protocol #10121). Demographic data including clinical signs, duration of signs, head conformation, sex, body condition score, and comorbidities were acquired from the medical record. Results of heartworm testing were either acquired from referring veterinarian records (previous 6 months) or performed in hospital using heartworm antigen by point-of-care, SNAP® Heartworm RT Test (SNAP; IDEXX Laboratories, Inc., Westbrook, ME, USA).

Videofluoroscopy

Standardized VFSS including baseline survey respiratory fluoroscopy was performed using an unrestrained, free-feeding protocol and plexiglass kennels in RESP and CON dogs with details provided in a prior publication.[12] Dogs were fasted for 12 hours prior to study. Studies were performed at 30 frames per second using a GE OEC 9900 Elite Mobile C-Arm system (GE Healthcare OEC, Salt Lake City, UT). A distance calibration marker (19 mm stainless steel ball secured to a plastic coil collar fitted around the neck) was positioned at the midline ventral neck of each dog to facilitate objective quantification of swallowing function. Baseline survey images prior to feeding were obtained focusing on the oropharynx and upper airways (oropharynx, nasopharynx, larynx, extra-thoracic trachea), lower airways (intrathoracic trachea and mainstem bronchi), esophagus, and stomach. Subjective and objective VFSS metrics using liquid

and slurry with iohexol (Omnipaque 350, GE Healthcare; diluted to 25% using canned chicken broth or canned pureed food) and barium extruded kibble (40% w/v) were assessed individually. The bowl was positioned ~6 inches below wither height, which allowed the fluoroscope to remain at a constant height while following swallowed boluses through the upper GI tract.[12] After consuming all three consistencies, additional cine loops were obtained while radiology personnel applied abdominal pressure to evaluate for pathologic reflux or a sliding hiatal hernia.

Subjective metrics included abnormalities of: (1) oral-preparatory phase (jaw excursion, mastication, collection of the food bolus at the tongue base) (2) pharyngeal phase (pharyngeal constriction, transfer of the bolus from the pharynx through the UES) (3) esophageal stage (efficacy of contraction and peristalsis for aboral bolus transport, beginning in the proximal esophagus and initiated by pharyngeal swallows), (4) megaesophagus (esophageal dilation with poor or absent motility) (5) GER (reflux originating in the stomach traversing into the esophagus, with pathologic reflux defined as retrograde movement of ingesta into the proximal or middle third of the esophagus or outside the esophagus [extra-esophageal reflux]) (6) nasopharyngeal reflux (movement of contrast from the pharynx to nasopharynx during pharyngeal swallow or with esophago-oropharyngeal reflux) (7) excessive aerophagia (swallowing marked volumes of air resulting in greater than one third of the end gastric volume) and (8) hiatal hernia (passive or induced herniation of the stomach into the thoracic cavity through the esophageal hiatus). Analysis of subjective and objective metrics was performed by one blinded investigator (M Grobman) with experience in interpretation of VFSS who reviewed

videos frame by frame using Pinnacle Studio 25 video editing software (Corel, Ontario, Canada).

Objective metrics included PCR, PAS and ETT and when possible, were assessed for each food consistency. PCR was determined from a representative still frame of the larynx that at rest, was within the fluoroscope field of view for objective quantification purposes with the calibration marker in view. A region of interest (ROI) was digitally drawn using commercially available freeware (NIH ImageJ, National Institute of Health, Bethesda, MA) to encompass the supraglottic airspace within the pharynx. The ROI was defined dorsally by the dorsal pharyngeal wall and extended from the hyoid apparatus rostrally to the UES caudally. Next, a maximum constriction frame was identified in which the dorsal pharyngeal wall had assumed its most caudoventral position. An ROI was drawn in the corresponding image to outline any residual barium or airspace remaining within the pharynx at this point. Both ROIs were expressed in pixel number. Pharyngeal constriction ratio was calculated as the ratio of pixels in the maximum constriction frame ROI divided by the pixels in the hold frame ROI. To assess laryngeal airway protection, a PAS was assigned on a scale of 1 to 7, with 1 considered normal, 2-4 considered penetration and 5-7 considered aspiration as previously published.[64] Pathologic PAS was defined as a score ≥3.[64] The ETT was defined as the duration of time from the bolus entering the esophagus after pharyngeal swallow until the tail of the bolus entered the stomach.[12]

Respiratory diagnostics

For dogs receiving thoracic radiography, 3-view radiographs were obtained and all were reviewed by a board-certified radiologist. For dogs receiving echocardiography, transthoracic echocardiography was performed using an Artida Aplio (Toshiba Medical Systems Corporation, Otawara, Japan) with standard imaging planes[65] by a board-certified cardiologist or a directly-supervised cardiology resident. When present, tricuspid regurgitation velocity (TRV) was interrogated; supportive clinical signs and a TRV>3.4 m/s were compatible with intermediate to high probability of pulmonary hypertension.[66]

Anesthesia (protocols at the discretion of a board-certified anesthesiologist) was required for advanced imaging. Anesthetic induction took place in the room with the CT scanner using intravenous propofol (Diprivan, Frenenius Kabi USA, LLC, Lake Zurich IL) titrated to effect (induction: 6 mg/kg IV once; maintenance 0.2-0.5 mg/kg/min). A subset of dogs had functional upper airway examination using Doxapram hydrochloride (Dopram, Hikma Pharmaceuticals USA, Eatontown NJ) 1 mg/kg intravenously to stimulate deep inhalation. Appearance of mucosa, soft palate length, and presence of tonsillar eversion, epiglottic retroversion, laryngeal paresis, and laryngeal paralysis were recorded. Dogs were intubated with a sterile endotracheal tube for CT scans.

Thoracic CT was accomplished with assistance from a mechanical ventilator (Engstrom Carestation ventilator, GE Healthcare, Chicago, IL) in the volume-controlled ventilation setting with the following standardized parameters: 40% fraction inspired oxygen, tidal volume of 10 ml/kg, respiratory rate of 10 breaths per minute, inspiratory:expiratory ratio of 1:3.5, and positive end expiratory pressure (PEEP) of 5 cm H_2O. Ventilator settings were adjusted to meet patient needs. CT images were acquired

using inspiratory and expiratory breath holds with the expiratory breath hold having PEEP=0 cm H20. Additional inspiratory and expiratory breath hold series were performed following administration of an intravenous contrast agent (Omnipaque, GE Healthcare Inc., Marlborough, MA). Following acquisition of CT images, patients were transferred to a gas anesthetic machine and maintained on 100% oxygen while moved to the endoscopy suite for tracheobronchoscopy and BALF. CT studies were interpreted by board-certified radiologists.

Dogs were positioned in sternal recumbency and prior to bronchoscopy a sterile endotracheal tube was replaced with a sterile red rubber catheter to provide oxygen. A sterilized flexible fiberoptic bronchoscope (Model EB-450t & FB-120p Fujinion, Toyko, Japan) was passed through the larynx into the tracheobronchial tree. The tracheobronchial tree was evaluated in a standard fashion and site for BAL was chosen based on findings from thoracic CT, bronchoscopy, or both. Collection of BAL was performed by instilling one to three 20ml aliquots of sterile saline through the channel of the bronchoscope when wedged in an airway. Samples were pooled, placed on ice, and delivered to the University of Missouri Veterinary Health Center's clinical pathology laboratory and diagnostic laboratory for culture and bacterial culture, respectively.

Total nucleated cell count (TNCC) was determined using an automated cell counter (Advia 120; Siemens, Deerfield, IL). A 100 to 500 cell count differential was performed by a board-certified clinical pathologist on Wright-stained cytospin material. Reference ranges for normal BAL cytology were as follows: TNCC <500 cell/ µl, >78% macrophages, <7% lymphocytes, <5% neutrophils, <6% eosinophils, <1% mast cells and <1% epithelial cells.[67] Samples submitted for culture were plated on MacConkey and

blood agar for aerobic, and chocolate agar for capnophilic cultures. Dogs that had received antimicrobials prior to airway lavage had BAL inoculated into a growth medium with antimicrobial removal devices. Organisms were identified and reported semi-quantitatively using CFU/ml.

