Transcutaneous ultrasonographic characteristics of the canine cervical esophagus

Transcutane echografische kenmerken van de cervicale slokdarm bij honden

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ABSTRACT

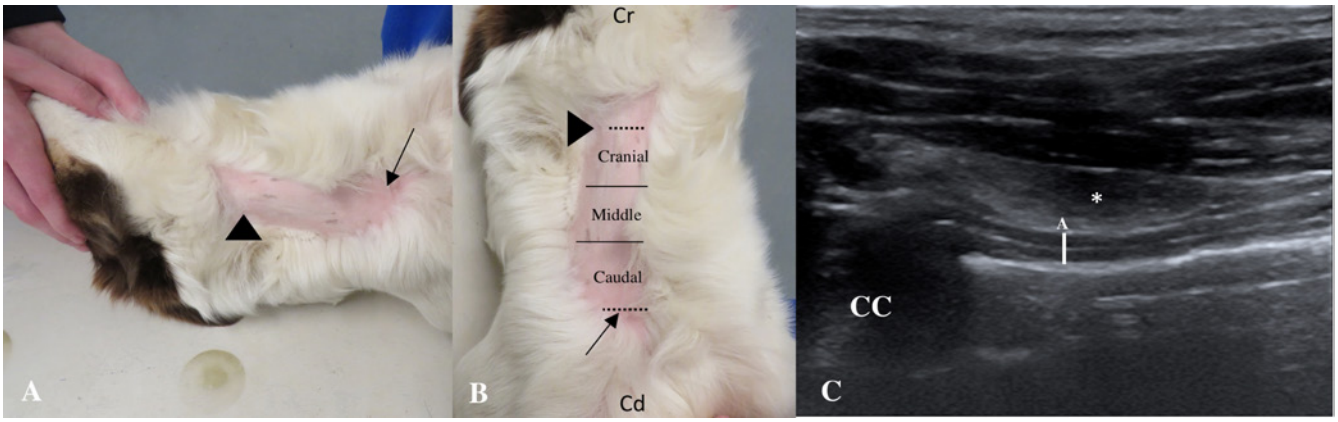
Ultrasound is a readily available imaging modality that allows detailed visualization of the canine esophageal wall. However, in the literature, there are few reports of its use for this purpose. The aim of the present study was to document the sonographic appearance and measurements in normal dogs. Seven cadavers and ten healthy live dogs were examined using a 14MHz transducer. Two, four or six wall layers were identified using ultrasound. To the authors' knowledge, the identification of six layers using ultrasound has not been reported before; this is apparently due to fibrous tissue located between the muscle layers as determined using histology. The mean ultrasonographic wall thickness for the live dogs was 2.8 mm ± 1 mm. These findings support the use of transcutaneous ultrasonography to evaluate the cervical esophagus, but further research is required to determine its use in clinical cases.

SAMENVATTING

Echografie is een in ruime mate beschikbare beeldvormende modaliteit waarmee gedetailleerde visualisatie van de slokdarmwand mogelijk is. Desondanks zijn er weinig meldingen in de literatuur wat betreft het gebruik van echografie voor dit doeleinde bij honden. De doelstelling van het voorliggende onderzoek was om de echografische kenmerken en afmetingen van de slokdarmwand bij normale honden te beschrijven. Zeven kadavers en tien gezonde, levende honden werden onderzocht met een 14MHz-transducer. Twee, vier en zes wandlagen werden geïdentificeerd via echografie. Volgens de auteurs werd de echografische identificatie van zes wandlagen niet eerder gerapporteerd; uit histologisch onderzoek is gebleken dat dit te wijten is aan bindweefsel dat zich tussen de spierlagen bevindt. De gemiddelde echografische wanddikte bij de normale honden in het voorliggende onderzoek was 2,8 mm ± 1 mm; dit was significant gecorreleerd met gewicht ($P<0,05$). Deze bevindingen ondersteunen het gebruik van transcutane echografie om de cervicale slokdarm te beoordelen. Verder onderzoek is echter nodig om de klinische toepassing hiervan te bepalen.

