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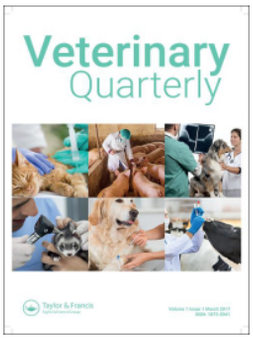

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Ultrasonographic features of gallbladder wall thickening in dogs with hypoalbuminemia

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ABSTRACT

Hypoalbuminemia has been considered as a cause of gallbladder wall thickening in humans and dogs. Recent study revealed that gallbladder wall thickening in dogs with hypoalbuminemia may not be associated with serum albumin/plasma levels within 48 h of ultrasound study. However, gallbladder wall edema may change within 48 h, and the ultrasonographic features of gallbladder wall thickening in dogs with hypoalbuminemia have not been reported. The purpose of this study is to describe the relationship between serum albumin levels within 24 h of ultrasound and gallbladder wall thickening, and to describe the ultrasonographic features of thickened gallbladder walls in dogs with hypoalbuminemia. 37 hypoalbuminemic dogs with gallbladder ultrasound images were retrospectively included. Ultrasound studies were reviewed, and gallbladder wall thickness, layering appearance, echogenicity, echotexture, distribution, evidence of gallbladder mucocele, and presence of peritoneal effusion were recorded. Additionally, serum albumin levels within 24 h of ultrasound study and the administerd sedation were recorded. The prevalence of gallbladder wall thickening in dogs with hypoalbuminemia was 13.5%. The 3-layer appearance of the gallbladder wall was observed in 4 dogs, and a single-layer gallbladder wall thickening in one dog. Diffuse thickening was observed in all 5 dogs. The serum albumin level of dogs with gallbladder wall thickening was not different ($p = 0.14$) from dogs without thickening. Gallbladder wall thickening was not common, occurring only with mild hypoalbuminemia, and was commonly associated with a 3-layer appearance and considered as gallbladder wall subserosal edema. Causes other than hypoalbuminemia may be responsible for thickening of the gallbladder wall in dogs with hypoalbuminemia.

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Gallbladder wall edema; canine; hypoproteinemia; ultrasound

Introduction

Gallbladders in both humans and dogs are usually evaluated by ultrasonography. In humans, diffuse gallbladder wall thickening can be observed ultrasonographically in a wide range of gallbladder diseases (e.g. cholecystitis), and extracholecystic conditions, such as liver disease, portal hypertension, extracholecystic inflammation (e.g. acute hepatitis, pancreatitis, or peritonitis), right-sided heart failure, renal failure, neoplasia, adenomyomatosis, sedation-induced (Seitz et al. 2021; Bini et al. 2022) or hypoalbuminemia (Fiske et al. 1980; Runner et al. 2014). Diffuse gallbladder wall thickening has also been reported in dogs with cholecystitis (Bargellini et al. 2016), infectious canine hepatitis (De Jonge et al. 2020), shock induced by intravenous administration of heartworm extract (Kitoh et al. 1998), leptospirosis (Sonet et al. 2018), anaphylaxis (Quantz et al. 2009; Haworth et al. 2019), right-sided heart disease (Vientos-Plotts et al. 2019; Lisciandro et al. 2021), and sedation-induced (Seitz et al. 2021; Bini et al. 2022). Other possible causes and disease associated with gallbladder wall thickening in dogs include cystic mucosal hyperplasia, hepatopathy, hepatobiliary neoplasia, pancreatitis, chronic bile duct obstruction, renal failure, overhydration, recent transfusion, immune-mediated disease, hypoalbuminemia, hypovolemic shock, systemic inflammatory response syndrome, sepsis, and disseminated intravascular coagulation (Spaulding 1993; Woodworth et al. 2021). Hypoalbuminemia is known to cause edema of multiple organs, including gastrointestinal wall edema (Peterson and Willard 2003; Murakami et al. 2019, 2020, 2022) and pitting edema of the limbs, scrotum, or face (Dossin and Lavoue 2011).

