



Standard Article

J Vet Intern Med 2018;32:188–194

Gallbladder Agenesis in 17 Dogs: 2006–2016

K. Sato , M. Sakai, S. Hayakawa, Y. Sakamoto , Y. Kagawa, K. Kutara, K. Teshima, K. Asano, and T. Watari**Background:** Gallbladder agenesis (GBA) is extremely rare in dogs.**Hypothesis/Objectives:** To describe the history, clinical signs, diagnosis, treatment, and outcomes of dogs with GBA.**Animals:** Seventeen client-owned dogs with GBA.**Methods:** Medical records from 2006 through 2016 were retrospectively reviewed. Dogs were included when GBA was suspected on abdominal ultrasonography and confirmed by gross evaluation. Signalment, clinical signs, clinicopathological data, diagnostic imaging, histopathology, treatment, and outcome were recorded.**Results:** Dogs were of 6 different breeds, and Chihuahuas (10 of 17) were most common. Median age at presentation was 1.9 (range, 0.7–7.4) years. Clinical signs included vomiting (5 of 17), anorexia (2 of 17), ascites (2 of 17), diarrhea (1 of 17), lethargy (1 of 17), and seizures (1 of 17). All dogs had increased serum activity of at least 1 liver enzyme, most commonly alanine aminotransferase (15 of 17). Fifteen dogs underwent computed tomography (CT) cholangiography; common bile duct (CBD) dilatation was confirmed in 12, without evidence of bile duct obstruction. Gross evaluation confirmed malformation of the liver lobes in 14 of 17 dogs and acquired portosystemic collaterals in 5 of 17. Ductal plate malformation was confirmed histologically in 16 of 17 dogs. During follow-up (range, 4–3,379 days), 16 of 17 dogs remained alive.**Conclusions and Clinical Importance:** Dogs with GBA exhibit clinicopathological signs of hepatobiliary injury and hepatic histopathological changes consistent with a ductal plate abnormality. Computed tomography cholangiography was superior to ultrasound examination in identifying accompanying nonobstructive CBD distention. Computed tomography cholangiography combined with laparoscopic liver biopsy is the preferable approach to characterize the full disease spectrum accompanying GBA in dogs.**Key words:** Canine; Cholangiography; Computed tomography; Gallbladder.

Gallbladder agenesis (GBA) is an extremely rare entity in humans and is characterized by the absence of the gallbladder without atresia of the extrahepatic biliary system.¹ In humans, GBA has an incidence ranging from 0.01 to 0.065% and is often asymptomatic and diagnosed incidentally.¹ It is considered to be an embryological disorder and possibly hereditary.^{2,3} However, most information about GBA in humans arises from isolated case reports and the etiology remains unclear.^{2–11} Guidelines have been established for the diagnosis of GBA.^{4,12} The recommendation in humans is that GBA should be diagnosed preoperatively to avoid unnecessary surgical exploration and to minimize the risk of iatrogenic injury.^{4,9,12} In humans, symptoms associated with GBA are suggestive of biliary colic, and as a result of a general lack of awareness of GBA, patients have been

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The findings of this study were presented, in part, at the 25th Annual Congress of the European College of Veterinary Internal Medicine—Companion Animals, September 10–12, 2015, Lisbon, Portugal.

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Submitted June 12, 2017; Revised November 18, 2017; Accepted November 30, 2017.

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DOI: 10.1111/jvim.15034

Abbreviations:

APSCs	acquired portosystemic collaterals
CBD	common bile duct
CPSS	congenital portosystemic shunt
CT	computed tomography
DPM	ductal plate malformation
GBA	gallbladder agenesis
mgI/kg	milligrams of iodine per kilogram
PHPV	primary hypoplasia of the portal vein
SPP	splenic pulp pressure

misdiagnosed with cholecystitis and cystic duct obstruction or with a scleroatrophic gallbladder, resulting in unnecessary surgical intervention.^{4,7} Diagnostic computed tomography (CT), magnetic resonance cholangiography, or endoscopic retrograde cholangiopancreatography is recommended when a gallbladder is not found on abdominal ultrasonography.^{4,12}

Clinical and prognostic information about GBA in dogs is sparse. Nevertheless, existing literature indicates that some dogs with GBA have accompanying increases in serum liver enzyme activity, in addition to histopathological changes in the liver such as bile duct proliferation and fibrosis.^{13–15} These findings suggest that GBA is not necessarily an asymptomatic condition in some dogs. Accordingly, our study aimed to review history, clinical signs, diagnosis, treatment, and outcomes of dogs with GBA.

