The standard technique for cholecystectomy requires dissection of the gallbladder from the hepatic fossa in the quadrate lobe.\textsuperscript{1–5} The dissection is carried out between the wall of the gallbladder and the liver parenchyma. The subserosal layer dissection (SLD) technique has recently been reported as an option for cholecystectomy in humans.\textsuperscript{6–8} In the SLD technique, the gallbladder is dissected within the subserosal layer within a cystic plate.\textsuperscript{9,10} Examination of the thickness of the different components of gallbladder wall in cases with mucocele and/or cholecystitis

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OBJECTIVE
To assess the thickness of each layer of the gallbladder wall with different diseases in dogs.

SAMPLE
72 gallbladders.

METHODS
Retrospective study of dogs that underwent cholecystectomy. Histopathological specimens of the gallbladders were reviewed. Histopathological diagnosis was made as gallbladder mucocele or cholecystitis, and cholecystitis was further categorized into chronic cholecystitis, acute-on-chronic cholecystitis, acute cholecystitis, and necrotic cholecystitis. The thickness of each layer of the gallbladder wall was measured.

RESULTS
22 dogs were diagnosed with gallbladder mucocele without cholecystitis, 24 with gallbladder mucocele and cholecystitis, 20 with only cholecystitis, and 6 as normal. Histopathological subclassification of cholecystitis in 44 gallbladders led to diagnosis of chronic cholecystitis in 21 gallbladders, acute-on-chronic cholecystitis in 10 gallbladders, acute cholecystitis in 6 gallbladders, and necrotic cholecystitis in 7 gallbladders. The thickness of the entire wall of the gallbladder ($P < .0001$) and the thickness of the mucosa ($P < .0001$) and subserosa ($P < .0001$) were affected by the different disease processes.

CLINICAL RELEVANCE
Layers of the gallbladder wall were affected by diseases present in the gallbladder. It resulted in a difference in the thickness of the wall of the gallbladder among the gallbladder diseases in this study. Histopathological changes should be taken into consideration before surgery while deciding what technique to use to perform a cholecystectomy.

Keywords: gallbladder, histopathology, dog, thickness, cholecystectomy

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To the author’s knowledge, the histopathological change within the gallbladder wall and variation of the thickness of the different layers of the gallbladder wall with the disease process in dogs have not been well documented.

Therefore, the objective of this study is to assess the thickness of each layer of the gallbladder with different diseases in dogs.

We hypothesized the wall thickness of the different layers of the gallbladder is not affected by diseases present in the gallbladder.

## Methods

### Gallbladders

Six normal gallbladders without gross abnormalities were collected from dogs without a history of biliary disease who died for reasons unrelated to this study. Electronic medical records at Colorado State University Veterinary Teaching Hospital were searched for all dogs that had cholecystectomy between May 2015 and January 2022, and they were included in this study. The gallbladders were included only when cholecystectomy was performed with the standard technique. The histopathological slides had to be available for review by the authors. The gallbladders with tumors or polyps were excluded from this study.

### Clinical data

Information collected from medical records included breed; gender; age; body weight; clinical signs; duration of clinical signs; preoperative CBC, bilirubin, ALP, ALT, and GGT; ultrasound diagnosis; preoperative medical treatment; duration of the preoperative medical treatment; laparotomy or laparoscopy; elective surgery or emergency surgery; and bacterial culture (aerobic and anaerobic).