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This work has orally presented at European College of Veterinary Diagnostic Imaging Online Conference 2020.

Gallbladder wall thickening secondary to hypoalbuminemia is speculated to be gallbladder wall edema due to decreased oncotic pressure, which is associated with serum albumin levels (Fiske et al. 1980). However, there are few publications on gallbladder wall thickening due to hypoalbuminemia in dogs (Spaulding 1993; Sparago et al. 2021). Recently, one publication investigated the relationship between gallbladder wall thickening and serum/plasma albumin levels in dogs, but it did not discuss gallbladder wall edema or the ultrasonographic appearance of gallbladder wall thickening (Sparago et al. 2021). Additionally, the serum/plasma albumin levels reported in the previous study were measured within 48 h of the ultrasound study, and gallbladder wall edema may resolve or change within 48 h based on the acute changes of gastric wall edema reported on the previous studies (Murakami et al. 2019, 2020). The ultrasonographic characteristics of gallbladder wall edema are considered to be a 3-layer appearance with thin inner and outer hyperechoic layers and a thick middle hypoechoic layer (Teefey et al. 1991; Lisciandro et al. 2021). Thus, the aims of this retrospective study are to describe the relationship between serum albumin levels within 24 h of ultrasound and gallbladder wall thickening, and further describe the ultrasonographic features of thickened gallbladder walls in dogs with hypoalbuminemia.

Materials and methods

Experimental design

This is a retrospective case series study.

Case selection

The Purdue University Veterinary Teaching Hospital Medical Record database from 1 April 2018, through 31 March 2019, was electronically searched to identify dogs that had both hypoalbuminemia (<2.3 g/dl) and abdominal ultrasound performed within 24 h from the blood sample collection. All ultrasonographic studies were performed by board-certified veterinary radiologists and radiology residents using a same ultrasound machine (Aplio™ i800, Canon Medical Systems USA, Inc., Tustin, CA). A linear array (5–18 MHz) or microconvex array (4–11 MHz) transducer was used. Sedation used during the ultrasound study was recorded. Case histories were reviewed by a board certified internist (S.S.) and causes of hypoalbuminemia were classified into following categories: hypoalbuminemia secondary to gastrointestinal disease including protein losing enteropathy, protein losing nephropathy, inflammatory or septic process, liver failure, neoplastic disease, or other (unknown cause or combination of diseases makes it difficult to assign category). Histopathological findings of the gallbladder were recorded when available. Dogs with suboptimal recorded ultrasound image quality of the gallbladder wall were excluded from this study.

Ultrasonographic findings

Ultrasonographic still images of the gallbladder wall in the form of digital files were reviewed by board-certified radiologists (M.M. and H.G.H.) using an image viewing workstation (Asteris Keystone viewer, Asteris Inc., Stephentown, NY).

Several references provide ranges for normal gallbladder wall thickness in dogs, with values ranging from 2 mm (Kealy et al. 2011; Schiborra et al. 2017) to up to 3 mm (Spaulding 1993). Using these references, we separated the dogs into three groups based on their gallbladder wall thickness: Group A included dogs with thickness less than or equal to 2 mm, which was considered normal; Dogs with thickness greater than 2 mm and less than 3 mm were included in Group B, indicating possible gallbladder wall thickening (Kealy et al. 2011; Schiborra et al. 2017); Group C included dogs with thickness greater than 3 mm, which was considered gallbladder wall thickening (Spaulding 1993; Kealy et al. 2011; Schiborra et al. 2017).

The following criteria of the ultrasonographic gallbladder wall changes were evaluated: (i) thickness (mm), (ii) layering appearance (single or more than 1 layer, number of layers described if more than 1 layer), (iii) echogenicity of the layer(s) (hypoechoic, isoechoic or hyperechoic), (iv) echotexture of the layers (homogeneous or heterogeneous), (v) distribution of lesion (diffuse vs. focal), and (vi) ultrasonographic evidence of gallbladder mucocele (yes or no), and vii) presence of peritoneal effusion (yes or no).