Materials and Methods

Case Selection

The medical records database at Nihon University Animal Medical Center was reviewed retrospectively from 2006 through

2016 to identify dogs with GBA. Dogs were included in the study if the gallbladder was undetectable or extremely small on abdominal ultrasonography, and GBA was confirmed by gross evaluation at laparoscopy or laparotomy. The study was performed in accordance with the Guide for Animal Experimentation published by the College of Bioresource Sciences, Nihon University.

Review of Medical Records

Information regarding breed, sex, age, clinical signs, clinicopathologic data, diagnostic imaging, histopathologic findings, treatment, and outcome was retrieved from the medical records. Dogs with incomplete medical data were excluded. When information regarding clinical signs, survival time after diagnosis, serum liver enzyme activity, and treatment in a case was not available at our hospital, follow-up data were obtained by telephone interview with the referring veterinarian.

Computed Tomography Cholangiography and Portography

Computed tomography cholangiography was performed under general anesthesia. An over-the-needle catheter was inserted into a cephalic vein, and the contrast agent, meglumine iotroxate,^a was administered at a dosage of 100 mgI/kg over a 30-minute period followed by administration of 0.9% NaCl solution at a constant rate for 30 minutes with an infusion pump. Induction of anesthesia was performed during the infusion of saline, and CT scanning was performed 60 minutes after injection of meglumine iotroxate. The method of administration and dosage of the contrast medium were based on the previous reports involving human patients.^{16,17} The dogs were positioned in ventral recumbency and imaged with a multi-detector CT scanner.^{b,c} The scanning parameters were as follows: X-ray tube potential, 120 kV; X-ray tube current, 200 mA; slice thickness, 0.5 mm; reconstruction interval, 0.5 mm; rotation time, 0.5 seconds; table speed, 7.5 mm/rotation; and helical pitch, 15.0. Computed tomography portography was performed after cholangiography if a portosystemic shunt was suspected. Iohexol^d was used as the contrast medium and administered at a dosage of 750 mgI/kg via a cephalic vein with a power injector.^e The scanning parameters and contrast medium injection time were as described previously.¹⁸ All CT scanning was performed during apnea.

The CT images were analyzed by 2 observers (KS, KK) using a Virtual Place workstation.^f The morphology of the biliary tree, including the gallbladder, common bile duct (CBD), and portal vein, was evaluated on volume-rendered images. The diameter of the CBD was measured by curved planar reformation described previously.¹⁹ If the maximum diameter of the CBD was >3 mm, it was considered to be dilated, as reported elsewhere.²⁰

Laparoscopy and Laparotomy

Laparoscopy or laparotomy was performed under general anesthesia for a definitive diagnosis of GBA. Gross evaluation of the gallbladder and liver was performed by a veterinary laparoscopist (MS) and either 1 of 2 surgeons (KA, KT). The abdominal cavity was observed with a laparoscope (5-mm diameter) after insufflation with CO₂ gas and placement of 2 or 3 cannulae in the abdomen. Liver samples were obtained with 5-mm cup forceps under laparoscopy or by a wedge biopsy technique under laparotomy. At least 1 liver sample was obtained from 1 liver lobe in each dog for histopathological analysis. Tissue from the same sample also was subjected to bacterial culture if infection was suspected. Splenic pulp pressure (SPP) was measured under laparoscopy as previously

described.²¹ Ovariohysterectomy was performed concomitantly if desired by the owner.

Histopathology

Liver samples collected by laparoscopy or laparotomy were fixed in 10% neutral buffered formalin and embedded in paraffin. All sections were prepared and stained with hematoxylin and eosin. Azan staining was used to assess the extent and distribution of fibrosis in the portal tracts. Rhodanine-stained slides were used to assess the presence and severity of hepatocellular accumulation of copper. Immunohistochemistry labeling with antibodies against cytokeratin 19 antibody^g was used to identify the bile ducts and differentiate them from arterioles, and Ki-67^h was used to determine whether or not the bile ducts were proliferating. Azan staining, rhodanine staining, and immunohistochemistry were performed in cases for which paraffin-embedded tissue was available.