INTRODUCTION

Esophageal disease is associated with regurgitation and vomiting (Bright et al., 1990; Washabau, 2005; Elwood, 2006; Evans and de Lahunta, 2013; Marks, 2017), but can be difficult to diagnose. Although endoscopy is the imaging modality of choice (Noh et al., 1995; Elwood, 2006; Mateen et al., 2006; Ridgeway 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), is not readily available to veterinary practitioners. Ultrasound can provide detailed information about esophageal wall architecture, luminal contents and surrounding soft tissue (Baloi et al., 2013; Gory et al., 2014; Bristow, 2015) and is avail-



Figures 1 A. and B. Illustration of the attributed neck regions in a dog (cranial, middle and caudal) and area of the neck scanned. Cranial (Cr) and caudal (Cd). Larynx (arrow head) and thoracic inlet (black arrow). C. Longitudinal ultrasound image of the cranial region of a normal canine cervical esophagus in a cadaver. Cranial is to the left of the image. Esophageal wall thickness (solid line at A); Cricoid cartilage of larynx (CC); Thyroid gland (*).

able in most veterinary practices. Although the thoracic esophagus is inaccessible due to the surrounding air-filled lungs, the cervical and abdominal sections can both be examined.

Ultrasound of the normal canine cervical esophagus was first reported in 1991 (Wisner et al., 1991). Although more recent textbooks include images, no more detailed information about its appearance has been published since (Zhu, et al., 2004; Neelis et al., 2015). Transcutaneous ultrasound of the abdominal esophagus (Gory et al., 2014) and endoscopic ultrasound of the cervical and thoracic esophagus (Baloi et al., 2013) have also been described.

The histological layers of the gastrointestinal tract, the tunica mucosa, tela submucosa, tunica muscularis and tunica adventitia, are all visible sonographically (Baloi et al., 2013; Gory et al., 2014; Neelis et al., 2015). In humans, sometimes six esophageal layers can be demonstrated due to the presence of a connective tissue layer between the inner circular and outer longitudinal muscular layers (Capitani et al., 2014); however, this has not been reported in dogs.

Transcutaneous ultrasound has been used to assess the human esophagus (Capitani et al., 2014) but there are few reports in the veterinary literature (Zhu, et al., 2004; Gory et al., 2014; Bristow, 2015; Neelis et al., 2015; Zwingenberger and Taeymans, 2015). Normal values for canine gastrointestinal tract and abdominal esophageal wall thickness have been reported (Neelis et al., 2015; Zwingenberger and Taeymans, 2015), but there are currently no normal values for the cervical esophagus in dogs.

The aim of this study was to provide a more current ultrasonographic description of the normal canine cervical esophagus and correlate this with histological findings; determine whether conventional transcutaneous ultrasonography could be used to evaluate the entire cervical esophagus in conscious healthy dogs.

The authors hypothesized that transcutaneous ultrasonography could be used to document the appearance and thickness of the canine cervical esophagus.

MATERIAL AND METHODS

This is a prospective, anatomic observational study performed from 2016 to 2017 at the University of Glasgow, School of Veterinary Medicine. Ethics approval was obtained (Ref 09a/16). Sample sizes were determined by the availability of appropriate dogs during the period of study. The ultrasonographic esophageal study was performed in fresh cadavers (Part A) and live healthy dogs (Part B).

Part A

Seven fresh cadavers donated to University of Glasgow, School of Veterinary Medicine for teaching and research proposes, were used. No signalment was available but sex, breed type and estimated age were recorded.

Ultrasound was performed within eight hours after euthanasia. The left side of the neck was clipped, then water and ultrasonographic gel were 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 esophagus were visible. For the longitudinal images, the transducer was placed in the median plane over the caudal aspect of the larynx and ventral to the trachea and then moved to the left until the esophagus was visible between the trachea and common carotid artery. The neck, from the caudal aspect of the larynx to the thoracic inlet, was divided into equal cranial, middle and caudal sections. Transverse and longitudinal images from each section were digitally stored to a PACS (Figure 1).

The esophagus was resected on the same day, 5-10 minutes after ultrasonography for all cadavers, and

were immediately after examined ultrasonographically in a water bath. The esophagus was resected from caudal to the larynx and at the thoracic inlet in all dogs. Water bath ultrasonography was performed immediately after the esophageal dissections using tap water. The osmolarity of the water was not measured.

A histological section was prepared from the cranial, middle and caudal regions of the esophagus in all seven cadavers. All histological sections were fixed individually in 10% neutral buffered formalin after the water bath ultrasonography and kept for less than twenty-four hours. Samples were processed on a Thermo Shandon (Excelsior AS processor). Tissues were embedded into paraffin blocks and sections, cut at 2.5µm and floated out on a 56°C water bath and picked upon positively charged slides. All sections were baked in a 56°C oven for one hour. The esophageal sections were deparaffinized using Histoclear (Histological clearing agent, National Diagnostics, Atlanta) for two minutes, then taken to water through three changes of graded alcohol. The nucleus was stained in Gills hematoxylin (made up in house) for five minutes, then differentiated in 1% acid alcohol. The sections were then counterstained with Putts Eosin (made up in house) for five minutes, then dehydrated through graded alcohols back into Histoclear, then mounted with glass coverslips.