Gallbladder wall thickness was measured at the thickest part perpendicular to the gallbladder wall from the most inner part to the most outer part of the wall. The inability differentiating gallbladder intraluminal hypoechoic or hyperechoic content from the gallbladder wall was excluded from wall thickness measurement. The echogenicity of individual layer thickness was described as hypoechoic, isoechoic, and hypoechoic relative to adjacent hepatic parenchyma. The echotexture was further classified as homogeneous or heterogeneous. The distribution of the abnormal gallbladder wall thickening such as diffuse or focal was noted. The presence or absence of ultrasonographic evidence supporting a gallbladder mucocele was evaluated based on the previous publications (Besso et al. 2000; Choi et al. 2014). The presence or absence of peritoneal effusion was recorded.

Data analysis

Relationships between serum albumin levels of dogs and gallbladder wall thickness were analyzed using Pearson's correlation model. Statistical significance was set at $p < 0.05$.

The prevalences of gallbladder wall thickening (more than 2 mm; groups B and C, and more than 3 mm; group C) in dogs with hypoalbuminemia were calculated using the following formula: Prevalence (%) = (number of dogs with hypoalbuminemia and

ultrasonographic gallbladder wall thickening/total number of dogs with hypoalbuminemia) × 100 (%). The mean serum albumin level of dogs with ultrasonographic gallbladder wall thickening was compared with the mean serum albumin level of dogs without ultrasonographic evidence of gallbladder wall thickening, and analyzed statistically using the Mann-Whitney *U* test.

Results

A total of 44 dogs had an abdominal ultrasound within 24 h from blood sample collection with the diagnosis of hypoalbuminemia (<2.3 g/dl). One dog was excluded from the present study due to previous cholecystectomy, and 6 dogs were excluded due to suboptimal ultrasound image quality or missing gallbladder wall images. In the rest of the dogs, gallbladder mucocele or intraluminal gallbladder contents did not affect the evaluation of the gallbladder wall. After exclusion, 37 dogs were included in the present study. During ultrasound, sedation was administered to a total of 31 dogs. The sedation protocol involved the administration of dexmedetomidine in 12 dogs, butorphanol in 26 dogs, midazolam in 4 dogs, hydromorphone in 2 dogs, and acepromazine in 1 dog.

Gastrointestinal disease was the cause of hypoalbuminemia in 9 dogs and was the most common cause, followed by inflammatory or septic process in 8 dogs, protein losing nephropathy in 6 dogs, neoplastic disease in 5 dogs, and liver failure in 2 dogs. The rest of 7 dogs were categorized as other and a single specific cause of hypoalbuminemia could not be determined.

The mean gallbladder wall thickness in all 37 dogs was 1.19 ± 0.70; 0.4–3.4 (mean ± SD; range). Thirty-two dogs were classified into Group A (normal), 3 dogs in Group B (possible gallbladder wall thickening; gallbladder wall thicknesses of 2.2, 2.4, and 2.6 mm), and 2 dogs in Group C (gallbladder wall thickening; gallbladder wall thicknesses of 3.3 and 3.4 mm). Using 3 mm as a cut-off for gallbladder thickening (Group C), the prevalence of gallbladder wall thickening in dogs with hypoalbuminemia was 5.4% (2/37 dogs). When using 2 mm as a cut-off (Groups B and C), the prevalence was 13.5% (5/37 dogs). In these 5 dogs, with gallbladder wall thickness more than 2 mm, the final diagnoses associated with the liver or the gallbladder were as follows: hepatic metastatic carcinoma (1), caudal vena cava obstruction due to neoplasia associated thrombosis with hepatic congestion (1), lymphoma (2), and cholecystitis (1). One of the lymphoma dogs had confirmed liver infiltrates. The other lymphoma dog was diagnosed as multicentric lymphoma based on fine needle aspiration of enlarged lymph nodes, and hepatic infiltration of lymphoma was suspected due to hepatomegaly; however, histological confirmation of the liver was not performed. Causes of hypoalbuminemia in these 5 dogs were neoplastic disease in 2 dogs, inflammatory disease in 1 dog, and other in 2 dogs. One of the dogs classified under the "other" category exhibited caudal vena cava obstruction due to neoplasia associated thrombosis, accompanied by panhypoproteinemia. However, further investigation into the precise cause of hypoalbuminemia was not conducted for this particular dog. Another dog classified under the "other" category had lymphoma but also presented with sepsis and acute kidney injury. The exact cause of hypoalbuminemia in this dog remains unknown.