Liver biopsy specimens were examined histologically by a board-certified pathologist (YK) according to the criteria developed by the World Small Animal Veterinary Association Liver Standardization Group.^{22,23} Ductal plate malformation (DPM) was diagnosed by a previously reported method.²⁴ Each liver sample was graded as being normal or having mild, moderate, or severe histopathology. The histological features graded were as follows: narrowing of the portal vein lumen; proliferation of bile duct epithelium; proliferation of arterioles; portal fibrosis; infiltration of inflammatory cells; increased numbers of spindle cells; atrophy of hepatocytes; and accumulation of copper. Quantitative copper analysis was performed when samples were available.

Results including age, body weight, clinicopathological data, diameter of the CBD, SPP measurements, quantitative copper results, and follow-up period were reported as median (range).

Results

A review of the medical records database identified 21 dogs with suspected GBA on abdominal ultrasonography. Four dogs were excluded because the owners refused gross evaluation by laparoscopy or laparotomy, leaving 17 dogs in which a definitive diagnosis was made for inclusion in the study. The median age of the 17 dogs at presentation was 1.9 (range, 0.7–7.4) years and 4 of 17 dogs were <1 year of age at the time of referral. There were 9 of 17 intact females, 3 of 17 neutered males, 3 of 17 intact males, and 2 of 17 spayed females. Median body weight at the time of initial examination was 3.3 kg (range, 1.5–28.5 kg). Breeds included Chihuahua (10 of 17), Toy Poodle (3 of 17), German Shepherd (1 of 17), Shiba (1 of 17), and Jack Russell terrier (1 of 17); 1 dog was of mixed breed (1 of 17). All dogs were referred for the investigation of persistently increased serum liver enzyme activities. Clinical signs at the time of initial examination included vomiting (5 of 17), anorexia (2 of 17), ascites (2 of 17), diarrhea (1 of 17), lethargy (1 of 17), and seizures (1 of 17). Eight of 17 dogs were asymptomatic and had been brought to the referring veterinarians for a routine medical evaluation or elective neutering. Before referral, 6 of 8 asymptomatic dogs were treated with ursodeoxycholic acid. Seven of the 9 symptomatic dogs were treated with a variety of medications, including ursodeoxycholic acid (5 of 7), metronidazole (3 of 7), and amoxicillin (2 of 7). One of 7 dogs was treated with lactulose for hyperammonemia and seizures.

A CBC and serum biochemistry panel were performed in all dogs at the time of diagnosis (Table 1). Three of 17 dogs had mild thrombocytopenia ($160\text{--}180 \times 10^3/\mu\text{L}$). All dogs were found to have increased serum liver enzyme activity, most commonly alanine transaminase (15 of 17). Low blood urea nitrogen concentration (6 mg/dL), hypoalbuminemia (2.1 g/dL), and hypofibrinogenemia (67 mg/dL) were detected in 1 of 17 dogs. None of the dogs had hyperbilirubinemia at the time of presentation to our facility.

Radiographic and ultrasonographic examinations of the abdomen were performed in all 17 dogs. Findings on thoracic radiography were normal in all cases. On abdominal radiography, the liver was small in 6 of 17 dogs, and abdominal detail was decreased in 1 of 17 dogs. On abdominal ultrasonography, the gallbladder was not detectable in 15 of 17 dogs and noted to be vestigial (10.2 and 10.3 mm in diameter) in 2 of 17 dogs. The CBD was dilated in 2 of 17 dogs (5.1 and 3.8 mm in luminal diameter). Five of the 17 dogs had a liver with an irregular surface or peripheral edge, and the liver parenchyma was diffusely hyperechoic in 4 of 17 and hypoechoic in 2 of 17. Cholelithiasis and acquired portosystemic collaterals (APSCs) were identified in 1 of 17 and 3 of 17 dogs, respectively.

Computed tomography cholangiography was performed in 15 of the 17 dogs and revealed no gallbladder in 11 of 15 cases and a vestigial gallbladder in 4 of 15 (Fig 1). The biliary tree, including the hepatic duct and CBD, was uniformly enhanced by the contrast medium. Curved planar reformation identified a dilated CBD in 12 of the 15 dogs (median diameter, 4.97 mm, range, 3.04–9.56 mm), with no findings of biliary tract obstruction. The hepatic ducts were normal in all cases. Contrast medium was observed in the intestinal tract in 14 of the 15 dogs. Computed tomography portography was performed in 7 of 17 dogs, and detected

cholelithiasis in 1 of 7 dogs, APSCs in 2 of 7 dogs, and a congenital portosystemic shunt (CPSS) in 1 of 7 dogs. Computed tomography imaging did not identify any instances of ectopic gallbladder or any cystic lesions in the abdominal organs.

