Canine oesophageal diseases

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Abstract

Oesophageal diseases in the dog can be challenging to diagnose. Multiple diagnostic imaging modalities are necessary for the assessment of the canine oesophagus. This study was divided into prospective and retrospective parts. The purpose of the prospective study was to determine whether conventional transcutaneous ultrasonography can be used to evaluate the canine cervical oesophagus and describe the sonographic appearance and measurements in normal dogs and those with clinical signs associated with the oesophagus. Seven canine cadavers, ten healthy staff owned dogs and eleven client owned dogs with vomiting and regurgitation were examined using a 14MHz transducer. Transcutaneous ultrasonography of the cervical oesophagus was performed using a left lateral approach. Ultrasonography allowed visualization of the entire cervical oesophagus. Four or six sonographic layers were identified which corresponded with histology. An additional thin hyperechoic layer was present within the muscular layer in some dogs which corresponded to fibrous tissue located between the inner circular and outer longitudinal muscle layers. Mean ultrasonographic wall thickness for normal dogs 2.7 ± 1 mm and was significantly correlated with weight in the live dogs *($P<0.05$).* No sonographic abnormalities were identified in the clinical cases.

The purpose of the retrospective study was to document the occurrence of oesophageal abnormalities in brachycephalic dogs using multiple diagnostic imaging modalities (radiography, fluoroscopy, computed tomography and magnetic resonance imaging). Record the incidence of oesophageal redundancy in brachycephalic breeds with or without oesophageal disease. Hospital records between November 2009 to December 2016 identified fifty-one brachycephalic dogs with oesophageal abnormalities. Megaoesophagus was the most prevalent oesophageal abnormality in the brachycephalic breeds. Hiatal hernia, oesophageal dysmotility and GOR were the most prevalent oesophageal diseases in dogs with BOAS and megaoesophagus, dysmotility and hiatal herniation in dogs without BOAS. The occurrence of BOAS was highest for English bulldogs, followed by French bulldogs

and Pugs, however there was no significant correlation between the presence or absence of BOAS in dogs with oesophageal abnormalities. There was no significant correlation between breed, weight, sex and clinical signs or oesophageal abnormalities present. Oesophageal redundancy incidence was low in the brachycephalic dogs in this study with and without concomitant oesophageal disease.

These studies suggest that multiple diagnostic imaging modalities can be used to evaluate the oesophagus. However, further studies are warranted with a larger study sample to expand the clinical use of transcutaneous ultrasonography in dogs with oesophagitis and to determine the prevalence of oesophageal abnormalities in brachycephalic breeds with statistical significance.

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Dedication

I dedicate this dissertation to my family and Gordon, for always being there for me. Thank you!

Declaration

I, Bárbara Andreia Jardim Gomes, declare that the work in this thesis is original and was carried out solely by myself or with due acknowledgements. It has not been submitted in any form for another degree or professional qualification.

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Abbreviations

BOAS - Brachycephalic obstructive airway syndrome

CKCS – Cavalier King Charles Spaniel

CT – Computed Tomography

DICOM - Digital Imaging and Communications of Medicine

DV – dorsoventral

GOR – Gastro-oesophageal reflux

H&E - Haematoxylin and eosin

MHz – Megahertz

MRI – Magnetic Resonance Imaging

PACS - Picture Archiving and Communication System

SNR – Signal-to-noise ratio

V30R-DLeO -ventral 30 degrees right – dorsal left oblique

VD – ventrodorsal

Chapter 1 - General Introduction

Disease of the oesophagus can be challenging to diagnose due to its structure and its location as it passes through the neck and thorax to reach the abdomen. As a consequence, multiple non-invasive diagnostic imaging methods have been reported in an attempt to obtain a complete assessment. Additionally, the involvement of concomitant abnormalities of the upper and lower respiratory tracts, which might be secondary to the oesophageal condition, may mask the origin of the disease and limit the investigation (Gaschen, 2018).

1 Oesophagus

1.1 Anatomy

The alimentary system (*apparatus digestorius*) includes the oral cavity, pharynx, alimentary canal and accessory organs (including teeth, tongue, salivary glands, liver, gallbladder, pancreas and paranal sinuses) (Evans & Lahunta, 2013). The oesophagus is the tubular structure that connects the pharynx to the stomach. According to Evans & Lahunta (2013), in a medium sized dog, the empty oesophagus is approximately 30 cm long and 2 cm in diameter.

The oesophagus is divided into three segments: cervical (*pars cervicalis*), thoracic (*pars thoracica*) and abdominal (*par abdominalis*). The cervical oesophagus runs within the visceral space of the neck and begins cranially at the cricopharyngeal sphincter or cranial oesophageal sphincter. This incorporates striated muscle (Jergens, 2010), is formed by the cricopharyngeus and thyropharyngeus muscles (Dyce, et al., 2010) and lies dorsal to the cricoid cartilage of the larynx in a position that is dorsal and slightly to the left of the longus colli and longus capitis muscles (Evans & Lahunta, 2013). The cervical oesophagus follows the trachea dorsally, becoming completely left sided at the thoracic inlet (Dyce, et al., 2010; Gaschen, 2018). The left common carotid artery, vagosympathetic trunk, internal jugular vein, and tracheal duct course between the oesophagus and the longus capitis muscle while the corresponding structures on the right are lateral to the trachea (Evans & Lahunta, 2013).

Within the thoracic cavity the oesophagus runs in the mediastinum. The thoracic portion of the oesophagus is in close proximity to the trachea (Evans & Lahunta, 2013; Gaschen, 2018). From the thoracic inlet where the oesophagus lies to the left of the trachea, it gradually progresses dorsally over the tracheal bifurcation at the level of the fifth and sixth thoracic vertebrae. It lies to the right of the aortic arch and ventral to the right and left longus colli muscles (Dyce, et al., 2010; Evans & Lahunta, 2013). Throughout its course to this point, the oesophagus is separated from these muscles by the pre-vertebral fascia (Evans & Lahunta, 2013).

After the left mainstem bronchus, the oesophagus sits over the left atrium and the accessory lung lobe before reaching the oesophageal hiatus in the diaphragm below the 10^{th} thoracic vertebrae. The caudal oesophageal sphincter, located at the oesophageal hiatus, is formed by the focal muscular thickening of the oesophagus with the transverse oriented gastric folds, the muscularis sling which is created by the right crus of the diaphragm and the deep oblique muscle of the lesser curvature of the stomach (Gaschen, 2013). Between the tracheal bifurcation and caudal oesophageal sphincter, the oesophagus lies in the median plane (Evans & Lahunta, 2013; Gaschen, 2018).

The short abdominal section (*pars abdominalis*) of the oesophagus has a wedge-shape which joins the stomach dorsally at the cardia (Dyce, et al., 2010; Gaschen, 2018) and ventrally, notches the thin dorsal border of the caudate lobe of the liver (Evans & Lahunta, 2013).

The canine oesophagus is composed of four histological layers: the mucosa, submucosa, muscularis and tunica adventitia. According to Baloi, Kircher & Kook (2003) the mucosa histologically is composed of three layers: stratified squamous epithelium, lamina propria and lamina muscularis mucosae. The submucosa is known to have multiple mucus-secreting tubule-acinar glands (Baloi, et al., 2013). Both the mucosal and submucosal layers are separated by a fenestrated muscularis mucosae, but this is only present in the caudal half of the oesophagus (Evans & Lahunta, 2013). The muscularis layer contains two oblique muscles layers of striated muscle, but moving distally towards the stomach, the outer and inner layers become progressively more longitudinal and circular respectively (Dyce, et al., 2010; Pollard, 2012; Evans & Lahunta, 2013). The muscularis layer along the entire length of the canine oesophagus is mainly composed of striated muscle whereas, the distal third in felines is composed of smooth muscle (Jergens, 2010; Gaschen, 2018). In humans, it has been reported that more than half of the muscularis layer of the oesophagus is formed by smooth muscle (Meyer, et al., 1986).

1.2 Oesophageal nerves

The innervation of the oesophagus is complex and involves twenty-five paired spinal ganglia from C2 to L5 (Dyce, et al., 2010). Three major regions of innervation are recognised in the dog: the cervical region is supplied by the paired para-recurrent laryngeal nerves; the cranial thoracic region is supplied by the left para-recurrent laryngeal nerve and the caudal thoracic and abdominal region is supplied by the vagal trunk (Elwood, 2006; Dyce, et al., 2010; Venker-van-Haagen, 2013)

The striated muscle of the oesophagus is under the control of somatic motor neurons of the vagus nerve. A myenteric plexus exists throughout the entire length of the oesophagus. This plexus serves as a sensory function to the regions of striated muscle and acts to synchronize the movements of the striated muscle portion with the smooth muscle of the stomach (Dyce, et al., 2010).

The dorsal branches of the right and left vagal nerves run dorsocaudally alongside the oesophagus and connect to each other at the dorsal aspect of the oesophagus, 2-4 centimetres cranial to the oesophageal hiatus (Evans & Lahunta, 2013; Gaschen, 2018). This vagosympathetic trunk then divides into a dorsal and ventral vagal trunk before it passes throughout the hiatus (Evans & Lahunta, 2013).

1.3 Oesophageal Vasculature and Lymphatic Drainage

The arterial supply of the cervical portion of the oesophagus originates from the cranial and caudal thyroid arteries and oesophageal branches of the carotid arteries (Evans & Lahunta, 2013; Venker-van-Haagen, 2013). Several branches of the cranial thyroid artery supply the glandular layer at the cranial oesophageal sphincter (pharyngoesphageal limen). A long and small descending branch at the thoracic inlet on the left side, exiting from the left caudal thyroid artery anastomoses with an ascending branch from the broncho-oesophageal artery. From this small anastomotic trunk, branches go to the oesophagus (Evans & Lahunta, 2013).

The oesophageal portion of the broncho-oesophageal artery is the main source of blood supply to the cranial two-thirds of the thoracic portion of the oesophagus (Evans & Lahunta,

2013; Venker-van-Haagen, 2013). The caudal thoracic oesophagus is supplied by branches of the aorta or dorsal intercostal arteries. However, the terminal portion is supplied by the oesophageal branch of the left gastric artery (Evans & Lahunta, 2013; Gaschen, 2018).

The veins that drain the oesophagus are satellites of the arteries. The venous drainage is via the external jugular and azygos veins (Venker-van-Haagen, 2013). Adjacent veins anastomose with each other on the oesophagus (Evans & Lahunta, 2013; Venker-van-Haagen, 2013).

The lymph vessels from the oesophagus drain into the medial retropharyngeal, deep cervical, cranial mediastinal, bronchial, portal, splenic, gastric and jejunal lymph nodes (Evans & Lahunta, 2013; Venker-van-Haagen, 2013).

1.4 Physiology

The oesophagus in the dog is responsible for transporting the ingesta and liquids between the pharynx and the stomach (Jergens, 2010; Gaschen, 2018). Normal swallowing or deglutition is a functional mechanism that involves the tongue, hard and soft palates, pharyngeal muscles, oesophagus, and gastro-oesophageal junction.

Deglutition involves a first voluntary and a second involuntary stage. The voluntary stage occurs when the food is shaped into a bolus by the tongue, impelled caudally into the oropharynx and contacts the pharyngeal mucosa (Cunningham, 2007; Dyce, et al., 2010). The involuntary movement happens primarily between the pharynx and the oesophagus (Cunningham, 2007). As the food moves caudally, the soft palate is elevated, and the free margin is drawn toward the dorsocaudal pharyngeal wall, closing the pharyngeal opening of the nasopharynx (Cunningham, 2007; Dyce, et al., 2010). At the same time, the hyoid bones and larynx are pulled cranially which pulls the glottis under the epiglottis, covering the laryngeal opening. The pharynx then contracts, making the paired cricopharyngeus and thyropharyngeus muscles relax, to allow passage of the bolus into the proximal oesophagus by a peristaltic wave (Cunningham, 2007; Dyce, et al., 2010; Pollard, 2012). Once the food bolus passes the cranial oesophageal sphincter, this closes to prevent retrograde movement of ingesta (Pollard, 2012). The bolus is then propelled along the oesophagus into the stomach by a primary or secondary peristaltic wave. The primary peristaltic wave is generated in the pharynx and propagated through the oesophagus to transport the bolus to the caudal

oesophageal sphincter (Cunningham, 2007; Jergens, 2010; Pollard, 2012). If primary peristalsis fails, sensation of local oesophageal distension triggers a secondary peristaltic wave. As the food bolus reaches the distal oesophagus, the caudal oesophageal sphincter relaxes allowing passage of the bolus into the stomach (Elwood, 2006; Cunningham, 2007; Jergens, 2010; Pollard, 2012). After the bolus reaches the stomach the caudal oesophageal sphincter contracts to prevent gastro-oesophageal reflux (Jergens, 2010).

The swallowing reflex is coordinated by multiple cranial nerves including the trigeminal (V), facial (VII), glossopharyngeal (IX), vagus (X), hypoglossal (XII) and those nuclei that are controlled by the reticular formation known as swallowing centre (Pollard, 2012; Gaschen, 2018).

2 Diagnostic Imaging

There are a number of diagnostic imaging techniques that can be used to assess the canine oesophagus including radiography, contrast radiography (contrast oesophagram), fluoroscopy, ultrasonography, nuclear scintigraphy, Computed Tomography (CT) and Magnetic Resonance Imaging (MRI). However, radiography and ultrasound are the modalities that are currently most readily available in small animal veterinary practices (Wisner, et al., 1991; Pollard, 2012; Bristow, 2015; Gaschen, 2018). Endoscopy is a useful and minimally invasive technique used for the diagnosis and treatment of oesophageal diseases (Gualtieri, 2001; Tolbert, 2017)

2.1 Radiography

Survey radiographs of the cervical and thoracic region of the oesophagus from the base of the tongue to the cardia of the stomach are indicated in dogs with clinical signs of oesophageal disease (Gaschen, 2018). Ventrodorsal (VD) and lateral projections of the neck and thorax are necessary to examine the entire oesophagus. A ventroright-dorsal left oblique (V30ºR-DLeO) projection of the thorax provides an alternative view of the thoracic oesophagus without overlapping vertebrae and sternebrae (Kleine & Lamb, 1989).

The oesophagus is not radiographically visible when empty in the normal dog. However,

plain radiographs provide information regarding oesophageal content and are useful for detecting oesophageal dilation and foreign bodies (Elwood, 2006). Moreover, they can also be used to identify secondary complications, such as aspiration pneumonia and oesophageal perforation (e.g. pneumothorax, pneumomediastinum, mediastinitis, pleural effusion) (Venker-van-Haagen, 2013).

In addition to survey radiographs, a static oesophagram can be performed using oral positive contrast media alone (e.g. barium sulphate suspension or paste) or a mixture of barium and food and will provide structural information (Bradley, 2005; Elwood, 2006; Gaschen, 2018). Barium paste adheres to the mucosal wall for longer than liquid barium, however liquid barium can better outline space occupying lesions or narrowing of the lumen (Kleine & Lamb, 1989). Lateral contrast views of the cervical and thoracic oesophagus are often the most useful for its evaluation. However, additional views for the thoracic oesophagus, such as dorsoventral a (DV) may add further information (Bradley, 2005). Contrast radiography delineates the oesophagus and can therefore provide additional information about its size and content, as well as defining the cardia and helping outline radiolucent foreign bodies (Gaschen, 2018; Elwood, 2006). Moreover, these barium studies allow characterization of oesophageal masses, peri-oesophageal masses, oesophageal strictures, vascular ring anomalies, oesophageal perforation, oesophageal diverticulum, tracheo or bronchoesophageal fistulae and hiatal hernias (Venker-van-Haagen, 2013; Gaschen, 2018). Additionally, a contrast oesophagram can be used to subjectively evaluate oesophageal motility by observing a temporal or persistent retention of barium in sequential radiographs. This can arise due to a lack of secondary peristalsis resulting from primary motility disorders or underlying diseases such as infiltrative neoplasia, foreign bodies or vascular ring anomalies (Gaschen, 2018). Negative oesophagraphy, using room air can be helpful to outline soft tissue masses in the wall (Gaschen, 2018).

A potential complication of positive contrast radiography of the oesophagus is barium aspiration. Aspiration of small amounts is likely to be insignificant although it can lead to chronic inflammation with the development of pneumonia and lung granulomas when it enters the airways or mediastinum (Elwood, 2006; Gaschen, 2018). Iodinated water-soluble contrast media is preferred when a perforation is suspected (Elwood, 2006; Gaschen, 2018).

2.2 Fluoroscopy

Fluoroscopy is a dynamic radiographic study that, in conjunction with the administration of contrast media, allows real time evaluation of swallowing, oesophageal motility and functional anatomy of the gastroesophageal sphincter (e.g. gastro-oesophageal reflux, transient sliding hiatal hernias) (Elwood, 2006; Gaschen, 2018). It is also useful to document oesophageal dilation or oesophageal redundancy (Bright, et al., 1990; Washabau, 2005; Elwood, 2006; Reeve, et al., 2017; Gaschen, 2018).

Contrast fluoroscopy appears to be more sensitive in identifying oesophageal dysfunction and subtle or transient abnormalities that normally would go unnoticed on contrast radiography (Elwood, 2006; Gaschen, 2018)

The barium meal study involves the use of barium-coated food (e.g. soft food or kibble) or liquid barium (Elwood, 2006; Bonadio, et al., 2009; Gaschen, 2018). It allows evaluation of swallowing and then passage of the bolus of barium meal along the length of the oesophagus and through the gastro-oesophageal sphincter into the stomach (Elwood, 2006). The study can be done with the dog in a sternal or standing position or restrained in lateral recumbency (Bonadio, et al., 2009; Gaschen, 2018). A radiolucent squeeze box can be used to restrain the dogs in a standing or sternal position (Bonadio, et al., 2009; Gaschen, 2018).

The positioning of the dog can affect the oesophageal transit time therefore sternal positioning is preferred for evaluation of the swallowing and oesophageal transit times (Venker-van-Haagen, 2013). Bonadio et al (2009) demonstrated that the cervical oesophageal transit time was significantly shorter in dogs positioned in sternal recumbency due to a different frequency of the type of peristaltic wave trigged by swallowing (Bonadio, et al., 2009).

2.3 Nuclear scintigraphy

Nuclear scintigraphy has the ability to offer dynamic and quantitative information about organ morphology and function (Koblik & Hornof, 1985; Kleine & Lamb, 1989). Oesophageal scintigraphy can be more accurate than other diagnostic imaging procedures to measure oesophageal function (Kleine & Lamb, 1989). It is also suitable for monitoring the response to the treatment of neuromuscular disease in animals (Kleine & Lamb, 1989).

This method has the advantage of exposing the patient to less radiation than with standard fluoroscopy studies (Koblik & Hornof, 1985; Kleine & Lamb, 1989) and is more sensitive than contrast radiography for documenting oesophageal fistulae or perforation (Koblik & Hornof, 1985). Also, there is no side effect to extravasation of the contrast medium if there is a perforation (Koblik & Hornof, 1985; Kleine & Lamb, 1989). According to Kleine & Lamb (1989) non-perforating oesophageal ulceration may be detected using ^{99m}technetium-labelled sulcrafe. In humans with gastro-oesophageal reflux, scintigraphy has been used to quantify refluxed levels, duration and frequency, measure gastric emptying and evaluate possible lung aspiration (Codreanu, et al., 2013). Current gastrointestinal nuclear medicine studies are used to evaluate oesophageal motility, gastroesophageal reflux, gastric emptying, gastric secretory function, infectious and inflammatory gastrointestinal conditions, gastrointestinal bleeding and to detect oesophageal or gastric ulceration (Koblik & Hornof, 1985; Kleine & Lamb, 1989; Gaschen, 2018).

2.4 Computed tomography

CT is a valuable modality for the characterisation of oesophageal masses, allowing evaluation of the surrounding tissues and vessels (Kirberger, et al., 2014; Gaschen, 2018). CT of the oesophagus has been shown to be useful in the detection of metastatic neoplasia, characterisation of oesophageal spirocerca nodules (Kirberger, et al., 2014) and the diagnosis of vascular ring anomalies and oesophageal varices (Ledda, et al., 2015; Gaschen, 2018).