The serum albumin level of the dogs in Group A (1.82 ± 0.33 g/dl) and the dogs in Groups B and C (2.10 ± 0.17 g/dl) was not significantly different ($p = 0.14$; Figure 1). The mean serum albumin levels of dogs in Group B and Group C were 2.07 and 2.15 g/dl, respectively. There was no correlation between serum albumin levels and gallbladder wall thickness ($p = 0.12$; Figure 1).

In all dogs with normal gallbladder wall thickness (Group A), the gallbladder wall was single-layer and homogeneously hyperechoic. There was ultrasonographic evidence of a gallbladder mucocele in 5 dogs and the presence of peritoneal effusion in 13 dogs.

In Group B, a single-layer homogeneously hyperechoic gallbladder wall was present in one dog (Figure 2A) and a 3-layer appearance with thin inner and outer hyperechoic layers and a thicker central hypoechoic layer was present in the other two dogs. In Group C, a 3-layer appearance with thin inner and outer hyperechoic layers and a thicker central hypoechoic layer (Figure 2B) was present in the both dogs. One dog with the 3-layer appearance in Group C also had hyperechoic lacy striations within the central hypoechoic layer (Figure 2C). All 5 dogs in Groups B and C had diffuse thickening of the gallbladder wall. None of these 5 dogs had ultrasonographic evidence of a gallbladder mucocele and 4 dogs had peritoneal effusion. The ultrasonographic characteristics, clinical information, as well as the sedation used

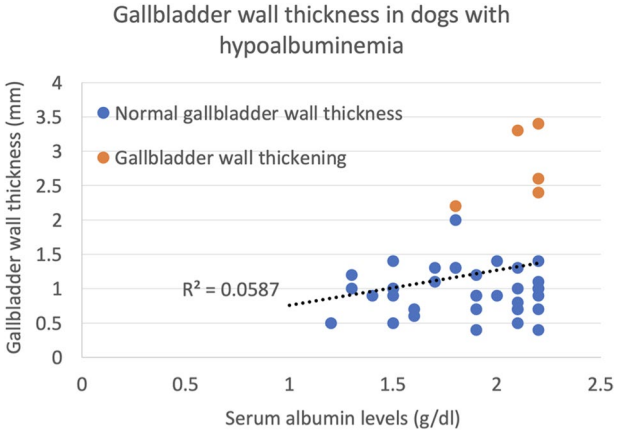

Figure 1. Gallbladder wall thickness in dogs with hypoalbuminemia. The serum albumin level of the dogs with normal gallbladder wall thickness (<2 mm; Group A) was shown in blue dots and with gallbladder wall thickening (Group B and C) was shown in orange dots. The serum albumin level of the dogs in Group A (1.82 ± 0.33 g/dl) and the dogs in Groups B and C (2.10 ± 0.17 g/dl) was not significantly different ($p = 0.14$). No correlation was present between the serum albumin level and the gallbladder wall thickness ($p = 0.12$).