Final respiratory diagnoses

A comprehensive evaluation of the medical record, including signalment, prior referring veterinary records, history, physical examination, respiratory diagnostics (including review of all CT images and, when available, video clips of tracheobronchoscopic examination), and reports of lung histology (2 antemortem, 1 post-mortem) was performed by one author with expertise in respiratory disease (C Reinero) to assign final diagnoses. Final diagnoses determined for each RESP dog were based on previously published definitions (Gamracy, J. et al, 2022 Supplementary Table 1[68]) and additional diagnoses in Table 1. A classification scheme for anatomic site of pathology in RESP dogs based on airway and parenchymal involvement is shown in Table 2 with examples of thoracic CTs underlying each category shown in Figure 1. For statistical comparisons, RESP dogs with airway disease and airway predominant disease were grouped together in the airway disease group, and dogs with parenchymal disease and parenchymal predominant disease were grouped together in the parenchymal disease group.

Statistical Analysis

Statistical analyses were performed using SigmaPlot data analysis software (version 12.0, Systat Software Inc. Chicago, IL) Descriptive statistics were performed where appropriate. All data were found to be non-normally distributed using a Sharpio-Wilk test. Data were evaluated non-parametrically with results presented as median and interquartile ranges (IQR). A Mann-Whitney rank sum test was used to determine the difference between RESP and CON groups in terms of presence or absence of evaluated metrics. A Fischer's exact test was used to compare the presence or absence of evaluated metrics between subgroups of RESP dogs (those with predominating airway disease or parenchymal disease) and CON. Significance was set at $p < 0.05$.

Results

Animals

One-hundred and forty-seven cases were identified for inclusion over the two-year period. A flowchart of inclusion and exclusion criteria is shown in Figure 2. Forty-five dogs met enrollment criteria for the RESP group. The median (IQR) age of RESP dogs was 9 (4.3-11.0) years and included 24 castrated males, 15 spayed females, and 5 intact males. The median (IQR) body condition score was 6.0 (5.0-7.8) and body weight was 20.8 (9.0-31.3) kilograms. Breeds included in the RESP dog group were Labrador Retriever (n=8), Australian shepherd (5), mixed breed (5), Golden retriever (3), Yorkshire terrier (3), Pug (2), French bulldog (2), Miniature poodle (2), and one each of Dachshund, Border collie, Beagle, Havanese, Pomeranian, German shepherd, Shih Tzu, Norwegian elkhound, Miniature pinscher, Chihuahua, Shetland sheepdog, Tibetan terrier, West Highland white terrier, and Siberian Husky. Head conformations included

mesaticephalic (n=34), brachycephalic (7), and dolichocephalic (4). Median (IQR) for body temperature, heart rate and respiratory rate were 38.8 (38.3-39.1) °C, 120 (100-127) beats per minute) and 32 (24-42) breaths per minute. Presenting respiratory signs included cough (n=36), excessive panting (7), collapse (7), exercise intolerance (5), sneeze (1) and nasal discharge (1). When owners were specifically questioned, 3 dogs coughed with eating or drinking. The median (IQR) for duration of clinical signs was 5 months (2-12 months).

Fifteen dogs met the inclusion and exclusion criteria for enrollment in the CON group. Median (IQR) for age was 4 (3-8 years) and included 8 castrated males, 6 spayed females, and 1 intact male dog. Median (IQR) for weight and body condition score were 19 (10.75-24.9) kilograms and 5 (5-6.5), respectively. Breeds included mixed breed (n=10), Miniature poodle (2), American Staffordshire terrier (1), Labrador retriever (1), and Catahoula leopard dog (1). Fourteen dogs had a mesaticephalic head conformation and one was dolichocephalic. Median (IQR) for body temperature, heart rate, and respiratory rate were 37.8 (38.4-39) °C, 120 (105-122) beats per minute, and 24 (22-28) breaths per minute. Comorbid diseases included atopic dermatitis (n=2) and ACVIM Stage B2 degenerative mitral valve disease (1). Follow up on all healthy controls 1 year after VFSS revealed that 4 dogs had developed respiratory signs; 3 had developed a cough and 1 dog developed a single episode of aspiration pneumonia.

When comparing age, body weight, body condition score, temperature, pulse and respiration, the only significant difference between RESP and CON dogs was that the RESP dogs were significantly older ($p=0.045$).

Fluoroscopy

Baseline (pre-feeding) respiratory fluoroscopic images revealed respiratory abnormalities in 19 RESP dogs. Abnormalities in RESP dogs included narrowed mainstem bronchial diameters spontaneously or with cough (n=14), dynamic pharyngeal collapse/narrowing (5), cervical lung herniation (5), elongated soft palate (5), tracheal collapse (5), caudal movement of the epiglottis on inspiration (2 persistent, 1 intermittent), and a tracheal opacity (1). For CON dogs, baseline respiratory fluoroscopy identified mainstem bronchial collapse in 1 dog. RESP dogs were significantly more likely to have baseline abnormalities on respiratory fluoroscopy than CON dogs ($p=0.023$).

Abnormal subjective metrics on VFSS were noted in 34/45 (75%) RESP dogs. Oral preparatory defects were noted in 9 dogs and pharyngeal phase defects in 7 dogs. Esophageal weakness was present in 10 dogs, but no dogs had megaesophagus. For RESP dogs with pathologic reflux (n=16), maximal margination sites were extraesophageal (n=6), proximal esophagus (4), mid-esophagus (4), and esophagopharyngeal (2). Physiologic reflux (considered a variant of normal) was noted in 11/45 RESP dogs. Pathologic aerophagia was identified in 21 dogs. Four dogs had a hiatal hernia. Comparatively, only 2 CON dogs had abnormalities on VFSS, one with pathologic reflux marginating to the mid-esophagus with pathologic reflux, and the other with pathologic reflex marginating to the mid-esophagus, esophageal weakness, and hiatal hernia. Follow up at 1 year in these 2 CON dogs revealed both had developed respiratory clinical signs. Physiologic reflux was noted in 6/15 CON dogs. Overall, RESP

dogs were significantly more likely to have abnormal subjective VFSS metrics than CON dogs ($p = 0.006$).

Pharyngeal constriction ratio measurements were able to be measured in 27 RESP dogs and 10 CON dogs with a median (IQR) for PCR being 0.0961 (0.0057-.0259) and 0.0098 (0.0044-0.0129), respectively. There was no significant difference in PCR measurements between groups. Twelve RESP dogs had pathologic PAS (score≥3). The median (IQR) for PAS in all RESP dogs was 1 (1-2) and for pathologic PAS, 7 (3-7). No CON dog had penetration or aspiration noted. When comparing RESP and CON dogs, the former were significantly more likely to have pathologic PAS ($p=0.027$). In RESP dogs, ETT was able to be measured for puree (n=15), liquid (9), and kibble (18) with the median (IQR) being 4.6 (4.0-5.4) seconds, 3.1 (2.2-3.7) seconds, and 4.9 (3.8-5.5) seconds, respectively. Esophageal transit times in the CON group were only measurable for 3 dogs with puree and 2 dogs each with kibble and liquid. Statistical comparisons between RESP and CON groups were not performed due to low sample size for this metric.

Respiratory diagnostics

Thirteen dogs had thoracic radiographs performed. Radiographic patterns included bronchial (n=10), interstitial (3), and alveolar (1). Three dogs had radiographic evidence of cardiomegaly, and one had both tracheal and mainstem bronchial collapse. Two dogs had unremarkable thoracic radiographs. Three of 22 dogs had echocardiography that showed intermediate to high probability of pulmonary

hypertension. Heartworm testing was available for all dogs with two (2/45, 4%) being positive.

Sixteen dogs had a functional upper airway examination performed. Findings included laryngeal erythema (7), laryngeal paresis (5), laryngeal paralysis (4), and epiglottic retroversion (2). Findings on thoracic CT included both airway and parenchymal changes, with most dogs having greater than one type of pattern. Airway changes included peribronchial cuffing (19), bronchiectasis (17), bronchomalacia (16), tracheal collapse (9) and mainstem bronchial collapse (3). Major pulmonary patterns included increased attenuation (28), linear pattern (22), decreased attenuation (9) and a nodular/mixed pattern (1). Examples of these patterns are shown in Figure 3.