2.5 Magnetic resonance imaging

MRI is a minimally invasive technique that provides excellent soft tissue contrast but is considered a technical challenge when evaluating the oesophagus (van Rossum, et al., 2013; Rossum, et al., 2015). This technique is therefore not routinely used for oesophageal examination in animals but is used frequently to assess oesophageal tumours in humans (Rossum, et al., 2015; Gaschen, 2018). The oesophagus is difficult to visualise on MRI due to its localisation within the mediastinum, respiratory movement, cardiac motion, blood flow in the aorta and pulmonary vessels and peristalsis, all combined with the relative slow acquisition time of the MRI volume data, resulting in a degraded signal-to-noise ratio (SNR) (Riddell, et al., 2006; van Rossum, et al., 2013; Rossum, et al., 2015). Several technical

innovations have been developed to reduce these image artefacts and enable proper visualisation of the oesophagus in humans. However, there is no data available in the literature relating to their application in veterinary medicine. MRI has been reported in one veterinary case involving the detection of a cervical wooden foreign body caused by a stick penetration injury. However, in this case, MRI was only selected as the first imaging modality because there was suspicion of a spinal problem (Young, et al., 2004).

2.6 Ultrasonography

Conventional ultrasonography can be used for evaluation of the cervical and abdominal parts of the canine oesophagus (Wisner, et al., 1991; Gory, et al., 2014; Zwingenberger & Taeymans, 2015; Gaschen, 2018). Trans-thoracic ultrasound in humans has been described by Shang-Yong et al. (2005) but only allowed partial visualisation of the thoracic region of the oesophagus. Limitations encountered with this technique in both dog and humans include the localisation of the oesophagus, the surrounding skeletal system, gas within the lungs and the patient's body condition which can all interfere with its visualisation (Shang-Yong, et al., 2005).

The abdominal oesophagus and cardia can be identified using trans-abdominal ultrasonography through the acoustic window of the left hepatic lobe in both humans and dogs (Shang-Yong, et al., 2005; Gory, et al., 2014). Many diseases of the gastroesophageal junction including gastro-oesophageal reflux, carcinoma, varices, hiatal hernia and leiomyoma can be detected using this technique in humans (Shang-Yong et al., 2004). In a study by Gory et al. (2014) the oesophageal layering in this region could be visualised in 89% of the dogs.

Endoscopic ultrasonography can be performed in the dog and cats using a trans-oesophageal transducer (Capitani, et al., 2014). This technique is less commonly used in veterinary medicine than in humans due to the requirement for expensive specialist equipment and the need for heavy sedation or general anaesthesia to prevent equipment damage from the patient's teeth. Indications for this procedure include mural infiltration, fistula, diverticula, and peri-oesophageal masses (Gaschen, 2018).

2.7 Endoscopy

Oesophagoscopy is a technique that allows evaluation of the lumen and mucosal lining of the oropharynx and oesophagus (Gualtieri, 2001; Tolbert, 2017). It is useful to identify mucosal lesions, foreign bodies, oesophagitis, strictures, detect early oesophageal dysfunction and acquisition of sample biopsies (Gualtieri, 2001; Elwood, 2006; Tolbert, 2017). Also, oesophageal ulcers, fistulae and masses, however they are unfrequently encountered in dogs (Gualtieri, 2001). This technique can provide a more accurate and accessory information to contrast radiography and videofluorosocopy in the investigation of megaoesophagus, oesophageal diverticula, vascular ring anomalies and hiatal disorders (Gualtieri, 2001).

3 Oesophageal abnormalities

3.1 Clinical signs

The most common clinical signs in dogs with oesophageal disease is regurgitation (solids or liquids) (Elwood, 2006; Marks, 2017). Other reported clinical signs including hypersalivation, anorexia, chronic vomiting, odynophagia, dysphagia, nasal discharge or coughing (secondary to aspiration pneumonia) can be seen depending on the disease progression and/or secondary complications (Washabau, 2005; Elwood, 2006; Marks, 2017). An acute history of regurgitation is usually seen with oesophageal foreign bodies, acute oesophagitis, strictures and gastro-oesophageal intussusception. However, a chronic history of regurgitation is more consistent with megaoesophagus, vascular ring anomalies, diverticulae, hiatal hernias, chronic reflux oesophagitis, a mature stricture or oesophageal neoplasia. Intermittent occurrence can be seen with hiatal hernias and reflux oesophagitis (Venker-van-Haagen, 2013).

3.2 Anatomic malformations

3.2.1 Hiatal herniation

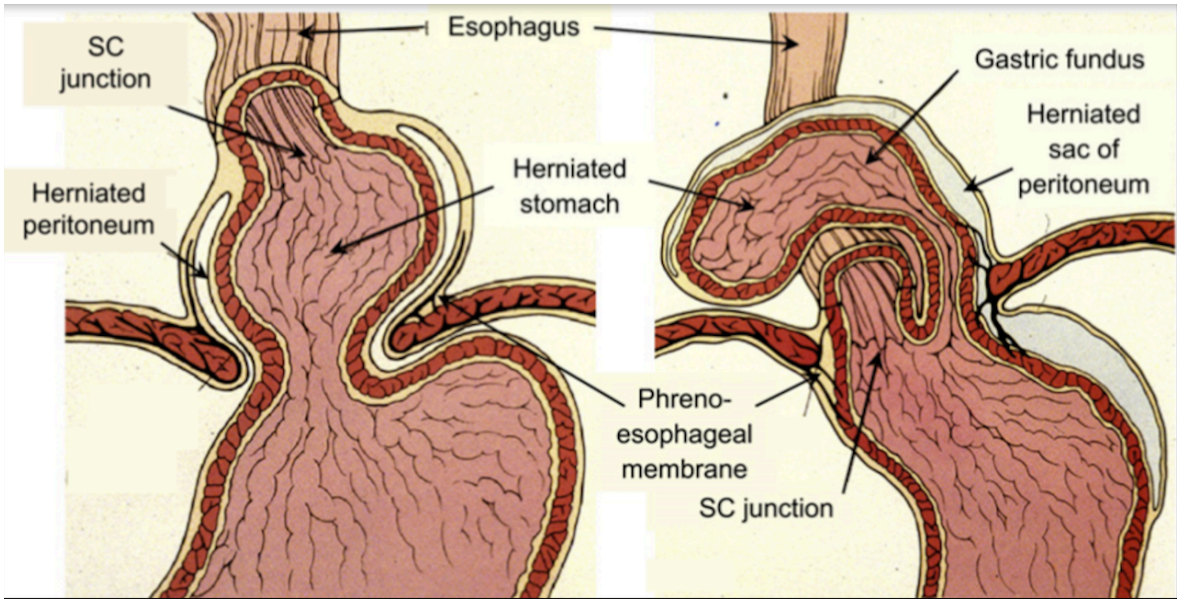
Oesophageal hiatal herniation is an uncommon condition in dogs and cats that is defined as the transposition of any abdominal structure through the diaphragmatic oesophageal hiatus. Hiatal hernias are a result of stretching the phrenic oesophageal ligament allowing herniation of the abdominal oesophagus, gastroesophageal junction, stomach and other abdominal organs. (Dvir, et al., 2003; Pollard, 2012). Clinical signs may be absent, or dogs may have recurrent gastrointestinal signs such as regurgitation, retching, and possibly vomiting.

Oesophageal hernias can be congenital or acquired. The congenital form has been described previously in certain brachycephalic breeds, such as the Chinese shar-pei dogs, English Bulldog and Chow Chow (Jergens, 2010; Dvir, et al., 2003; Reeve, et al., 2017). According to Poncet et al. (2005) the French Bulldog it is more likely to suffer from an acquired form caused by high abdominal and low intra-oesophageal pressures. Weakness of the diaphragm, increased abdominal pressure, and upper airway obstruction are predisposing factors in acquired hiatal hernia (Jergens, 2010; Reeve, et al., 2017).

There is a classification scheme that recognizes four types of hiatal hernia (Kahrilas , et al., 2008). Type I axial or sliding hernias, where there is a widening of the muscular hiatal tunnel and circumferential laxity of the phrenoesophageal membrane, cause the caudal oesophageal sphincter and a portion of the gastric cardia to move in and out of the caudal mediastinum (Kahrilas , et al., 2008; Jergens, 2010). Type I is the most common hiatal hernia seen in people and small animals (Kirkby, et al., 2005) **(Figure 1)**.

Type II paraoesophageal or “rolling” hiatal hernias result from a localized defect of the phrenoesophageal membrane while the gastroesophageal junction remains fixed to the pre-aortic fascia and the median arcuate ligament (Kahrilas , et al., 2008) causing the fundus to herniate within the mediastinum alongside the oesophagus while the caudal oesophageal sphincter remains within the abdomen (Callan, et al., 1993; Kirkby, et al., 2005; Kahrilas , et al., 2008; Gaschen, 2018) **(Figure 1)**.

Figure 1. *Difference between a sliding hiatal hernia (type I) and paraoesophageal hernia (type II) (Kahrilas , et al., 2008). Squamocolumnar junction (SC) represents the oesophagogastric junction.*



Type III hiatal hernias are a combination of type I and II hernias (Kirkby, et al., 2005; Kahrilas , et al., 2008; Gaschen, 2018), where there is a progressive enlargement of the hernia through the hiatus due to the stretching of the phrenoesophageal membrane, displacing the gastroesophageal junction above the diaphragm, thereby adding a sliding component to the type II hernia (Kahrilas , et al., 2008). Type IV hiatal hernias are also a mixed type I and II hiatal hernia, where the phrenoesophageal membrane defect is larger allowing herniation of other abdominal organs (Kahrilas , et al., 2008). However, Pollard (2012) defined type IV hiatal hernias as a combination of type III with herniation of abdominal organs other than the stomach. Hiatal hernias are frequently congenital but can also be seen secondary to trauma, upper airway obstruction, or tetanus (Pollard, 2012).

3.2.1.1 Diagnostic imaging

Sliding oesophageal herniation appears as a soft tissue or mixed soft tissue and gas opacity between the aorta and caudal vena cava, in the dorsocaudal mediastinum on lateral radiographs, silhouetting with the craniodorsal diaphragmatic cupula (Venker-van-Haagen, 2013; Gaschen, 2018). On the VD projection, the opacity is located on midline or slightly to the left. Type I hiatal hernias can also be identified using a static barium oesophagram or contrast fluoroscopy (Gaschen, 2018). On VD views, the herniated fundus is localised to the

left of the oesophagus and the content may also move in and out with respiration in type II hiatal hernias (Gaschen, 2018).

Endoscopy can be helpful in the diagnosis of a sliding hiatal hernia, but not always possible if transient in nature (Gualtieri, 2001). The cardia can be followed by a dilated section covered by gastric mucosa (intra-thoracic stomach) which terminates with a narrowing of the lumen by the oesophageal hiatus. In a paraoeosphageal hernia, the gastro-oesophageal junction appears normal and part of the stomach can be seen dislocating through the oesophageal hiatus when the endoscope is in retroflexed position (Gualtieri, 2001).

3.3 Oesophageal motility disorders

3.3.1 Megaoesophagus

Megaoesophagus is characterized by a focal or diffusely dilated and hypomotile oesophagus (Marks, 2017; Gaschen, 2018). Megaoesophagus can be congenital or acquired, with the latter being idiopathic or secondary to a recognised disease **(Table 1)** (Dennis, et al., 2010; Wagner, 2008; Marks, 2017; Gaschen, 2018). The most common cause of megaoesophagus is idiopathic (Wagner, 2008; Gaschen, 2018).

Megaoesophagus can also be transient, secondary to aerophagia, heavy sedation or general anaesthesia (Dennis, et al., 2010).

Table 1. *Causes for acquired non-idiopathic megaoesophagus in the dog (Wagner, 2008; Dennis, et al., 2010).*

Acquired megaoesophagus
Immune-mediated myopathies and neuropathies: - Polymyositis - Acquired myasthenia gravis - Acute polyradiculoneuritis - Systemic lupus erythematosus - Polyneuritis - Dermatomyositis
Metabolic neuropathies and myopathies: - Hypoadrenocorticism - Hypothyroidism - Corticosteroid-induced polymyopathy - Diabetes mellitus - Hyperinsulinism - Uraemia
Toxic neuropathies: - Organophosphates - Heavy metals (e.g. zin, cadmium, thallium) - Chlorinated hydrocarbons - Anticholinesterases - Herbicides - Acrylamide - Botulism - Tetanus
Secondary to: - Foreign body - Stricture - Vascular ring anomaly - Neoplasia - Acute gastric dilation and volvulus - Snake bite - Oesophagitis

The most common clinical sign of megaoesophagus is regurgitation but it can also be asymptomatic (Wagner, 2008; Gaschen, 2018). A congenital predisposition has been suggested in the following breeds: Irish Setter, Great Dane, German Shepherd, Labrador Retriever, Chinese shar-pei, Newfoundland, Miniature Schnauzer and Wired hair Fox terrier breeds (Wagner, 2008; Marks, 2017). Congenital segmental oesophageal dysfunction has been reported in the Chinese shar-pei and Newfoundland Retriever (Wagner, 2008). Irish Setters, Golden retrievers and German Shepherd have been documented to have an increased risk of developing acquired megaoesophagus (Wagner, 2008). Aspiration pneumonia is a common complication (Wagner, 2008; Marks, 2017; Gaschen, 2018).

3.3.1.1 Diagnostic imaging

Radiographic findings of megaoesophagus can include a generalised to focal dilation of the oesophagus with gas, fluid or ingesta, a tracheal stripe sign, a sharp interphase between the oesophagus and *longus colli* muscle, ventral displacement of trachea, ventral displacement of the heart and an increased amount of gas in the stomach (Wagner, 2008; Gaschen, 2018). On the lateral view, the oesophagus when dilated with gas appears as two soft tissue opaque, parallel bands in the dorsal aspect of the thorax, representing the walls of the oesophagus (Gaschen, 2018). On the DV view, the gas filled oesophagus is identified as a thick soft tissue band on the left side of the vertebral column, however when severely dilated, a second band can also be seen on the right side (Gaschen, 2018).

The degree of oesophageal dilation, function and the extent of structural abnormalities can also be evaluated using contrast radiography (Wagner, 2008). Fluoroscopy is indicated in early disease, mild or segmental disease and oesophageal dysmotility (Wagner, 2008). Contrast fluoroscopy would show aperistalsis and impaired oesophageal bolus transportation without the presence of an oesophageal obstruction (Venker-van-Haagen, 2013). Additionally, scintigraphy has been shown to be more sensitive in the detection of subtle oesophageal motility abnormalities when no abnormalities are detected on routine diagnostic imaging (Wagner, 2008).

Endoscopy is not routinely necessary for the diagnosis of megaoesophagus (Gualtieri, 2001). A mild dilation of the oesophagus can be normal on endoscopy. In more severe cases, the oesophagus appears dilated and flaccid, containing food, fluid or saliva (Gualtieri, 2001).

3.3.2 Oesophageal dysmotility

Oesophageal dysmotility is characterised by reduced oesophageal peristalsis, food retention and regurgitation (Venker-van-Haagen, 2013). Subclinical oesophageal motility disorder has been reported in the Chinese shar-Pei and Bouvier des Flanders (Bexfield, et al., 2006). Causes of oesophageal dysmotility are thought to be similar to those of megaoesophagus. It has been suggested that a motility disorder may occur prior to oesophageal dilation in many cases of megaoesophagus (Bexfield, et al., 2006). Also, the presence of oesophageal dysmotility in the absence of megaoesophagus has been characterised by Gaschen (2018) as an abnormal primary wave that moves the bolus less that 5 cm aborally and abnormal secondary waves that cause bolus retention (Gaschen, 2018).

Oesophageal dysmotility has been reported in young dogs secondary to a delayed maturation of the oesophageal function (Bexfield, et al., 2006; Venker-van-Haagen, 2013; Gaschen, 2018), muscular dystrophy, myasthenia gravis, inflammatory myopathy and transient dysfunction following general anaesthesia (Venker-van-Haagen, 2013). Delayed maturation of the oesophageal function has been recognised as a cause of swallowing dysfunction and oesophageal motility disorders in human infants (Bexfield, et al., 2006). Similar to humans, the dog's oesophagus matures at 1 year of age (Bexfield, et al., 2006). Spontaneous improvement has been documented in dogs after 1 year of age, due to maturation of the neuromuscular system (Bexfield, et al., 2006; Gaschen, 2018). Oesophageal dysmotility have been documented in terrier breeds without presenting evident clinical signs (Bexfield, et al., 2006).

Oesophagitis secondary to gastro-oesophageal reflux (GOR) can lead to dysmotility (Bexfield, et al., 2006; Venker-van-Haagen, 2013; Gaschen, 2018).

A study by Bexfield et al. (2006) recognised oesophageal dysmotility in dogs without megaoesophagus, in both symptomatic and non-symptomatic dogs. It was also suggested to occur most commonly in young terrier breeds (Bexfield, et al., 2006).

Regurgitation, anorexia and weight loss are some of the clinical signs noted in dogs with oesophageal dysmotility. However, Bexfield at al. (2006) reported four terriers with evidence of dysmotility on fluoroscopy but without clinical signs.

3.3.2.1 Diagnostic imaging

A segmental or generalised dilation of the oesophagus with abnormal content may be detected on survey radiographs due to absence of normal peristalsis, producing similar changes to megaoesophagus. Fluoroscopy is the most accurate method to assess the oesophageal motility (Gaschen, 2018).

3.4 Oesophageal obstruction

3.4.1 Oesophageal foreign bodies

Foreign bodies are common in young dogs and some terrier breeds appear to be predisposed (Marks, 2017; Gaschen, 2018). Dogs with foreign bodies present with dysphagia (Marks, 2017). The most common oesophageal foreign bodies are bones, fish-hooks, needles and sticks (Marks, 2017). These foreign bodies may cause a partial or complete obstruction (Elwood, 2006; Wagner, 2008). They are typically lodged at the thoracic inlet, the base of the heart or the diaphragmatic hiatus, which are all sites of minimal distension (Wagner, 2008; Marks, 2017; Gaschen, 2018). Non-obstructive foreign bodies, such as fish hooks and other sharp objects, tend to lodge in the pharyngeal region (Gaschen, 2018). Sharp objects may perforate the oesophageal wall and lead to the formation of a pneumomediastinum, pneumothorax, mediastinitis, pleuritis and trachea-oesophageal fistula (Elwood, 2006; Wagner, 2008). Oesophageal stricture and aspiration pneumonia are secondary complications which may occur if the foreign material remains in situ (Elwood, 2006).

3.4.1.1 Diagnostic imaging

Survey radiographs should be taken from the base of the tongue to the cranial abdomen, including the stomach (Elwood, 2006; Wagner, 2008; Gaschen, 2018). Radiographic features include visibility of the foreign material, gas dilation proximally to the obstruction, delineation of the foreign body by gas / gas surrounding the foreign body and displacement of the trachea (e.g. ventrally on the lateral views and to the right on the DV view) (Wagner, 2008; Gaschen, 2018). Additionally, pneumomediastinum and pleural effusion may be

evident as a complication secondary to perforation (Wagner, 2008; Gaschen, 2018). Some foreign bodies can be recognised on the survey radiographs as a focal radiopaque structure. Static positive contrast oesophography is a useful technique for the characterisation of oesophageal masses, non-radiopaque foreign bodies, oesophageal strictures and vascular ring anomalies (Gaschen, 2018). This technique is contra-indicated when there are changes on the survey radiographs suggestive of oesophageal perforation (Gaschen, 2018). The use of non-ionic, iodinated, low-osmolar, water soluble contrast media is indicated when perforation is suspected (Wagner, 2008).

Oesophagoscopy is indicated to confirm and remove the foreign body, also to assess the oesophageal mucosa (Gualtieri, 2001).

3.4.2 Gastro-oesophageal intussusception

Gastro-oesophageal intussusception is a rare, potentially life-threatening condition characterised by the invagination of the stomach and occasional other abdominal structures including the spleen, pancreas, proximal duodenum and omentum into the caudal thoracic oesophagus (Pietra, et al., 2003; Venker-van-Haagen, 2013; Murphy, et al., 2015; Brady, et al., 2017; Gaschen, 2018). Gastro-oesophageal intussusception is considered a surgical emergency due to possible ischaemia of the stomach and other associated organs (Brady, et al., 2017). Clinical signs include vomiting or regurgitation, haematemesis, abdominal pain and dyspnoea (Pietra, et al., 2003).

The aetiology of gastro-oesophageal intussusception is not well understood. Theories include a gradual weakening of the oesophagus, disease causing underlying weakness of the oesophagus (e.g. myasthenia gravis) and abnormal development of the oesophageal hiatus (Brady, et al., 2017). Additionally, idiopathic megaoesophagus and hiatal herniation have been reported associated with gastro-oesophageal intussusception (Venker-van-Haagen, 2013).

This condition usually affects young male medium to large breed dogs, with a higher incidence reported in German Shepherds (Pietra, et al., 2003; Venker-van-Haagen, 2013). Risk factors for developing gastro-oesophageal intussusception in humans depends on the elevation of the abdominal pressure, excess food with intense physical activity or chronic dyspepsia (Pietra, et al., 2003).