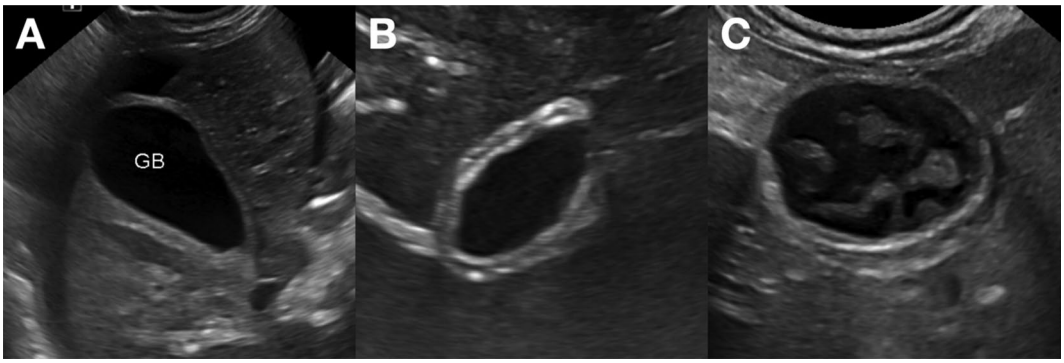

Figure 2. Ultrasound images of gallbladder wall thickening in dogs with hypoalbuminemia. (A) A mild and diffuse homogeneously hyperechoic gallbladder wall thickening is present. This dog was diagnosed with lymphoma. (B) A mild and diffuse gallbladder wall thickening with a 3-layer appearance. There are thin inner and outer hyperechoic layers and a central hypoechoic layer. This dog was diagnosed with hepatic metastatic carcinoma. (C) A mild and diffuse gallbladder wall thickening with a 3-layer appearance. Hyperechoic lacy striations are present within the central hypoechoic layer. This dog was diagnosed with the caudal vena cava obstruction due to neoplasia associated thrombosis with hepatic congestion and ascites.

during the ultrasound study for dogs with gallbladder wall thickening (>2 mm: Groups B and C) and hypoalbuminemia were shown in Table 1.

One dog with gallbladder wall thickening (2.2 mm) underwent necropsy a day after the ultrasound study. However, no information regarding the gallbladder was mentioned in the necropsy report.

Discussion

This study describes the relationship between serum albumin levels within 24 h of ultrasonographic evidence and features of gallbladder wall thickening in dogs with hypoalbuminemia. Additionally, the study describes the ultrasonographic features of gallbladder wall thickening in these dogs. Gallbladder wall thickening in dogs with hypoalbuminemia was a rare finding in this study, estimated to be between 5.4 and 13.5% (with 5.4% having obvious gallbladder wall thickening of >3 mm and 13.5% having possible gallbladder wall thickening of >2 mm). However, of the dogs with gallbladder wall thickening and hypoalbuminemia, 80% (4/5) had ultrasonographic features of gallbladder wall edema. Thus, some of the gallbladder wall thickening in dogs with hypoalbuminemia is considered to be subserosal edema. However, the causal relationship between the edema and hypoalbuminemia was not confirmed because there is no correlation between serum albumin level and gallbladder wall thickening.

Ultrasound is commonly used to measure gallbladder wall thickness in dogs, with several reference values reported at 1–2 mm (Kealy et al. 2011) or 2–3 mm (Spaulding 1993). However, accurately defining gallbladder wall thickening using ultrasound can be challenging due to the variable range of normal wall thickness, which may be influenced by the flexibility of the gallbladder wall. To address this issue, we classified our dogs into Group B (wall thickness 2–3 mm as possible gallbladder wall thickening) and C (wall thickness greater than 3 mm as obvious gallbladder wall thickening) according to different references. In humans, gallbladder volume and wall thickness are combined to evaluate gallbladder wall thickness because of the flexibility of the gallbladder wall (Handler 1979), and further studies may be needed to establish normal gallbladder wall thickness in dogs, taking into account gallbladder volume, for more accurate evaluation of gallbladder wall thickening. Due to the retrospective nature of the present study, accurate evaluation of gallbladder volume was not possible, and we did not consider it for analysis. However, we observed a 3-layer appearance, with outer and inner hyperechoic layers and a central hypoechoic layer, in four dogs with gallbladder wall thickening greater than 2 mm. This finding is similar to the reported gallbladder wall subserosal edema observed on ultrasound (Teefey et al. 1991; Lisciandro et al. 2021) and CT (van Breda Vriesman et al. 2007), indicating the presence of pathological gallbladder wall thickening, even when the thickness is less than 3 mm in some dogs. Conversely, we did not observe a 3-layer appearance in any dogs with gallbladder wall thickness less than 2 mm.