Bronchoscopic findings included erythema of the trachea, bronchi, or both (n=40), bronchomalacia (16), bronchiectasis (13), tracheal collapse (9), and mainstem bronchial collapse (3). Total nucleated cell count median (IQR) of BAL was 210/µl (140-380/ µl). Nineteen dogs (19/45, 42%) had neutrophilic inflammation (3 were septic), 9 dogs had eosinophilic inflammation (9/45, 20%), 6 had mixed inflammation (6/45 13%), 5 dogs had a normal cytology (5/45, 11%), 4 had macrophagic inflammation (4/45, 8%), one dog had mastocytic inflammation (1/45, 2%) and one had lymphocytic (1/45, 2%). Twenty-five dogs had a BAL culture performed. While 16 cultures revealed isolates of one or more organisms, only 2 dogs had growth that was considered clinically relevant as these had colony forming units >100,00 with septic neutrophilic inflammation on cytology. In all other dog with positive cultures, the organisms were not considered pathogenic due to only being recovered on enrichment broth and lack of septic inflammation on BAL cytology.[69]

Final diagnoses

Thirty-seven dogs in the RESP group had more than one final diagnosis (range, 2-7 diagnoses). Overall, final diagnoses included bronchiectasis (17), bronchomalacia (16), chronic bronchitis (n= 14), uncharacterized parenchymal disease (11), tracheal collapse (10), bronchiolar disease (9), suspect pulmonary fibrosis (8), eosinophilic bronchitis (6), laryngeal paresis (5), laryngeal paralysis (4), eosinophilic bronchopneumopathy (4), aspiration pneumonia (3), mainstem bronchial collapse (3), epiglottic retroversion (2), and one each of eosinophilic pneumonia, pyothorax, pulmonary nodule, pulmonary bulla (cystic lung disease), BOAS, interstitial lung disease, other pulmonary vascular disease, and developmental lung disease. Intermediate or high probability of pulmonary hypertension (which is not a disease per se but a sequel to a variety of cardiac, vascular, and respiratory disorders) was noted in 3 dogs.[66]

Nineteen dogs were considered to have an airway or airway predominant disorder, and 13 dogs had a parenchymal or parenchymal predominant disorder. Eleven dogs had equal distribution between airway and parenchymal disorders and 3 dogs had no airway or parenchymal lesions and were diagnosed with idiopathic cough. All dogs with idiopathic cough had VFSS abnormalities including extra-esophageal reflux (n=2) and sliding hiatal hernia (1).

Presence of pathologic reflux was not significantly different ($p = 0.281$) between CON dogs (2/15), RESP dogs with airway disease (7/19), and RESP dogs with parenchymal disease (3/13), nor was there a difference in presence of pathologic reflux between RESP dogs with airway disease and RESP dogs with parenchymal disease ($p =$

0.467). Presence of pathologic PAS was significantly different (p = 0.015) between CON dogs (0/15), RESP dogs with airway disease (7/19), and RESP dogs with parenchymal disease (2/13). Post-hoc analysis showed RESP dogs with airway disease were more likely to have pathologic PAS than CON dogs (p = 0.011). There was no significant difference in presence of pathologic reflux between RESP dogs with airway disease versus RESP dogs with parenchymal disease (p = 0.246).

Discussion

In dogs presenting for evaluation of respiratory clinical signs without owner-reported dysphagia, regurgitation, or vomiting, 75% had abnormalities on VFSS, underscoring the critical interplay between the shared respiratory and upper digestive tracts. A prior retrospective study documented a high rate (81%) of VFSS abnormalities in dogs presenting solely for cough, and the current study supports similar findings in dogs with more diverse respiratory clinical signs and in a prospective manner, with healthy control dogs and using uniform and standardized advanced diagnostics.[60] Pathologic reflux did not differ between RESP (15/45, 33%) and CON (2/15, 13%) dogs, but interestingly, both CON dogs with pathologic reflux developed overt respiratory clinical signs within a year. Further study is warranted to determine if pathologic reflux noted on VFSS is an early predictor for development of clinical respiratory disease. While there were significantly more subjective VFSS abnormalities in RESP versus CON dogs (p=0.006), the only objective VFSS metric that significantly differed between RESP and CON dogs was pathologic PAS (p=0.027). When comparing pathologic PAS between RESP dogs with airway disease, RESP dogs with parenchymal disease, and

CON dogs, there was a significant difference attributable to RESP dogs with airway disease having a higher pathologic PAS than CON dogs. Thus, higher PAS in dogs with airway disease suggests a contribution of penetration-aspiration to the pathogenesis of disease in these patients. Collectively, results of this study emphasize the need to identify, understand, and address silent alimentary tract disease as a contributor to a large spectrum of respiratory disorders.

Although RESP dogs were significantly older than CON dogs, a prior study documented lack of age-related changes on VFSS in healthy dogs.[12] In humans, obesity is shown to increase the risk of development of AeroD.[70] However, neither body weight nor BCS were shown to be significantly different between study groups. Brachycephalic breeds are predisposed to aspiration-related respiratory disease such as aspiration pneumonia and additionally have a high prevalence of tandem digestive tract abnormalities.[24,41] Our study could not statistically compare differences in head conformation between groups as our CON group lacked brachycephalic dogs, perhaps because the majority of brachycephalic dogs have at least some evidence of respiratory or digestive clinical signs and thus would fail inclusion criteria as set out in the current study.

A thorough diagnostic evaluation revealed that most dogs (37/45, 82%) had multiple respiratory disorders and that VFSS was key to provide evidence of overlap between pathology of the digestive and respiratory tracts. Airway diseases identified included functional, structural, and inflammatory etiologies. Functional laryngeal examination showed 7 dogs with laryngeal erythema, 5 dogs with laryngeal paresis, and 4 dogs with laryngeal paralysis. In humans, repetitive, silent aspiration is a cause of

laryngeal paresis or paralysis.[71] While a prior study showed a subset of dogs presenting for cough had abnormalities on laryngeal examination suggestive of abnormal airway defenses that the authors speculated could lead to micro- or microaspiration, it was not proposed that the laryngeal abnormalities themselves resulted from repetitive micro- or macroaspiration.[30] Bi-directional pathology is likely. Inflammatory airway diseases were commonly identified in the current study including bronchiectasis (17/45, 38% dogs), chronic bronchitis (14/45, 31% dogs), and eosinophilic bronchitis and bronchopneumopathy (6/45, 13% dogs and 4/45, 9% dogs, respectively).

Thirteen RESP dogs had evidence of a parenchymal predominate disorder, perhaps suggesting swallowing abnormalities including aspiration of gastric acid and digestive enzymes can incite inflammation and potentially lead to parenchymal pathology. Numerous studies in humans have shown higher prevalence of GER, anatomic abnormalities predisposing to GER, and dysphagia in patients with idiopathic pulmonary fibrosis and interstitial pneumonia when compared to those without pulmonary disease.[72-74] It is possible that GER and other digestive abnormalities in dogs can similarly contribute to parenchymal pathology such as pulmonary fibrosis or other uncharacterized parenchymal diseases including interstitial lung diseases, which are comparatively poorly described and uncommonly recognized in veterinary medicine.[75]

In support of our hypothesis, RESP dogs had more subjective abnormalities on VFSS than CON dogs, with the top 3 abnormalities being pathologic aerophagia (21/45, 47%), reflux (16/45, 36%), and esophageal dysmotility (10/45, 22%). It is worth noting that absence of observing a VFSS abnormality does not rule out its presence, as cine loops were relatively brief, and anomalies could be intermittent and therefore evade video

capture. Pathologic aerophagia is defined as swallowing excessive air with consumption of food or liquid leading to air accounting for >1/3rd of the end gastric volume.[60] While not studied in dogs, in people with obstructive sleep apnea treated with continuous positive airway pressure, those with aerophagia have a greater presence of GER.[76] This is thought to be induced by gastric distension exacerbating LES relaxations.[76] In people, a higher prevalence of GER compared to the general population has been documented in those with COPD and asthma.[77,78] Similarly, dysphagia and/or esophageal weakness have a high prevalence in people with chronic respiratory diseases such as COPD or sleep apnea.[79] Given the high number of RESP dogs with these abnormalities noted on VFSS, it is possible that they may be contributing to respiratory disease.

