

American Cocker Spaniel Chronic Hepatitis in Japan

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Background: American Cocker Spaniels are predisposed to chronic hepatitis.

Objective: To describe the clinical and histological features of chronic hepatitis in Japanese American Cocker Spaniels.

Animals: Thirteen cases examined from 2003 to 2009.

Methods: Retrospective study. Medical records were searched for American Cocker Spaniels with chronic liver diseases. History, physical examination, clinicopathologic features, hepatic ultrasonographic findings, hepatic histopathology, and immunohistochemistry were evaluated.

Results: The median age was 4.6 (1.9–10.7) years. Clinical signs included inappetence (11/13), ascites (11/13), lethargy (9/13), diarrhea (7/13), and melena (2/13). Only 1/13 dogs was jaundiced. Clinicopathological abnormalities were increased liver enzymes (gamma-glutamyl transpeptidase: 9/12, aspartate aminotransferase: 7/10, alanine aminotransferase: 6/13, alkaline phosphatase: 6/13), increased total serum bile acid concentrations (10/12), and hypoalbuminemia (10/13). The liver had an irregular surface in all dogs and acquired portosystemic collaterals were verified in 11/13 dogs by abdominal ultrasound (2), laparoscopy (4), or both (5). Liver histology revealed severe fibrosis and cirrhosis in all cases, subdivided in lobular dissecting hepatitis (7), periportal fibrosis (1), micronodular cirrhosis (3), and macronodular cirrhosis (2). Inflammatory activity was low to mild. Immunohistochemical stains showed ductular proliferation. The median survival time was 913 (range: 63–1981) days.

Conclusion and Clinical Importance: Hepatitis in Japanese American Cocker Spaniels is clinically silent until an advanced stage and is associated with severe hepatic fibrosis leading to cirrhosis, extensive ductular/putative hepatic progenitor cell proliferation, portal hypertension, and acquired portosystemic collateral shunting, but relatively long survival times. Lobular dissecting hepatitis seems more prevalent than in previously reported cases from other countries.

Key words: Breed-related hepatitis; Liver progenitor cells; Portal hypertension.

Hepatitis is a common disease in the dog. One of the most frequent forms is chronic hepatitis, which is characterized by hepatocellular apoptosis or necrosis, a variable mononuclear or mixed inflammatory infiltrate, regeneration, and fibrosis.¹ In dogs, recognized causes of chronic hepatitis may be infectious, autoimmune, drug-induced, or copper-associated. Fibrosis and cirrhosis, the end stage of this disease, may result in portal hypertension (PH). The clinical consequences of chronic PH include the development of acquired portal systemic collaterals (APSCs) and/or ascites.²

In dogs, chronic hepatitis and cirrhosis were histopathologically categorized by the WSAVA Liver Standardization Group according to etiology (idiopathic or copper-associated), time course (acute, chronic, or cirrhotic), or by having special morphological characteristics

Abbreviations:

ACSH	American Cocker Spaniel hepatitis
APSCs	acquired portal systemic collaterals
CK	cytokeratin
ECM	extracellular matrix
LPCs	liver progenitor cells
PH	portal hypertension

(eg, lobular dissecting hepatitis). However, different cases even within the same category show clinical and morphological diversity. Predisposition to chronic hepatitis has been reported in a wide range of breeds such as Bedlington Terrier,³ West Highland White Terrier,⁴ Skye Terrier,⁵ Dalmatian,⁶ Labrador Retriever,^{7,8} Standard Poodle,⁹ English Springer Spaniels,^{10,11} and Doberman Pinschers,^{12–18} some of which are associated with copper storage. In addition, American and English Cocker Spaniels were reported to have high incidence of chronic liver disease in Europe, United States, and Japan.^{19–21,a} However, clinical features of hepatitis in the Japanese population of American Cocker Spaniels were not previously reported.

In this study, we describe the distinct clinical and morphological features of chronic liver disease in American Cocker Spaniels in Japan.