Gallbladder wall thickening due to hypoalbuminemia was first reported in humans (Fiske et al. 1980) and subsequently extrapolated to dogs (Spaulding 1993). However, a recent publication revealed that there is no correlation between serum/plasma albumin levels and gallbladder wall thickness in dogs with hypoalbuminemia, and 24.3% of dogs with severe hypoalbuminemia (<1.5 g/dL) showed gallbladder wall thickening (>2 mm) (Sparago et al. 2021). Notably, in that study, serum/plasma albumin levels were measured within 48 h of the ultrasound study (Sparago et al. 2021). Based on previous reports of gastric wall edema in hypoalbuminemic dogs (Murakami et al. 2019, 2020), gallbladder wall edema may resolve or change within this timeframe. In the present study, we used stricter inclusion criteria, selecting cases with serum albumin levels measured within 24 h of the ultrasound study, which limits the number of cases included in the study. Our results showed similar findings to the previous publication (Sparago et al. 2021), with no correlation observed between gallbladder wall thickness

Table 1. Ultrasonographic characterics and other information of the dogs with gallbladder wall thickening (>2 mm) and hypoalbuminemia.

Dog #	Group	Serum albumin level (g/dl)	GB wall thickness (mm)	# of layers	Ultrasonographic appearance	Lacy appearance	GB mucocele	Peritoneal effusion	Diagnosis	Causes of hypoalbuminemia	Sedation used
1	B	2.2	2.6	3-layer	Outer and inner hyperechoic and central hypoechoic layers	No	No	Yes	Lymphoma	Neoplastic disease	Butorphanol
2	B	2.2	2.4	3-layer	Outer and inner hyperechoic and central hypoechoic layers	No	No	Yes	Hepatic metastatic carcinoma	Neoplastic disease	Dexmedetomidine
3	B	1.8	2.2	1-layer	Hyperechoic layer	No	No	No	Lymphoma	Other	No
4	C	2.2	3.4	3-layer	Outer and inner hyperechoic and central hypoechoic layers	Hyperechoic lacy striations within central layer	No	Yes	Caudal vena cava obstruction	Other	No
5	C	2.1	3.3	3-layer	Outer and inner hyperechoic and central hypoechoic layers	No	No	Yes	Cholecystitis	Inflammatory disease or sepsis	No

and serum albumin levels. Moreover, 13.5% of dogs with hypoalbuminemia (<2.3 g/dL) exhibited possible gallbladder wall thickening (>2 mm), indicating that while hypoalbuminemia may not directly cause gallbladder wall thickening, it may be associated with other causes of gallbladder wall thickening in dogs.

When performing an ultrasound of the gallbladder, a single-layer thickening of the gallbladder wall may indicate cholecystitis (Tamborini et al. 2016), neoplasia (MacLeod et al. 2023), or physiologic thickening resulting from partial wall contracture (Spaulding 1993). Gallbladder wall subserosal edema is considered when there is a 3-layer appearance, with thin inner and outer layers and a thick hypoechoic middle layer (Spaulding 1993). Since a small amount of peritoneal effusion around the gallbladder can sometimes mimic gallbladder wall edema (Spaulding 1993), we only classified cases with diffuse circumferential gallbladder wall thickening and an obvious 3-layer appearance as having 3-layer wall thickening, indicative of gallbladder wall subserosal edema.