Hematoxylin and eosin (H&E) stain was used in all twenty-one samples and additional staining with Masson´s trichrome was applied in three dogs (nine samples). All histologic samples were examined and the wall thickness was measured three times for each region. This information was then correlated with the ultrasound images.

Part B

Ten healthy dogs owned by clinical staff were recruited. 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 were also recorded.

Analysis

Image analysis was performed using a DICOM -viewer (OsiriX MD; Pixmeo, Bernex, Switzerland) Electronic calipers (0.03 mm) were used to measure esophageal wall thickness in all regions in both longitudinal and transverse planes (Figure 1). The wall nearest to the transducer was measured from the mucosal-lumen interface to the outer surface of the tunica adventitia. Three measurements were performed for each region and plane at three different time points. An overall mean wall thickness was calculated using cadavers in Part A and live dogs in part B.

Images were also evaluated for visibility of the wall layering, echogenicity and the number of the wall layers, and were compared to the corresponding histological images obtained from the cadavers. Identification of the wall layering and the number of layers were documented in all histological samples. All histological samples were classified as normal or abnormal.

Statistical analysis was performed using statistics software (Minitab; version 17.1.0.0 Minitab Ltd., Coventry). Bland-Altman (BA) plots were used to identify the degree of agreement between different methods used to measure the transverse wall thickness of the esophageal wall. Pairwise BA plots were created for ultrasonography versus ultrasonography in water bath; ultrasonography versus histology; and ultrasonography in water bath versus histological measurements.

The average of the three measurements of esophageal wall thickness acquired from ultrasonographic measurements for each dog at each cranial, middle and caudal region was compared between Part A and Part B. Normality tests indicated evidence of a lack of normality ($p=0.037$), hence Mann-Whitney tests were used for this analysis. Regression analysis was used to investigate for Part B only if body weight, age or sex was associated with esophageal wall thickness. Statistical significance was $P<0.05$. 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).

RESULTS

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

Part A

All seven dogs were adult, three were neutered males (two mixed breed and one miniature poodle) and four were entire males (three mixed breed and one English bulldog).

The H&E histological sections demonstrated four distinct layers: tunica mucosa, tela submucosa, tunica muscularis and tunica adventitia (Figure 2B). The mucosa was composed of a stratified squamous epithelium, a lamina propria mucosae and in some samples a

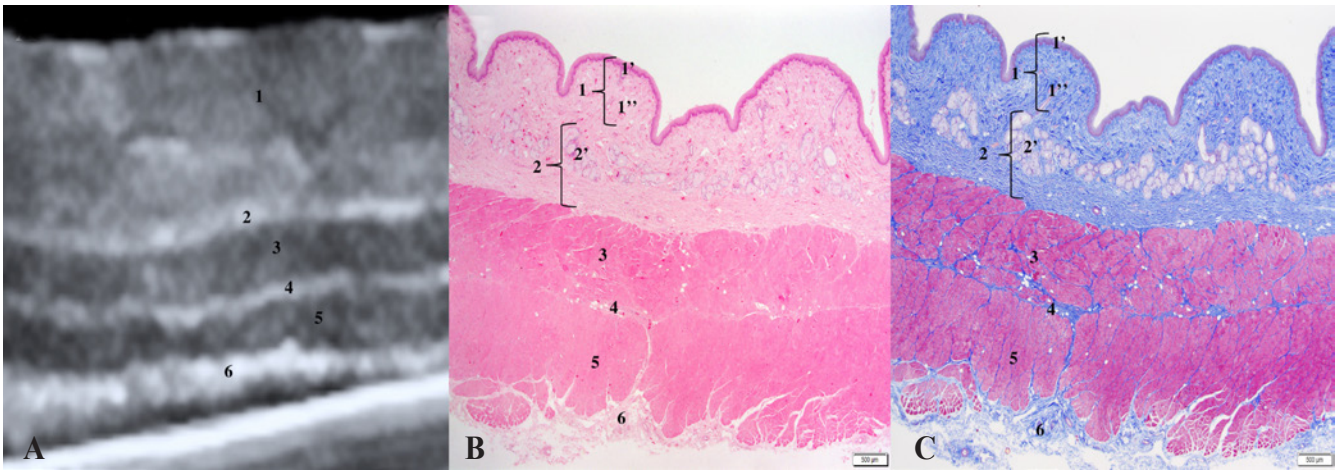


Figure 2. A. Longitudinal ultrasonographic and B. and C. Histological sections from the cranial region of a normal canine esophagus (cadaver five). B. Stained with hematoxylin and eosin (H&E) and C. Stained with Masson's trichrome. Section thickness 500 mm. Note that (4) the fibrous connective tissue is more evident using Masson's trichrome stain. (1) Tunica mucosa composed by (1') stratified squamous epithelium and (1") lamina propria mucosae, (2) tela submucosa with (2') a glandular portion, (3) inner circular tunica muscularis, (4) fibrous connective tissue, (5) outer longitudinal tunica muscularis, (6) tela adventitia.