3.4.2.1 Diagnostic imaging

Survey radiographs reveal a proximal gas dilation of the oesophagus associated with a homogeneous, ovoid, soft tissue opacity or a mixed soft tissue and gas opacity along the mid to caudal portion of the thoracic oesophagus (Venker-van-Haagen, 2013; Gaschen, 2018). The sharp demarcation between the cranial edge of the intussusceptum with the gas filled oesophageal lumen is a feature used to differentiate gastro-oesophageal intussusception from a sliding para-oesophageal hiatal hernia (Gaschen, 2018). Contrast radiographs usually show a retention of the barium at the proximal aspect of the oesophagus, but it not reaching the caudal thoracic oesophagus or stomach (Venker-van-Haagen, 2013). Gastric rugal folds are often identified within the oesophageal lumen using contrast radiography (Venker-van-Haagen, 2013). CT is a useful diagnostic imaging method used for pre-operative diagnosis and surgical planning (Shum, et al., 2007).

Oesophagoscopy can reveal a dilated oesophagus filled with gastric mucosal folds (Gualtieri, 2001).

3.5 Oesophageal neoplasia

Neoplasia of the oesophagus is very rare (Venker-van-Haagen, 2013; Marks, 2017). Tumours may be of primary oesophageal, peri-oesophageal (e.g. lymph nodes, thyroid, heart base and thymus) or metastatic origin (Marks, 2017). Fibrosarcoma and osteosarcoma are the most common tumours affecting the oesophagus of the dog resulting from the malignant development of *Spirocerca lupi* (Venker-van-Haagen, 2013; Wagner, 2008; Marks, 2017). Other less common tumours include leiomyoma, leiomyosarcoma, chondrosarcoma, adenocarcinomas, undifferentiated carcinoma, lymphoma and metastatic carcinoma (Venker-van-Haagen, 2013; Wagner, 2008; Marks, 2017). Gastric metaplasia caused by GOR (Barret's oesophagus) is a relevant cause of oesophageal carcinoma in humans but has only been reported in three cats (Venker-van-Haagen, 2013). Oesophageal tumours are commonly located in the caudal thoracic oesophagus; however, leiomyomas are known to occur closest to the lower oesophageal sphincter (Venker-van-Haagen, 2013). Oesophageal tumours are locally invasive and occasionally metastasise to the regional lymph nodes (Venker-van-Haagen, 2013).

3.5.1.1 Diagnostic imaging

A soft tissue mass lesion with or without mineralisation along the region of the oesophagus may be recognised on survey radiographs (Gaschen, 2018). The oesophagus may be dilated with gas cranial to the mass or displaced if a peri-oesophageal mass lesion is present (Wagner, 2008). Contrast radiography, CT or endoscopy may be required to differentiate oesophageal masses from oesophageal foreign bodies or non-oesophageal masses (Venker-van-Haagen, 2013; Gaschen, 2018). Temporary or persistent retention of barium may occur due to the absence of a secondary peristaltic wave associated with the tumour infiltration (Gaschen, 2018). Additionally, mural thickening, asymmetry or narrowing of the lumen and irregular mucosa suggestive of ulceration may be present with barium retention (Gaschen, 2018).

Dystrophic mineralisation of masses is rare but can be associated with neoplasia or *S. lupi* infection (Gaschen, 2018). Dogs infected with *S. lupi* may also have thoracic spondylosis (Venker-van-Haagen, 2013). CT angiography perfusion analysis of oesophageal nodules can be used to differentiate non-neoplastic from neoplastic nodules associated with *S. lupi* (van der Merwe, et al., 2008). Oesophageal sarcomas associated with *S. lupi* are less well perfused (van der Merwe, et al., 2008; Gaschen, 2018; Pazzi, et al., 2018)

Endoscopic ultrasonography is an alternative diagnostic imaging modality that can be used to evaluate the oesophagus in these cases (Gaschen, 2018). Oesophagoscopy may show the esophageal lumen partially or completely obstructed by an irregular and firm annular growth of the mucosa (Gualtieri, 2001). The proximal portion of the oesophagus can be dilated and contain fluid, ingesta or gas if the lumen is stenotic (Gualtieri, 2001).

3.6 Redundant oesophagus

Redundant oesophagus is often an incidental finding in young brachycephalic breeds such as English and French bulldogs and Chinese shar-peis (Wagner, 2008; Gaschen, 2018). It is usually a ventral, but occasionally a lateral deviation of the oesophagus at the thoracic inlet (Wagner, 2008).

3.6.1.1 Diagnostic imaging

Oesophageal redundancy may not be visible on survey radiographs or appear as focal gas dilation of the oesophagus at the level of the thoracic inlet (Gaschen, 2018). Contrast radiography and fluoroscopy (e.g. barium meal) shows the redundancy as a tortuous or U shape course of the oesophagus ventral to the trachea (Wagner, 2008; Gaschen, 2018). The redundant segment has a normal motility, therefore, contrast accumulation at the redundancy is only temporary and the oesophagus can appear normal on the subsequent radiographs (Gaschen, 2018).

3.7 Inflammatory disease

3.7.1 Gastro-oesophageal reflux

Gastro-oesophageal reflux disease (GOR) is a disorder of the gastro-oesophageal sphincter in human and dogs, characterised by reflux of the gastric or oesophageal content (e.g. food or liquid) leading to regurgitation (Venker-van-Haagen, 2013; Muenster, et al., 2017; Torrente, et al., 2017). Oesophageal inflammation, regurgitation and pain can occur secondary to GOR (Muenster, et al., 2017). Additionally, development of oesophageal ulcerations, strictures and epithelial metaplasia may appear as a complication to GOR (Muenster, et al., 2017).

3.7.1.1 Diagnostic imaging

Survey radiographs are usually normal, or an increased soft tissue opacity can be identified between the aorta and cauda vena cava on the lateral projection due to retention of fluid material in the oesophagus. The caudal thoracic oesophagus may become distended with air or fluid as it accumulates (Gaschen, 2018). Static contrast oesophagrams are also usually negative with GOR. However, when severe ulceration is present, the contrast medium will adhere to the mucosa highlighting the ulceration (Gaschen, 2018). GOR can also be identified using fluoroscopy by visualisation of the barium meal being refluxed from the stomach back into the oesophagus (Gaschen, 2018).

On oesophagoscopy, the lower oesophageal sphincter can be been open due to the constant reflux of gastric content towards the oesophagus. In mild cases, the diagnosis can be really challenging (Gualtieri, 2001).

3.7.2 Oesophagitis

Oesophagitis is a common sequel of an underlying disease or secondary consequence of oesophageal disease, which may cause oesophageal hypomotility and, in severe cases, oesophageal strictures (Jergens, 2010; Marks, 2017; Reeve, et al., 2017). It consists of an acute or chronic inflammation of the oesophageal mucosa, involving occasionally the submucosa and muscularis (Marks, 2017). It may be caused by GOR alone or associated with structural abnormalities (e.g. hiatal hernia, neoplasm), foreign bodies, trauma, ingestion of caustic agents, chronic vomiting (Elwood, 2006; Wagner, 2008; Marks, 2017; Gaschen, 2018) radiation injury, idiopathic generalized megaoesophagus, inflammation associated with malignancy (Venker-van-Haagen, 2013) and general anaesthesia (Torrente, et al., 2017). Another cause for oesophagitis is abnormal oesophageal motility (Gaschen, 2018). Additionally, oesophagitis has been documented to increase the risk for the development of megaoesophagus in dogs (Wagner, 2008).

3.7.2.1 Diagnostic imaging methods

Survey radiographs are usually unremarkable, and endoscopy is required for diagnosis (Venker-van-Haagen, 2013; Gaschen, 2018). Severe oesophagitis may lead to the development of ulcerations, motility disorders, segmental narrowing, irregular mucosa or thickening of the wall, which could be highlighted by barium contrast studies (Gaschen, 2018). The mucosal surface may appear irregular with secondary hypomotility and segmental narrowing secondary to inflammation (Venker-van-Haagen, 2013). Transient GOR or intermittent hiatal herniation may be evident on fluoroscopy as the cause for oesophagitis (Venker-van-Haagen, 2013). Oesophagitis secondary to GOR can be recognised on fluoroscopy as a segmental spasticity and minor dilation (Gaschen, 2018).

Oesophagoscopy is the best way to reach the definitive diagnosis of oesophagitis (Gualtieri, 2001).

4 Aims of the study

The aims of this study were to:

- Determine whether conventional transcutaneous ultrasonography can be used to evaluate the entire canine cervical oesophagus
- Describe the sonographic appearance and measurements of the cervical oesophagus in normal dogs and those with clinical signs associated with the oesophagus.
- Document the occurrence of oesophageal abnormalities in brachycephalic dogs presenting to a veterinary tertiary referral centre.
- Record the incidence of oesophageal redundancy in brachycephalic breeds with or without oesophageal disease.

The entire study was performed in accordance with ethical approval granted by the University of Glasgow ethics committee (Ref 09a/16 & Ref 10a/16).

Chapter 2 - Ultrasonography study

1 Introduction

The canine oesophagus is a tubular structure that connects the pharynx to the stomach and is divided into cervical, thoracic and abdominal sections. Its wall is composed of four histological layers, the mucosa, submucosa, muscularis and adventitia, and these are consistent along the entire length of the gastrointestinal tract (Evans and de Lahunta, 2013).

The most common clinical sign associated with oesophageal disease is regurgitation (Bright et al., 1990; Washabau, 2005; Elwood, 2006; Marks, 2017) but conditions affecting the oesophagus can be difficult to diagnose. Current diagnostic imaging modalities for examination of the canine oesophagus include survey radiography, contrast radiography, fluoroscopy, magnetic resonance imaging, endoscopic and transcutaneous ultrasound, computed tomography (CT) and endoscopy, with endoscopy being the modality of choice (Noh et al., 1995; Elwood, 2006; Mateen et al., 2006; Ridgway and Graves, 2010; Baloi et al., 2013; Jagmohan and Goh, 2013; Venker-van-Haagen, 2013; Gory et al., 2014; Kirberger et al., 2014; Bristow, 2015; Marks, 2017; Gaschen, 2018). However, not all of these are readily available to practitioners.

Unlike other imaging modalities, ultrasound provides detailed information about the internal architecture of the oesophageal wall in addition to its luminal contents and surrounding soft tissue structures (Baloi et al., 2013; Gory et al., 2014, Bristow, 2015). Endoscopic ultrasound is not readily available in clinical settings, but most veterinary practices now have access to transcutaneous ultrasound, which is a relatively inexpensive, non-invasive imaging method that does not require the use of ionising radiation. Although the thoracic portion of the oesophagus is inaccessible to transcutaneous ultrasound due to the surrounding air-filled lungs, the cervical and abdominal sections can be both be examined.

The ultrasonographic appearance of the normal canine cervical oesophagus was first reported by Wisner et al. (1991) but although more recent textbooks include images, no more detailed information about its appearance has been published (Neelis et al., 2015; Zwingenberger and Taeymans, 2015). The appearance of the abdominal oesophagus was described using transcutaneous abdominal ultrasound (Gory et al., 2014) and the cervical and thoracic oesophagus using endoscopic ultrasound (Baloi et al., 2013). The visible

ultrasonographic layers are the mucosa, submucosa, muscularis and adventitia (Baloi et al., 2013; Gory et al., 2014; Neelis et al., 2015) which correspond with the histological layers demonstrated in the abdominal oesophagus and throughout the rest of the canine gastrointestinal tract (Gory, et al., 2014). In humans, there are usually also four ultrasonographic layers visible but sometimes six layers can be demonstrated (Shang-Yong et al., 2004). Comparison with histological specimens identified a connective tissue layer between the inner circular and outer longitudinal muscular layers in humans which was responsible for this extra layer (Shang-Yong et al., 2004).

Transcutaneous ultrasound has been used to assess the normal and diseased human oesophagus (Shang-Yong et al., 2004) but there are only a few reports of its use in the veterinary literature (Capitani et al., 2014; Gory et al., 2014; Bristow, 2015; Neelis et al., 2015; Zwingenberger and Taeymans, 2015). Likewise, although there are published tables of normal wall thickness for the canine abdominal oesophagus and the rest of the gastrointestinal tract (Neelis et al., 2015; Zwingenberger and Taeymans, 2015), there are currently no normal values for the cervical oesophagus or reports of the use of transcutaneous ultrasound to prospectively investigate dogs with clinical signs associated with the oesophagus.

2 Aim of the study

The aims of this prospective study were therefore to determine whether conventional transcutaneous ultrasonography could be used to evaluate the entire cervical oesophagus in conscious dogs; to describe the sonographic appearance of the normal canine cervical oesophagus and compare this to histological findings in cadavers; to determine parameters for cervical oesophageal wall thickness in relation to histology in normal dogs; and to investigate the use of transcutaneous ultrasound prospectively in dogs with clinical signs associated with the oesophagus.

We hypothesised that transcutaneous ultrasonography could be used to document the appearance and thickness of the canine cervical oesophagus but that its use in clinically affected dogs would be limited to those with gross wall changes, with subtle histological changes not being identifiable.

3 Material and Methods

This was a prospective, anatomic observational study performed between January 2016 to August 2017 at the University of Glasgow, School of Veterinary Medicine. Approval was obtained from University of Glasgow, School of Veterinary Medicine Ethical Committee (Ref 09a/16). The sample size in each part was determined by the availability of appropriate dogs during the period of study.

3.1 Part A: Cadaver anatomy

Seven fresh cadavers donated to the University of Glasgow, School of Veterinary Medicine for teaching and research purposes, were selected based on freshness by an experienced anatomic technician. No clinical history or patient details were available for any of the cadavers, but sex, breed type and estimated age were recorded.

Ultrasound was performed within eight hours of euthanasia by the same second year diagnostic imaging resident. The left side of the neck was clipped then water and ultrasonographic gel applied. Each cadaver was positioned in dorsal recumbency with the head to the sonographers right and scanned using an ultrasound machine (LOGIQ 9; GE Healthcare, Solingen, Germany) with a linear-array 14 MHz transducer. The transducer was placed in a transverse plane at the level of the larynx then moved slightly towards the left side of the neck and caudally until the trachea, thyroid and oesophagus were visible. For the longitudinal images, the transducer was placed in mid-sagittal plane over the caudal aspect of larynx/cranial aspect of the trachea and then moved slightly laterally until the oesophagus was visible between the trachea and common carotid artery. Transverse and longitudinal images were acquired of the cranial (caudal to the larynx), middle and caudal (cranial to the thoracic inlet) regions of the cervical oesophagus **(**Fig. 1**)**. The neck length variation between dogs did not allow standardisation of the regions of the cervical oesophagus. Therefore, the neck was divided into three equal sections from the caudal aspect of the larynx to the thoracic inlet, which were then referred as cranial, middle and caudal regions.

Static images were captured and digitally stored to a Picture Archiving and Communication System (PACS).

The oesophagus was then resected from all cadavers and examined ultrasonographically in a water bath. Transverse and longitudinal images were digitally stored to the PACS. A section was removed from each of the three regions of the oesophagus (cranial, middle and caudal) in all seven cadavers and prepared for histology. Haematoxylin and eosin (H&E) stain was used to stain all twenty-one samples and additional staining with Masson´s trichrome was applied in three dogs (nine samples) to allow better identification of collagen fibres. The same final year pathology resident examined all histologic samples and measured the wall thickness three times for each region. This information was then compared with the ultrasound images.

3.2 Part B: Normal live dogs

Ten healthy dogs owned by clinical staff were recruited by the diagnostic imaging resident (BJG). Inclusion criteria were dogs without any history of gastrointestinal disease, vomiting or regurgitation in the two months prior to the study. Informed written owner consent was obtained and the dogs were scanned unsedated. Ultrasound equipment, patient preparation, positioning, ultrasonographic evaluation and measurements were the same as in part A. Body weight, age and breed for each dog was also recorded.

3.3 Part C: Dogs with clinical signs relating to the oesophagus

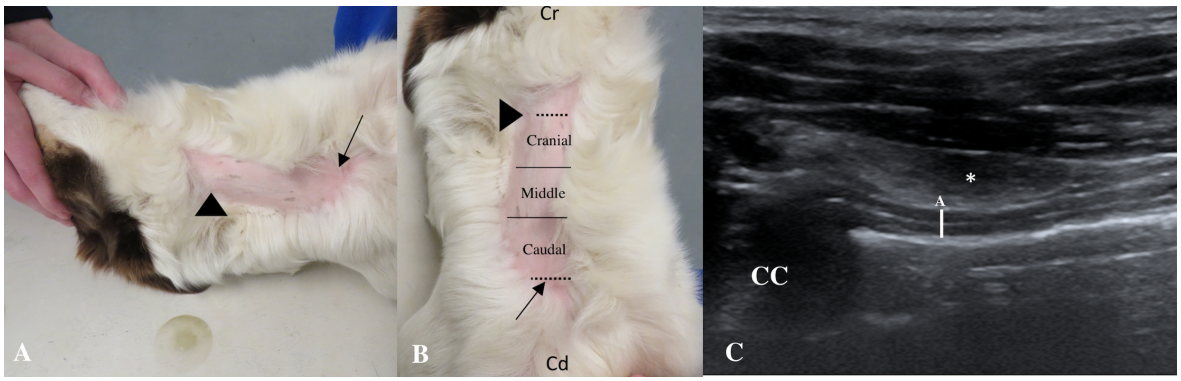
Eleven client owned dogs that were referred to University of Glasgow Small Animal Hospital during the period of the study for the investigation of gastrointestinal disorders were selected by the diagnostic imaging resident (BJG). Inclusion criteria were dogs with a history of regurgitation or chronic vomiting that were undergoing an abdominal ultrasound examination while the resident was on that rotation. Dogs with chronic vomiting were included in the study based on the presumption that chronic acid reflux could potentially lead to inflammation and erosion of the oesophagus. Informed written owner consent was obtained using the generic Small Animal Hospital clinical consent form. Some of the dogs required sedation for the abdominal ultrasound examination but none were sedated specifically for examination of the oesophagus. Ultrasound equipment, patient preparation, positioning, ultrasonographic evaluation and measurements were the same as in part A and

B. Body weight, signalment, history, clinical signs and final diagnosis in each case were also recorded.

4 Image Analysis

Image analysis was performed using Digital Imaging and Communications in Medicine (DICOM) -viewer (OsiriX MD; Pixmeo, Bernex, Switzerland). The images were displayed on a monitor (iMac, 2013, Apple inc, California, United States of America). The matrix of the images was 1024x768 pixels. The electronic calipers (size 0.03 mm) were used to make measurements of oesophageal wall thickness of the cranial, middle and caudal regions in the both longitudinal and transverse planes (**Figure 2**).

Figure 2. *Illustration of the attributed neck regions in a dog (cranial, middle and caudal) and area of the neck scanned (A and B). Cranial is to the left on image A and to the top on image B. Larynx (arrow head) and thoracic inlet (black arrow). Longitudinal ultrasound image of the cranial region of a normal canine cervical oesophagus in a cadaver (C). Cranial is to the left of the image. Oesophageal wall thickness (solid line at A); Cricoid cartilage of larynx (CC); Thyroid gland (*).*



Measurement of the thickness of the wall nearest to the transducer was performed from the mucosal-lumen interface to the adventitia. Three measurements were performed for each region and plane at three different times points. A total of six measurements were performed for each region on ultrasound at three different points. An overall group mean wall thickness was calculated using cadavers in Part A (ultrasound, water bath and histology) and dogs in part B.

Images were also evaluated for visibility of the wall layering and compared to the corresponding histological images obtained from the cadavers.

5 Statistical analysis

Statistical analysis was selected and performed by a Diplomate in Veterinary Epidemiology (TP) using statistics software (Minitab®; version 17.1.0.0 Minitab Ltd., Coventry). Correlation between the body weight, age and breed with the mean oesophageal wall thickness was calculated for parts B and C using the Pearson correlation coefficient ($P<0.05$). Regression analysis between the mean oesophageal wall thickness and the body weight in parts B and C was also performed. Statistical significance was set at $P<0.05$. Maximum, minimum, mean with standard deviation were calculated for the ultrasonographic wall thickness in each part of the study using commercially available software (Microsoft Excel for Mac, Microsoft Office, Redmond, WA).

6 Results

Transcutaneous ultrasonography of the canine cervical oesophagus using a left sided approach was possible in all the dogs and allowed the whole length of the cervical oesophagus to be clearly visualized. On transverse images the oesophagus appeared oval shaped and on longitudinal images appeared rectangular, which corresponded with its tubular nature. In the presence of intra-luminal gas only the wall nearest to the transducer could be visualised due to the presence of acoustic shadowing.

6.1 Part A: Cadaver anatomy

Although information regarding their specific age was not available, all seven dogs were adults. Three were neutered males (two mixed breed and one miniature poodle) and four were entire males (three mixed breed and one English bulldog). None of these dog's weight was available and therefore not comparable with normal live dogs.