Idiopathic cough is a syndrome recognized in people where the cause of cough is undetermined despite systematic evaluation. Though no cause can be found, multiple postulations exist including undetected GER or organ-specific autoimmune disease.[62,80] Three dogs in our study had unremarkable advanced respiratory diagnostics and all had abnormalities on VFSS; they were diagnosed with idiopathic cough. In these 3 dogs, the presence of tandem VFSS abnormalities and positive response to therapeutic intervention targeting alimentary tract disease for suspected GER (data not shown) support that as in human medicine, idiopathic cough in dogs could be related in part to digestive disorders.[80]

This study also demonstrated that RESP dogs were significantly more likely to have pathologic PAS than CON dogs, with overt macroaspiration being a clear risk factor for injury to the respiratory tract. Due to intermittent poor patient compliance and inability to capture desired frames (calibration marker for PCR, a complete clip from

pharyngeal swallow to gastric filling for ETT), we were unable to obtain PCR and ETT measurements on all dogs, thus limiting statistical comparisons. Both human and veterinary studies have shown that higher PCRs are associated with dysphagia.[47-50] Additionally, in humans, prolonged ETT has been associated with increased risk of aspiration and reflux.[81] Future studies in dogs with respiratory disease, including healthy control dogs in which these metrics can be consistently measured are needed to determine if they can be indicative of AeroD, even in the absence of other alimentary abnormalities.

Reflux and aspiration have been documented in humans with both airway and parenchymal diseases such as COPD and pulmonary fibrosis.[14,20-22] Investigating differences in pathologic reflux and pathologic PAS between RESP dogs and CON dogs, the only significant finding was that RESP dogs with airway disease were more likely to have pathologic PAS compared to CON dogs. We were thus unable to prove our hypothesis that dogs with parenchymal disease would have more severe reflux and a higher PAS than those with airway disease. This was based on the presumption that aspirated reflux contacts the airways first and, assuming a smaller volume and adequate mucociliary function, it should be cleared before damaging the parenchyma.[71] Additionally, airway defenses may be overwhelmed in dogs with repetitive micro- or macro-aspiration, leading to parenchymal damage that may also occur with a large volume of aspirated material. While neither pathologic reflux or pathologic PAS differed between dogs with airway and parenchymal disease, this could have been due to small sample size, capturing small durations of time on VFSS clips that may miss penetration or aspiration events, or because there is no association with higher PAS based on airway versus parenchymal location.

Limitations of study include the aforementioned short cine loops of data capture and sporadic presence of some VFSS abnormalities. Additionally, VFSS is not capable of identifying micro-aspiration which may be a key contributor to respiratory pathology when repetitive. Thus, the current study, despite finding a high incidence of VFSS abnormalities, may have underestimated the true incidence of aerodigestive pathology. Another important limitation was that we were not able to obtain PCR and ETT for all dogs due to poor patient compliance (movement within the kennel, refusal to eat or finish a particular food or liquid) or inability to record the needed measured metrics within the captured frame. This prevented certain statistical comparisons between groups. Finally, while our goal was to encompass a diverse population of dogs with varied respiratory disorders, in doing so the number of dogs with each disease was limited. Future studies could investigate a uniform respiratory disease population (e.g., bronchiectasis or pulmonary fibrosis) to characterize VFSS abnormalities more specifically with that disorder.

In conclusion, in dogs presenting for evaluation of a spectrum of respiratory disorders with advanced diagnostic imaging including VFSS, a pathologic link between the alimentary and respiratory tracts (i.e., AeroD) was observed. Importantly, maintaining a high index of suspicion for AeroD is important as dogs with respiratory disease may frequently have silent alimentary disease as is seen in people.[71,82] The majority (75%) of RESP dogs had subjective VFSS abnormalities, and RESP dogs with airway disease had a significantly higher PAS than CON dogs. At present, VFSS is an underutilized tool in respiratory disease evaluation. Identification of AeroD is the first step to improving medical management of respiratory disease by developing a comprehensive treatment

plan targeting both respiratory and alimentary tract pathology. Abnormalities on VFSS can allow the clinician to tailor specific treatments to patients with AeroD, including optimizing food or water consistency to decrease risk of aspiration or recommending surgical intervention for disorders such as hiatal hernias or LES achalasia-like syndrome.[60] This study shows that VFSS in an invaluable tool in identifying AeroD in dogs and should be considered as part of a thorough diagnostic evaluation of dogs with respiratory disease, even in absence of alimentary signs.

CHAPTER 3

CONCLUSIONS & FUTURE DIRECTIONS

AeroDs are likely more common than currently recognized in veterinary medicine. The study described in the second chapter of this thesis demonstrates that there is a clear association between dogs with respiratory disease and alimentary pathology. Importantly, it also highlights that alimentary disease is often silent in the canine population as no patients in this study had owner reported alimentary signs. Respiratory disease is an important cause of morbidity and mortality in dogs.[26,83,84] Early recognition and treatment of disease is key to improving quality of life. However, AeroDs are often not recognized or even thought of as cause of respiratory signs or cough. In dogs with AeroD, clinicians may not improve respiratory disease without first recognizing alimentary pathology and its sequalae on the respiratory tract. Currently, investigation of AeroDs with diagnostic modalities such as VFSS are underutilized in dogs presenting for evaluation of cough, and as shown in this thesis, other respiratory clinical signs.

Chapter two of this thesis illustrates that VFSS is a vital diagnostic for investigation of AeroDs. VFSS allows clinicians to determine if structural pathology is present that may be remedied with surgical correction or even tailor optimal food consistency in patients with dysphagia. Information gathered from VFSS can be used for direct intervention that benefits both the alimentary and respiratory tracts. Correction or treatment of these diseases can lessen or stop injury to the airways and pulmonary parenchyma, preserving lung health.

Future studies should attempt to elucidate the connection between the severity of alimentary disease such as GER and the presence of airway versus parenchymal disease.

The present study in chapter two included dogs with both airway and parenchymal disorders, therefore making the number of dogs with each disease low and precluding further statistical evaluation. As discussed in chapter one, there is a known link between the presence and severity of GER and parenchymal diseases such as COPD or asthma in humans.[14,77] It is likely that the same is true in dogs. Elucidation of this connection can allow for earlier detection of the disease and targeted intervention.

Current intervention for GER in dogs is limited. Treatment is aimed at reducing acid production with proton-pump inhibitors, minimizing a large gastric volume with smaller and more frequent feedings ideally with a low fat diet, and increasing gastric emptying time to avoid reflux.[85] If a structural abnormality is present (such as sliding hiatal hernia) and if medical management is unsuccessful, surgical intervention can be pursued. In humans, other treatment modalities to address reflux include Nissan fundoplication, which can strengthen the anti-reflux barrier at the gastroesophageal junction.[86] Interventional procedures including endoscopic radiofrequency heating of the gastroesophageal junction (Stretta procedure) and endoscopic gastroplasty have been described.[87] These are currently not performed in veterinary medicine. However, further investigation into the severity of GER and its potential damage to the airway may lead to novel procedures in dogs aimed to prevent GER and subsequent respiratory injury.

As described in the human literature, micro-aspiration may play a key role in the development of respiratory disorders, but these events would not be evident on VFSS. In humans, esophageal pH monitoring is employed to evaluate for GER with the possibility it may be linked to microaspiration.[88] In the future, advanced diagnostics such as pH monitoring in dogs could be considered to recognize clinically significant GER and

employ earlier treatment. Previously described advanced diagnostics such as nuclear scintigraphy that can better assess for micro-aspiration can be expanded upon and possibly used more routinely in a clinical setting.[9] Additionally, use of biomarkers assessing for specific proteins found in the alimentary tract, such as pepsin in oropharyngeal samples or bile acids in BALF, could be promising as alternative, potentially less invasive means of diagnosing GER in clinical patients.[10,11,89]

Lastly, as outlined in chapter two of this thesis, the authors were not able to obtain objective metric measurements for all patients including ETT and PCR. This could be improved with more optimal patient selection for compliance or possibly refining food given during the study to entice consumption. Further studies with optimal patient compliance can be pursued to hopefully determine if these metrics are correlated with increased risk of reflux, penetration, or aspiration. Determination of this would allow clinicians viewing short cine clips of VFSS to hopefully predict swallowing abnormalities. This would be particularly useful when pathology is highly suspected but not directly seen on cine loops, as it would support empirical therapy for a patient who may otherwise not receive treatment.