Agnesis or hypoplasia of the gallbladder was detected on gross observation in all dogs. No gallbladder was detected in 12 of 17 dogs and a vestigial gallbladder was confirmed in 5 of 17 dogs. These findings were consistent with those of CT cholangiography. Gross abnormalities of the liver were confirmed in 14 of 17 dogs; 12 of 13 by laparoscopy and 2 of 4 by laparotomy (Table 2). Absent or hypoplastic hepatic lobes were found in 10 of 17 dogs. The surface of the liver was rough in 5 of 17 dogs, and 1 dog each had adhesions in the abdominal wall, omentum, or liver lobes. Gross visualization identified APSCs around the left kidney in 5 of 17 dogs. The SPP was measured in 7 of 17 dogs and found to be in the range of 5–13 (median, 7) mmHg; 2 of 7 dogs with formation of APSCs had SPP of 8 and 13 mmHg, which is considered high in comparison with previously reported mean SPP of 6.2 ± 0.8 mmHg in healthy dogs.²⁵ There were no instances of positive bacterial growth in the 7 of 17 dogs in which liver tissue samples were obtained. Ovari-hysterectomy was performed concurrently in 8 of 17 dogs and an ameroid constrictor was placed in 1 of 17 dogs with CPSS.

Liver biopsy and histopathologic examination were performed in all 17 dogs. Nine of 17 dogs had ≥ 2 biopsy samples from multiple liver lobes, and 5 of 17 had multiple samples from a single lobe. One biopsy sample from a single lobe was obtained in 3 of 17 dogs, 2 of which were obtained by laparotomy. Paraffin-embedded tissues from 16 of 17 dogs were available for rhodanine staining, Azan staining, and immunohistochemistry. The most common feature was narrowing of the portal vein,

Table 1. Hematology and biochemistry results in 17 dogs with gallbladder agenesis.

Variable	Reference Range	Median (range)	Abnormal Results, n (%)	Dogs Tested, n
WBC ($\times 10^3/\mu\text{L}$)	6.0–17.0	9.8 (6.9–16.7)	0	17
PCV (%)	37–55	48 (37–56)	1 (6)	17
PLT ($\times 10^3/\mu\text{L}$)	200–500	330 (160–688)	7 (41)	17
ALT (U/L)	10–100	308 (38–1,374)	15 (88)	17
ALP (U/L)	23–212	126 (71–234)	1 (6)	17
AST (U/L)	0–50	68 (6–473)	10 (59)	17
GGT (U/L)	0–7	14 (3–25)	13 (76)	17
Albumin (g/dL)	2.3–4.0	3.0 (2.1–3.7)	1 (6)	17
Total bilirubin (mg/dL)	0–0.9	0.1 (0.1–0.3)	0	15
Glucose (mg/dL)	74–143	105 (85–145)	1 (6)	17
Ammonia ($\mu\text{g/dL}$)	0–98	17 (0–78)	0	16
BUN (mg/dL)	7–27	12 (6–22)	2 (12)	17
Cholesterol (mg/dL)	110–320	171 (111–364)	2 (12)	17
SBA-fasting ($\mu\text{mol/L}$)	5	18 (4.8–178.8)	7 (89)	8
SBA-postprandial ($\mu\text{mol/L}$)	20	56.1 (15.8–170.6)	3 (60)	5
Protrombin time (seconds)	6–8	7.9 (5.9–12.4)	8 (47)	17
Partial thromboplastin time (seconds)	10–16	13.4 (10–25.8)	1 (6)	17
Fibrinogen (mg/dL)	86–375	142 (67–266)	1 (6)	17
Antithrombin (%)	102–156	117 (70–140)	3 (18)	17

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltranspeptidase; BUN, blood urea nitrogen; PCV, packed cell volume; PLT, platelets; SBA, serum bile acid.