6.1.1 Histological findings

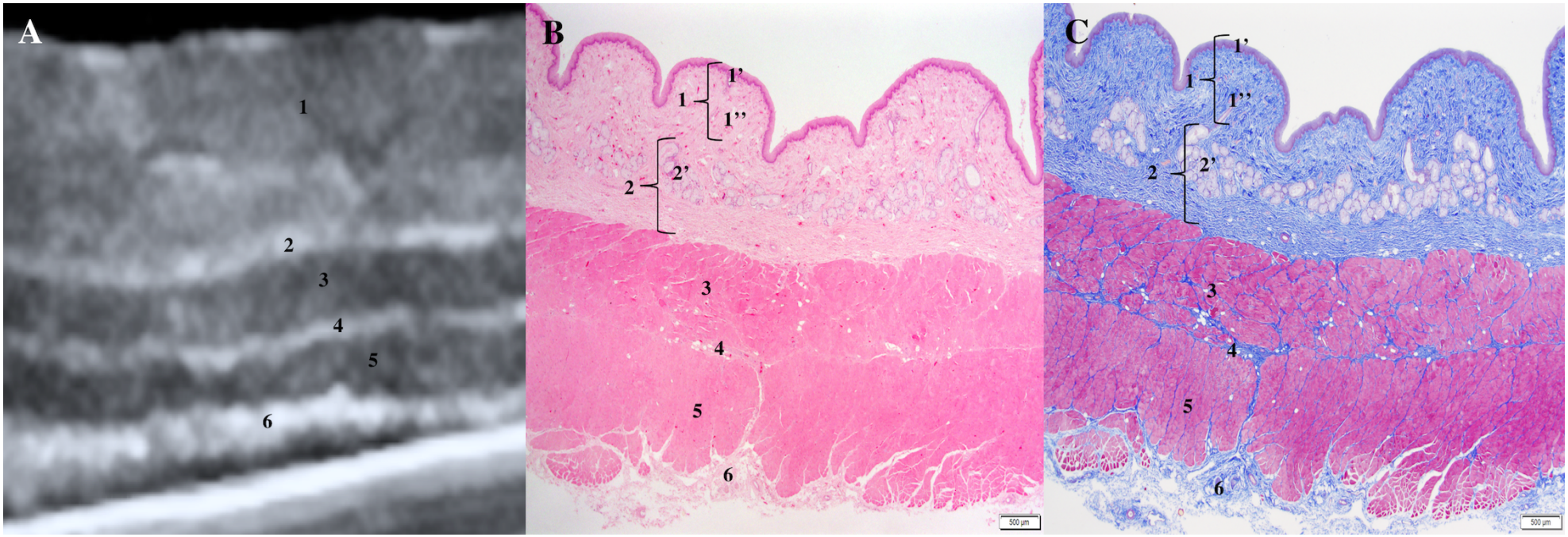
The oesophageal sections stained with H&E had four distinct layers: mucosa, submucosa, muscularis and adventitia (**Figure 3B**). The mucosa was composed of a stratified squamous epithelium, a lamina propria and in some samples a muscularis mucosae. Occasional mitotic figures were present in the basal layer of the epithelium in all seven cadavers. The lamina propria contained small, multifocal, mostly perivascular and

periductal, infiltrates of lymphocytes and plasma cells. Sporadic macrophages (three cadavers), mast cells (one cadaver) and neutrophils (three cadavers) were also observed. In one cadaver, moderate numbers of eosinophils were present. The muscularis mucosae was absent in most of the sections but was present as inconspicuous isolated bundles in the middle (four cadavers) and caudal sections (five cadavers) of the oesophagus.

The submucosa was composed of abundant lobules of mucus secreting glands. Clusters of plasma cells were common between the glands in all cadavers. Admixed with these inflammatory cells were occasional lymphocytes (three cadavers), macrophages (three cadavers) and neutrophils (one cadaver). Mild to moderate infiltration of adipose tissue between the collagen bundles of the submucosa, especially near the muscularis, was present in five cadavers.

The muscularis externa was composed of two layers of skeletal muscle but in most cases the fibres were haphazardly arranged, without a clear distinction between the inner circular and outer longitudinal layer**.** It was also common to see a few degenerate fibres (four cadavers), occasional isolated necrotic fibres being infiltrated by lymphocytes and macrophages (four cadavers), as well as, a small amount of adipose tissue between the inner and outer muscular layers (two cadavers). In the samples stained with Masson's trichrome, a very thin and inconspicuous sheet of fibrous tissue was visible between the inner circular and outer longitudinal muscle layers (**Figure 3C**).

Figure 3. *Longitudinal water bath ultrasonographic (A) and histological sections (B and C) from the cranial region of a normal canine oesophagus (Cadaver five). Image (B) is stained with Haematoxylin and Eosin (H&E) and (C) is stained with Masson's Trichrome. Section thickness 500 µm. Note that the fibrous connective tissue (4) is more evident using Masson's Trichrome stain. Mucosal layer (1) composed of stratified squamous epithelium (1') and lamina propria (1''), submucosa layer (2) with a glandular portion (2'), inner circular muscle (3), fibrous connective tissue (4), outer longitudinal muscle (5), adventitia (6).*



In all samples, the adventitia was present as a loose and discontinuous layer of collagen, adipocytes, blood vessels, lymphatics and nerves. This layer was frequently incomplete due to the technical and chemical procedures used for preparation of the histological sections. In consequence, oesophageal wall thickness in the histological sections was measured from the muscularis layer to the mucosal-lumen interface. The results are presented in **Table 2**.

Table 2. *Histological measurements of canine cervical oesophageal wall thickness (from muscularis to mucosal-lumen interface) in cadavers.*

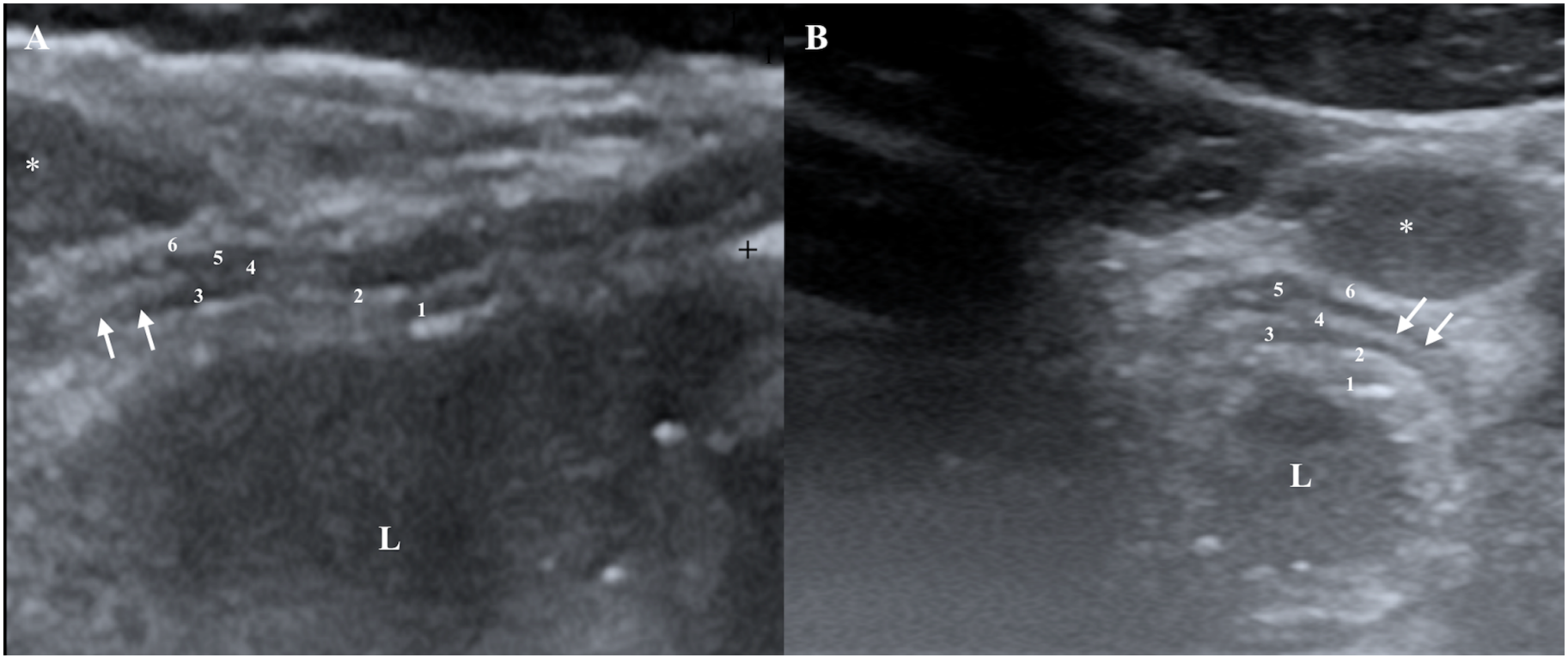
	Histological oesophageal wall thickness		
Region of neck	**Maximum (mm)**	**Minimum (mm)**	**Mean ± SD (mm)**
Cranial	6.8	1.5	3.7 ± 1.5
Middle	4.9	1.1	3.0 ± 1.1
Caudal	5.6	1.4	3.2 ± 1.2

Despite the changes reported on histological examination, all cadavers were considered by the pathologist to be within normal limits with the exception of the cadaver with the eosinophilic infiltration in the lamina propria. The mean wall thickness for this cadaver was 2.8 mm in situ on ultrasound, 2.96 mm in the water bath and 3.48 mm on histology. This cadaver was excluded from the results in Table 2 and further calculation of wall thickness.

6.1.2 Ultrasonographic findings

Oesophageal wall layers were identified in all seven cadavers (**Figure 4**).

Figure 4. *Longitudinal ultrasound image of the middle region of a normal canine cervical oesophagus in situ in cadaver four (A). Cranial is to the left of the image. Transverse ultrasound image of the cranial region of a normal canine cervical oesophagus in situ in cadaver four (B). Lateral is to the right of the image. 1 Mucosa; 2 Submucosa; 3 Inner circular muscle; 4 Fibrous connective tissue; 5 Outer longitudinal muscle; 6 Adventitia. The hyperechoic fibrous layer between the muscular layers (white arrows) is clearly evident on image (B), but faintly seen on image (A). Fluid filled oesophageal lumen (L); Mucosal-lumen interface with reverberation artefact (black +); Thyroid gland (*).*



The entire length of the oesophagus appeared to have four wall layers in one cadaver and six layers in three. The other three cadavers had inconsistent wall layering with either four or six layers being visible. In the water bath, six layers were consistently identified along the length of the oesophagus in five of the cadavers **(Figures 5)** but a similar variation between four and six wall layers was observed in the remaining two cadavers.

The four-wall layer appearance was characterised by alternating hyperechoic and hypoechoic layers, from the inner mucosal-lumen interface, through the mucosa, submucosa and muscularis to the outer adventitia and corresponded with the main layers reported on histological examination. The six-wall layer appearance was produced by the presence of an additional thin hyperechoic layer in the centre of the hypoechoic muscularis layer, which corresponded with the thin sheet of fibrous tissue identified on the histological sections stained using the Masson's trichrome. The small amount of adipose tissue detected between the inner and outer muscular layer on histology of two cadavers was not perceptible on ultrasound. This was likely associated with the adipose tissue bordering with sheet of fibrous tissue, therefore becoming indistinguishable on ultrasound due to similar echogenicity.

A variation in the echogenicity of the mucosal layer was noted in some regions. In three cadavers this layer was uniformly hypoechoic as expected but in four it appeared echogenic in some regions. This was also the case in all seven cadavers examined in the water bath, with some areas of the mucosal layer appearing echogenic. Wall thickness measurements are shown in **Table 3** and **Table 4**. Undefined correlation between the ultrasonographic oesophageal regions and histology.

Figure 5. *Longitudinal (A) and transverse (B) ultrasound images of the middle region of a normal canine cervical oesophagus in a water bath (Cadaver five). 1 Mucosa; 2 Submucosa; 3 Inner circular muscle; 4 Fibrous connective tissue; 5 Outer longitudinal muscle; 6 Adventitia. The mucosa is thicker than the adjacent layers and echogenic with multiple hyperechoic speckles, consistent with a collapsed oesophagus and its longitudinal folds. Fluid filled oesophageal lumen (L).*

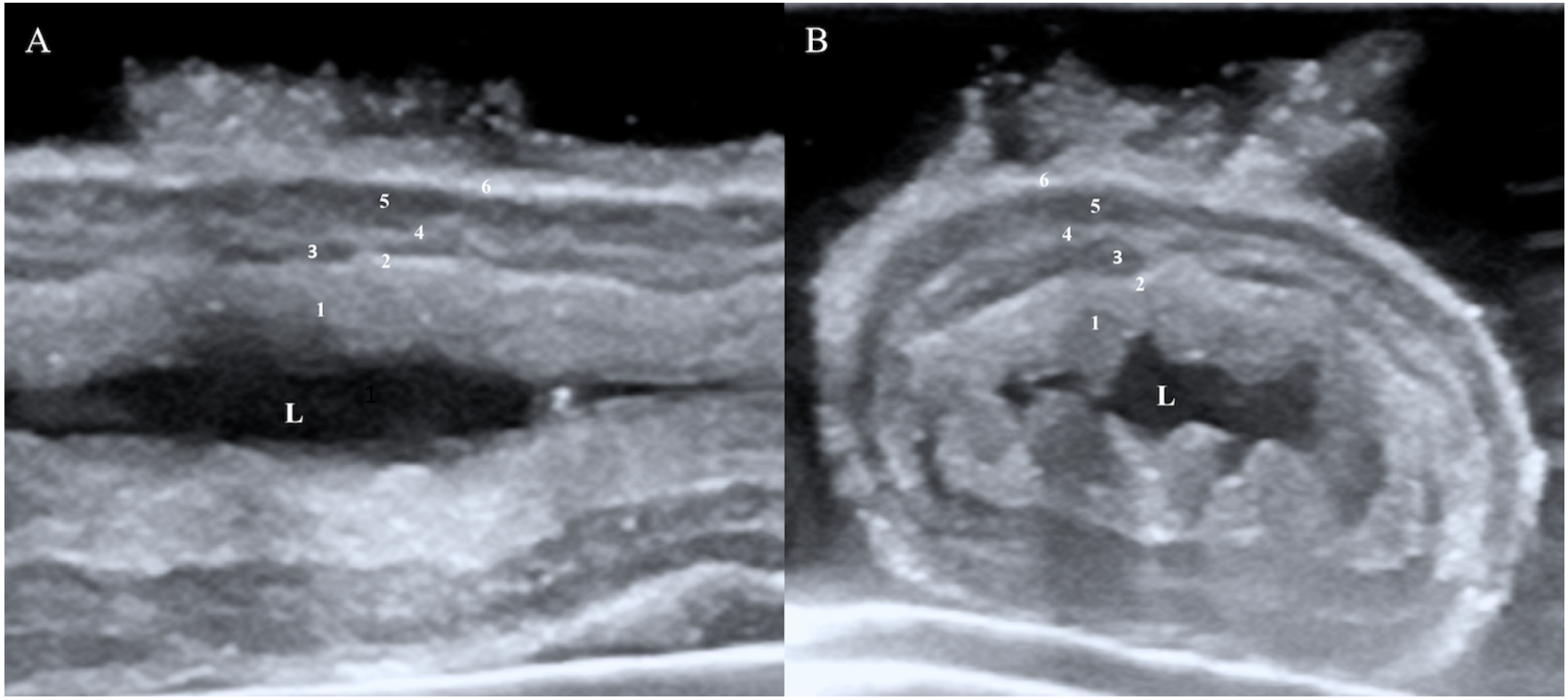


Table 3. *Overall ultrasonographic measurements of canine cervical oesophageal wall thickness (from adventitia to mucosal-lumen interface) and standard deviation (SD) in cadavers and live dogs. Part A - cadavers in situ and cadaver samples in a water bath, Part B - normal live dogs and Part C - dogs presenting with clinical signs relating to the oesophagus.*

Ultrasonographic oesophageal wall thickness			
	Maximum (mm)	**Minimum (mm)**	**Mean ± SD (mm)**
Part A - Cadavers	6.3	1.1	3 ± 1.4
Part A – Water bath	6.4	1.4	2.9 ± 0.9
Part B – Normal live dogs	5.1	1.4	2.5 ± 0.6
Part C – Dogs with clinical signs relating to the oesophagus	4.6	1.3	2.3 ± 0.5

Table 4. *Ultrasonographic regional measurements of canine cervical oesophageal wall thickness (from adventitia to mucosal-lumen interface) and standard deviation (SD) in Part A - cadavers in situ and cadaver samples in a water bath, Part B - normal live dogs and Part C - dogs presenting with clinical signs relating to the oesophagus.*

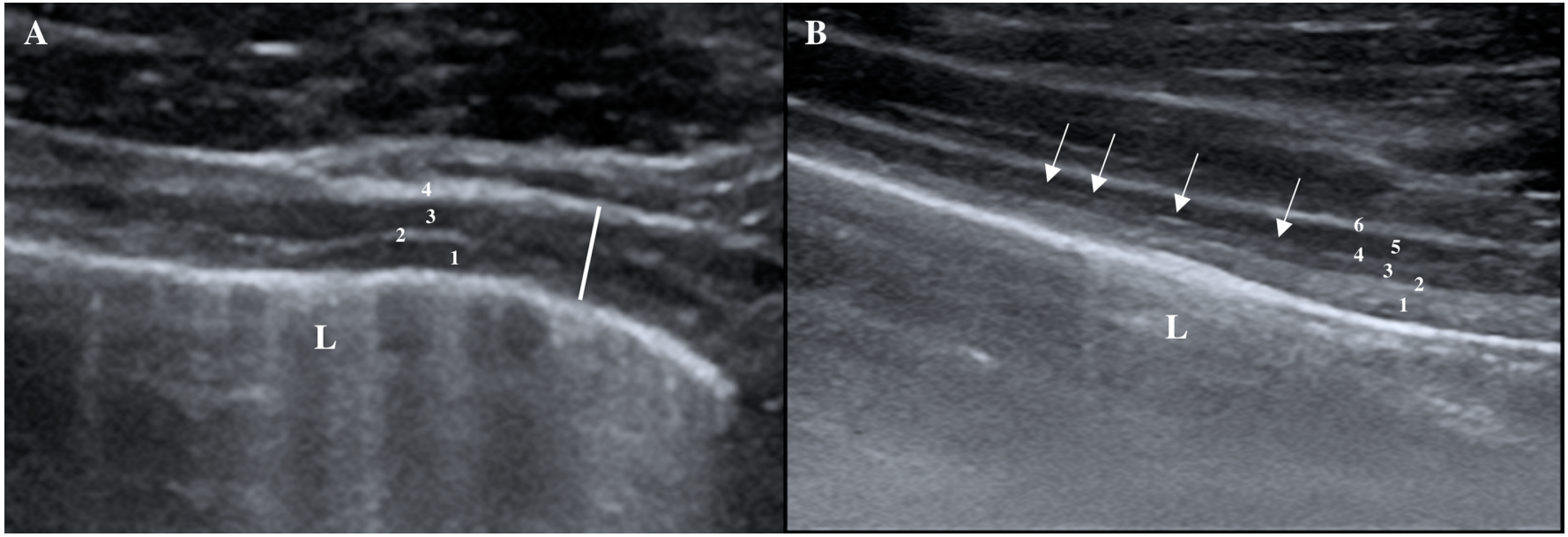
Part A - Cadavers			
	Maximum (mm)	**Minimum (mm)**	**Mean ± SD**
Cranial	6.1	1.7	3.2 ± 1.4
Middle	5.9	1.1	2.8 ± 1.5
Caudal	6.3	1.5	3.0 ± 1.3
Part A - Water bath			
	Maximum (mm)	**Minimum (mm)**	**Mean ± SD**
Cranial	4.7	1.4	2.9 ± 0.9
Middle	6.4	1.6	2.9 ± 0.9
Caudal	4.7	1.5	3.0 ± 0.8
Part B - Normal live dogs			
	Maximum (mm)	**Minimum (mm)**	**Mean ± SD**
Cranial	5.1	1.4	2.6 ± 0.7
Middle	3.9	1.6	2.4 ± 0.6
Caudal	3.7	1.4	2.4 ± 0.6
Part C - Dogs with clinical signs relating to the oesophagus			
	Maximum (mm)	**Minimum (mm)**	**Mean ± SD**
Cranial	4.6	1.5	2.3 ± 0.5
Middle	3.6	1.3	2.3 ± 0.5
Caudal	3.6	1.6	2.4 ± 0.5

6.2 Part B: Normal live dogs

Ten dogs met the inclusion criteria, two mixed breeds and one Jack Russell, Flat Coat Retriever, Corgi, Chihuahua, Border Collie, Golden retriever and Labrador. The mean age was five years (range 1.5 – 12 years), mean body weight was 18.8 kg (range 1.8 – 35.5 kg), six were neutered females and four were neutered males.