Materials and Methods

Cases and Clinical Features

Paraffin blocks of American Cocker Spaniels with chronic hepatitis from Nihon University and the University of Tokyo were obtained between May 2003 and February 2009. Cases were selected manually from the records and represented all cases in the study period. The selection was all Cocker Spaniels with well

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recorded diagnosis of liver disease through histopathological examination. Therefore, accurate prevalence could not be calculated. Medical records included information on history, findings of physical examination, clinicopathology, radiography, and abdominal ultrasonography,^{b,c,d} laparoscopy or laparotomy, as well as treatment and clinical outcome. Because of the different reference values for blood examination between the 2 animal hospital laboratories, some of the data were converted into a ratio with the upper (liver enzyme activity and ammonia) or lower (albumin) 95% confidence interval of the local reference range. The median survival time (time from 1st presentation at the universities until death) was estimated by the Kaplan–Meier method.

Histological Examination

Representative samples from multiple liver lobes were collected by laparoscopy or laparotomy and fixed in 10% neutral buffered formalin. Biopsies were subsequently sliced and histological sections were stained with HE, rubeanic acid (for copper detection), and reticulin staining as described elsewhere.^{1,22,23} All the histology was interpreted by 1 pathologist (TSGAMvI), with semiquantitative evaluation of necro-inflammatory activity (0 = none, 1 = slight, 2 = mild, 3 = moderate, 4 = marked, 5 = severe) and the degree of fibrosis (0 = none, 1 = mild, focal, 2 = moderate, with some bridging fibrosis, 3 = marked fibrosis with marked bridging fibrosis, 4 = bridging with architectural distortion or cirrhosis) as reported elsewhere.²² Immunohistochemistry was performed using antibodies for cytokeratin (CK) 19, CK 7, pan-CK, and alpha smooth muscle actin (α SMA). The details of the protocols are summarized in Table S1. After deparaffinization, antigen retrieval was performed. For heat-induced antigen retrieval, sections were incubated in citrate buffer or Tris EDTA-HCl buffer for 40 minutes in a water bath at 96°C followed by cooling down for 30 minutes at RT. For proteinase-mediated antigen retrieval, sections were incubated with Proteinase K^e for 10 minutes at room temperature. Endogenous peroxidase activity was blocked by Dual Enzyme Block Agent (DAKO) for 10 minutes at room temperature, and nonspecific binding of antibody was blocked by 10% normal goat serum. Sections were incubated with primary antibody for 1 hour at RT or at 4°C overnight and with the appropriate ready-to-use, peroxidase-based secondary antibody (EnVision^g) for 45 minutes or 1 hour (Table 1). The positive staining reaction was visualized by DAB solution (3/3'-diaminobenzidine^e) with hematoxylin-stained nuclei. Between the steps, sections were washed 3 times for 5 minutes with PBS or TBS with (before secondary antibody reaction) or without (after secondary antibody reaction) 0.2% Tween 20. Sections were mounted with VectaMount.^f

Immunohistochemical staining for CK19 was assessed semiquantitatively; CK19-positive ductular reaction (ie, small clusters of epithelial-like cells with strong positive staining of CK19) and putative intermediate cells (ie, cells with hepatocyte-like morphology and weak or membranous CK19 staining compared with the surrounding ductular reaction) were scored in the fibrous septa and in the parenchyma as 0 (absent), 1 (mild, 0–5 foci/10× objective), 2 (moderate, 5–10 foci/10× objective), and 3 (severe, >10 foci/10× objective).

Results

Cases

Thirteen cases were included in this study (3 intact males, 4 intact females, 4 castrated males, and 2 spayed females). The median age was 4.6 (range: 1.9–10.7) years and the body weights varied from 6.7 to 12.1 (median 8.6) kg. All cases had been regularly vaccinated (at least

Table 1. Summary of the clinical pathology in 13 American Cocker Spaniels.