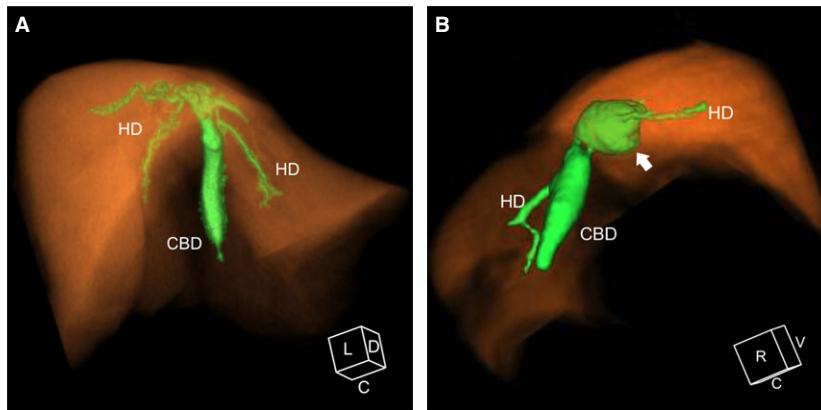


Fig 1. Volume-rendered images obtained by computed tomographic cholangiography. The biliary tree is represented in a 3-dimensional image colored in light green. (A) A volume-rendered image of a dog with gallbladder agenesis. (B) A volume-rendered image of a dog with a vestigial gallbladder (arrow). The common bile duct was dilated in this dog (8.2 mm). C, caudal; CBD, common bile duct; D, dorsal; HD, hepatic duct; L, left; R, right; V, ventral.

Table 2. Gross evaluation of the liver via laparotomy (n = 4) or laparoscopy (n = 13) in 17 dogs with gallbladder agenesis.

Characteristics of Gross Evaluation	Dogs, n (%)
Gallbladder agenesis	12 (71)
Gallbladder hypoplasia	5 (29)
Liver lobe agenesis	
Quadrato lobe	5 (29)
Multiple lobe	1 (6)
Liver lobe hypoplasia	
Quadrato lobe	3 (18)
Multiple lobe	1 (6)
Liver lobe surface	
Smooth	12 (71)
Rough	5 (29)
Liver lobe adhesions	3 (18)

which was confirmed in all but 1 dog (Fig 2A); severity was assessed as mild in 6 of 17 dogs, moderate in 4 of 17, and severe in 6 of 17. Mild to moderate portal fibrosis and proliferation of the intrahepatic arterioles were confirmed in 6 of 17 and 11 of 17 dogs, respectively (Fig 2B). Infiltration of inflammatory cells, consisting of lymphocytes and plasma cells, was detected in the portal area in 1 of 17 dogs. Other features included infiltration of spindle cells (8 of 17) and atrophy of hepatocytes (2 of 17). An increased bile duct profile was confirmed in all 16 dogs (Fig 2C); severity was assessed as mild, moderate, and severe in 5 of 16, 8 of 16, and 3 of 16 dogs, respectively. Active ductal proliferation was excluded by negative immunoreactivity for Ki-67 in the portal tracts in all 16 dogs (Fig 2D). Quantitative copper analysis was performed in 3 of 17 dogs, with a median of 483.4 $\mu\text{g/g}$ (range, 242.9–892.9 $\mu\text{g/g}$) dry weight liver. Mild copper accumulation was detected on rhodanine-stained slides in 2 of 16 dogs. On the basis of these results, 16 of 17 dogs were histologically diagnosed with DPM. One dog (1 of 17) was assessed to be normal from the samples obtained.

Laparoscopy and laparotomy were performed without major complications. Thirteen of the 17 dogs were treated postoperatively with medication and dietary management. Nine of 13 dogs were treated with ursodeoxycholic acid (5–20 mg/kg PO q12–24h) and 3 of 13 with antibiotics (metronidazole 20 mg/kg PO q12h [n = 1]; amoxicillin 20 mg/kg PO q12h [n = 1]; cephalexin 20 mg/kg PO q12h [n = 1]). Three of 13 dogs were treated with lactulose (1 mL/kg PO q12h) to prevent hepatic encephalopathy. One of 13 dogs was treated with diuretics (furosemide and spironolactone, each at 0.5 mg/kg PO q12h). In addition, a commercial low-fat diet,^{l,j} and a liver prescription diet^k were prescribed in 4 of 17 dogs and 1 of 17 dogs, respectively. Seven of the 9 symptomatic dogs improved after treatment. Clinical signs resolved in 2 of 9 dogs without treatment. The dog that was treated surgically for CPSS recovered uneventfully without clinical signs and exhibited a normal serum bile acid concentration at the time of its final presentation to our facility.