### Gallbladder histopathological diagnosis and gallbladder wall thickness

The gallbladder histopathological slides were reviewed by a boarded pathologist. Tissue samples were stained with hematoxylin and eosin. The histopathology samples were scanned and reviewed using the Philips IntelliSite Pathology Solution (Philips North America Corporation), and histopathological diagnosis was made retrospectively (gallbladder mucocele, cholecystitis). Cholecystitis was further categorized into subgroups. Chronic cholecystitis was characterized by wall thickening by fibrosis and the presence of chronic inflammatory cells only without neutrophils. Acute chronic cholecystitis was characterized by superimposition of neutrophilic infiltration on chronic cholecystitis. Acute cholecystitis was characterized by acute inflammation without significant fibrous thickening of the gallbladder wall. Necrotic cholecystitis was defined when acute inflammation was associated with evidence of necrosis of the gallbladder wall. When cholecystitis was observed with a mucocele, the case was entered into the cholecystitis group according to their degree of severity. The degree of inflammation was histopathologically graded based on the number of inflammatory cells in the mucosa and the subserosa: mild, not more than 10 inflammatory cells at an area of 0.237 mm$^2$; moderate, between 11 and 30 inflammatory cells at an area of 0.237 mm$^2$; and severe, more than 31 inflammatory cells at an area of 0.237 mm$^2$. Hemorrhage, fibrosis, and epithelial hyperplasia were assessed as the other histopathological variables in this study. Hemorrhage in the mucosa and the subserosa was graded as mild (RBC in 20% or less than 20% of the mucosal and the subserosal layer of whole section), moderate (RBC in 21% to 70% of the mucosal and the subserosal layer of whole section), and severe (RBC in more than 70% of the mucosal and the subserosal layer of whole section). Fibrosis in the mucosa and the subserosa was graded as mild (collagen deposition in 20% or less than 20% of the mucosal and the subserosal layer of whole section), moderate (collagen deposition in 21% to 70% of the mucosal and the subserosal layer of whole section), and severe (collagen deposition in more than 70% of the mucosal and the subserosal layer of whole section). The thickness of each layer of the gallbladder wall was measured.

Grading of inflammation, hemorrhage, and fibrosis and measurement of the thickness of each layer were performed at randomly selected 10 different sites sliced in the most nearly perpendicular to the wall, and the mean value was calculated from these measurements.

### Statistical analyses

The Shapiro-Wilk test was used to assess the distribution of the thickness of each layer of the gallbladder mucosa, subserosa, and total gallbladder wall. The thickness of each layer of the gallbladder was not normally distributed and was analyzed using Wilcoxon rank-sum test to compare median thickness among normal gallbladder, gallbladder mucocele, chronic cholecystitis, acute-on-chronic cholecystitis, acute cholecystitis, and necrotic cholecystitis. A $\chi^2$-analysis was used to compare the distribution of severity of fibrosis in the mucosal and the subserosal layer between the histopathological diagnosis and also the hyperplasia of the epithelium among the different histopathological diagnoses. The data are presented as median (range). A $P$ value less than .05 was considered statistically significant. All analyses were performed with JMP (SAS Institute).

### Results

#### Gallbladders

The histology of 6 gallbladders was reviewed from dogs without a history of biliary disease. The histopathology of 66 gallbladders was reviewed from dogs that required a cholecystectomy at Colorado State University Veterinary Teaching Hospital.
All the cholecystectomies were performed with a standard technique.

**Clinical data**

Breeds included mix breed (n = 9), Chihuahua (5), Labrador Retriever (5), Shetland Sheepdog (5), Cocker Spaniel (3), German Shepherd Dog (3), Miniature Schnauzer (3), Pug (3), Australian Shepherd (2), Border Collie (2), Border Terrier (2), Dachshund (2), Pomeranian (2), American Pit Bull Terrier (1), Australian Heeler (1), Belgian Malinois (1), Bernese Mountain Dog (1), Brittany Spaniel (1), Cavalier King Charles Spaniel (1), Exotic Canine (1), Jack Russell Terrier (1), Maltese (1), Miniature Pinscher (1), Miniature Poodle (1), Papillon (1), Scottish Terrier (1), Shiba Inu (1), Shih Tzu (1), Silky Terrier (1), Soft-Coated Wheaten Terrier (1), Standard Poodle (1), Tolling Dog (1), and West Highland White Terrier (1).

Thirty-two dogs were spayed female, 27 dogs were castrated male, 6 dogs were intact male, and 1 dog was intact female. The age of dogs was 10 years (range, 1 to 15 years). The weight of the dogs was 9.5 kg (range, 1.6 to 43.8 kg). Clinical signs at presentation included vomiting (n = 20), anorexia (17), lethargy (9), diarrhea (1), abdominal pain (1), pyrexia (1), retching (1), distended abdomen (1), tachypnea (1), ataxia (1), and collapse (1). Thirty-two dogs (48%) had no clinical signs. Duration of clinical signs was 2 days (range, 1 to 28 days).