	n	Mean	Range	95% Confidence Interval
AST	7/10	1.24	0.4–2.04	0.97–1.51
ALT	6/13	0.55	0.49–2.29	0.80–1.41
ALP	6/13	1.37	0.08–3.63	0.70–2.10
GGT	9/12	2.46	0–10	1.00–3.92
Albumin	10/13	0.84	0.61–1.09	0.76–0.92
Ammonia	3/11	0.90	0–3.8	0.33–1.48
WBC ($\times 10^3$ /mL)	7/13	196	103–371	154–238
PCV (%)	7/13	35	25–43	33–38
Total cholesterol (mg/dL)	3/10	118.8	87–189	108–140
Bilirubin (mg/dL)	1/12	0.36	0–2.3	0.00–0.72
BUN (mg/dL)	5/13	14	2.9–69.0	4.2–23
Creatinine (mg/dL)	0/13	0.7	0.3–1.1	0.6–0.8
TBA-pre (μ M)	9/12	65.2	5.3–224.0	33.5–96.9
TBA-post (μ M)	10/12	126	9.7–249.0	80.6–172
PT (seconds)	2/13	9.3	8–11	8.9–9.8
APTT (seconds)	10/13	25.5	16–63	18.9–32.1
Fibrinogen (mg/dL)	9/11	142	51–396	87.9–196
Antithrombin (%)	9/10	60.9	46–92	53.3–68.6
CRP (mg/dL)	8/13	1.89	0.1–6.4	0.86–2.91

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; APTT, activated partial thromboplastin time; BUN, blood urea nitrogen; CRP: C-reactive protein; PT, prothrombin time; PCV, packed cell volume; TBA, total bile acid.

every 3 years) against canine adenovirus 1 and 2, parvovirus, distemper, parainfluenza virus, and rabies virus. None of the dogs in the study had a history of exposure to known hepatotoxic drugs or any other form of intoxication that could lead to hepatic injury. All the dogs were fed a commercial dry food. No test for presence of infectious disease was performed.

Clinical Signs and History

At the time of 1st presentation, clinical signs had been present for 2 weeks–14 months (median 3 months). Clinical signs were inappetence (n = 11), lethargy (n = 9), diarrhea (n = 7), melena (n = 1), abdominal distention (n = 6), weight loss (n = 3), vomiting (n = 1), alopecia, and pain on distal portion of extremities (n = 1). Neurological signs such as circling (n = 2), tremor (n = 1), and decreased activity after feeding (n = 1) were seen in 4 dogs. Three dogs had ascites that had been resolved after previous diuretic treatment. One dog was treated with an anti-inflammatory dose of corticosteroids for 3 months.

Clinical Pathology

Results of blood examination are summarized in Table 1. The abnormalities noted on blood examination were hypoalbuminemia (10/13), increased total bile acid concentration (preprandial: 9/12; postprandial: 10/12), and hyperammonemia (preprandial:

3/11). Increase in liver enzyme activities was variable: gamma-glutamyl transpeptidase (GGT): 9/12, aspartate aminotransferase (AST): 7/10, alanine aminotransferase (ALT): 6/13, and alkaline phosphatase (ALP): 6/13. Mild anemia and thrombocytopenia were found in 7 and 2 of 13 dogs, respectively. Mild increases in C-reactive protein concentration and white blood cell count were present in 8/13 and 7/13 dogs (neutrophilia 7/11, monocytosis 2/11), respectively, indicating an active inflammatory process. Hyperbilirubinemia was noted only in 1 dog (1/13). Urinalysis was performed in 8 dogs. Examination of urine revealed bilirubinuria in 7 dogs and ammonium biurate crystals in 2 dogs.

Diagnostic Imaging

On abdominal radiography, the size of the liver was small ($n = 7$) or normal ($n = 3$). In 3 dogs, radiography was not performed. Thoracic radiography revealed cardiomegaly in 2 cases, both of which had mitral regurgitation.

Ultrasonographic examination of the abdomen was performed in all cases. Liver parenchyma was diffusely hyperechoic ($n = 9$) or irregularly hyperechoic with hypoechoic nodules ($n = 4$). Eleven dogs had an irregular surface and/or peripheral edge of the liver. Abnormal multiple vessels caudal to the left kidney consistent with APSCs were detected in 7 dogs and ascites was present in 8 dogs. In 3 other dogs, the presence of ascites was reported by the referring veterinarian, but upon treatment with diuretics it had disappeared at the time of referral. Analysis of abdominal fluid revealed pure transudate in all cases (8/8). Other findings in the abdominal cavity included sludge in the urinary bladder ($n = 2$), increased thickness of the gallbladder wall and gallbladder sludge ($n = 1$), and fluid accumulation in the uterus ($n = 1$).