Follow-up data were obtained for all dogs. One dog developed severe intractable ascites and died within 4 days. This dog was histologically confirmed to have DPM with a high SPP (8 mmHg) and presence of APSCs. During the follow-up period, persistently increased serum liver enzyme activity was documented in 12 of 17 dogs, 2 of which experienced intermittent vomiting as a residual sign. Two of 8 dogs without clinical signs exhibited progressive hypoalbuminemia and low blood urea nitrogen concentrations, suggesting progressive hepatic failure. One of these 2 dogs initially was histologically diagnosed with DPM and accompanying APSCs formation. The other was diagnosed with DPM without evidence of portal hypertension. Sixteen of 17 dogs remained alive from the time of diagnosis to the censored time point, and each dog had a follow-up period of different duration until they were censored: 8 remained alive for >3 years in good condition, and the median follow-up period was 570 days (range, 4–3,379 days).

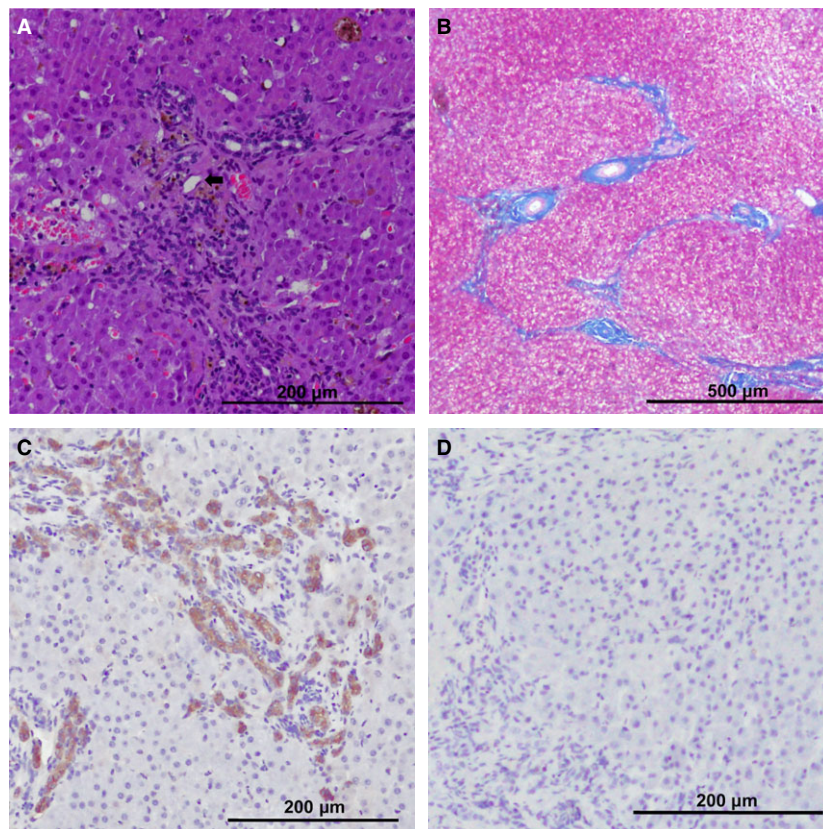


Fig 2. Histopathologic characteristics of a liver with gallbladder agenesis. Narrowing of the portal vein is observed (hematoxylin and eosin stain) (A). The portal vein is narrowed (arrow) with juvenile arteriolar proliferation and portal fibrosis. Mild amounts of fibrillar collagen and portal-to-portal bridging can be observed (Azán stain) (B). Bile ducts are positive for CK19 (C). The differentiated biliary epithelium was strongly positive for CK19. The biliary epithelium shows a negative reaction to Ki-67 staining (D). Each image was from a different dog.

Discussion

Gallbladder agenesis is an extremely rare condition in dogs and has been the subject of only 3 case reports.^{13–15} Although these cases had several similar clinical features, information about GBA in dogs is limited. Ours is the first study to present data for several dogs with GBA. Most dogs with GBA were small breed dogs with persistently increased liver enzymes activity and histologic abnormalities of the intrahepatic portal and biliary systems. In our study, 5 of 17 (29%) dogs were confirmed to have APSCs formation, which indicates the presence of accompanying portal hypertension. Furthermore, 2 of 17 (12%) dogs were presumed to have progressive liver failure.