lamina muscularis mucosae. In one cadaver, a moderate number of eosinophils were present in the lamina propria mucosae. The tela submucosa was composed of abundant lobules of mucus secreting glands. The tunica 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**.** A small amount of adipose tissue was present between the muscular layers in 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 2C).

The tunica 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 techniques used for preparation. The wall thickness in these sections was therefore measured from the mucosal-lumen interface to the tunica muscularis (Table 1).

The pathologist considered all the cadavers to be within normal limits except the one with the eosinophilic infiltrate in the lamina propria mucosae. The

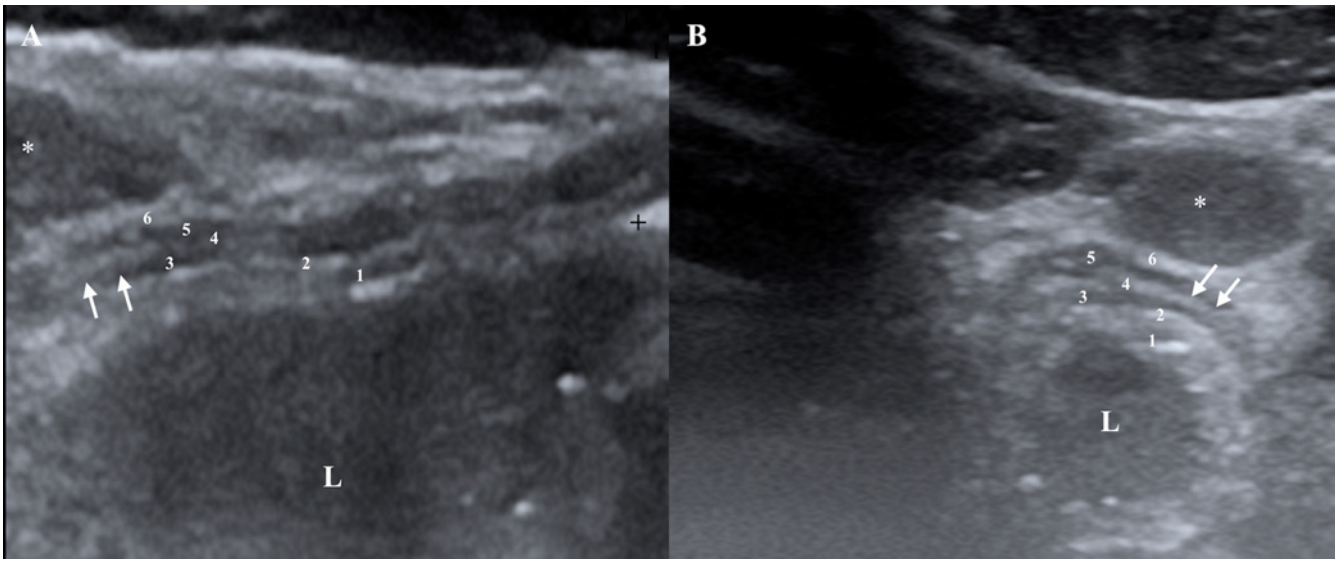


Figure 3. A. Longitudinal ultrasound image of the middle region of a normal canine cervical esophagus in situ in cadaver four. Cranial is to the left of the image. B. Transverse ultrasound image of the cranial region of a normal canine cervical esophagus in situ in cadaver four. Lateral is to the right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

Table 1. Histological measurements of canine cervical esophageal wall thickness (from muscularis to mucosal-lumen interface) in cadavers.

Region of neck	Histological esophageal wall thickness		
	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

mean wall thickness for this cadaver was 2.8 mm in situ, 2.96 mm in the water bath and 3.48 mm on histology. This cadaver was excluded from the results in Table 1 and further wall thickness calculations.

Using ultrasound, the entire length of the esophagus appeared to have four wall layers in one cadaver and six layers in three (Figure 3). 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 in five of the cadavers but a similar variation between four and six wall layers was observed in the remaining two cadavers (Figure 4).