Laparoscopy and Laparotomy

Twelve dogs had laparoscopic examination and concurrent liver biopsy and laparotomy including

sampling of liver tissue was done in 1 case, in which ovariohysterectomy was performed in the same procedure. The liver surface was irregular in all cases (Fig 1a). Grossly detectable multiple small nodules (several millimeters in diameter) and large nodules (more than 1 cm in diameter) were noted in 4 and 2 dogs, respectively. In some cases, a brownish to yellowish ($n = 6$) or dark reddish ($n = 2$) color of the liver surface and decreased liver size were also noted. APSCs were visually confirmed in 9 cases (Fig 1b).

Histological Examination and Immunohistochemistry

The histological and immunohistological findings are summarized in Table 2, and shown in Figures 2–4. All dogs had the highest possible fibrosis score (4). In 7 cases, histological examination after HE and reticulin staining revealed lobular dissecting hepatitis (LDH) characterized by a disrupted architecture of the liver attributable to the presence of diffuse, extensive fibrosis surrounding single or small groups of hepatocytes and profound ductular reaction, with ($n = 5$) or without ($n = 2$) regenerative nodules. The more usual periportal fibrosis ($n = 1$) and micronodular ($n = 3$) or macronodular ($n = 2$) cirrhosis were present in the other dogs.

In contrast to the fibrosis score, the necro-inflammatory activity was low in most cases (activity score: median 1, range: 0–3). The inflammatory infiltrate was scattered along the fibrous tissue in 12 of 13 cases (the exception was a case with macronodular cirrhosis), and consisted mainly of pigment loaded macrophages with moderate lymphocytic, plasmacytic, and neutrophilic infiltrate. A zonal distribution of necro-inflammatory activity could not be identified in most cases attributable to the distorted architecture.

Extramedullary hematopoiesis and dilated lymphatics were additionally seen in 4 and 3 cases, respectively. Marked to moderate ballooning of hepatocytes was present in 8 cases.

The rubeanic acid stain for copper deposition was negative in 11 dogs. In 2 dogs, occasional hepatocytes which were irregularly distributed through the tissue

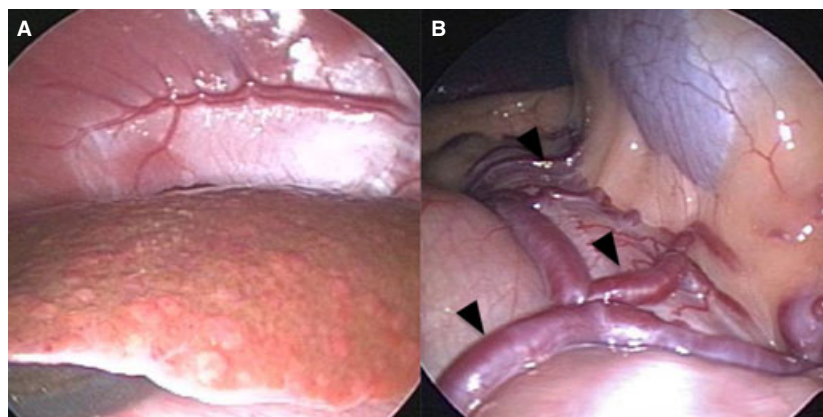


Fig 1. Laparoscopic findings of a representative case. The liver was stiff and atrophied, and appeared irregular. Some small regenerative nodules can also be recognized (A). Exploration around the left kidney revealed acquired portal systemic collaterals (B, arrowhead).

Table 2. Histologic findings in 13 cases of American Cocker Spaniel hepatopathy.

Category	Severity/Type	n
Fibrosis type	Diffuse (lobular dissecting)	7
	Micronodular cirrhosis	3
	Macronodular cirrhosis	2
	Periportal	1
Necro-inflammatory activity	Absent (grade 0)	1
	Slight (grade 1)	8
	Mild (grade 2)	0
	Moderate (grade 3)	4
	Marked (grade 4)	0
	Severe (grade 5)	0
Hepatocellular ballooning	Severe	3
	Moderate	5
	Absent	5
CK19 staining		
Parenchymal	Severe	5
	Moderate	1
	Mild	7
	Absent	0
Fibrous septa	Severe	4
	Moderate	2
	Mild	0
	Absent	0
Intermediate cells	Severe	1
	Moderate	0
	Mild	10
	Absent	2
Extramedullary hematopoiesis	Present	4
	Absent	9
Apoptotic hepatocytes	Present	3
	Absent	10
Dilated lymph vessels	Present	3
	Absent	10

(less than 3 per 10× objective) had few or moderate numbers of copper positive granules.