Preoperative blood work was available in 58 dogs (Supplementary Table S1).

Abdominal ultrasound was available in 40 dogs. Ultrasound diagnosis included gallbladder mucocele (n = 21), gallbladder mucocele and cholecystitis (10), cholelithiasis (4), gallbladder mucocele and mucous hyperplasia (2), cholecystitis (2), cholecystitis and mucous hyperplasia (1). Medical treatment at presentation included ursodiol (n = 17), denamarin (4), amoxicillin (1), enrofloxacin (1), and metronidazole (1). Duration of medical treatment was 35 days (range, 3 to 750 days). Forty-eight dogs (72%) had no medical treatment before surgery. Thirty-five dogs had elective surgery and 31 dogs had emergency surgery because of biliary obstruction. Forty-six dogs underwent open laparotomy, and 20 dogs were treated with laparoscopy, of these 6 dogs were converted to an open procedure. Within the cases treated with laparoscopy, 6 had a mucocele and 8 had chronic cholecystitis. Within the 46 dogs treated with laparotomy, 16 had a mucocele, 10 had chronic cholecystitis, 9 had acute-on-chronic cholecystitis, 5 had acute cholecystitis, and 6 had necrotic cholecystitis. Within the 6 cases converted to laparotomy, 3 had chronic cholecystitis, 1 had acute cholecystitis, 1 had acute-on-chronic cholecystitis, and 1 had necrotic cholecystitis. Distribution of the disease processes was different between the cases treated with laparoscopy and laparotomy (P = 0.014).

Bacterial culture results were available in 59 dogs, and the culture result was positive in 19 dogs (32%). It included *Escherichia coli* (6 cases), *Enterococcus* (6 cases), coagulase-negative *Staphylococcus* (3 cases), *Streptococcus* (2 cases), *Clostridium* (2 cases), *Klebsiella* (1 case), *Corynebacterium* (1 case), and *Bacillus* (1 case).

**Gallbladder histopathological diagnosis and gallbladder wall thickness**

Twenty-two dogs were diagnosed with gallbladder mucocele without cholecystitis, 24 with gallbladder mucocele and cholecystitis, and 20 with only cholecystitis. Six gallbladders were normal.

Histopathological subclassification of cholecystitis in 44 gallbladders led to diagnosis of chronic cholecystitis in 21 gallbladders, acute-on-chronic cholecystitis in 10 gallbladders, acute cholecystitis in 6 gallbladders, and necrotic cholecystitis in 7 gallbladders (Figure 1). Cholecystitis was ranked as mild in 18 cases, moderate in 17 cases, and severe in 9 cases. Lymphocytes and plasmacytes were the main cell populations in the acute, chronic, and acute-on-chronic cholecystitis in all the layers of the gallbladder wall, while macrophages and neutrophils were predominant in necrotic cholecystitis. The degree and the location of inflammation were as follows: for chronic cholecystitis: 12 mild, 5 moderate, and 3 severe cases in the mucosa and 17 mild cases and 1 moderate case in the subserosa; for acute-on-chronic cholecystitis: 1 mild case, 6 moderate cases, and 1 severe case in the mucosa and 6 mild cases, 3 moderate cases, and 1 severe case in the subserosa; for acute cholecystitis: 3 mild and 2 moderate cases in the mucosa and 2 mild, 2 moderate, and 2 severe cases in the subserosa; and for necrotic cholecystitis: 4 moderate and 2 severe cases in the mucosa and 5 moderate and 2 severe cases in the subserosa. The degree and the location of hemorrhage were as follows: for gallbladder mucocele: 6 mild cases in the mucosa and 5 mild cases in the subserosa; for chronic cholecystitis: 19 mild cases and 1 moderate case in the mucosa and 16 mild and 2 moderate cases in the subserosa; for acute-on-chronic cholecystitis: 6 mild and 2 moderate cases in the mucosa and 7 mild and 3 severe cases in the subserosa; for acute cholecystitis: 2 mild cases, 1 moderate case, and 2 severe cases in the mucosa and 2 mild and 4 moderate cases in the subserosa; and for necrotic cholecystitis: 2 mild, 3 moderate, and 2 severe cases in the mucosa and 1 mild case, 5 moderate cases, and 1 severe case in the subserosa. The degree and the location of fibrosis were as follows: for gallbladder mucocele: 7 mild cases in the mucosa and 5 mild cases in the subserosa; for chronic cholecystitis: 6 mild, 10 moderate, and 5 severe cases in the mucosa and 7 mild cases, 8 moderate cases, and 1 severe case in the subserosa; for acute-on-chronic cholecystitis: 1 mild case, 5 moderate cases, and 2 severe cases in the mucosa and 3 mild and 6 moderate cases in the subserosa; for acute cholecystitis: 5 mild cases in the mucosa and 1 mild case in the subserosa; and for necrotic cholecystitis: 6 mild cases and 1 severe case in the mucosa and 3 mild and 3 moderate cases in the subserosa. In the subserosal layer, the degree of fibrosis was different.
between the different histopathological diagnoses \((P < .0001)\). Chronic cholecystitis was the histopathological diagnosis with a higher grade of fibrosis within the mucosa. The degree of epithelial hyperplasia was different between the histopathological diagnoses \((P < .0001)\) with the mucocele and chronic cholecystitis having the highest degree of hyperplasia.