The four-wall layer appearance was characterized by alternating hyperechoic and hypoechoic layers, from the inner mucosal-lumen interface, through the tunica mucosa, tela submucosa and tunica muscularis to the outer tunica 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 center of the hypoechoic tunica muscularis, which corresponded with the thin sheet of fibrous tissue identified on the histological sections stained with Masson's trichrome. The small amount of adipose tissue detected between the inner and outer muscular layer on histology in two cadavers was not visible on ultrasound.

A variation in the echogenicity of the tunica mucosa 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 tunica mucosa appearing echogenic. Wall thickness measurements are shown in Table 2.

Table 2. Ultrasonographic measurements of canine cervical esophageal wall thickness (from adventitia to mucosal-lumen interface), standard deviation (SD) and total mean wall thickness in Part A: cadavers in situ and cadaver samples in a water bath, and Part B: live dogs.

Part A - Cadavers				
	Maximum (mm)	**Minimum (mm)**	**Mean ± SD**	**Total Mean ± SD**
Cranial	6.1	1.7	3.2 ± 1.4	
Middle	5.9	1.1	2.8 ± 1.5	3 ± 1.4
Caudal	6.3	1.5	3.0 ± 1.3	
Part A - Water bath				
	Maximum (mm)	**Minimum (mm)**	**Mean ± SD**	**Total Mean ± SD**
Cranial	4.7	1.4	2.9 ± 0.9	
Middle	6.4	1.6	2.9 ± 0.9	2.9 ± 0.9
Caudal	4.7	1.5	3.0 ± 0.8	
Part B – Live dogs				
	Maximum (mm)	**Minimum (mm)**	**Mean ± SD**	**Total Mean ± SD**
Cranial	5.1	1.4	2.6 ± 0.7	
Middle	3.9	1.6	2.4 ± 0.6	2.5 ± 0.6
Caudal	3.7	1.4	2.4 ± 0.6	

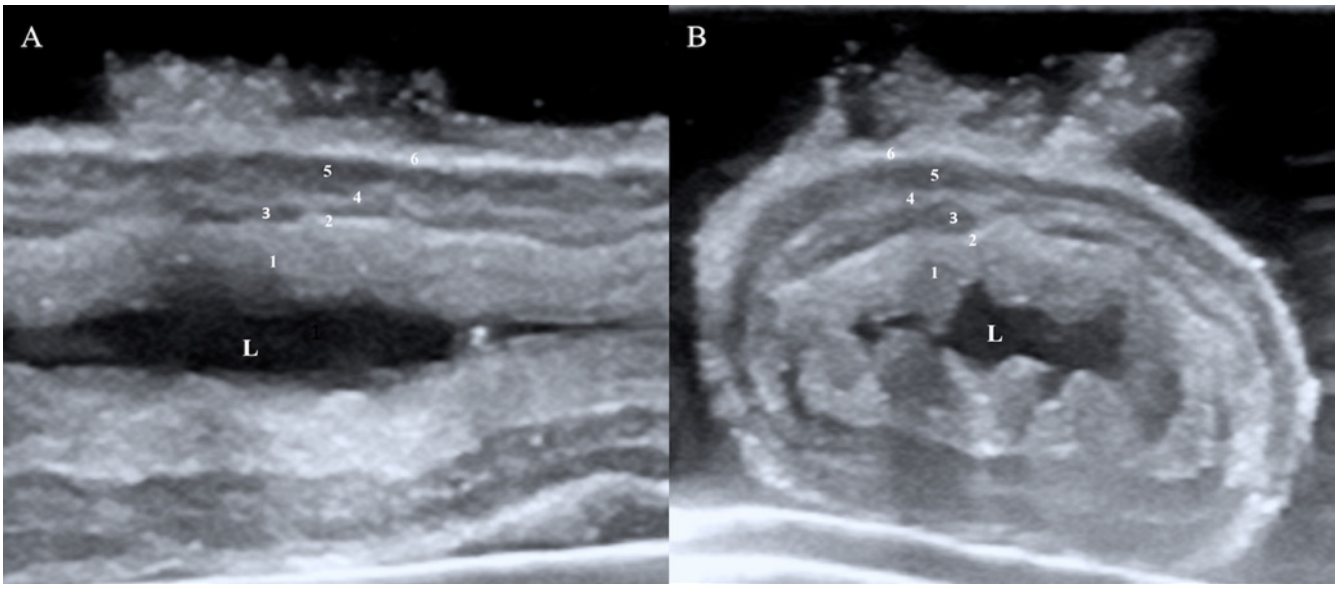


Figure 4. A. Longitudinal and B. Transverse ultrasound images of the middle region of a normal canine cervical esophagus in a water bath (cadaver five). (1) Tunica mucosa; (2) Tela submucosa; (3) Inner circular tunica muscularis; (4) Fibrous connective tissue; (5) Outer longitudinal tunica muscularis; (6) Tunica adventitia. The tunica mucosa is thicker than the adjacent layers and echogenic with multiple hyperechoic speckles, consistent with a collapsed esophagus and its longitudinal folds. Fluid filled esophageal lumen (L).