In two, a Corgi and a Chihuahua, only two wall layers were visible on ultrasound examination. These corresponded with the adventitia with a hypoechoic layer between representing an amalgamation of the mucosa, submucosa and muscularis which could not be distinguished from each other. The Corgi was obese (14.5kg) and the Chihuahua very small in size (1.8 kg). In the other eight dogs, two consistently demonstrated four wall layers, two consistently demonstrated six layers, and four varied intermittently between four and six layers. The appearance of these wall layers in the live dogs was the same as that observed in the cadavers in part A (**Figure 6B**). Likewise, a similar variation in the echogenicity of the mucosa was observed, with it appearing echogenic rather than hypoechoic in some regions in four of the ten live dogs. Wall thickness measurements are shown in Table 2 and Table 3. The mean wall thickness for Part A cadavers and Part B combined was 2.7 ± 1

Figure 6. *Longitudinal ultrasound image (A) of the caudal region of the cervical oesophagus in a dog with clinical signs associated with the oesophagus. Cranial is to the left of the image. The oesophageal lumen contains gas which is producing reverberation artefact (L). Alternating hyperechoic and hypoechoic layers are visible within the oesophageal wall (solid white line) with a four-wall layer pattern. The mucosa appears hypoechoic (1). 1 mucosa; 2 submucosa; 3 muscularis (inner circular muscle and outer longitudinal muscle); 4 adventitia. Longitudinal ultrasound image (B) of the caudal region of the cervical oesophagus in a normal live dog. The mucosa appears echogenic (1). The fibrous connective tissue that is present within the muscular layer is faintly visible as a thin hyperechoic layer in some areas (white arrows) producing an intermittent six wall-layer pattern to the oesophageal wall. 1 mucosa; 2 submucosa; 3 Inner circular muscle; 4 Fibrous connective tissue; 5 Outer longitudinal muscle; 6 Adventitia.*



6.3 Part C: Dogs with clinical signs relating to the oesophagus

Eleven dogs met the inclusion criteria, four French Bulldogs, four Labradors, two Border terriers and one mixed breed. The mean age was 4 years (range 6 months – 12 years), mean body weight was 19 kg (range 5.8 – 42 kg), there were five females (four neutered and two entire) and five males (one neutered and four entire).

The most common clinical signs were regurgitation (seven out of eleven dogs) and chronic vomiting (six) followed by retching (two), diarrhoea (two), acute vomiting (one), lethargy (one) and seizures (one). More than one clinical sign was present in each dog. The one dog with acute vomiting and the six dogs with chronic vomiting also had regurgitation. Three dogs were eventually diagnosed with lymphoplasmocytic enteritis, two with gastro-oesophageal reflux, one with eosinophilic enteritis, one with dietary responsive enteropathy, one with helicobacter gastritis, one with colitis, one with brachycephalic syndrome, one with a splenic mass and in one no diagnosis was reached. Two dogs had more than one diagnosis. In addition to the transcutaneous oesophageal and abdominal ultrasonography, all patients had additional imaging and diagnostic tests performed as part of their clinical investigation. Five of the dogs had endoscopy of the upper gastrointestinal tract with biopsies taken from the oesophagus, stomach and duodenum. The histological results of the oesophageal samples were normal (three dogs), inconclusive (one dog) or contained insufficient tissue for evaluation (one dog).

Four oesophageal wall layers were consistently visible on ultrasound examination in seven dogs and intermittently four or six layers in the remaining three dogs (**Figure 6A**). The layers observed were the same as those described in parts A and B of this study. The mucosal layer appeared echogenic in all seven dogs with four distinct layers visible and hypoechoic in all three dogs where a variable four to six wall layer pattern was present. No obvious oesophageal wall changes were identified in any of the eleven dogs. Wall thickness measurements are shown in **Table 3** and **Table 4**.

6.3.1 Statistics

Four or six wall layers were identified in 93% of the dogs in this study. There was no significant difference between the mean transverse and longitudinal wall thicknesses of the dogs in Part B and part C of the study ($P<0.46$) and therefore the measurements from these two groups were combined to increase the number of dogs included in this calculation.

There was a significant correlation between body weight and the mean oesophageal wall thickness as determined using ultrasound (transverse images $P<0.017$ and longitudinal images $P<0.02$). The regression analysis coefficient indicated that for every additional 1kg in the body weight of the dog, it is expected the oesophageal wall thickness will increase by 0.20 mm. There was no significant correlation between the mean oesophageal wall thickness and the other variables, age, sex and breed ($P>0.05$).

6.4 Discussion

This study demonstrated that conventional transcutaneous ultrasound can be used to evaluate the entire cervical oesophagus in conscious dogs using a left lateral approach. To the authors' knowledge this is the first study correlating the ultrasonographic and histological characteristics of the cervical oesophagus in normal dogs, determining parameters for cervical oesophageal wall thickness in relation to body weight in normal dogs and prospectively using transcutaneous ultrasound to investigate dogs with clinical signs associated with the oesophagus.

The results of this study indicate that two, four or six layers are visible sonographically in the canine cervical oesophagus. Histologically and sonographically, the canine oesophageal wall is composed of four layers, the mucosa, the submucosa, the muscularis and the adventitia (Evans and de Lahunta, 2013). Typically, five interfaces are visible ultrasonographically due to the additional innermost layer produced by mucosal-lumen interface (Neelis et al., 2015; Zwingenberger and Taeymans, 2015). Although, four ultrasonographic layers are usually also visible in the human cervical oesophagus, six layers have been reported due to the presence of connective tissue between the inner circular and outer longitudinal muscular layers producing an extra hyperechoic layer on the images (Shang-Yong et al., 2004). It would seem that the thin extra hyperechoic layer seen on ultrasound and demonstrated using Masson's trichrome stain in the present study is similar to this layer described in humans. This has not been previously reported in dogs, so this is the first time this extra wall layer has been described on ultrasonography in the veterinary literature. Masson´s trichrome stain produces better visualisation of collagen fibres which would explain why this layer was best appreciated using this technique.

Wall layer variation between on ultrasound and water bath could be explained by associated with no of acoustic interface being present between the mucosal surfaces because of the collapse and folding of the oesophageal lumen (Zhu, et al., 2004).

It is generally accepted that a minimum of 7.5MHz is required to consistently distinguish the four wall layers in the canine gastrointestinal tract (Nyland et al, 2015; Penninck and d'Anjou, 2015). Four wall layers were identified in the abdominal oesophagus in 89% of dogs using a 9.5 MHz transducer but six layers were not reported in any (Gory, et al., 2014). A human study demonstrated that stepping up the frequency to 12MHz increased the likelihood of six oesophageal wall layers being identified ultrasonographically (Shang-Yong et al., 2004). The use of a 14 MHz transducer in the present study is therefore likely to have resulted in the increased number of wall layers that were visible. Despite this, only two wall layers were visible ultrasonographically in two dogs. One was an obese 14.5 kg Corgi and the other a very small 1.8 kg Chihuahua. Large amounts of fat have been shown to reduce image quality (Mattoon and Nyland, 2015; d'Anjou and Penninck, 2015) and it was speculated that the miniature size of the Chihuahua may have resulted in oesophageal wall layers that were just too thin for the machine to resolve, despite the use of a 14MHz transducer. In both cases, this resulted in an inability to distinguish between the mucosa, submucosa and muscularis so only two layers were discernible. This demonstrates the effect patient factors can have on image quality. Furthermore, this factor could also be the reason for the constant perceptibility of the sixth-wall layer on the water compared to ultrasonography.

The ultrasonographic appearance of the normal gastrointestinal mucosa is generally considered to be uniformly hypoechoic (Nyland et al, 2015; Penninck and d'Anjou, 2015). However, in the present study it varied between hypoechoic and echogenic, which corresponded with the findings of Gory et al., (2014) who also reported an echogenic appearance to the mucosa in the canine abdominal oesophagus and suggested this was a result of the squamous nature of the mucosa. An echogenic appearance to the normal canine small intestinal mucosa has been reported due to the speculated accumulation of fluid, gas and small particles between the villi while mucosal speckles have been found in cases with intestinal inflammatory disease due to the possible focal accumulation of substances in the mucosal crypts including mucus, cellular debris, protein, mineralized or fibrous tissue or gas (Le Roux et al, 2016). Anatomically, a collapsed oesophagus has large and numerous longitudinal folds (Evans and de Lahunta, 2013), therefore the echogenicity of the oesophageal mucosa could also presumably be due to gas or small particles becoming trapped in these folds. Since no abnormalities were detected on histology in the present study that would explain an increased echogenicity of the mucosa, whatever the underlying cause,

this appearance can be considered a normal finding in the canine cervical oesophagus.

The canine cervical oesophagus wall thickness has been reported to measure approximately 4 mm (Evans and de Lahunta, 2013). In an oesophageal endoscopic ultrasonography study in healthy dogs, the thickness of the proximal third was reported to be approximately 2.19 mm (Baloi et al., 2013). In human studies, the normal cervical oesophagus was approximately 2.3 mm (Shang-Yong et al., 2004) and depending on the neck position and side of scanning, the thickness varied between 2.6 mm and 2.9 mm (Mateen et al, 2006). In the present study, the mean ultrasonographic wall thickness showed similar measurements to these studies but was lower than the values in a standard anatomical textbook (Evans and de Lahunta, 2013). The wall thickness in the cadavers and in the water bath samples corresponded well with each other but the values in the live dogs in parts B and C were lower. The increased oesophageal muscle tone in the live dogs in parts B and C could explain this difference in wall thickness. The lack of post mortem or changes in the histological sections could not confirm this difference. The histological measurements were higher than the corresponding ultrasonographic ones despite not including the adventitia. Similar findings were noticed in another study and are likely due to the technical and chemical procedures used for histologic sample preparation which can cause an artefactual loosening and clefting of the tissue (Baloi et al., 2013). There was a significant correlation between the mean ultrasound wall thickness measurements from the live dogs and their body weight, suggesting that larger dogs could have thicker oesophageal walls. Similar findings were seen in a study on the canine abdominal canine oesophagus (Gory, et al., 2014). Though, a large number of dogs with diverse body weights is required to support this finding and greater statistical power.

The histological features described in the cadaver samples in this study are typical of the range of finding that can be considered normal in the canine oesophagus (Goetsh, 1910; Long and Orlando, 1999; Kuo and Urma, 2006). However, the cadaver with multifocal eosinophilic infiltrates in the lamina propria layer of the mucosa was considered abnormal. Eosinophilic oesophagitis is commonly described in gastro-oesophageal reflux in humans and in patients with food or aeroallergen hypersensitivity (DeNardi and Riddell, 1991; Raheem et al, 2014) but is rare in the veterinary literature. It was described in one dog with dysphagia, regurgitation and coughing that also had oesophageal ulceration and granulation tissue formation (Mazzei et al, 2009). The cadaver in the present study had no such concurrent changes but whether the changes observed represents an early stage of the disease is uncertain as, unfortunately, no clinical data was available. However, despite the presence

of these changes, they did not result in any changes to the ultrasound image and therefore were not detected. These changes were felt to be similar to those described in inflammatory disease of the gastrointestinal tract, where the wall layering appeared normal on ultrasound in the presence of inflammation (Larson & Biller, 2009). This is presumably due to the resulting changes being too subtle to be identified using currently available ultrasound equipment.

An increase in the upper and lower oesophageal diameters and wall thickness of the cervical oesophagus has been documented in people with gastro-oesophageal reflux (Palabiyik et al., 2012). However, none of the dogs in the present study with clinical signs demonstrated an increase in wall thickness or changes in the ultrasonographic appearance of the oesophageal wall that were discernible even using a frequency as high as 14MHz. These findings therefore confirm our hypothesis that the use of ultrasound in clinically affected dogs would be limited to those with gross wall changes, with subtle histological changes not being identifiable. However, further work is still indicated in a larger number of cases with confirmed oesophageal disease and corresponding biopsy samples to determine the extent to which ultrasound could be used to investigate and manage these cases.

Transcutaneous ultrasonography of the cervical oesophagus is not routinely used in veterinary medicine. The abdominal and thoracic oesophagus are the common sites affected by oesophagitis secondary to gastro-oesophageal reflux in animals and in people (Gory et al., 2014; Marks, 2017), which might not be visualised in the cervical oesophagus using this technique. Inability to visualise the entire circumference of the oesophageal wall may be encountered with this technique, due to the presence of intra-luminal air.

There are several limitations in the study. A larger sample size would be necessary to increase the power of the study and correlate the body weight and/or breed specific factors. No signalment or clinical history was available for the cadavers and there was no clinical history or data to corroborate the eosinophilic infiltration in the affected cadaver.

Limitations of transcutaneous ultrasound for oesophageal examination include the inability to examine the thoracic oesophagus, which is most commonly affected by lesions (Sellon and Willard, 2003; Gory et al., 2014). Also, it was not possible to visualise the entire circumference of the oesophageal wall in some images due to the presence of intra-luminal air.

Unfortunately, none of the dogs in Part C with clinical signs relating to the oesophagus demonstrated identifiable pathological changes. Only five patients in part C had

oesophageal biopsies and none were supportive of oesophagitis. Finally, there was no standardization between part B and C with regards to sedation.

This study indicates that transcutaneous ultrasonography can be used to assess the canine cervical oesophagus using a left lateral approach. This allowed visualization of the wall layers, as confirmed by correlation with histological samples, with four or six layers being visible in 93% of the dogs using a 14 MHz transducer. The additional connective tissue layer within the muscularis which is responsible for the six-layer appearance has been previously reported in humans, but this is the first report in dogs. However, inherent patient factors could affect image quality, reducing the visible wall layers to two. The canine oesophageal mucosa often appears echogenic and this appears to be a normal finding. There were no changes identified on ultrasound in any of the dogs in this study despite the presence of histological changes or clinical signs associated with the oesophagus therefore further work is required in clinical cases to determine its use in such cases.

Chapter 3 – Occurrence of oesophageal abnormalities in brachycephalic breeds

1 Introduction

Oesophageal abnormalities in the dog have been widely documented in the literature (Elwood, 2006; Marks, 2017). Many anatomical malformations of the oesophagus have been recognised in brachycephalic dogs including hiatal hernias and oesophageal redundancy (Poncet, et al., 2005; Unzueta, et al., 2011). Hiatal hernias can be classified as either congenital or acquired. The congenital form has been described in the Chinese shar-pei, French bulldog and Chow chow (Poncet, et al., 2005; Reeve, et al., 2017). Developmental abnormalities of the oesophageal hiatus or of the phrenicoabdominal ligament are associated with the congenital form of the hiatal hernias (Reeve, et al., 2017). Acquired hiatal hernias have been documented secondary to diaphragmatic repair, trauma, oesophageal or upper respiratory tract disease or neuromuscular disorder (Reeve, et al., 2017). Four types of hiatal hernia have been described in the literature (Sivacolundhu, et al., 2002). Oesophageal redundancy is commonly recognized in young dogs or short-necked brachycephalic breeds, such as English and French bulldogs and Chinese shar-peis (Poncet, et al., 2005; Unzueta, et al., 2011; Gaschen, 2018). Oesophageal redundancy is often an incidental finding but has been previously documented in association with clinically significant motility disorders (Gaschen, 2018). Oesophageal deviation has also been previously described in English bulldogs with both gastro-intestinal and digestive problems (Poncet, et al., 2005). Congenital megaoesophagus has been well recognised in the Chinese shar-pei, but also in non-brachycephalic breeds, such as the Irish Setter, Great Dane, German Shepherd, Labrador Retriever, Newfoundland, miniature Schnauzer and Fox terrier. The prevalence of oesophageal diseases in brachycephalic breeds is yet to be documented.

Brachycephalic obstructive airway syndrome (BOAS) has been extensively described in dogs with shortened skulls and muzzles (Poncet, et al., 2006; Liu, et al., 2017). A previous

publication on upper respiratory syndrome in brachycephalic dogs, showed a correlation between respiratory and gastrointestinal disorders (Poncet, et al., 2005). These authors suggested that upper respiratory tract diseases had an influence on gastro-oesophageal diseases and vice versa (Poncet, et al., 2005). Dogs with an increased respiratory effort develop a high negative intra-thoracic pressure, which can trigger gastrointestinal signs such as regurgitation, vomiting (Poncet, et al., 2005; Liu, et al., 2017) leading to an increase positive abdominal pressure (Poncet, et al., 2005; Liu, et al., 2017; Reeve, et al., 2017). Weakness of the diaphragm, elevated abdominal pressure and upper airway obstruction are predisposing factors for acquired hiatal hernias (Gaschen, 2018).

Gastro-oesophageal reflux has been previously associated with BOAS, hiatal herniation and megaoesophagus (Lecoindre & Richard, 2004; Poncet, et al., 2005; Muenster, et al., 2017; Reeve, et al., 2017). According to Bright et al (1990), hiatal herniation is alleged to cause lower oesophageal incompetence and subsequent gastro-oesophageal reflux.

Oesophagitis is a common sequel of an underlying disease or secondary consequence of oesophageal disease which may cause oesophageal hypomotility and, in severe cases, oesophageal strictures (Jergens, 2010; Marks, 2017; Reeve, et al., 2017). Several factors can contribute to the development of reflux oesophagitis, such as incompetence of the lower oesophageal sphincter (LES), inadequacy of secondary peristalsis to clear reflux from the distal oesophagus and content of the refluxed material (Bright, et al., 1990).

2 Aims of the study

To the authors knowledge, this is the first study documenting the occurrence of oesophageal abnormalities in brachycephalic breeds. The aim of this study was to document the prevalence of oesophageal abnormalities in brachycephalic breeds with or without BOAS. Also, to determine the incidence of oesophageal redundancy in brachycephalic dogs with or without associated oesophageal abnormalities.

3 Material and methods

This was a retrospective cross-sectional study design. The medical records of brachycephalic dog breeds presented to the School of Veterinary Medicine, Small Animal Hospital of

Glasgow University, from November 2009 to December 2016 were reviewed. Approval was obtained from the University of Glasgow, College of MVLS ethical committee (Ref 10a/16).

Inclusion criteria were brachycephalic dogs with imaging diagnosis of any oesophageal abnormalities or oesophageal redundancy by at least one of the following diagnostic imaging methods: radiography, CT or fluoroscopic barium meal. The signalment, clinical signs, age, sex and weight of these dogs were recorded in addition to the oesophageal abnormality that was present. All images were reviewed by a Diagnostic Imaging resident (BJG.) using OsiriX MD DICOM-viewer (Pixmeo, Bernex, Switzerland). The studies were reviewed without knowledge of the final clinical diagnosis.

A single radiographic projection (right lateral) or two projections (right lateral and either left lateral or dorsoventral) of the thorax were acquired using a Siemens Multix Top Digital XR (Siemens, Muenchen, Germany). There was no standardised protocol for sedation. Sedation was used in patients without suspicion of megaoesophagus or if the patient was not compliant for the positioning and acquisition of the radiographs.

Computed Tomography (CT) of the thorax was performed with the dogs under general anaesthesia and positioned in sternal recumbency, using a dual slice CT scanner (Somatom Spirit, Siemens AG, Arlange, Germany) with a 512x512 matrix, 130 Kvp, 30 mA, pitch 1.4, 3 mm slice thickness and a medium and high reconstruction algorithm. Contrast was administered in all dogs undergoing CT by an intravenous infusion of iodinated non-ionic contrast medium (Ioversol, Optiray®, 300 mg iodine/ml, Guerbet, France).

Fluoroscopy was performed in conscious dogs using a C-Arm (BV Libra, Philips, Netherland). The barium meal study was performed with all the dogs in a standing position with a raised food bowl. The food used depended on the dog's appetite or food preference but was at room temperature and coated with 20 ml of liquid barium sulphate (Barium sulphate, 100% w/v, Baritop®, Sanochemia, Bristol, United Kingdom).

The total number of brachycephalic breed seen in hospital during November 2009 to December 2016 were documented.

4 Statistical analysis

Statistical analyses were performed by a Diplomate in Veterinary Epidemiology (TP) using statistics software (Stata® SE 12.1, StataCorp LLC, Texas). Logistic regression analysis was calculated between the body weight, age and breed for each oesophageal disease. Statistical significance was set at $P<0.05$.