Of the 21 cases with gallbladder mucocele on abdominal ultrasound, 12 were confirmed with only a mucocele on histopathology, 6 were confirmed with mucocele and cholecystitis, and 3 were confirmed with only cholecystitis. Of the 10 cases diagnosed with gallbladder mucocele and cholecystitis on ultrasound, 3 had only a mucocele on histopathology,
5 had a mucocele and cholecystitis, and 2 had only cholelithiasis. The 4 cases diagnosed with cholelithiasis and the 2 cases with cholecystitis diagnosed on ultrasound had cholecystitis on histopathology. Of the 2 cases with gallbladder mucocele and hyperplasia diagnosed on ultrasound, 1 had a mucocele and cholecystitis and 1 had cholecystitis on histopathology. One case with cholecystitis and hyperplasia on ultrasound had cholecystitis on histopathology.

The thickness of each layer of the gallbladder is reported (Supplementary Table S2). The thickness of the entire wall of the gallbladder \( (P < .0001) \) and the thickness of the mucosa \( (P < .0001) \) and subserosa \( (P < .0001) \) were affected by the different disease processes (Figure 2). The entire wall thickness of the gallbladder \( (P < .001) \) and the thickness of the mucosa \( (P < .0001) \) and subserosa \( (P < .0001) \) were affected by the severity of the cholecystitis (Figure 3).

**Figure 2**—Box plots of the thickness of mucosa, subserosa, and entire wall of the gallbladder in dogs with normal gallbladder, gallbladder mucocele, chronic cholecystitis, acute-on-chronic cholecystitis, acute cholecystitis, and necrotic cholecystitis. The cases in the gallbladder mucocele did not have cholecystitis. *Data points with the same superscripts are significantly different \( (P < .001) \).

**Figure 3**—Box plots of the thickness of mucosa, subserosa, and entire wall of the gallbladder in dogs with no cholecystitis (M No), mild cholecystitis (Mld), moderate cholecystitis, and severe cholecystitis. *Data points with the same superscripts are significantly different \( (P < .001) \).

**Discussion**

Layers of the gallbladder wall were affected by diseases present in the gallbladder. It resulted in a difference in the thickness of the wall of the gallbladder among the gallbladder diseases in this study. The degree of fibrosis was also different between histopathological diagnoses.

The population of dogs entered in this study is similar to the populations of dogs entered in other studies related to gallbladder surgery. Middle-aged dogs were the majority of the population. Also, small-breed dogs were more common. All the laparoscopic procedures were elective surgeries without obvious obstruction of the biliary system. More cases with cholecystitis were treated with laparotomy because most of those cases had signs of obstruction of the biliary tree at the time of presentation.
The bacterial population reported in this study is of intestinal origin, as reported with cholecystitis and cholangiohepatitis. In a retrospective study on 219 canine gallbladders, using ultrasonography a thickness of the gallbladder wall in dogs of less than 0.5 mm has been reported, which is similar to the wall thickness reported in this study. Mitsui et al. did not report the thickness of the different layers of the gallbladder wall since ultrasonography was used to measure the thickness.