Part B

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.

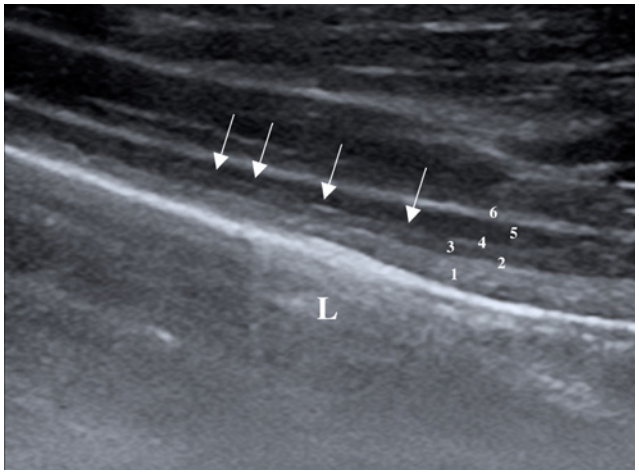


Figure 5. Longitudinal ultrasound image of the caudal region of the cervical esophagus in a normal live dog. The tunica mucosa appears echogenic (1). The fibrous connective tissue that is present within the muscular layer is faintly visible as a thin hyperechoic layer in some areas (white arrows) producing an intermittent six wall-layer pattern to the esophageal wall. (1) Tunica mucosa; (2) Tela submucosa; (3) Inner circular tunica muscularis; (4) Fibrous connective tissue; (5) Outer longitudinal tunica muscularis; (6) Tunica adventitia.

In two, a Corgi and a Chihuahua, only two wall layers were visible on ultrasound examination. These corresponded with the tunica adventitia and a hypoechoic layer representing an amalgamation of the tunica mucosa, tela submucosa and tunica 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). As for the other eight dogs, two of them 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 the appearance observed in the cadavers in part A (Figure 5). Likewise, a variation in the echogenicity of the tunica 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. The mean wall thickness for Part A cadavers and Part B combined was 2.7 ± 1 mm.

Statistics

Four or six wall layers were identified in 88.3% of the dogs in this study. The BA plot for ultrasonography versus ultrasonography in water-bath wall-thickness measurements showed an average difference or bias of 0.21 mm with limits of agreement between -1.76mm and +2.17mm. The distribution of data points suggests that at thinner measurements, ultrasonography estimates are consistently thinner than equivalent water bath estimates, but for thicker measurements, ultrasonography estimates are consistently thicker than the equivalent water bath estimates. The BA plot for ultrasonography versus histology wall thickness mea-

surements showed an average difference or bias of 0.2 mm with limits of agreement between -3.4mm and +3mm. The distribution of data points suggests that at thicker measurements, ultrasonography estimates tend to be thicker than the equivalent histological estimates. The BA plot for water bath versus histology wall thickness measurements showed an average difference or bias of 0.41 mm with limits of agreement between -2.84mm and +2.02mm. The distribution of data points suggests no particular trend with respect to differences between the two estimates related to thickness of the esophageal wall (Figure 6).

There was no evidence of a difference in the average of the ultrasonographic esophageal wall thicknesses between Part A and Part B (p=0.342).

Regression analysis with eight different breeds among the ten healthy dogs, including breed of dog as an independent variable with esophageal wall thickness as the dependent variable was not feasible. Neither age (p-value = 0.46) nor sex (p-value = 0.16) were associated with esophageal wall thickness. The weight of the dogs was also not significantly associated with esophageal wall thickness, but the p-value of 0.07 would suggest 'a trend' and the coefficient indicates that for each extra kg in weight, the thickness of the esophageal wall might be expected to increase by 0.03mm.

DISCUSSION

In this study, it is demonstrated that conventional transcutaneous ultrasound can be used to evaluate the entire cervical esophagus 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 esophagus in normal dogs, describing the cervical esophageal wall thickness in relation to body weight in a cohort of normal dogs and prospectively using transcutaneous ultrasound to assess the esophagus in dogs.