In dogs, subserosal edema of the gallbladder wall is known to be caused by inflammation (Bargellini et al. 2016; Sonet et al. 2018), allergic reactions (Kitoh et al. 1998; Quantz et al. 2009; Haworth et al. 2019; Seitz et al. 2021; Bini et al. 2022), or congestion (Vientos-Plotts et al. 2019; Lisciandro et al. 2021). Hypoalbuminemia is also considered a possible cause of gallbladder wall edema, as it is reported in humans (Fiske et al. 1980; Runner et al. 2014), and also commonly causes edema in other organs in dogs (Peterson and Willard 2003; Dossin and Lavoue 2011; Murakami et al. 2019, 2020, 2022). In a previous study, gallbladder wall thickening was observed in dogs with hypoalbuminemia, but the ultrasonographic appearance of the thickening was not described (Sparago et al. 2021). In the present study, 4 out of 5 hypoalbuminemic dogs with gallbladder wall thickening showed diffuse circumferential wall thickening with a 3-layer appearance, indicative of gallbladder wall edema.

In dogs with 3-layer gallbladder wall thickening observed in the present study, one dog had cholecystitis, which is known to cause gallbladder wall thickening (Teefey et al. 1991; Bargellini et al. 2016). Three other dogs with gallbladder wall thickening had diseases associated with the liver (lymphoma infiltration to the liver, hepatic metastatic carcinoma, and hepatic congestion from caval obstruction due to neoplasia associated thrombosis). One dog had multicentric lymphoma, and hepatic infiltration of lymphoma was suspected due to hepatomegaly; however, histological confirmation of the liver was not performed. None of the dogs with gallbladder wall thickening were categorized as having gastrointestinal diseases including protein-losing enteropathy, protein-losing nephropathy, or liver failure, as the causes of hypoalbuminemia. In the present study, most of the dogs received some kind of sedation during the ultrasound study, including

dexmedetomidine, which is known to cause gallbladder wall edema (Seitz et al. 2021; Bini et al. 2022). Dexmedetomidine was administered during the ultrasound study in 12 dogs in the present study. This may contribute to the presence of gallbladder wall edema: however, possible gallbladder wall thickening was observed only in one dog in Group B with dexmedetomidine administration. Additionally, the thickness of gallbladder wall and serum albumin levels were not correlated in the present study or the previous study (Sparago et al. 2021). Moreover, the serum albumin level in dogs with suspected gallbladder wall edema was not significantly different from the levels observed in dogs without gallbladder wall thickening in the present study. Therefore, the causes of gallbladder wall edema in the present study may not be solely due to hypoalbuminemia and could be attributed to other factors, especially a primary hepatobiliary disease or dexmedetomidine administration.

In some cases of gangrenous cholecystitis in humans, hyperechoic striations have been observed in the thickened gallbladder wall (Teefey et al. 1991). The cause of these striations is unknown, but they have been suggested to be associated with necrosis of the gallbladder wall due to severe cholecystitis (Teefey et al. 1991). In this study, we observed a hyperechoic lacy appearance in the thickened gallbladder wall of a dog with cholecystitis, which is similar to the reported striated gallbladder wall thickening in humans.

Although a necropsy was performed on one dog that exhibited gallbladder wall thickening on ultrasound, we were unable to histologically confirm this thickening, which was likely due to autolysis of the gallbladder mucosa and lacked cytomorphologic detail. This is not an uncommon occurrence in necropsy cases, as the gallbladder epithelium is susceptible to bile-associated autolysis (Argon et al. 2013).

In conclusion, gallbladder wall thickening of more than 2 mm, especially with a 3-layer appearance on ultrasound, was considered as gallbladder wall edema. However, gallbladder wall thickening/edema was not found to be correlated with serum albumin levels, and dogs with gallbladder wall thickening also had concurrent hepatobiliary diseases. Therefore, other factors, such as hepatobiliary disease, may be responsible for gallbladder wall thickening, and hypoalbuminemia may not be the sole cause of gallbladder wall thickening. Further investigation of the pathogenesis of gallbladder wall edema in dogs with hypoalbuminemia is needed, and careful consideration of these findings is necessary for clinical understanding.

Disclosure Statement

Authors declare no conflict of interest.

Off-Label Antimicrobial Declaration

Authors declare no off-label use of antimicrobials.

Institutional Animal Care and Use Committee (IACUC) or Other Approval Declaration

Authors declare IACUC approval was not needed due to the retrospective nature of the study.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author, MM, upon reasonable request.

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