Gallbladders with a mucocele without cholecystitis have a thinner wall than a normal gallbladder. The mucosa and subserosal layers were thinner than the same layers in normal gallbladders entered in this study. Mucoceles are the result of accumulation of mucus within the gallbladder resulting in a partial or complete obstruction of the cystic and or the common bile duct. The mucus is produced by the mucosa in the gallbladder. Since cholecystitis was not present, there was no lymphoplasmacytic infiltration in the mucoceles. The presence of a mucocele may stretch the wall of the gallbladder, resulting in a thinner wall when compared to normal gallbladder and gallbladder with cholecystitis.

The different types of cholecystitis affect the entire wall thickness of the gallbladder wall and mostly the subserosal layer. The necrotic cholecystitis was associated with the most profound effect on the mucosa and subserosa of the gallbladder. However, there was a lot of variability in the thickness of the mucosa when necrotic cholecystitis was present. This is more likely due to the distribution of the necrosis not being even in the entire gallbladder wall. The inflammation was lymphoplasmacytic, and neutrophils were more common with necrotic cholecystitis. In human patients, neutrophils are predominant with necrotic cholecystitis. Fibrosis in the mucosa and subserosal layers were more prominent with cholecystitis and especially chronic cholecystitis.

The reduction of the thickness of the wall of the gallbladder with a mucocele may explain the increased risk of perforation during laparoscopic cholecystectomy with SLD when a mucocele is present. Honda et al. reported successful utilization of the SLD technique to perform an LC in human patients. Human gallbladder wall thickness is 2.6 mm thicker than canine gallbladder wall. The thickness of the gallbladder wall in human patients increased with different forms of cholecystitis as in this study. Gallbladder mucoceles are rarely reported in human patients and seem to be the result of cholelithiasis. Therefore, SLD might be successful in humans, but it may not be as successful in dogs because of the major differences in gallbladder wall thickness. Kanai et al. reported a 21% perforation rate in their study while performing SLD for mostly gallbladder mucocele. Kondo et al. did not report perforation but an 8.8% conversion rate performing SLD for mostly chronic cholecystitis. Since chronic cholecystitis is associated with more fibrosis and thicker mucosa, it should reduce the risk of rupture of the gallbladder during LC, especially using SLD. Kondo et al. converted 8.8% of their cases because of severe adherence that precluded the SLD technique. Perforation rates of 4% to 10% have been reported with the standard technique to perform LC for gallbladder mucocele in dogs, which compares favorably to the SLD technique for mucocele.

Preoperatively only ultrasound diagnosis is available to the surgeon. Diagnosis of gallbladder mucocele is straightforward on ultrasound and a standard cholecystectomy should be recommended. If cholecystitis is present on ultrasound, it is usually diagnosed on histopathology and a subserosal dissection could be attempted for the cholecystectomy. The thickness of the wall of the gallbladder was not reported in most of the ultrasound reports available for this study and therefore could not be correlated with the measurements performed on histopathology. This could be evaluated in a prospective study with standardized imaging collected on ultrasound.

This study has several limitations. Only a part of the gallbladder wall was observed. The most severe macroscopic lesion is supposed to be included when the gallbladder is trimmed, but it cannot be excluded that lesions were missed. Histopathological analysis was only limited to Hematoxylin and eosin stains since this is a retrospective study. Tissue fragility cannot be evaluated because of the nature of this retrospective histopathological study. Tissue fragility needs to be assessed to further discuss the perforation rate of gallbladder diseases.

Ultrasound measurements could not be correlated with the histopathological measurements given the retrospective nature of this study with unstandardized ultrasonography.

Disease processes present in the gallbladder affect differently the thickness of the different layers of the wall of the gallbladder. The mucosa is associated with the thinning of the wall while cholecystitis is associated with the thickening wall. The subserosa is the layer most commonly affected by different disease processes. Therefore, when a gallbladder mucocele is diagnosed on ultrasound, it would be prudent not to use the SLD technique to perform a cholecystectomy; however, the SLD technique might be useful for a gallbladder with cholecystitis, especially chronic cholecystitis.

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Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org.