In humans, GBA is considered to be a congenital embryologic disorder and a consequence of failure of vacuolation in the pars cystica.^{2–4} However, GBA may result from failure of development of the hepatic diverticulum or an abnormality in concurrent development of the pars hepatica and cystica.¹³ During embryogenesis, development of the liver and gallbladder starts as the hepatic diverticulum, which divides into the pars hepatica and pars cystica. The pars hepatica develops into hepatocytes, the intrahepatic biliary system, and the CBD, whereas the pars cystica develops into the gallbladder.² Earlier cases of GBA in dogs were confirmed to have liver lobe hypoplasia

and malformation with portal fibrosis.^{13,15} Similar to these reports, the dogs in our study were confirmed histologically to have high rates of macroscopic and microscopic liver abnormalities, including liver lobe agenesis and increased bile duct profiles. Developmental failure of the pars cystica may lead to agenesis of the gallbladder, but it is unlikely to lead to liver abnormalities. Thus, concomitant abnormalities of the gallbladder, liver lobes, and liver parenchyma in dogs with GBA may result from a more complex developmental failure in the embryological phase, rather than pars cystica alone, which also has been documented in humans.^{2–4}

Familial and genetic factors have been implicated in the etiology of GBA in humans.^{2,3} The majority of our cases were young at the time of presentation and were mainly small pure-bred dogs. The previous 2 cases were Maltese^{13,14} and there has been another report of a Chihuahua with GBA.¹⁵ Therefore, genetic factors may be important in the etiology of GBA in dogs.

All of the dogs in our report presented with increased liver enzyme activity, and the most frequent clinical sign was vomiting (56%). Sixteen of the 17 dogs in our series were histologically diagnosed with DPM. This congenital liver disease is characterized by high serum liver enzyme activity and mild gastrointestinal signs, ascites, and seizures.^{24,26} Therefore, the increased liver enzyme

activity and clinical signs that occur in dogs with GBA could stem from malformation of the intrahepatic portal and biliary systems.

Histopathologic findings in our cases suggest an association of GBA in dogs with an embryologic abnormality of the intrahepatic biliary and portal veins. Histopathological evaluation identified DPM in 94% (16 of 17) of these dogs. A recent retrospective report involving 30 Boxer dogs with DPM confirmed a missing gallbladder in 8 cases as well as liver lobe malformations.²⁴ Furthermore, in a previous study involving dogs with primary hypoplasia of the portal vein (PHPV), 1 with GBA was documented and considered to be an incidental finding.²⁷ Although the association between GBA and DPM/PHPV was not clear in previous studies, our study suggests a relationship between these congenital abnormalities.

Eighty percent of the dogs in our study presented with dilatation of the bile duct, which could prompt clinicians to suspect obstruction. Bile duct obstruction in dogs often requires surgical intervention but CT cholangiography indicated that none of these dogs had evidence of biliary obstruction. A previous study indicated that dilatation of the CBD could occur when the CBD compensated functionally for the missing gallbladder, or when there was pre-existing cholelithiasis.¹⁵ Considering the prevalence of cholelithiasis in our study, the former appears to be more likely. Thus, the dilatation of CBD in GBA dogs is likely a result of the congenital abnormality and requires no surgical intervention.

A minimally invasive method is needed for affected dogs, not only to confirm the missing gallbladder, but also to rule out biliary obstruction and diagnose the underlying histopathological abnormality. In previously reported affected dogs, GBA was diagnosed by exploratory laparotomy.¹³⁻¹⁵ The rarity of GBA in dogs may have prompted previous investigators to confirm the absence of the gallbladder grossly or by retrograde cholangiography and also to perform liver biopsy. Exploratory laparotomy is an invasive diagnostic procedure and therefore a minimally invasive procedure is required for the diagnosis of GBA in dogs, as suggested in human medicine.^{4,9,12} In our study, we determined that a combination of CT cholangiography and laparoscopy was an optional minimally invasive method to diagnose GBA in dogs.