5 Results

A total of 51 dogs met the inclusion criteria. The breeds included were French Bulldog (12), English Bulldog (12), Boxer (6), Bullmastiff (1), Chihuahua (1), Lhasa Apso (4), Pug (3), Cavalier King Charles Spaniel (CKCS) (7), Shih-Tzu (2), Boston terrier (1), Dogue de Bordeaux (1) and Chinese shar-pei (1). There were 28 males (12 male entire and 16 male neutered) and 23 females (11 female entire and 12 females neutered). The mean body weight was 15.6 kg (range 430 gr – 45 kg) and mean age 3 years (range 5 weeks – 11.5 years). Clinical signs were regurgitation (15), vomiting (12), retching (6), lethargy (6), cough (4), tachypnoea (3), exercise intolerance (3), stertor (3), difficulty breathing (2), gagging (3), dyspnoea, pyrexia, anorexia, weakness. More than one clinical sign was present in 18 of the dogs.

Oesophageal abnormalities observed were megaoesophagus (16) **(Figure 7)**, oesophageal dysmotility (16), oesophageal hiatal hernia (15), gastro-oesophageal reflux (GOR) (13), foreign body (4), transient gastro-oesophageal intussusception (1) and neoplasia (1) **(Table 5 and Appendix 1)**. Some of the oesophageal abnormalities were detected incidentally (6/51), in dogs presenting for further investigation of BOAS, lymphoma, hepatic mass, tetraparesis, hypothyroidism, hind limb ataxia and pyometra.

Figure 7. *A and B, Right lateral radiographic images of megaoesophagus in 2 different dogs. Note the presence of fluid lines within the oesophagus and stomach on image B (horizontal beam projection); Dilated oesophagus with air (*). C and D, Fluoroscopic images of fluid/food line from the same dog in image B (white arrows). Stomach (S). Fluid/gas interface in the thoracic oesophagus and stomach demonstrating the fluid lines (white arrows). On image D, the fluid line consists of an interface of liquid barium meal with gas. Cranial is to the left of all the images.*

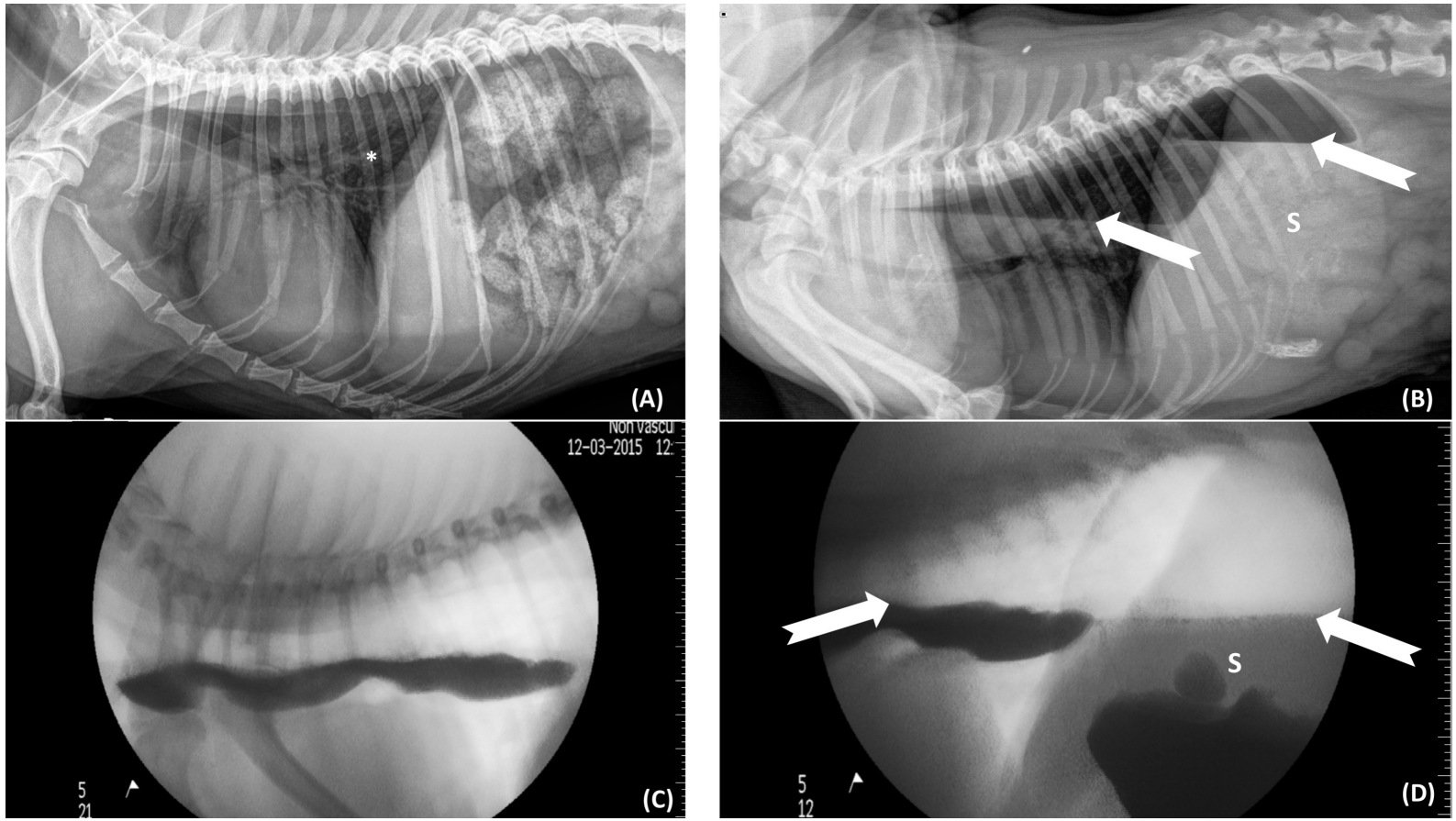
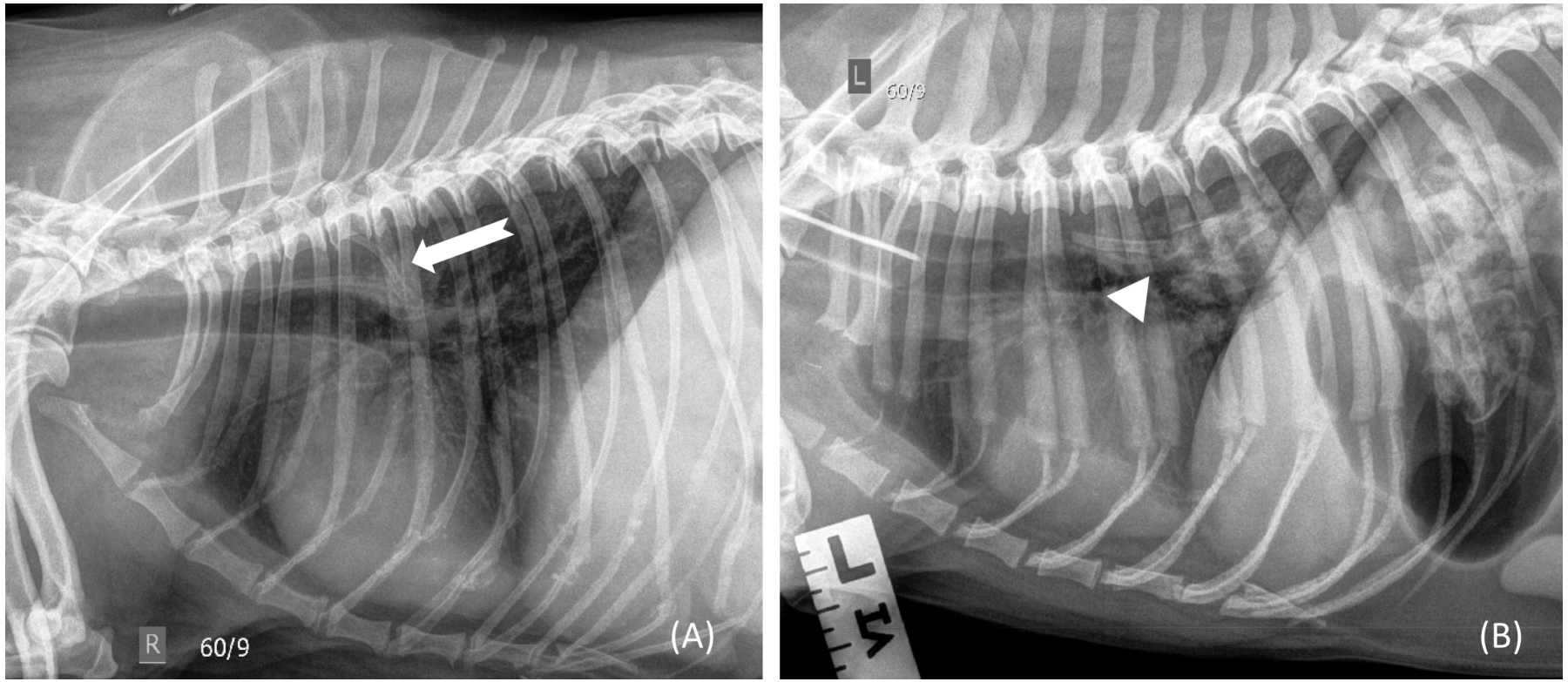


Table 5. *Number of oesophageal abnormalities identified in different brachycephalic breeds of dog. Twenty dogs had more than one abnormality diagnosed. GOR = Gastro-oesophageal reflux; FB = Foreign body; incidental * = total number of dogs that were not reported as demonstrating clinical signs associated with the oesophagus.*

Breed	Megaoesophagus	Hiatal Hernia	Dysmotility	GOR	FB	Gastro-oesophageal Intussusception	Neoplasia	Oesophageal redundancy	Total
	Number affected								
French Bulldog	1	6	2	5	0	0	0	5	19
English Bulldog	1	4	7	6	0	0	0	4	22
Boxer	3	1	2	0	0	0	1	0	7
Bullmastiff	1	0	0	0	0	0	0	0	1
Chihuahua	1	0	0	0	0	0	0	0	1
Lhasa Apso	2	0	1	0	1	0	0	0	4
Pug	1	2	2	1	0	0	0	1	7
CKCS	4	0	1	0	2	1	0	1	9
Shih-Tzu	1	0	0	0	1	0	0	0	2
Boston Terrier	0	1	0	0	0	0	0	0	1
Dogue de Bordeaux	0	0	1	1	0	0	0	0	2
Chinese shar-pei	1	1	0	0	0	0	0	0	2
Total	**16**	**15**	**16**	**13**	**4**	**1**	**1**	**11**	
Incidental *	4	5	4	4	0	1	0	5	

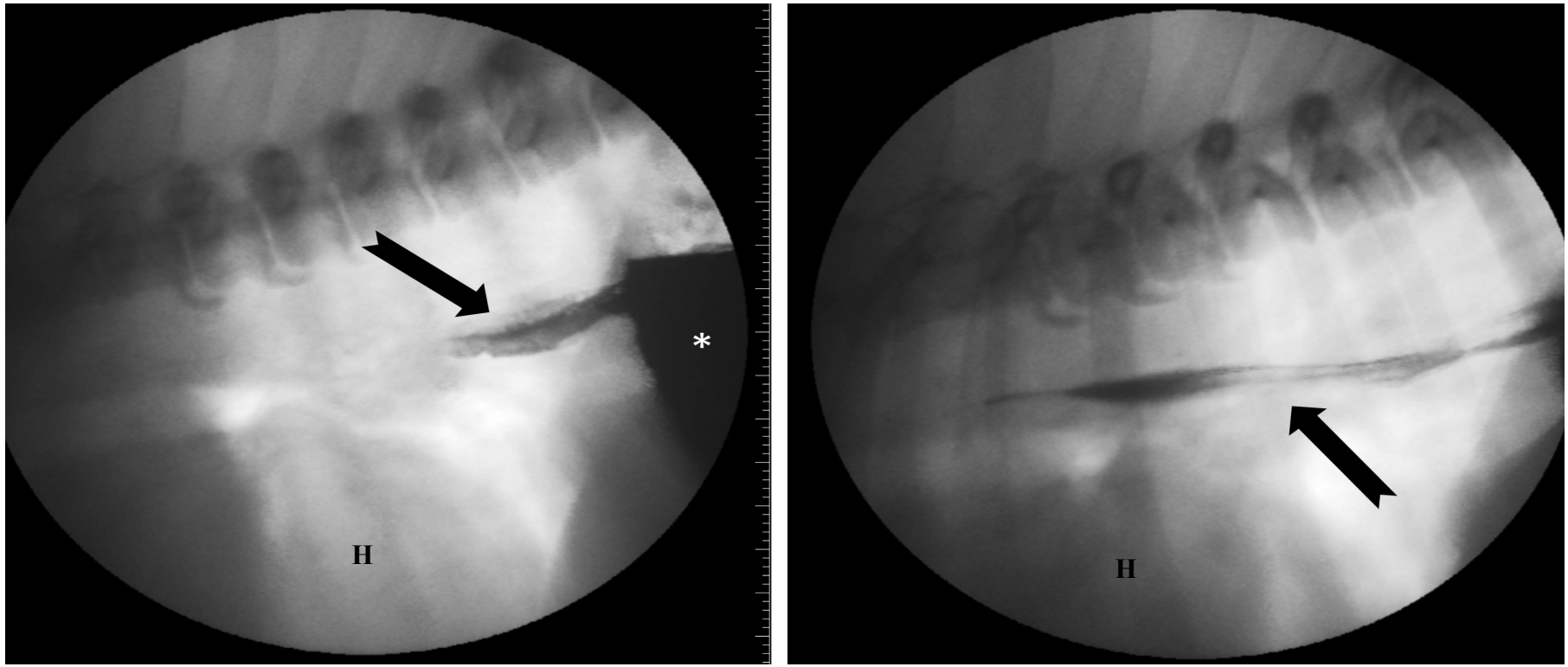
The diagnostic imaging methods selected and used for the diagnosis of the oesophageal abnormalities were based on the dog's clinical signs and presentation. In this study, radiography was the only modality used to diagnose a foreign body **(Figure 8).**

Figure 8. *A, Right lateral radiographic projection of the thorax with a mineralised tubular foreign body lodged in the mid thoracic oesophagus at the level of the base of the heart (white arrow); B, Left lateral projection of the thorax of a different dog with multiple tubular to irregular mineralised foreign bodies within the caudal aspect of the thoracic oesophagus (white arrow head) and also within the stomach. Cranial is to the left of both images.*



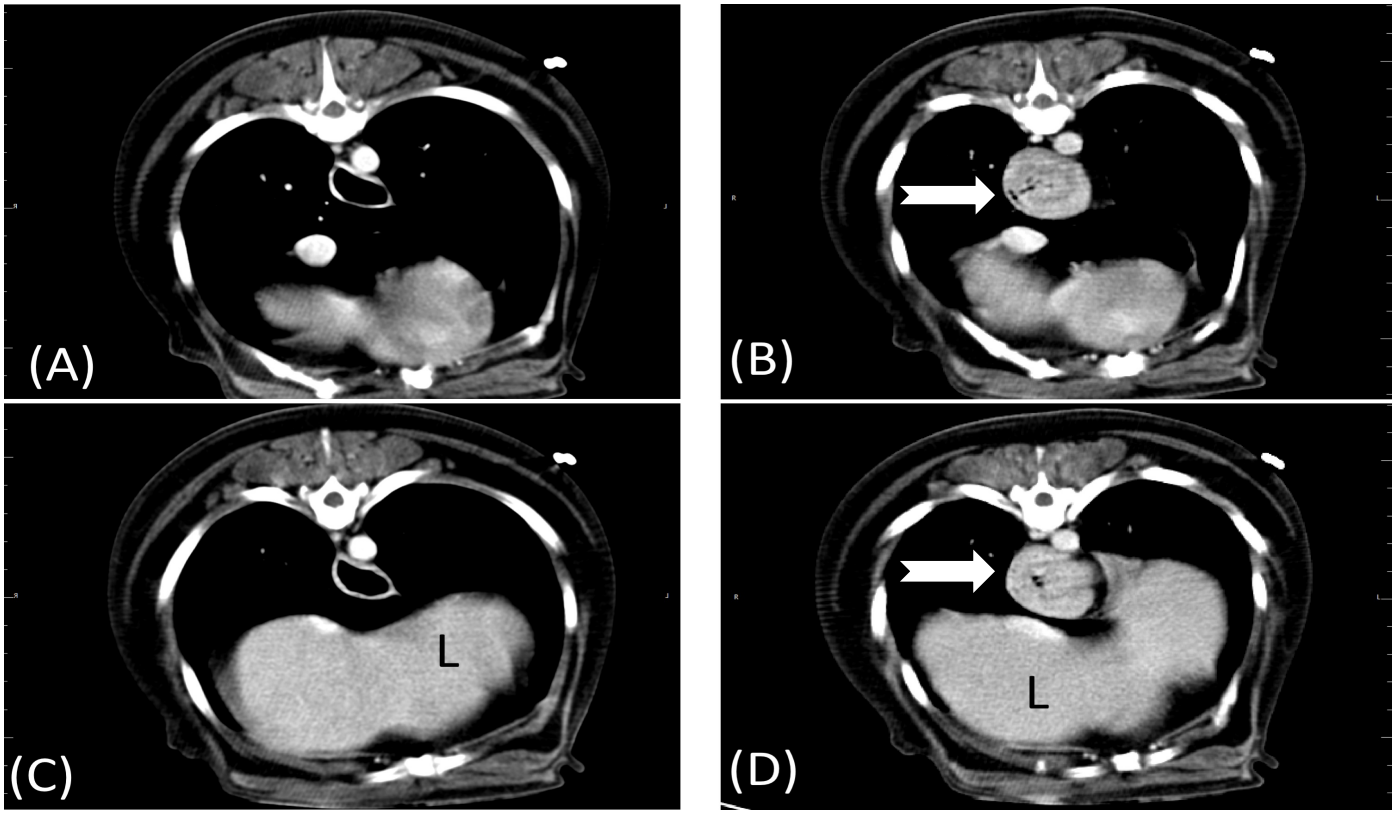
Fluoroscopy was used to diagnose GOR or dysmotility **(Figure 9)**.

Figure 9*. Fluoroscopy images (two images of the same dog) showing refluxed barium meal (liquid) within the caudal thoracic oesophagus (black arrows). Stomach filled with barium meal (*). H – cardiac silhouette. Cranial is to the left of both images.*



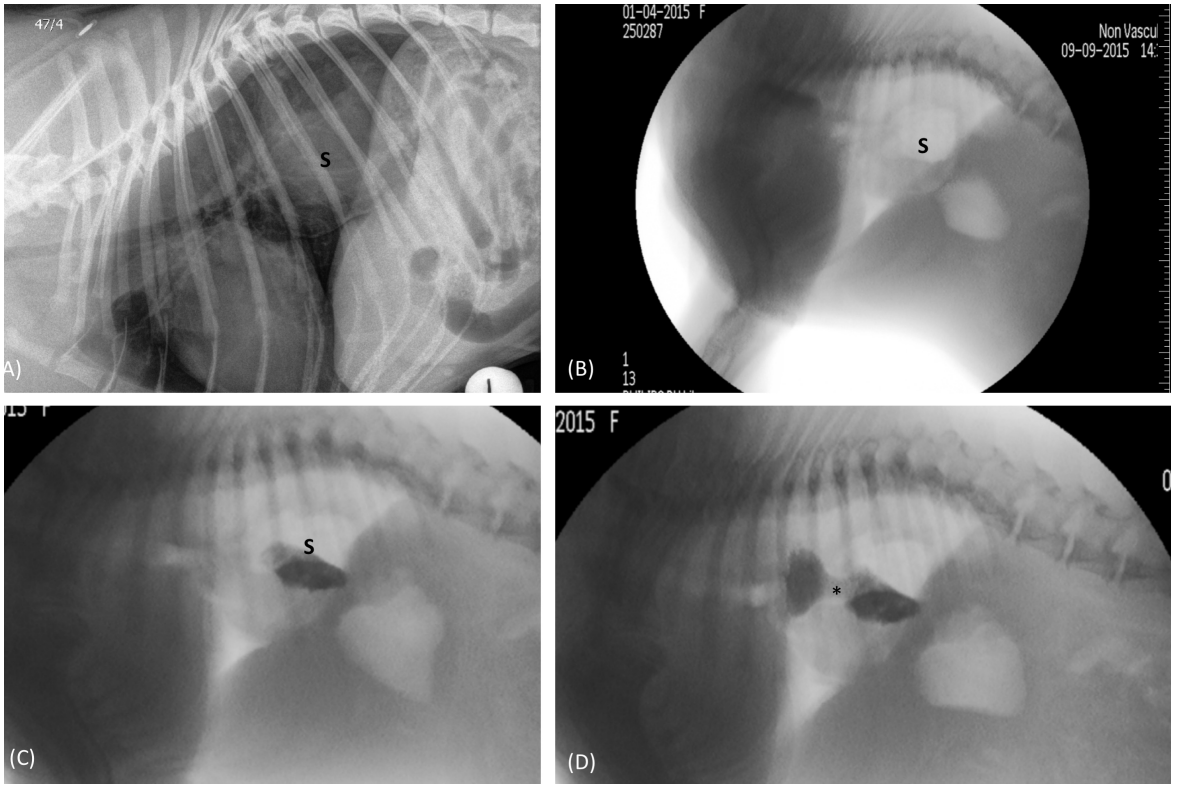
CT was only used in two dogs, one with oesophageal neoplasia and another dog with transient gastro-oesophageal intussusception **(Figure 10).**

Figure 10*. Transverse CT images (all of the same dog) demonstrating transient gastro-oesophageal intussusception. Images acquired 30 seconds (A and B) and 120 seconds after intravenous contrast medium administrated (B and D). Dilated oesophagus with air (*). L – Liver. Gastro-oesophageal intussusception (white arrows)*



Radiography and fluoroscopy were used intermittently together or individually to diagnose megaoesophagus, oesophageal redundancy and hiatal hernia **(Figure 11)**.

