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Original Article

Relationship between ultrasonographic and histopathological measurements of small intestinal wall layers in fresh cat cadavers

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ABSTRACT

The relationship between histological and ultrasonographic thickness of the intestinal wall and its layers in cats is unknown so far. The aims of this study were to establish the relationship between ultrasonographic measurements in the transverse and longitudinal planes of the small intestine and to establish the agreement between ultrasonographic and histologic thickness of the overall intestinal wall and layers in cat cadavers. Seventeen adult cats were euthanased for reasons unrelated to gastrointestinal tract disease and ultrasonography was performed immediately after death using a high-frequency linear transducer. Ultrasound images of the duodenum, jejunum, ileum, and distal ileum were acquired in both the longitudinal and transverse planes. Small intestinal samples were collected close to where ultrasonographic images were obtained, fixed in formalin, and histological sections were obtained. Measurements of the intestinal layers and the overall wall thickness were performed on the ultrasonographic images and histological sections.

No statistical differences were found between the ultrasonographic measurements of thickness obtained in the transverse and longitudinal planes except for the distal ileum ($P < 0.05$). There was good agreement between the ultrasonographic and histologic measurements of the overall wall thickness and the layers of the different intestinal segments except at the submucosa and muscularis of the duodenum. Immediate postmortem ultrasonographic and histological thickness measurements of the different layers of the small intestine obtained in this study could serve as a reference for ultrasonographic scans and histological samples in cats.

Introduction

Ultrasound (US) is a useful diagnostic tool to assess the gastrointestinal tract (GIT). The normal ultrasonographic appearance of different intestinal segments has been widely described in cats (Newell et al., 1999; Goggin et al., 2000; Besso et al., 2004; Di Donato et al., 2014; Winter et al., 2014; Hahn et al., 2017a). The normal wall layers are clearly visible and easy to assess using high-frequency transducers since the axial resolution of the US beam has been improved (Wiersema and Wiersema, 1993; Goggin et al., 2000; Nielsen et al., 2016).

Evaluation of the wall thickness of different segments of the small intestine and its layers is important to detect diseases involving the GIT (Zwingenberger et al., 2010). Several studies have reported thickening of the muscularis layer in cats with lymphoma, inflammatory bowel disease (IBD) (Zwingenberger et al., 2010; Daniaux et al., 2014), idiopathic intestinal smooth muscle hypertrophy (Diana et al., 2003), or eosinophilic enteritis (Tucker et al., 2014). Small intestinal muscularis thickening has been reported when the ratio of the width of muscularis to the submucosa is >1 in cats with lymphoma or IBD (Daniaux et al., 2014). Other studies have described ultrasonographic abnormalities at the level of the ileocecocolic junction (Taeymans et al., 2011; Hahn et al., 2017b), such as thickening of the cecum or the muscular layer of the ileum, hyperechoic fat, and lymphadenopathy in cats with GI signs (Taeymans et al., 2011). An overlap in caecal wall thickness between healthy cats and cats with typhlitis has been reported. Since there were significant differences in the proximal caecal wall thickness, abdominal ultrasonography is recommended to obtain proximal caecal wall measurements in cats (Hahn et al., 2017b). Based on these studies, reference intervals for the ultrasonographic wall thickness of the different segments of the small intestine and its layers in healthy cats have been established (Newell et al., 1999; Goggin et al., 2000; Di Donato et al., 2014; Winter et al., 2014).

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In dogs, several studies established ultrasonographic reference intervals for different segments of the small intestinal wall (Delaney et al., 2003; Stander et al., 2010; Gladwin et al., 2014). A good morphological correlation between the ultrasonographic and histological appearance of the intestinal wall layers except for the serosa has recently been reported in dogs (Le Roux et al., 2016). Nevertheless, to the best of our knowledge, no studies have described the correlation between the ultrasonographic and histological thickness of each wall layer in cats using high-resolution ultrasound.

The aims of this study were: (1) to evaluate the thickness of the different segments of the small intestinal wall layers by US using a high-resolution transducer in the longitudinal and transverse planes in cats and establish the relationship between those measurements; and (2) to establish the agreement between the ultrasonographic and histologic thickness of the individual and overall small intestinal wall layers.

Materials and methods

Animals

The study was approved by the Animal Care and Ethics Committee of the University of Murcia (Approval No. 401/2017; Approval date 5th February, 2018). Seventeen fresh cat cadavers (6 adult males and 11 adult females) with a mean weight of 3.6 kg (range 2.8–8.6 kg) were obtained from a local animal shelter and were humanely euthanased for reasons unrelated to gastrointestinal tract disorders, such as behavioural or orthopaedic problems. Age was unknown, but the cats were assumed to be young adults based on their dentition.

Ultrasonography

The US scans were performed immediately after euthanasia. All cats were fasted for at least 12 h prior to US. All ultrasonographic examinations were performed by the same operator (MM) using a US machine equipped with a 4 to 13-MHz linear array transducer (MyLab Twice LA523, Esaote). All scans were performed using the maximum frequency (13 MHz). The ventral abdomen of each cat was clipped, the skin cleaned, and acoustic coupling gel applied. The cadavers were positioned in dorsal, left and right lateral recumbency to assess the different segments of the GIT. Ultrasound images from the duodenum, jejunum, ileum, and distal ileum at the level of the ileocecocolic junction were obtained in the longitudinal and transverse planes. The landmarks used to localise each segment were the descending duodenum along the right body wall, ventral or ventrolateral to the right kidney, and the jejunum adjacent to the mesenteric lymph nodes. To localise the ileocecocolic junction from the descending duodenum, the transducer was moved to midline until a “wagon-wheel” sign was visualised, and from that point, the transducer was moved caudally (ileum: approximately 2–3 cm cranial to the ileocecocolic junction) and just at the level of the ileocecocolic junction (distal ileum).