In conclusion, this thesis provides evidence that AeroDs are common in dogs, and swallowing abnormalities can have significant impact on the airways and pulmonary parenchyma. Identification is key to treating these disorders, but current modalities such VFSS are underutilized. VFSS is an invaluable tool and can guide the clinician to recognize AeroDs, intervene, and preserve lung health to allow dogs to live happier and longer lives.

APPENDIX 1: FIGURES

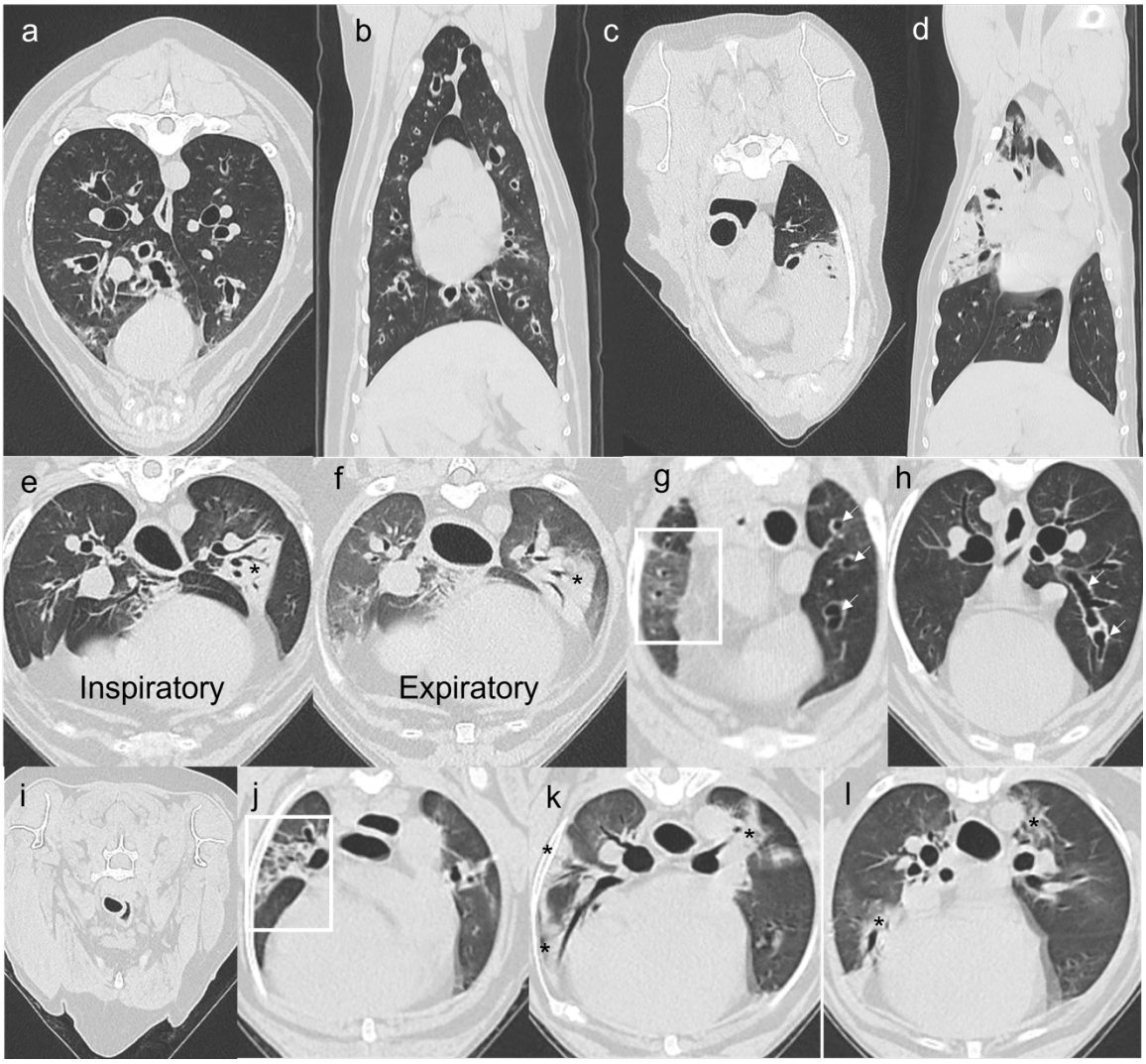

Fig. 1: Use of thoracic CT to aid in classification of final diagnosis in dogs with respiratory disease based on airway, parenchymal, or mixed airway and parenchymal involvement. (a-b) Transverse and dorsal sections from a 6-year-old MC Siberian Husky with airway disease. Note the marked peribronchovascular thickening creating an appearance of peribronchial cuffing in cross-section. Bronchiectasis was noted by dilation and lack of tapering of airways traversing to the periphery. The final diagnoses were eosinophilic bronchitis and bronchiectasis. (c-d) Transverse and dorsal sections

from a 10-year-old FS Labrador retriever with parenchymal disease. Note the extensive regions of consolidation. Histology and response to immunosuppression confirmed an immune-mediated lung disease. (e-f) Paired ventilator-assisted inspiratory:expiratory breath hold transverse sections from an 11-year-old MC French bulldog with both airway and parenchymal disease substantially contributing to clinical signs. On the inspiratory series, increased peribronchovascular thickening and focal consolidation (*) are noted. On the expiratory series, the caliber of the segmental and subsegmental airways are smaller than on the inspiratory series, with a corresponding loss of lung volume and presence of more global ground glass opacity due to downstream effects of bronchomalacia. The previously noted region of consolidation (*) remains present. The final diagnoses included BOAS, bronchomalacia, aspiration pneumonia, and suspect pulmonary fibrosis (corresponding lesions not shown). (g-h) Transverse images from a 13-year-old FS French bulldog with predominating airway lesions and mild, focal evidence of parenchymal disease. In (g), ground glass opacification in the mid-zone of the lung (within the white box), with Hounsfield units ranging from -425 to -550. The white arrows on the right side of the figure show bronchiectatic airways. Further evidence of cylindrical bronchiectasis is seen in (h) with a lack of tapering as shown by the white arrows. Using data from the clinical picture and other advanced diagnostics, the final diagnoses were chronic bronchitis, bronchiectasis, mainstem bronchial collapse, hiatal hernia, and uncharacterized parenchymal disease. (i-l) Transverse images from a 14-year-old FS Pomeranian with predominating parenchymal disease and a lesser contribution of airway disease. In (i) taken from the expiratory series, grade II tracheal collapse is demonstrated by a 50% reduction in luminal diameter with a flattened shape. In j, the box

outlines a region of architectural distortion characterized by traction bronchiectasis/bronchiolectasis superimposed on a background of reticulation and ground glass opacity, compatible with pulmonary fibrosis. In k and l, there are multifocal regions of ground glass opacity and consolidation (*). Final diagnoses were grade II tracheal collapse, grade I mainstem bronchial collapse, grade I bronchomalacia, extra-esophageal reflux, suspect pulmonary fibrosis, and uncharacterized parenchymal disease.