The results of this study indicate that two, four or six layers are visible sonographically in the canine cervical esophagus. Histologically and sonographically, the canine esophageal wall is composed of four layers, the tunica mucosa, the tela submucosa, the tunica muscularis and the tunica 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 esophagus, 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 (Zhu et al., 2004). It seems that the thin extra hyperechoic layer seen on ultrasound

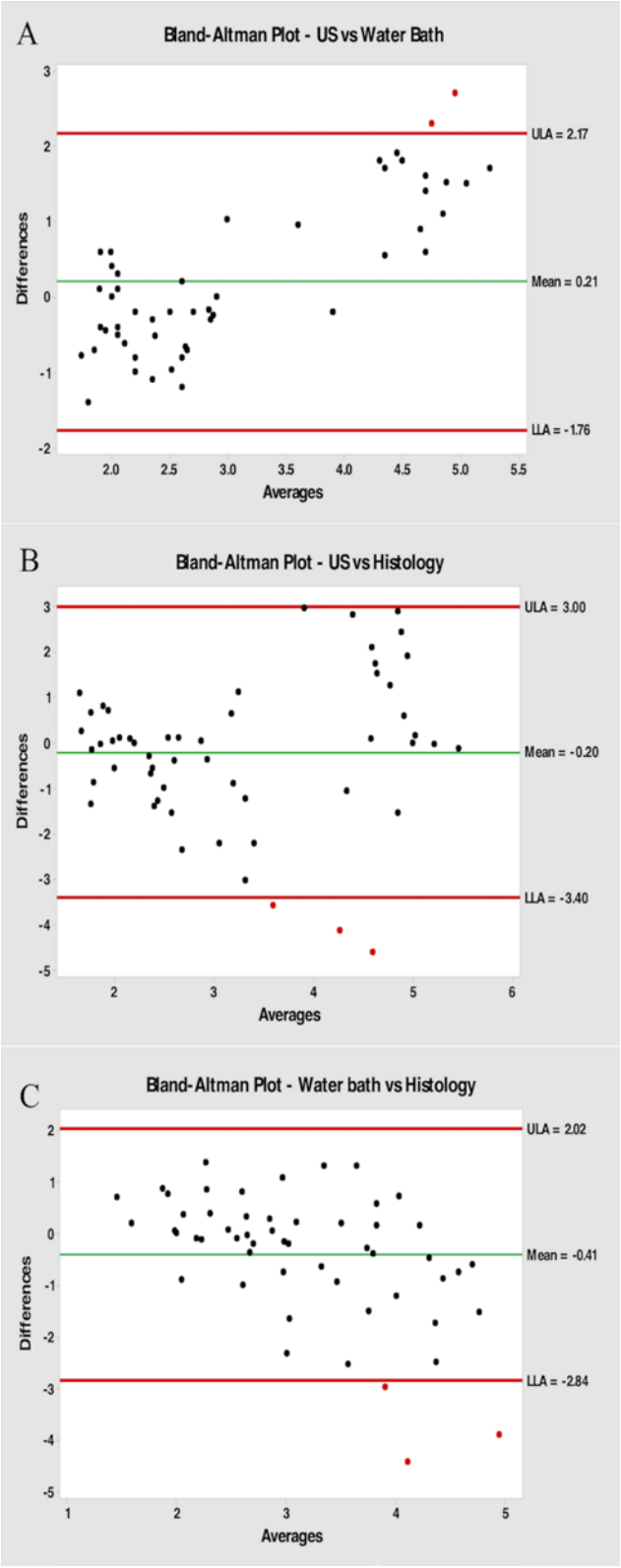


Figure 6. Bland-Altman (BA) plots showing the degree of agreement on Part A between ultrasonography (US), ultrasonography in water bath and histology of the esophageal wall thickness. A. BA for ultrasonography versus water bath; B. BA for ultrasonography versus histology; and C. Water bath versus histology. Upper limit of agreement (ULA); Lower limit of agreement (LLA).

and demonstrated using Masson's trichrome stain in the present study is similar to this layer described in humans. To the authors' knowledge, 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 visualization of collagen fibers which explains why this layer was best appreciated using this technique. Additionally, the small adipose tissue detected between muscular layers was not perceptible on ultrasound. This is likely due to bordering with a sheet of fibrous tissue, therefore becoming indistinguishable on ultrasound due to similar echogenicity.

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). In a study by Gory et al. (2014), four wall layers were identified in the abdominal esophagus in 89% of the dogs using a 9.5 MHz transducer, but six layers were not reported in any of them. In a study by Zhu et al. (2004), it has been demonstrated that stepping up the frequency to 12MHz increases the likelihood of six esophageal wall layers being identified ultrasonographically in humans. 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 Chihuahua of 1.8 kg. Large amounts of fat have been shown to reduce image quality (Mattoon and Nyland, 2015; d'Anjou and Penninck, 2015). In the present study, it was speculated that the miniature size of the Chihuahua might have resulted in esophageal wall layers that were just too thin for the machine to resolve, despite the use of a 14MHz transducer. In both cases, this led to 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.