Acquisition of data

Ultrasound images of the GI tract were saved in a DICOM format. An image analysis program (MIP 4.5 Advanced Version 5.01.02, Digital Image Systems S.L.) was used to measure the layers in the different segments of the small intestine. The images were displayed on a monitor (Eizo FlexScan MX210). The matrix of the images was 800×608 pixel. The ratio pixel/cm was 129, and 1 pixel in the image measured approximately 0.07 mm. The caliper size of the analysis program was 1 pixel. To ensure consistency, one investigator (MM) performed all measurements using the image analysis system. The measurements were obtained in segments that did not contain ingesta. Electronic callipers were placed at the outside edge of each individual intestinal layer, and leading edge to leading edge measurements were made from the serosal interface to the luminal interface of the mucosa (Fig. 1A).

Three consecutive measurements of each layer (mucosa, submucosa, muscularis, and serosa) were obtained in both the longitudinal and transverse planes for each segment of the small intestine. At the level of the distal ileum (ileocecocolic junction), two different measurements of the mucosa and submucosa were obtained on the transverse plane, one at the level of the fold and one between folds (Di Donato et al., 2014) (Fig. 1B). The measurements were not performed where presumed lymphatic tissue was detected. An average of the three measurements was calculated. The thickness of the entire intestinal wall was obtained using the sum of the measurements of each layer.

Histological measurements

Immediately after ultrasonographic examination, the abdominal cavity was opened at the level of the linea alba. Histological specimens of 2–3 cm in length from the duodenum, jejunum, ileum, and distal ileum (ileocecocolic junction) were taken from the part of the similar regions where the ultrasonographic images were obtained using the same landmarks as the US examination. They were gently rinsed in water to remove excess blood, mucus, and food particles and then pinned, without stretching, onto small cardboard squares. The sections of the intestine were fixed in 10% neutral-buffered formalin, embedded in paraffin wax, sectioned at 4 μm, and stained with haematoxylin and eosin. One of the authors (FJP) evaluated the slides with a Zeiss Axioskop 40 photomicroscope equipped with a Spot Insight 2 FireWire digital camera using Spot Version 4.0.5 software for Windows.

The thickness of the mucosa, submucosa, and muscularis of each intestinal segment in regions without lymphoid aggregates were measured (Fig. 1C). Each measurement was obtained three times and the average was calculated.

At the level of the distal ileum (ileocecocolic junction), two different measurements of the mucosa and submucosa were obtained, one at the level of the fold and one between folds (Fig. 1D). The thickness of the entire intestinal wall was obtained using the sum of the measurements of each layer. In addition, histological analysis was considered normal if there was no evidence of abnormal cellular infiltrates or histological abnormalities.

Statistical analysis

Statistical tests were performed using R 3.2.2 software. Descriptive statistics were obtained (mean, standard deviation [SD], maximum and minimum values) for each of the duodenal, jejunal, and ileal variables. All the data were examined for normality using the Shapiro–Wilk test. Mixed analysis of variance (ANOVA) and post-hoc Tukey's tests were performed to compare the histological and ultrasonographic measurements of the thickness of each layer in different segments of the small intestine. A Welch two sample t test was used to compare the ultrasonographic measurements on transverse and longitudinal planes. P values < 0.05 were considered statistically significant.

Bland–Altman analysis was used to assess the limit of agreement between the ultrasonographic and histopathological measurements of the layers of the duodenum, jejunum, and ileum on the longitudinal plane and for the ileocecocolic junction on the transverse plane. An agreement was considered ‘good’ if 95% of the absolute differences were within two SDs ($SD \pm 1.96$).

Results

Ultrasonographic measurements

The ultrasonographic measurements of the different layers and overall wall thickness in the longitudinal and transverse planes of the duodenum, jejunum, ileum, and distal ileum (ileocecocolic junction) are summarised in Table 1. No statistical difference was found for overall wall thickness in the duodenum, jejunum, and ileum in the transverse and longitudinal planes. However, there were differences ($P < 0.05$) between the measurements obtained in the transverse and longitudinal planes of the distal ileum. The distal ileum at the level of the fold had the thickest wall, followed by the ileum, duodenum, and jejunum, respectively.

When each layer was considered, the mucosal layer was thicker ($P < 0.05$) than the submucosa, muscularis, and serosal layers in all intestinal segments, except for the distal ileum. The mucosa was thicker ($P < 0.05$) in the duodenum compared to the other segments and decreased progressively along the different portions of the intestine.

The muscularis layer was thicker ($P < 0.05$) than the submucosa and serosal layers in all segments except the distal ileum. In addition, the muscularis layer in the ileum was thicker ($P < 0.05$) than the duodenum and jejunum. The submucosal layer was thicker ($P < 0.05$) than the serosal layer at each intestinal segment. The submucosal layer was the thickest layer in the distal ileum at the level of the fold. The measurements of the serosal layer were constant along the different segments but thinner ($P < 0.05$) in the duodenum than in the ileum.

Histological measurements

None of the intestinal samples showed histological abnormalities. Histological measurements of the different layers and the overall wall thickness of each intestinal segment are shown in Table 2. The distal ileum at the level of the fold was the thickest