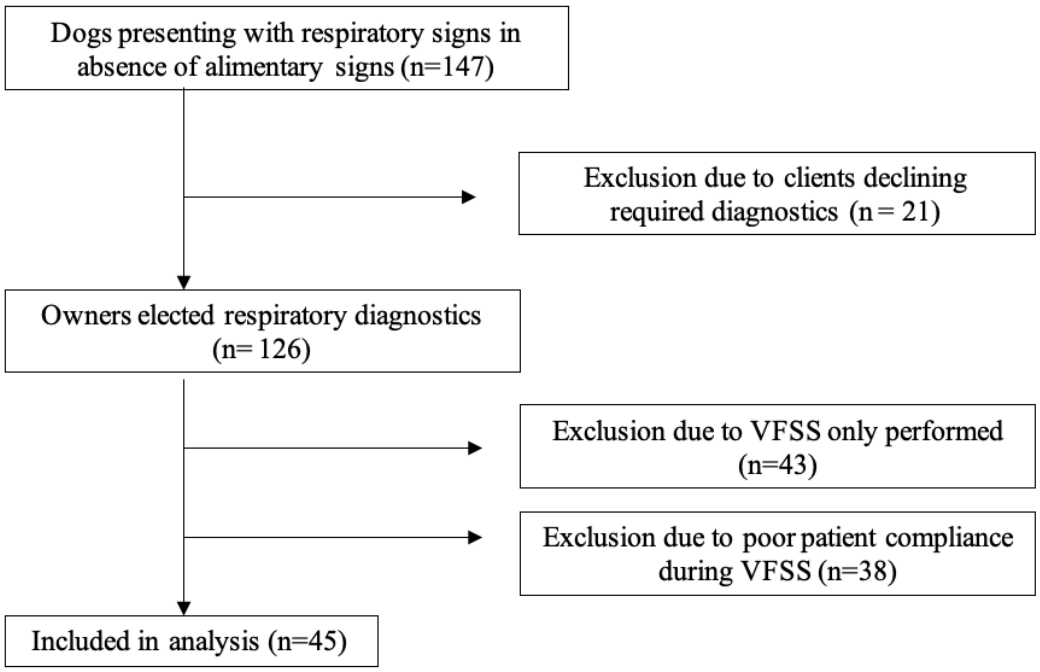

Fig. 2: Flow chart showing inclusion and exclusion of dogs with respiratory clinical signs in the absence of alimentary signs.

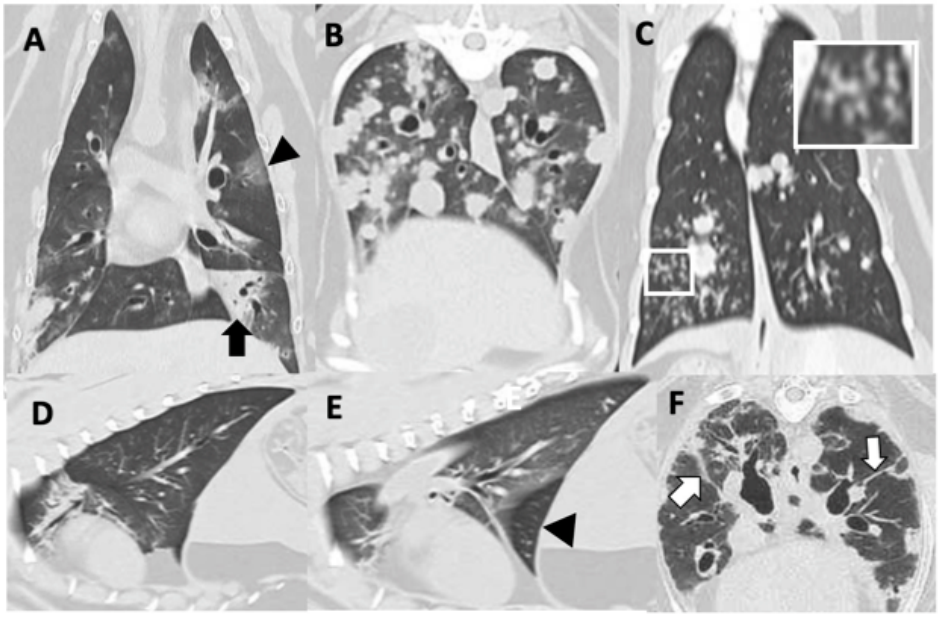

Fig. 3: Examples of the four primary pulmonary patterns on thoracic CT. (A) 666 A transverse image showing a three-density pattern as demonstrated by differing Hounsfield 667 units (HU) consisting of normal lung attenuation (HU=-850), ground glass opacity (HU=-640) 668 and consolidation (HU=35). Ground glass opacity and consolidation fall into the category of 669 increased lung attenuation. (B) A transverse image demonstrating the decreased attenuation 670 pattern (HU=-1000). On the central and right side of the image, the lungs also display a linear 671 pattern. (C) A transverse image illustrating a diffuse nodular pattern. (D) A section from a 672 transverse image showing a linear (reticular) pattern (arrows).

APPENDIX 2: TABLES

Table 1: Additional definitions for respiratory diagnoses and pathologic lesions[a]

Diagnosis	Definition
Epiglottic retroversion	Caudal displacement of the epiglottis into the rima glottidis during inspiration causing upper airway obstruction. Documented by respiratory fluoroscopy and the criterion standard test of functional upper airway examination during robust inspiratory effort.
Laryngeal paresis	Symmetric or asymmetric submaximal abduction of the arytenoids during inspiration. Documented by functional laryngeal examination during robust inspiratory effort.[b]
Uncharacterized parenchymal disease	Evidence of increased opacity or attenuation of the pulmonary parenchyma on thoracic radiography or computed tomography, without or with inflammatory airway cytology lacking obvious infectious organisms or neoplastic cells, and with negative bacterial culture of airway lavage. There is no histologic confirmation of a specific etiology including aspiration pneumonia, bacterial or other infectious pneumonia, interstitial lung disease, or neoplasia.
Developmental lung disease	Histologic evidence of airway dysplasia often with diminished terminal bronchioles and associated alveolar dilation.
Pyothorax	Septic purulent exudate within the pleural space identified via cytologic examination with positive aerobic or anaerobic bacterial culture.
Idiopathic cough	Chronic cough with absence of abnormalities on thoracic radiography, respiratory fluoroscopy, functional upper airway examination, thoracic computed tomography, tracheobronchoscopy and bronchoalveolar lavage and culture. Suspected to be caused by extra-esophageal reflux.
Cystic Lung disease	Thoracic radiography or computed tomographic evidence of air-filled lucencies bordered by a thin wall.

Pulmonary nodule(s)	Solitary or multiple round opacities of varying size (from micronodules to masses) observed on thoracic radiography or computed tomography that reflect infection, non-infectious inflammation, fibrosis, or neoplasia.

These definitions are intended to supplement those provided in Gamracy, J. et al, 2022 Supplementary Table 1[68]

[b]Stimulation of deep inspiration during functional upper airway examination is performed by administration of doxopram

Table 2: Proposed anatomic category of respiratory disease in dogs presenting without alimentary signs

Category	Definition
Airway disease	Laryngeal, tracheal, or bronchial pathology
Parenchymal disease	Pathology focused on the interstitium and alveoli
Both airway & parenchymal disease	Subjective assessment that magnitude of airway and parenchymal pathology are substantial and equally responsible for clinical signs
Airway predominant disease but with both airway and parenchymal pathology	Subjective assessment that the magnitude of airway pathology was responsible for clinical signs, with subtle evidence of parenchymal pathology
Parenchyma predominant disease but with both airway and parenchymal pathology	Subjective assessment that the magnitude of parenchymal pathology was responsible for clinical signs, with subtle evidence of airway pathology
No evidence of airway or parenchymal pathology	No evidence of respiratory disease; may need to consider clinical signs are attributable to a primary digestive disorder (i.e., idiopathic cough)