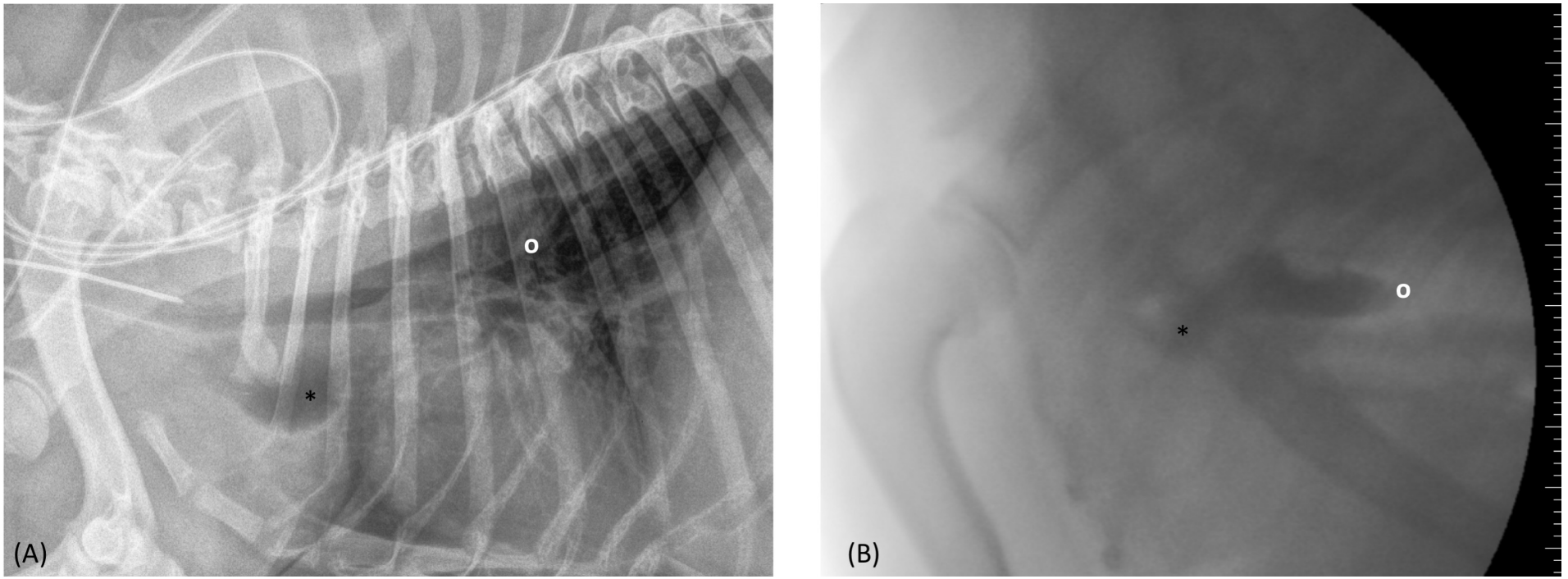
Figure 11. *A, Radiograph (left lateral projection) of a dog with a type IV hiatal hernia. Fluoroscopy (B, C and D) images of the same dog with sequential movement of the barium meal. Part of the stomach (fundus and body) (S) is herniated alongside the oesophagus and is seen cranial to the diaphragm. Lower oesophageal sphincter displaced cranially (*). Cranial is to the left of all the images.*



Combinations of oesophageal diseases were present in some dogs; GOR and oesophageal dysmotility (5/51), oesophageal dysmotility and hiatal hernia (4/51), hiatal hernia and GOR (3/51) all 3 (hiatal hernia, GOR and oesophageal dysmotility) (1/51) and hiatal hernia and megaoesophagus (1/51). Oesophageal redundancy was detected in 11 dogs **(Figure 12)**.

Three of these had no other oesophageal abnormalities but 8 also had gastroesophageal reflux, hiatal herniation or oesophageal dysmotility.

Figure 12. *Right Lateral Radiographic (A) and fluoroscopic (B) images of the same dog demonstrating an oesophageal redundancy at the level of the thoracic inlet. Redundant oesophagus (*). Mildly dilated thoracic oesophagus with air (O) on image A and with air and barium meal (O) on image B. Cranial is to the left of all the images.*



There were 22 dogs diagnosed with BOAS; 9 French bulldogs, 10 English bulldogs, 2 Pugs and 1 Boston terrier. There was no grading available for the BOAS. There was no significant correlation between the presence or absence of BOAS in dogs with oesophageal abnormalities. In addition to BOAS these dogs also had gastro-oesophageal reflux, hiatal herniation, oesophageal dysmotility, megaoesophagus and oesophageal redundancy **(Table 6)**.

Table 6. *Brachycephalic breeds of dog with BOAS and concurrent oesophageal disease.*

Breed	Megaoesophagus	Hiatal hernia	Dysmotility	Gastro-oesophageal Reflux	Oesophageal redundancy
Pug		1		1	1
Pug		1	1		
French Bulldog				1	
French Bulldog			1	1	
French Bulldog		1			
French Bulldog		1			
French Bulldog			1	1	1
French Bulldog		1			1
French Bulldog					1
French Bulldog				1	1
French Bulldog		1			1
English Bulldog	1		1		
English Bulldog		1	1	1	

English Bulldog			1	1	
English Bulldog		1	1		1
English Bulldog			1	1	
English Bulldog			1	1	
English Bulldog				1	1
English Bulldog					1
English Bulldog		1		1	
English Bulldog					1
Boston Terrier		1			
Total	1	10	9	11	10

English bulldog and French bulldog were the breeds with the highest occurrence of oesophageal abnormalities in this study, with GOR, dysmotility and hiatal herniation.

A minor incidence of oesophageal redundancy was identified in the French bulldog and English bulldog. Considering the total number of brachycephalic breeds seen during this period (6664 between April 2009 and December 2016), these findings were considered not to be clinically significant.

There was no significant correlation between breed, weight, sex and clinical signs or oesophageal abnormalities present. Regression analysis showed that dysmotility (odds ratio 0.75, P value 0.04, 95% Cl, 0.58-0.98), gastro-oesophageal reflux (odds ratio 0.68, P value 0.04, 95% Cl, 0.48-0.98), and BOAS (odds ratio 0.69, P value 0.006, 95% CI, 0.54-0.90) were more likely to occur in younger dogs.

6 Discussion

This study showed megaoesophagus and oesophageal dysmotility to be the most common oesophageal abnormality amongst the brachycephalic population, followed by hiatal hernia, and GOR. Most prevalent oesophageal abnormalities were oesophageal dysmotility and GOR in English bulldogs and hiatal herniation in French bulldogs. In agreement to previous studies, our study showed that gastro-oesophageal intussusception and oesophageal neoplasia occur uncommonly (Ranen, et al., 2008; Murphy, et al., 2015; Gaschen, 2018). Megaoesophagus was observed in most of the brachycephalic breeds represented in the present study, particularly in CKCS (4/51). This an unexpected finding with only a few documented in the literature (Summer, et al., 2015). All dogs in this study with megaoesophagus were diagnosed based on radiography but only 3 dogs had oesophageal dysmotility confirmed using fluoroscopy. Therefore, these dogs were not included in the dysmotility classification of this study. Megaoesophagus is the most common cause of regurgitation in dogs (Bexfield, et al., 2006), though no GOR was noted in this study in dogs with megaoesophagus, presumably due to the transient nature of the regurgitation and the diagnostic technique used.

BOAS is well described in the literature. A short and broad head, stenotic nares, narrowed and winding nasal cavities, elongated and thickened soft palate, everted laryngeal saccules and hypoplastic trachea are anatomical abnormalities described in brachycephalic breeds that aggravate the obstructive respiratory syndrome (Lecoindre & Richard, 2004). Female Pugs and male French bulldogs have a higher risk of developing BOAS (Liu, et al., 2017). The common brachycephalic breeds described in the literature that are predisposed to developing BOAS (Meola, 2013) are similar to those represented in this current study population. Increasing body weight has been reported as a risk factor for BOAS due to its impact on respiratory function (Liu, et al., 2017). A recent study reported 15% of underweight French bulldogs to have frequent regurgitation (Liu, et al., 2017). However, no statistical correlation was encountered in this study between breed, their weight and incidence of oesophageal abnormalities with or without BOAS.

The relationship between the presence of an obstructive pathology of the respiratory tract and a hiatal hernia or GOR has already been suggested in the literature (Lecoindre & Richard, 2004). Hiatal hernia is common in brachycephalic dogs and has been reported previously to be more prevalent in French bulldogs with BOAS (Reeve, et al., 2017). A similar breed prevalence was noted in this study. A significant relationship has been

documented between the severity of the respiratory and digestive signs (Poncet, et al., 2005; Reeve, et al., 2017). The presence of oesophageal redundancy and the increase in thoracic negative pressure during respiratory distress could trigger gastrointestinal signs (Lecoindre & Richard, 2004; Liu, et al., 2017; Reeve, et al., 2017). Dogs can develop regurgitation and/or vomiting secondary to GOR and temporary hiatal hernia (Burnie, et al., 1989; Lecoindre & Richard, 2004; Liu, et al., 2017). A study revealed that 81% of 51 dogs showed an improvement in digestive clinical signs after upper respiratory surgery and gastrointestinal management (Poncet, et al., 2006; Reeve, et al., 2017). Additionally, a previous study reported the development of GOR secondary to an obstructive nasal tumour (Lecoindre & Richard, 2004). Another study documented two cases of hiatal hernias associated with laryngeal paralysis (Burnie, et al., 1989; Lecoindre & Richard, 2004). Similar findings were appreciated in this study, where most of dogs in this study with BOAS presented either with hiatal hernia, GOR, dysmotility and/or oesophageal redundancy.

In this study oesophageal dysmotility was observed predominantly in English bulldogs (6/51) and associated with either hiatal hernia and/or GOR. According to Bexfield et al. (2006) oesophageal motility abnormalities are suspected to be similar to those of megaoesophagus, where the oesophageal motility is disrupted but the dilation is yet to occur. This hypothesis could not be verified in our study, since none of the dogs had a follow up recorded. Another probable mechanism suggested by Bexfield et al. (2006) for oesophageal dysmotility was a delayed maturation of the oesophageal function (Bexfield, et al., 2006; Reeve, et al., 2017). This is a common cause in human infants under a year old with swallowing dysfunction and gastrointestinal symptoms (Bexfield, et al., 2006). In the present study, 7/13 of the dogs with oesophageal dysmotility alone or with GOR were over 1 year old and 6/13 were under a year old. Therefore, delayed maturation should still be considered as a potential cause for reduced motility in this study. Similar to a previous study by Reeve et al. (2017), 4/13 dogs in this current study had a delayed oesophageal transit time and hiatal herniation, and 1 dog also had concurrent GOR. Hiatal herniation and its association with GOR has been widely recognized in the human and veterinary literature (Poncet, et al., 2005; Conrado, et al., 2011). The association between oesophageal dysmotility and hiatal herniation has also been suggested in humans. Four dogs (4/13) with oesophageal dysmotility also had hiatal herniation in this study. A human study suggested that hiatal hernias influence the lower oesophageal sphincter and decrease the amplitude of peristaltic waves in the distal oesophagus (Conrado, et al., 2011). In a study by Conrado et al. (2001),

humans with hiatal hernias had a significantly higher prevalence of dysmotility (14.8%) compared to the group without (7.7%).

Additionally, in humans there is a possible relationship between altered oesophageal motor function and GOR (Conrado, et al., 2011). There were 5/51 dogs in the present study with oesophageal dysmotility in association with GOR. Furthermore, GOR can indirectly lead to oesophageal dysmotility as a consequence of the presence of oesophagitis in both human and dogs (Bexfield, et al., 2006; Elwood, 2006).

Oesophageal redundancy is classified as an incidental deviation of the oesophagus at the thoracic inlet (Gaschen, 2018) and was only identified in 11/51dogs in the present study. The number of dogs diagnosed with oesophageal redundancy could have been underestimated in this study, because of the method used to select the cases. Eight of the 11 dogs were diagnosed with a concomitant oesophageal disease, such as GOR, hiatal hernia or oesophageal hypomotility. Oesophageal redundancy has been previous documented as an incidental finding or alongside motility and/or gastrointestinal disorders (Poncet, et al., 2005; Unzueta, et al., 2011; Gaschen, 2018). However, no statistical significance was encountered in this study to support a correlation between oesophageal disease and oesophageal redundancy. French and English bulldogs were over-represented in this study which was suspected to be due to breed popularity in the referral area. The most common breeds with oesophageal redundancy were French Bulldogs (5/11) and English bulldogs (4/11) in this study. However, it was also recognized in a Pug and CKCS. Oesophageal redundancy has been only described in English bulldogs, French bulldogs and Chinese shar-pei in the literature (Poncet, et al., 2005; Unzueta, et al., 2011; Gaschen, 2018).

One limitation of this study was the retrospective nature of the study, due to possible limitation of the data used in the search system. The low number of cases that met the inclusion criteria despite the large number of brachycephalic breeds seen during the period of the data collection. This was thought to be associated with the hospital being a referral centre and the dogs seen during this period of time being referred for conditions other than oesophageal disorders.

In conclusion, megaoesophagus and oesophageal dysmotility were the most common oesophageal abnormalities in this brachycephalic population. Hiatal hernia, oesophageal dysmotility and GOR were the most prevalent oesophageal diseases in the breed with BOAS and megaoesophagus, dysmotility and hiatal herniation in dogs without BOAS. The presence

of these conditions individually or concurrently is of clinical significance, considering these dogs present a higher risk of developing aspiration pneumonia when under general anaesthesia (Poncet, et al., 2006; Wagner, 2008; Marks, 2017; Gaschen, 2018). Oesophageal redundancy incidence was minimal and likely underestimated in this study. Further studies are warranted with a larger study sample to corroborate these findings with statistical significance.

General Discussion

Disease of the oesophagus can be challenging to diagnose. Dogs with oesophageal disease present commonly with regurgitation and/or vomiting (Bright, et al., 1990; Elwood, 2006; Marks, 2017). Similar clinical signs were also seen in our studies. Other clinical signs of a gastrointestinal or respiratory nature may also be present depending on the disease progression and/or secondary complications (Washabau, 2005; Elwood, 2006; Marks, 2017).

Oesophageal diseases in the dog include anatomical malformations, motility disorders, neoplasia, inflammatory disorders and oesophageal obstruction (Gaschen, 2018). A variety of diagnostic imaging methods are usually required to attempt a complete assessment of the oesophagus (Gaschen, 2018) with the choice of imaging modality being dependent on the clinical presentation of the patient and the information required by the clinician (Kleine & Lamb, 1989). The prospective part of this study focusses on ultrasound as this modality is currently readily available in small animal veterinary practices.

Despite the increasingly widespread use of transcutaneous ultrasound in small animal veterinary practice, it is not currently routinely used for examination of the oesophagus. Although there are a few reports of its use alongside other modalities to evaluate the oesophagus in dogs (Neelis et al., 2015; Zwingenberger, & Taeymans, 2015) there do not appear to be any detailed reports documenting its appearance or wall thickness. In our study, transcutaneous ultrasonography allowed evaluation of the whole length of the cervical oesophagus down to the thoracic inlet although visualisation of the entire oesophageal circumference was limited due to the presence of intra-luminal gas. It allowed visualisation of the wall layers, which correlated well with the histological samples that were taken for comparison. In addition to the usual 4 histological layers that are present along the length of the gastrointestinal tract (Evans & Lahunta, 2013), a connective tissue layer was identified within the muscularis layer that in some dogs resulted in a six-layer pattern on ultrasound which is similar to that reported in humans (Shang-Yong et al., 2004). This extra sonographic layer has also been reported in the canine colon as fibrous tissue in either the myenteric plexus or the tunica muscularis (Heng et al., 2015), but to the authors knowledge this is the first report of it in the canine oesophagus. Despite this additional layer being present in all the histological sections, its visibility was influenced by the equipment used as well as the size and body condition of the dog and was therefore was not consistently imaged in all dogs.

To the authors knowledge this is also the first report suggesting normal wall thickness measurements for the canine oesophagus using ultrasound and comparing with histology, with a significant correlation being identified between the wall thickness and the weight of the dog. However, additional studies with a larger study sample are required to increase the statistical significance of these findings.

The lack of discernible ultrasonographic changes in the dogs presenting with clinical signs and also in the cadaver with the abnormal histological findings supports our hypothesis that transcutaneous ultrasonography can be used to document the appearance and thickness of the canine cervical oesophagus but that its use in clinically affected dogs would be limited to those with gross wall changes and that subtle histological changes would not be identifiable.

In the second part of this study, the occurrence of oesophageal abnormalities in brachycephalic breeds was determined and again, to the authors knowledge, this is the first study reporting this. Radiography and/or fluoroscopy were the modalities of choice used in our study for their diagnosis (Bright, et al., 1990; Washabau, 2005; Elwood, 2006; Reeve, et al., 2017; Gaschen, 2018). Megaoesophagus was the most prevalent oesophageal disease amongst this brachycephalic population, followed by dysmotility and GOR

An overlap of oesophageal abnormalities was apparent in this study in dogs with and without BOAS. In the breeds with BOAS, hiatal hernia, oesophageal dysmotility and GOR were the most prevalent oesophageal diseases while in the dogs without BOAS, megaoesophagus, dysmotility and hiatal herniation were the most prevalent. This could be explained by the French and English bulldogs' over-representation in this study and predisposition to BOAS (Meola, 2013). Additionally, it has been suggested by several authors that upper respiratory tract disease can influence gastro-oesophageal disease and vice versa (Poncet, et al., 2005; Liu, et al., 2017; Reeve, et al., 2017). Megaoesophagus, however was most common in CKCS and Boxers, which could justify their prevalence in dogs without BOAS.

The presence of these conditions individually or concurrently is of clinical significance, considering these dogs present a higher risk of developing aspiration pneumonia when under general anaesthesia (Wagner, 2008; Marks, 2017; Gaschen, 2018).

The incidence of oesophageal redundancy was 11/51 in the brachycephalic dogs in this study, although concurrent oesophageal disease such as GOR, hiatal hernia or oesophageal hypomotility was present in 8 of these 11 dogs. Oesophageal redundancy has only been described in English bulldogs, French bulldogs and Chinese shar-pei in the literature, mostly as an incidental finding, but also occasionally associated with clinical signs (Poncet, et al., 2005; Unzueta, et al., 2011; Gaschen, 2018). However, there was not statistical association encountered in this study between the brachycephalic breeds with oesophageal redundancy.

Conclusion

To the author's knowledge this is the first study describing transcutaneous ultrasound examination of the canine cervical oesophagus in detail, providing normal wall thickness measurements and documenting the appearance of an additional histological layer that is visible when image quality and patient factors are good. Transcutaneous ultrasonography permitted evaluation of the entire cervical oesophagus in dogs by using a left lateral approach. This method allowed visualisation of the normal ultrasonographic wall layers with histological correlation. An additional thin hyperechoic echoic layer between the inner circular and outer longitudinal muscular layer was noticed presenting the oesophageal wall with an overall six-wall layer pattern. This study showed oesophageal wall layering to be composed of four or six-wall layer pattern.

To the author's knowledge this is also the first study documenting the occurrence of oesophageal conditions in brachycephalic dogs. Megaoesophagus was the most prevalent oesophageal abnormality in the brachycephalic breeds in this study. Hiatal hernia, oesophageal dysmotility and GOR were the most predominant oesophageal diseases in dogs with BOAS and megaoesophagus, dysmotility and hiatal herniation in dogs without BOAS. The incidence of oesophageal redundancy was low in this study with a trend towards the presence of concomitant oesophageal disease and/or BOAS.