Computed tomography cholangiography enabled us to confirm a missing or vestigial gallbladder in these dogs. This method was noninvasive and the patency of bile flow was evaluated by the presence of contrast medium in the duodenum. Additionally, this method confirmed dilatation of the CBD in 80% (12 of 15) of the dogs, whereas dilatation was identified in 12% (2 of 17) by abdominal ultrasound examination. The results obtained by CT and ultrasound examination were clearly discrepant. This may be explained by the operator-dependent nature of ultrasound examination, and some limitations in detecting the biliary tract have been reported previously.²⁸ From these results, CT cholangiography appears to be a reliable tool to evaluate the extrahepatic biliary system in dogs with GBA.

In dogs, laparoscopy is a minimally invasive method for gross evaluation, and liver biopsy samples may be

obtained from multiple liver lobes.²⁹ In our study, liver lobe abnormalities were grossly confirmed in 92% (12 of 13) of the dogs, and 92% (12 of 13) dogs had multiple liver samples obtained by laparoscopy. In a previous study, the severity of the lesions of DPM was reported to differ among liver lobes,²⁴ and the diagnosis of DPM and PHPV could be difficult to make in dogs with only a single biopsy specimen. Therefore, we believe a combination of CT cholangiography and laparoscopy is the least invasive method for the diagnosis of GBA in dogs.

Concurrent portal hypertension may affect the prognosis of dogs with GBA. In our series, 29% (5 of 17) of the dogs were confirmed to have portal hypertension. One dog with accompanying APSCs developed progressive liver dysfunction, indicated by progressive hypoalbuminemia and low blood urea nitrogen concentrations during the follow-up period. Another dog diagnosed with DPM and accompanying APSCs formation initially presented with severe liver failure and died within 4 days. In a previous study, dogs with DPM-associated portal hypertension or formation of APSCs had shorter survival time compared to dogs without portal hypertension.²⁴ Studies in humans also suggest possible progression in patients with DPM.³⁰ Therefore, in dogs with GBA, regular evaluations are needed to monitor for possible development of liver failure or portal hypertension. Furthermore, the severity of portal hypertension should be evaluated in dogs with GBA, which can be achieved by SPP measurement via laparoscopy, as previously reported.²¹

The main limitations of our study were its retrospective design and the low number of cases included. Because of the study design (ie, descriptive and retrospective), the development of treatment strategies, determination of risk factors, and prognosis of dogs with GBA were not evaluated. The final diagnosis of DPM was based on information from a previous report,²⁴ although the histopathological characteristics of DPM and PHPV are similar.^{22-24,26,27,31,32} To date, differentiation remains controversial and, moreover, the lack of a sufficient sample number may have made differentiation more difficult. No data regarding family history were collected and sampling for genetic studies was not performed. Although more than 50% of the dogs in our series were Chihuahuas, because of lack of hospital population data, it is uncertain whether this particular breed is predisposed to GBA. A previous study suggested that GBA in dogs may be progressive, based on histology findings.¹⁵ However, a second liver biopsy was not performed in any of these dogs to confirm progression of liver histopathology.

In conclusion, our study characterized the clinical, clinicopathological, and histological features of GBA in dogs. Veterinary clinicians should be aware of this rare disease and consider the possibility of GBA if no gallbladder is detected on abdominal ultrasonography. When suspected, however, clinicians should inform their clients of the importance of a definitive diagnosis and close monitoring because of the risk for possible progression of liver dysfunction. A combination of CT cholangiography and laparoscopy may be the least invasive diagnostic approach to confirm and thoroughly

evaluate dogs with GBA. Future genetic research and long-term survival data are required to elucidate the etiology and clarify the prognosis of dogs with GBA.

Footnotes

- ^a Biliscopin DIC 50; Bayer, Osaka, Japan
^b Aquilion 16; Toshiba, Tokyo, Japan
^c Aquilion ONE; Toshiba
^d Ioverin 300; Teva Pharma Japan Inc., Nagoya, Japan
^e Auto Enhance A-60; Nemoto-Kyorindo, Tokyo, Japan
^f Virtual Place, AZE, Tokyo, Japan
^g Monoclonal anticytokeratin 19 antibody; Leica Biosystems, Newcastle-upon-Tyne, UK
^h Mouse anti-Ki-67 antibody; Invitrogen, CA
ⁱ Prescription Diet w/d Canine; Hill's-Colgate (Japan) Ltd, Tokyo, Japan
^j Veterinary Diet Gastrointestinal Low Fat; Royal Canin Japon, Inc., Tokyo, Japan
^k Veterinary Diet Canine Hepatic; Royal Canin Japon, Inc.

Acknowledgments

Grant support: None.

Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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