BIBLIOGRAPHY

1. Grobman M. Aerodigestive Disease in Dogs. Vet Clin North Am Small Anim Pract 2021;51:17-32.
2. Jadcherla SR. Pathophysiology of aerodigestive pulmonary disorders in the neonate. Clin Perinatol 2012;39:639-654.
3. Matsuo K, Palmer JB. Anatomy and physiology of feeding and swallowing: normal and abnormal. Phys Med Rehabil Clin N Am 2008;19:691-707, vii.
4. Ibrahim WH, Gheriani HA, Almohamed AA, et al. Paradoxical vocal cord motion disorder: past, present and future. Postgrad Med J 2007;83:164-172.
5. Houghton LA, Lee AS, Badri H, et al. Respiratory disease and the oesophagus: reflux, reflexes and microaspiration. Nat Rev Gastroenterol Hepatol 2016;13:445-460.
6. Hu X, Lee J, Pianosi P. Aspiration-related pulmonary syndromes. Chest 2015;147:815-823.
7. Kato R, Iwamuro M, Hiraoka S, et al. Evaluation of the Upper Gastrointestinal Tract in Ulcerative Colitis Patients. Acta Med Okayama 2018;72:105-113.
8. Donn SM, Sinha SK. Pulmonary diagnostics. Semin Fetal Neonatal Med 2017;22:200-205.
9. Grobman ME, Maitz CA, Reinero CR. Detection of silent reflux events by nuclear scintigraphy in healthy dogs. J Vet Intern Med 2020;34:1432-1439.
10. Grobman M, Rindt H, Reinero CR. Proteomic Characterization of Canine Gastric Fluid by Liquid Chromatography-Mass Spectrometry for Development of Protein Biomarkers in Regurgitation, Vomiting, and Cough. Front Vet Sci 2021;8:670007.
11. Krishnan U, Mitchell JD, Messina I, et al. Assay of tracheal pepsin as a marker of reflux aspiration. J Pediatr Gastroenterol Nutr 2002;35:303-308.
12. Harris RA, Grobman ME, Allen MJ, et al. Standardization of a Videofluoroscopic Swallow Study Protocol to Investigate Dysphagia in Dogs. J Vet Intern Med 2017;31:383-393.
13. Rofes L, Arreola V, Mukherjee R, et al. Sensitivity and specificity of the Eating Assessment Tool and the Volume-Viscosity Swallow Test for clinical evaluation of oropharyngeal dysphagia. Neurogastroenterol Motil 2014;26:1256-1265.
14. Lee AL, Goldstein RS. Gastroesophageal reflux disease in COPD: links and risks. Int J Chron Obstruct Pulmon Dis 2015;10:1935-1949.
15. Martinucci I, de Bortoli N, Savarino E, et al. Optimal treatment of laryngopharyngeal reflux disease. Ther Adv Chronic Dis 2013;4:287-301.
16. Michaudet C, Malaty J. Chronic Cough: Evaluation and Management. Am Fam Physician 2017;96:575-580.
17. Petrov AA. Vocal Cord Dysfunction: The Spectrum Across the Ages. Immunol Allergy Clin North Am 2019;39:547-560.
18. Vertigan AE, Theodoros DG, Gibson PG, et al. The relationship between chronic cough and paradoxical vocal fold movement: a review of the literature. J Voice 2006;20:466-480.
19. Burbidge HM. A review of laryngeal paralysis in dogs. Br Vet J 1995;151:71-82.

20. Murry T, Tabaee A, Owczarzak V, et al. Respiratory retraining therapy and management of laryngopharyngeal reflux in the treatment of patients with cough and paradoxical vocal fold movement disorder. Ann Otol Rhinol Laryngol 2006;115:754-758.
21. Maret-Ouda J, Markar SR, Lagergren J. Gastroesophageal Reflux Disease: A Review. Jama 2020;324:2536-2547.
22. Weinberger M, Hurvitz M. Diagnosis and management of chronic cough: similarities and differences between children and adults. F1000Res 2020;9.
23. Tart KM, Babski DM, Lee JA. Potential risks, prognostic indicators, and diagnostic and treatment modalities affecting survival in dogs with presumptive aspiration pneumonia: 125 cases (2005-2008). J Vet Emerg Crit Care (San Antonio) 2010;20:319-329.
24. Ovbey DH, Wilson DV, Bednarski RM, et al. Prevalence and risk factors for canine post-anesthetic aspiration pneumonia (1999-2009): a multicenter study. Vet Anaesth Analg 2014;41:127-136.
25. Nafe LA GM, Masseau I, et al. Aspiration-related respiratory disorders in dogs. Journal of the American Veterinary Medical Association 2018;253:292-300.
26. Prather A, Smith T, Poletto D. Aspiration-related lung diseases. J Thorac Imaging 2014;29:304-309.
27. Smith J, Houghton L. The oesophagus and cough: laryngopharyngeal reflux, microaspiration and vagal reflexes. Cough 2013;9.
28. Fass R, Dickman R. Clinical consequences of silent gastroesophageal reflux disease. Curr Gastroenterol Rep 2006;8:195-201.
29. Johnston N, Ondrey F, Rosen R. Airway reflux. Ann N Y Acad Sci 2016;1381:5-13.
30. Johnson L. Laryngeal structure and function in dogs with cough. J Am Vet Med Assoc 2016;249:195-201.
31. Muenster M, Hoerauf A, Vieth M. Gastro-oesophageal reflux disease in 20 dogs (2012 to 2014). J Small Anim Pract 2017;58:276-283.
32. Lux CN, Archer TM, Lunsford KV. Gastroesophageal reflux and laryngeal dysfunction in a dog. J Am Vet Med Assoc 2012;240:1100-1103.
33. Reinero CR, Masseau I, Grobman M, et al. Perspectives in veterinary medicine: Description and classification of bronchiolar disorders in cats. J Vet Intern Med 2019;33:1201-1221.
34. Matsuse T, Fukuchi Y, Oka T. Importance of diffuse aspiration bronchiolitis caused by chronic occult aspiration in the elderly. Chest 1996;110:1289-1293.
35. Hu X, Yi E, Ryu J. Diffuse aspiration bronchiolitis: analysis of 20 consecutive patients. J Bras Pneumol 2015;41:161-166.
36. Hawkins E, Basseches J, Berry C. Demographic, clinical, and radiographic features of bronchiectasis in dogs: 316 cases (1988–2000). J Am Vet Med Assoc 2003;223:1628-1635.
37. Johannson K, Collard H. Acute exacerbation of idiopathic pulmonary fibrosis: a proposal. Curr Respir Care Rep 2013;2:233-240.
38. Reinero C, Cohn L. Interstitial lung diseases. Vet Clin North Am Small Anim Pract 2007;37:937-947.

39. Stanley BJ, Hauptman JG, Fritz MC, et al. Esophageal dysfunction in dogs with idiopathic laryngeal paralysis: a controlled cohort study. Vet Surg 2010;39:139-149.
40. Eivers C, Chicon Rueda R, Liuti T, et al. Retrospective analysis of esophageal imaging features in brachycephalic versus non-brachycephalic dogs based on videofluoroscopic swallowing studies. J Vet Intern Med 2019;33:1740-1746.
41. Poncet CM, Dupre GP, Freiche VG, et al. Prevalence of gastrointestinal tract lesions in 73 brachycephalic dogs with upper respiratory syndrome. J Small Anim Pract 2005;46:273-279.
42. Appelgrein C, Hosgood G, Thompson M, et al. Quantification of gastroesophageal regurgitation in brachycephalic dogs. J Vet Intern Med 2022;36:927-934.
43. Reeve EJ, Sutton D, Friend EJ, et al. Documenting the prevalence of hiatal hernia and oesophageal abnormalities in brachycephalic dogs using fluoroscopy. J Small Anim Pract 2017;58:703-708.
44. Luciani E, Reinero C, Grobman M. Evaluation of aerodigestive disease and diagnosis of sliding hiatal hernia in brachycephalic and nonbrachycephalic dogs. J Vet Intern Med 2022;36:1229-1236.
45. Pollard RE. Videofluoroscopic Evaluation of the Pharynx and Upper Esophageal Sphincter in the Dog: A Systematic Review of the Literature. Front Vet Sci 2019;6:117.
46. Watrous BJ. Clinical presentation and diagnosis of dysphagia. Vet Clin North Am Small Anim Pract 1983;13:437-459.
47. Pollard RE, Marks SL, Davidson A, et al. Quantitative videofluoroscopic evaluation of pharyngeal function in the dog. Vet Radiol Ultrasound 2000;41:409-412.
48. Pollard RE, Marks SL, Cheney DM, et al. Diagnostic outcome of contrast videofluoroscopic swallowing studies in 216 dysphagic dogs. Vet Radiol Ultrasound 2017;58:373-380.
49. Pollard RE, Marks SL, Leonard R, et al. Preliminary evaluation of the pharyngeal constriction ratio (PCR) for fluoroscopic determination of pharyngeal constriction in dysphagic dogs. Vet Radiol Ultrasound 2007;48:221-226.
50. Leonard R, Belafsky PC, Rees CJ. Relationship between fluoroscopic and manometric measures of pharyngeal constriction: the pharyngeal constriction ratio. Ann Otol Rhinol Laryngol 2006;115:897-901.
51. Leonard R, Rees CJ, Belafsky P, et al. Fluoroscopic surrogate for pharyngeal strength: the pharyngeal constriction ratio (PCR). Dysphagia 2011;26:13-17.
52. O'Brien JA, Harvey CE, Kelly AM, et al. Neurogenic atrophy of the laryngeal muscles of the dog. J Small Anim Pract 1973;14:521-532.
53. Polizopoulou ZS, Koutinas AF, Papadopoulos GC, et al. Juvenile laryngeal paralysis in three Siberian husky x Alaskan malamute puppies. Vet Rec 2003;153:624-627.
54. Ubbink GJ, Knol BW, Bouw J. The relationship between homozygosity and the occurrence of specific diseases in Bouvier Belge des Flandres dogs in The Netherlands. Vet Q 1992;14:137-140.
55. Jeffery ND, Talbot CE, Smith PM, et al. Acquired idiopathic laryngeal paralysis as a prominent feature of generalised neuromuscular disease in 39 dogs. Vet Rec 2006;158:17.