The ultrasonographic appearance of the normal gastrointestinal tunica 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 corresponds with the findings of Gory et al., (2014), who also reported an echogenic appearance to the mucosa in the canine abdominal esophagus 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 esophagus has large and numerous longitudinal folds (Evans and de Lahunta, 2013); therefore, the echogenicity of the esophageal tunica mucosa could also presumably be due to gas or small particles becoming trapped in these folds. Since in the present study, no abnormalities were detected on histology that would explain an increased echogenicity of the tunica mucosa, whatever the underlying cause, this appearance can be considered a normal finding in the canine cervical esophagus.

The canine cervical esophagus wall thickness has been reported to measure approximately 4 mm (Evans and de Lahunta, 2013). In an esophageal 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 medicine, the normal cervical esophagus was found to be approximately 2.3 mm (Zhu, et al., 2004) and depending on the neck position and side of scanning, the thickness can vary 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 the mentioned studies but was lower than the values in a standard anatomical textbook by Evans and de Lahunta (2013). In this study, there was a minor variation of the esophageal wall thickness between ultrasonography, water bath and histology; however, with no significant statistical deviation, which was likely to be associated with the small sample size. Additionally, there was no evidence of a difference in the mean ultrasonographic measurements of the esophageal wall thicknesses between Part A and Part B. There was no significant association between the body weight of the dogs and the ultrasonographic wall thickness; however, the p-value was 0.07, suggesting that larger dogs might have thicker esophageal walls. Similar findings were seen in a study on the canine abdominal canine esophagus (Gory et al., 2014). However, more dogs with diverse body weights would be required to support this finding.

The cadaver with multifocal eosinophilic infiltrates in the lamina propria mucosae of the mucosa was considered abnormal. Eosinophilic esophagitis is commonly described in gastro-esophageal reflux in human medicine, and more specifically, in patients with food or aeroallergen hypersensitivity (DeNardi and Riddell, 1991; Raheem et al., 2014), but is rare in the veterinary literature. It has been described in one dog with dysphagia, regurgitation and coughing that also had esophageal ulceration and granulation tissue formation (Mazzei et al., 2009). The cadaver in the present study had no such concurrent changes. Whether the changes observed represent an early stage of the disease is however uncertain as no clinical data were available. Despite the presence of these histological changes, they were not identified on the ultrasound images. A similar situation has been described, where the intestinal wall layering appeared

normal on ultrasound in the presence of inflammation (Larson and Biller, 2009). This is presumably due to the resulting changes being too subtle to be identified using currently available ultrasound equipment.

Transcutaneous ultrasonography of the cervical esophagus is not routinely used in veterinary medicine. The abdominal and thoracic esophagus are the common sites affected by esophagitis secondary to gastroesophageal reflux in animals and in humans (Gory et al., 2014; Marks, 2017) with the latter not usually being accessible using this technique. Another limitation is the inability to visualize the entire circumference of the esophageal wall in the presence of intraluminal air.

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

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

In this study, it is shown that transcutaneous ultrasonography can be used to assess the canine cervical esophagus using a left lateral approach. This allows visualization of the wall layers, as confirmed by correlation with histological samples, with four or six layers being visible in 88.3% of the dogs in the present study, using a 14 MHz transducer. The additional connective tissue layer within the tunica muscularis, which is responsible for the six-layer appearance, has been previously reported in humans, but to the authors' knowledge, this is the first report in dogs. However, inherent patient factors also affect image quality, reducing the visible wall layers to two. The canine esophageal tunica mucosa often appears echogenic, which 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; therefore, further research is required to determine its use in clinical cases.

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Uit het verleden

GENEESKRACHTIGE KATTENPIS

Om den cancker te genezen
'Nemt catten pisse van eenen swerten katere, ende men sal den cancker daermede wassen, het daechs drijmael, ende dan sal men die pisse met schoon doecken daerop leggen, ende als die doecken drooge sijn, soo sal men die wederom in die pisse nat maecken. Hiermede sijn twee oft drij personen genesen.'

Uit: Braeckman, W.L. (1985). Een Oostvlaams 'medecyn boeck' uit de zestiende eeuw. In: *Oost-Vlaamse Zanten*, 60, p. 83.
De meeste aangehaalde ingrediënten zijn plantaardig en zeer divers van aard. De bereidingen hebben één kenmerk gemeen: ze zijn allen zeer efficiënt. Maar hoe hulpeloos en wanhopig moet men zijn om zijn toevlucht te willen nemen tot kattenpis?

Luc Devriese