Further studies with larger sample sizes are warranted for both parts of this study. The next step with the ultrasound part of the study would be to identify dogs with oesophageal abnormalities for evaluation to determine what type of changes ultrasound will be able to identify. Increasing the numbers of cases for inclusion in the retrospective multi-modality part of the study would allow more meaningful statistical relationships to be determined. Seeking collaboration with other institutes would be an appropriate way to address this going forwards.

Appendix

Appendix 1. *Number of oesophageal abnormalities identified in different brachycephalic breeds of dog.*

Breed	Megaoesophagus	Hiatal hernia	Dysmotility	GOR	Oesophageal redundancy	Foreign Body	GE Intussusception	Neoplasia
Boston Terrier		1						
Boxer	1							
Boxer	1							
Boxer								1
Boxer	1							
Boxer			1					
Boxer		1	1					
Bullmastiff	1							
Chihuahua	1							
Chinese Shar-pei	1	1						
CKCS							1	
CKCS	1		1		1			
CKCS						1		
CKCS						1		
CKCS	1							
CKCS	1							
CKCS	1							
Dogue de Bordeaux			1	1				

English Bulldog	1		1					
English Bulldog		1	1	1				
English Bulldog			1	1				
English Bulldog			1					
English Bulldog		1	1		1			
English Bulldog			1	1				
English Bulldog			1	1				
English Bulldog				1	1			
English Bulldog					1			
English Bulldog		1		1				
English Bulldog					1			
English Bulldog		1						

French Bulldog				1				
French Bulldog			1	1				
French Bulldog		1						
French Bulldog		1						
French Bulldog		1						
French Bulldog	1							
French Bulldog			1	1	1			
French Bulldog		1		1				
French Bulldog		1			1			
French Bulldog					1			
French Bulldog				1	1			
French Bulldog		1			1			

Lhasa Apso						1		
Lhasa Apso	1							
Lhasa Apso			1					
Lhasa Apso	1							
Pug		1		1	1			
Pug	1		1					
Pug		1	1					
Shih-tzu						1		
Shih-tzu	1							

References

Baloi, P. A., Kircher, P. R. & Kook, P. H., 2013. Endoscopic ultrasonographic evaluation of the esophagus in healthy dogs. *American Journal of Veterinary Research,* 74(7), pp. 1005-1009.

Bexfield, N. H., Watson, P. J. & Herrtage, M. E., 2006. Esophageal Dysmotility in Young Dogs. *Journal of Veterinary Internal Medicine,* 20(6), p. 1314–1318.

Bonadio, C. M., Pollard, R. E., Dayton, P. A., Leonard, C. D. & Marks, S. L., 2009. Effects of Body Positioning on Swallowing and Esophageal Transit in Healthy Dogs. *Journal of Veterinary Internal Medicine,* 23(4), pp. 801–805.

Bradley, K., 2005. Practical contrast radiography 2. Gastrointestinal studies. *In Practice,* 27(8), pp. 412-417.

Brady, R., Biskup, J. & Latimer, C., 2017. Gastro-oesophageal intussusception with splenic involvement in an adult dog. *Veterinary Record Case Reports,* 4(2), pp. 1-4.

Bright, R. M., Sackman, J. E., DeNovo, C. & Toal, C., 1990. Hiatal hernia in the dog and cat: A retrospective study of 16 cases. *Journal of Small Animal Practice ,* Volume 31, pp. 244-250.

Bristow, P., 2015. Cervical masses in dogs and cats 1. Investigation and management.. *In Practice,* 37(6), pp. 267-274.

Burnie, A. G. .. G., Simpson, J. W. & Corcoran, B. M., 1989. Gastro-oesophageal reflux and hiatus hernia associated with laryngeal paralysis in a dog. *Journal of Small Animal Practice,* 30(7), pp. 414-416.

Callan, M. B. et al., 1993. Congenital Esophageal Hiatal Hernia in the Chinese Shar-Pei Dog. *Journal of veterinary internal medicine,* 7(4), pp. 210-215.

Capitani, O., Spinella, G., Fiorelli, F., Conte, M., Vagnini, M. & Gualtieri, M., 2014. Trans-Endoscopic Ultrasonography of the Oesophagus and Gastrointestinal Tract in Dogs and Cats: Pathological Findings. *Pakistan Veterinary Journal,* 34(3), pp. 319-323.

Codreanu, I., Chamroonrat, H., Edwards, K. & Zhuang, H., 2013. Effects of the frame acquisition rate on the sensitivity of gastro-oesophageal reflux scintigraphy. *The British Journal of Radiology,* Volume 86, pp. 1-8.

Conrado, L. M., Gurski, R. R., da Rosa, A. R., Simic, A. P. & Callegari-Jacques, S. M., 2011. Is There an Association Between Hiatal Hernia and Ineffective Esophageal Motility in Patients with Gastroesophageal Reflux Disease?. *Journal of Gastrointestinal Surgery,* Volume 15, pp. 1756-1761.

Cunningham, J. G. &. K. K. G., 2007. Movements of the Gatrointestinal Tract. In: *Textbook of Veterinary Physiology.* Missouri: Saunders Elsevier, pp. 311-325.

d'Anjou, Marc-André, Pennick, D., 2015. Practical physical concepts and artifacts. In D. Pennick, & M.-A. d'Anjou, *Atlas of Small Animal Ultrasonography*, Second Edn. Wiley Blackwell, Iowa, pp. 1-18.

Denardi, F. & Riddell, R. H., 1991. The normal esophagus. *American Journal Surgery Pathology ,* 15(3), pp. 96-309.

Dennis, R., Kirberger, R. M., Barr, F. & Wrigley, R. H., 2010. *Handbook of small animal radiology and ultrasound.* 2nd Edition ed. Edingurgh: Elsevier Saunders, Edingurgh, pp. 199-228.

Dvir, E., Spotswood, T. C., Lambrechts, N. E. & Lobetti, R. G., 2003. Congenital narrowing of the intrapharyngeal opening in a dog with concurrent oesophageal hiatal hernia. *Journal of Small Animal Practice,* Volume 44, p. 359–362.

Dyce, K. M., Sack, W. O. & Wensing, C. J. G., 2010. The digestive apparatus. In: *Textbook of Veterinary Anatomy.* Missouri: Saunders Elsevier, pp. 119-124.

Elwood, C., 2006. Diagnosis and management of canine oesophageal disease and regurgitation. *In Practice,* Volume 28, pp. 14-21.

Evans, H. E. & Lahunta, A., 2013. Miller's Anatomy of the Dog. In: *The digestive apparatus and abdomen.* 4th Edition ed. Missouri: Elsevier Saunders, pp. 281-337.

Gaschen, L., 2018. The Canine and Feline esophagus. In: *Textbook of Veterinary Diagnostic Radiology.* 7th ed. Missouri: Elsevier Saunders, pp. 596-617.

Goetsh, E., 1910. The structure of the mammalian esophagus. *American Journal of Anatomy,* 10(1), pp. 1-40.

Gory, G., Rault, D. N., Gatel, L., Dally, C., Belli, P., Couturier, L. & Cauvin, E., 2014. Ultrasonographic characteristics of the abdominal esophagus and cardia in dogs. *American College of Veterinary Radiology,* 55(5), pp. 552–560.

Gualtieri, M., 2001. Esophagoscopy. *Veterinary Clinics of North America: Small Animal Practice,* 31(4), pp. 605-630.

Heng, H. G., Lim, C. K., Miller, M. A. & Broman, M. M., 2015. Prevalence and significance of an ultrasonographic colonic muscularis hyperechoic band paralleling the serosal layering in dogs. *American College of Veterinary Radiology,* 56(6), pp. 666-669.

Jergens, A. E., 2010. Diseases of the Esophagus. In: *Textbook of Veterinary Internal Medicine.* 7th Edition ed. Missouri: Saunders Elsevier, pp. 1487-1499.

Kahrilas, P. J., Kim, H. C. & Pandolfino, J. E., 2008. Approaches to the diagnosis and grading of hiatal hernia. *Best Practice & Research Clinical Gastroenterology,* 22(4), p. 601–616.

Kirberger, R. M., Cassel, N., Stander, N., McLean, M. & Dvir, E., 2014. Triple phase dynamic computed tomographic perfusion characteristics of spirocercosis induced esophageal nodules in non-neoplastic versus neoplatic canine cases. *American College of Veterinary Radiology,* 56(3), pp. 257–263.

Kirkby, K. A., Bright, R. M. & Owen, H. D., 2005. Paraoesophageal hiatal hernia and megaoesophagus in a three-week-old Alaskan malamute. *Journal of Small Animal Practice,* Volume 46, p. 402–405.

Kleine, L. & Lamb, C., 1989. Comparative organ imaging: The gastrointestinal tract. *Veterinary Radiology,* 30(3), pp. 133-141.

Koblik, P. D. & Hornof, W. J., 1985. Gastrointestinal nuclear medicine. *Veterinary Radiology,* 26(5), pp. 138-142.

Kuo, B. & Urma, D., 2006. Esophagus - anatomy and development. Part 1 Oral cavity, pharynx and esophagus. *GI Motility online,* pp. 1-20.

Larson, M. M. & Biller, D. S., 2009. Ultrasound of the gastrointestinal tract. *Veterinary Clinics of North America: Small Animal Practice,* 39(4), p. 747–759.

Le Roux, A. B., Granger, L. A., Wakamatsu, N., Kearney, M. & Gaschen, L., 2016. Ex vivo correlation of ultrasonographic small intestinal wall layering with histology in dogs. *Veterinary Radiology and Ultrasound,* 57(5), pp. 534-545.

Lecoindre, P. & Richard, S., 2004. Digestive disorders associated with the chronic obstructive respiratory syndrome of brachycephalic dogs : 30 cases (1999-2001). *Revue de Médecine Vétérinaire,* 155(3), pp. 141-146.

Ledda, G., Caldin , M., Mezzalira, G. & Bertolini, G., 2015. Multidetector-row computed tomography patterns of bronchoesophageal artery hypertrophy and systemic-to-pulmonary fistula in dogs. *Veterinary Radiology & Ultrasound,* 56(4), pp. 347-358.

Liu, N.-C., Troconis, E. L., Kalmar, L., Price, D. J., Wright, H. E., Adams, V. J., Sargan, D. R. & Ladlow, J. F., 2017. Conformational risk factors of brachycephalic obstructive airway syndrome (BOAS) in pugs, French bulldogs, and bulldogs. *PLoS One,* 12(8), pp. 1-24.

Long, J. D. & Orlando, R. C., 1999. Esophageal submucosal glands: Structure and function. *American Journal of Gastroenterology,* 94(10), pp. 2818-2824.

Marks, S. L., 2017. Diseases of the pharynx and esophagus. In: S. J. Ettinger & E. C. Feldman, eds. *Textbook of veterinary internal medicine.* 8th Ed, Elsevier, Missouri, pp. 1476-1490.

Mateen, M. A., Kaffes, A. J., Sriram, P. VJ., Rao, G. V. & Reddy, D. N., 2006. Modified technique of high-resolution ultrasonography of the normal cervical esophagus. *Journal Gastroenterology and Hepatology ,* 21(11), pp. 1660-1663.

Mazzei, M. J., Sally, A. B., Murphy, K. M., Hunter, S., Neel, J. A., Eosinophilic esophagitis in a dog. *Journal American Veterinary Medicine Association,* 235(1), pp. 61-65.

Meola, S. D., 2013. Brachycephalic Airway Syndrome. *Topics in Companion Animal Medicine,* 28(3), pp. 91-96.

Meyer, G. W., Austin, R. M., Brady, C. E. & Castell, D. O., 1986. Muscle anatomy of the human esophagus. *Journal Clinical Gastroenterology,* 8(2), pp. 131-134.

Muenster, M., Hoerauf, A. & Vieth, V., 2017. Gastro-oesophageal reflux disease in 20 dogs (2012 to 2014). *Journal of Small Animal Practice,* 58(5), p. 276–283.

Murphy, L. A., Nakamura, R. K. & Miller, J. M., 2015. Surgical correction of gastro-oesophageal intussusception with bilateral incisional gastropexy in three dogs. *Journal of Small Animal Practice ,* 56(10), p. 630–63

Neelis, D. A., Matton, J. S. & Nyland, T. G., 2015. Neck. In: J. Matton & T. G. Nyland, eds. *Small Animal Diagnostic Ultrasound.* 3rd Ed, Elsevier Saunders, St. Louis, pp. 155-187.

Noh, T. M., Fishman, E. K., Forastiere, A. A., Bliss, D. F., Calhoun, P. S., 1995. CT of the esophagus: Spectrum of disease with emphasis on esophageal carcinoma. Radiographics, 15, pp. 1113-134.

Nyland, T. G., Neelis, D. A., Mattoon, J. S., 2015. Gastrointestinal tract. In: J. S. Mattoon, & T. G. Nyland eds. *Small Animal Diagnostic Ultrasound*. 3rd Ed, Elsevier Saunders, St. Louis, pp. 468-500.

Palabiyik, F. B., Bayramoglu, S., Nurten, T. G., Daglar, S. & Cimilli, T., 2012. Use of sonography for evaluation of the cervical and thoracic esophagus in children. *Journal Ultrasound Medicine,* 31(9), pp. 1375-1379.

Pazzi, P., Kavkovsky, A., Shipov, A., Segev, G. & Dvir, E., 2018. Spirocerca lupi induced oesophageal neoplasia: Predictors of surgical outcome. *Veterinary Parasitology,* 250(30), pp. 71-77.

Pennick, D. & d'Anjou, M-A., 2015. Gastrointestinal tract. In: D. Pennick & M. A. d'Anjou. *Atlas of Small Animal Ultrasonography,* 2nd Ed., Willey Blackwell, Iowa, pp. 259-308.

Pietra, M., Gentilini, F., Pinna, S. & Fracassi, F., 2003. Intermittent Gastroesophageal Intussusception in a Dog: Clinical Features, Radiographic and Endoscopic Findings, and Surgical Management. *Veterinary Research Communications,* 27(1), p. 783–786

Pollard, R. E., 2012. Imaging Evaluation of Dogs and Cats with Dysphagia. *International Scholarly Research Network Veterinary Science,* pp. 1-15.

Poncet, C. M., Dupre, G. P., Freiche, V. G. & Bouvy, B. M., 2006. Long-term results of upper respiratory syndrome surgery and gastrointestinal tract medical treatment in 51 brachycephalic dogs. *Journal of Small Animal Practice,* 47(3), p. 137–142.

Poncet, C. M., Dupre, G. P., Freiche, V. G., Estrada, M. M., Poubanne, Y. A. & Bouvy, B. M., 2005. Prevalence of gastrointestinal tract lesions in 73 brachycephalic dogs with upper respiratory syndrome. *Journal of Small Animal Practice ,* 46(6), p. 273–279.

Raheem, M., Leach, S. T., Day, A. S. & Lemberg, D. A., 2014. The pathophysiology of eosinophilic esophagitis. *Frontier in Pediatrics,* Volume 2, pp. 1-9.

Ranen, E., Dank, G., Lavy, E., Perl, S., Lahav, D. & Orgad, U., 2008. Oesophageal sarcomas in dogs: Histological and clinical evaluation. *The Veterinary Journal,* 178(1), pp. 78-84.

Reeve, E. J., Sutton, D., Friend, E. J. & Warren-Smith, C. M. R., 2017. Documenting the prevalence of hiatal hernia and oesophageal abnormalities in brachycephalic dogs using fluoroscopy. *Journal of Small Animal Practice (2017) 58, ,* 58(12), p. 703–708.

Riddell, A. M., Hiller, J., Brown, G., King, D. M., Wotherspoon, A. C., Thompson, J. N., Cunningham, D. & Allum, W. H., 2006. Potential of Surface-Coil MRI for Staging of Esophageal Cancer. *American Journal of Roentgenology,* 187(5), pp. 1280–1287.

Ridgway, M. D. & Graves, T. K. (November 2010). Megaoesophagus. *NAVC Clinician's Brief,* pp.43-48.

Rossum, P. S. N., van Lier, A. L. H. M. W., Lips, I. M., Meijer, G. J., Reerink, O., van Vulpen, M., Lam, M. G. E. H., van Hillegersberg, R. & Ruurda, J. P., 2015. Imaging of oesophageal cancer with FDG-PET/CT and MRI. *Clinical Radiology,* Volume 70, pp. 81-95.

Sellon, R. K. & Willard, M. D., 2003. Esophagitis and esophageal strictures. *Veterinary Clinics of North America: Small Animal Practice*, 33, pp. 945-967.

Shang-Yong, Z, Ruo-Chuan, L., Li-Hong, C., Hong, Y. Xu, F. & Xin-Hong, L., 2004. Sonographic anatomy of the cervical esophagus. *Journal Clinical Ultrasound,* 32(4), pp. 163-171.

Shum, J. S. F. et al., 2007. Gastroduodenal intussusception. *Abdominal Imaging,* 32(6), p. 698–700.

Sivacolundhu, R. K., Read, R. A. & Marchevsky, A. M., 2002. Hiatal hernia controversies – a review of pathophysiology and treatment options. *Australian Veterinary Journal,* 80(1-2), pp. 48-53.

Summer, J. F., O'Neil, D. G., Church, D. B., Thomson, P. C., McGreevy, P. D. & Brodbelt, D. C., 2015. Prevalence of disorders recorded in Cavalier King Charles Spaniels attending primary-care veterinary practices in England. *Canine Genetics and Epidemiology,* Issue 2, pp. 1-15.

Tolbert, M. K., 2017. Gastrointestinal endoscopy. In: S. J. Ettinger, E. C. Feldman & E. Côté, eds. *Textbook of veterinary internal medicine.* Canada: Elsevier, pp. 437-440.

Torrente, C., Vigueras, I., Manzanilla, E. G., Villaverde, C. Fresno, L., Carvajal, B., Fiñana, M. & Costa-Farré, 2017. Prevalence of and risk factors for intraoperative gastroesophageal reflux and postanesthetic vomiting and diarrhea in dogs undergoing general anesthesia. *Journal of Veterinary Emergency and Critical Care ,* 27(4), pp. 397–408.

Unzueta, A., Villegas, A., Aceña, M. C. & Garcia-Belenguer, S., 2011. Estudio de prevalencia de redundancia esofágica en la raza Bulldog Francés. *Clínica Veterinaria de Pequeños Animales,* 31(3), pp. 157-160.

van der Merwe, L. L. et al., 2008. Spirocerca lupi infection in the dog: A review. *The veterinary journal,* 176(3), pp. 294-309.

van Rossum, P. S., van Hillegersberg, R., Lever, F. M., Lips, I. M., van Lier, A. L., Meijer, G. J., van Leeuwen, M. S., van Vulpen, M. & Ruurda, J. P., 2013. Imaging strategies in the management of oesophageal cancer: what's the role of MRI?. *European Radiology,* 23(7), pp. 1753- 1765.

Venker-van-Haagen, A., 2013. Esophagus. In: R. J. Washabau & M. J. Day, eds. *Canine and Feline Gastroenterology.* St. Louis, Missouri: Saunders Elsevier, pp. 570-605.

Wagner, W. M., 2008. The oesophagus. In: T. Schwarz & V. Johnson, eds. *BSAVA Manual of Canine and Feline Thoracic Imaging.* Gloucester: British Small Animal Veterinary Association, pp. 200-212.

Washabau, R. J., 2005. Dysphagia and regurgitation. In: E. J. Hall, J. W. Simpson & D. A. Williams, eds. *BSAVA Manual of Canine and Feline gastroenterology.* 2nd Ed., British Small Animals Veterinary Association, Gloucester, pp. 69-72.

Wisner, E. R., Mattoon, J. S., Nyland, T. G. & Baker, T. W., 1991. Normal ultrasonographic anatomy of the canine neck. *Veterinary Radiology,* 32(4), pp. 185-190.

Young, B., Kloop, L., Albrecht, M. & Kraft, S., 2004. Imaging diagnosis: magnetic resonance imaging of a cervical wooden foreign body in a dog. *Veterinary Radiology & Ultrasound,* 45(6), p. 538–541.

Zhu, S.-Y., Liu, R-C., Chen, L-H., Luo, F., Yang, H., Feng, X. & Liao, X-H.,et al., 2004. Sonographic anatomy of the cervical esophagus. *Journal Clinical Ultrasound,* 32(4), pp. 163-171.

Zwingenberger, A. & Taeymans, O., 2015. Neck. In: D. Penninck & M. D'Anjou, eds. *Atlas of Small Animal Ultrasonography.* Iowa: Wiley Blackwell, pp. 55-80.