56. Braund KG, Steinberg HS, Shores A, et al. Laryngeal paralysis in immature and mature dogs as one sign of a more diffuse polyneuropathy. J Am Vet Med Assoc 1989;194:1735-1740.
57. Venker-van Haagen AJ. Diseases of the larynx. Vet Clin North Am Small Anim Pract 1992;22:1155-1172.
58. MacPhail CM, Monnet E. Outcome of and postoperative complications in dogs undergoing surgical treatment of laryngeal paralysis: 140 cases (1985-1998). J Am Vet Med Assoc 2001;218:1949-1956.
59. Tarvin KM, Twedt DC, Monnet E. Prospective Controlled Study of Gastroesophageal Reflux in Dogs with Naturally Occurring Laryngeal Paralysis. Vet Surg 2016;45:916-921.
60. Grobman ME, Masseau I, Reinero CR. Aerodigestive disorders in dogs evaluated for cough using respiratory fluoroscopy and videofluoroscopic swallow studies. Vet J 2019;251:105344.
61. Poncet CM, Dupre GP, Freiche VG, et al. Long-term results of upper respiratory syndrome surgery and gastrointestinal tract medical treatment in 51 brachycephalic dogs. Journal of Small Animal Practice 2006;47:137-142.
62. McGarvey LP, Ing AJ. Idiopathic cough, prevalence and underlying mechanisms. Pulm Pharmacol Ther 2004;17:435-439.
63. Grobman ME, Schachtel J, Gyawali CP, et al. Videofluoroscopic swallow study features of lower esophageal sphincter achalasia-like syndrome in dogs. J Vet Intern Med 2019;33:1954-1963.
64. Holman SD, Campbell-Malone R, Ding P, et al. Development, reliability, and validation of an infant mammalian penetration-aspiration scale. Dysphagia 2013;28:178-187.
65. Thomas WP, Gaber CE, Jacobs GJ, et al. Recommendations for standards in transthoracic two-dimensional echocardiography in the dog and cat. Echocardiography Committee of the Specialty of Cardiology, American College of Veterinary Internal Medicine. J Vet Intern Med 1993;7:247-252.
66. Reinero C, Visser LC, Kellihan HB, et al. ACVIM consensus statement guidelines for the diagnosis, classification, treatment, and monitoring of pulmonary hypertension in dogs. J Vet Intern Med 2020;34:549-573.
67. Hawkins EC, DeNicola DB, Kuehn NF. Bronchoalveolar lavage in the evaluation of pulmonary disease in the dog and cat. State of the art. J Vet Intern Med 1990;4:267-274.
68. Gamracy J, Wiggen K, Vientós-Plotts A, et al. Clinicopathologic features, comorbid diseases, and prevalence of pulmonary hypertension in dogs with bronchomalacia. J Vet Intern Med 2022;36:417-428.
69. Peeters DE, McKiernan BC, Weisiger RM, et al. Quantitative bacterial cultures and cytological examination of bronchoalveolar lavage specimens in dogs. J Vet Intern Med 2000;14:534-541.
70. McClean KM, Kee F, Young IS, et al. Obesity and the lung: 1. Epidemiology. Thorax 2008;63:649-654.
71. Ramsey D, Smithard D, Kalra L. Silent aspiration: what do we know? Dysphagia 2005;20:218-225.

72. Gnanapandithan K, Popkin JH, Devadoss R, et al. Gastroesophageal reflux and idiopathic pulmonary fibrosis: A long term relationship. Respir Med Case Rep 2016;17:40-43.
73. Sweet MP, Patti MG, Hoopes C, et al. Gastro-oesophageal reflux and aspiration in patients with advanced lung disease. Thorax 2009;64:167-173.
74. Ghisa M, Marinelli C, Savarino V, et al. Idiopathic pulmonary fibrosis and GER: links and risks. Ther Clin Risk Manag 2019;15:1081-1093.
75. Reinero C. Interstitial lung diseases in dogs and cats part I: The idiopathic interstitial pneumonias. Vet J 2019;243:48-54.
76. Shepherd K, Hillman D, Eastwood P. Symptoms of aerophagia are common in patients on continuous positive airway pressure therapy and are related to the presence of nighttime gastroesophageal reflux. J Clin Sleep Med 2013;9:13-17.
77. Liang B, Wang M, Yi Q, et al. Association of gastroesophageal reflux disease risk with exacerbations of chronic obstructive pulmonary disease. Dis Esophagus 2013;26:557-560.
78. Parsons JP, Mastronarde JG. Gastroesophageal reflux disease and asthma. Curr Opin Pulm Med 2010;16:60-63.
79. Li W, Gao M, Liu J, et al. The prevalence of oropharyngeal dysphagia in patients with chronic obstructive pulmonary disease: a systematic review and meta-analysis. Expert Rev Respir Med 2022;16:567-574.
80. McGarvey LP. Idiopathic chronic cough: a real disease or a failure of diagnosis? Cough 2005;1:9.
81. Miles A, Bennett K, Allen J. Esophageal Transit Times Vary with Underlying Comorbid Disease. Otolaryngol Head Neck Surg 2019;161:829-834.
82. Decalmer S, Stovold R, Houghton LA, et al. Chronic cough: relationship between microaspiration, gastroesophageal reflux, and cough frequency. Chest 2012;142:958-964.
83. Sherman R, Karagiannis M. Aspiration Pneumonia in the Dog: A Review. Top Companion Anim Med 2017;32:1-7.
84. Wilkins PA, Otto CM, Baumgardner JE, et al. Acute lung injury and acute respiratory distress syndromes in veterinary medicine: consensus definitions: The Dorothy Russell Havemeyer Working Group on ALI and ARDS in Veterinary Medicine. Journal of Veterinary Emergency and Critical Care 2007;17:333-339.
85. Marks SL, Kook PH, Papich MG, et al. ACVIM consensus statement: Support for rational administration of gastrointestinal protectants to dogs and cats. Journal of Veterinary Internal Medicine 2018;32:1823-1840.
86. Schwameis K, Oh D, Green KM, et al. Clinical outcome after laparoscopic Nissen fundoplication in patients with GER and PPI refractory heartburn. Dis Esophagus 2020;33.
87. Triadafilopoulos G, DiBaise JK, Nostrant TT, et al. The Stretta procedure for the treatment of GER: 6 and 12 month follow-up of the U.S. open label trial. Gastrointest Endosc 2002;55:149-156.

88. Oelschlager BK, Quiroga E, Isch JA, et al. Gastroesophageal and pharyngeal reflux detection using impedance and 24-hour pH monitoring in asymptomatic subjects: defining the normal environment. J Gastrointest Surg 2006;10:54-62.
89. Määttä OLM, Laurila HP, Holopainen S, et al. Reflux aspiration in lungs of dogs with respiratory disease and in healthy West Highland White Terriers. J Vet Intern Med 2018;32:2074-2081.