On immunohistochemical examination, ductular proliferation (DP) or putative LPC activation characterized by CK19- and CK7-positive clusters of small epithelial cells with marked cytoplasmic staining was clearly seen. DP was present throughout the lobules in all cases with LDH (Fig 3), but restricted to the fibrous septa in cases of micronodular or macronodular cirrhosis. In most of the cases (11/13), intermediate type hepatocytes were seen in the dissected lobules or at the periphery of the hyperplastic regenerative nodules in various degrees. In some areas, the intermediate hepatocytes were located in direct continuity to the ductular reaction. In 1 dog with mild periportal fibrosis, a moderate to marked ductular proliferation was present in the fibrotic region with marked presence of intermediate type hepatocytes in the in associated parenchyma. The scores of CK19 staining are summarized in Table 2. Within the fibrous tissue, numerous alpha-SMA positive hepatic stellate cells were observed in all cases (Fig 4).

Treatment and Clinical Outcome

All the dogs were treated with anti-inflammatory agents (prednisone, starting dosage of 0.5–1.25 mg/kg/day, with

or without azathioprine 1 mg/kg/day). Ursodeoxycholic acid (20 mg/kg/day) was also prescribed in most cases (n = 12). Lactulose (n = 9) and antibiotics (metronidazole (n = 9), amoxicillin (n = 5), ofloxacin (n = 3), kanamycin (n = 2), and enrofloxacin (n = 1)) were used in most cases to control or prevent hepatic encephalopathy. Ascites was controlled by diuretics (furosemide (n = 6), spironolactone (n = 5), or torsemide (n = 2)). Other treatment included S-adenosyl methionine (n = 4), vitamin E (n = 2), glycyrrhizin (n = 1), and vitamin K (n = 1). Dietary management included commercial Hepatic^g diet (n = 9) or 1/d^h (n = 4).

One dog showed severe lethargy, inappetence, increased ascites, melena, and diarrhea on day 25 (5 days after starting corticosteroid), suggesting that PH had progressed. No icterus was seen. Partial seizure was also seen, but plasma ammonium level remained normal. This case did not respond to intensive care and died at day 64. Eleven dogs improved clinically by showing increased activity and/or appetite after receiving treatment within 2–4 weeks. Ascites and hepatic encephalopathy were controllable in these 11 dogs. The activity of serum liver enzymes also decreased in 9 of these dogs, although the decreased serum albumin concentration did not return to the reference range. One additional dog deteriorated clinically and developed hepatic encephalopathy, which disappeared upon discontinuation of the corticosteroid medication. Of these 12 dogs, 4 remained stable for 4 months to 5 years, but then showed worsening general condition and liver-related problems such as uncontrollable ascites resulting in euthanasia or death at 165, 622, 913, and 1981 days after first examination. Of the 12 dogs, 2 dogs died of nonliver-related causes (intoxication) 180 and 140 days after initial visit at the university hospital. The remaining 6 dogs were in good general condition, the ascites disappeared, and the activities of plasma liver enzymes also decreased to the reference range. These 6 dogs were alive at the censored time point.

The median survival time was 913 days (Kaplan–Meier survival estimate, range: 63–1981 days, median: 913 days, 95% CI of lower range: 622 days).

Discussion

This study describes the clinical and histological features of a breed-associated form of chronic liver disease in American Cocker Spaniels in Japan. The main characteristics were a chronic disease course with signs of PH, extensive liver fibrosis associated with putative hepatic progenitor cell activation (ductular proliferation), and low degree inflammation. All dogs were clinically (ascites, hepatic encephalopathy) and histologically (different forms of cirrhosis) in an advanced stage of the disease. Lobular dissecting hepatitis, which has never been reported as a breed-associated form of cirrhosis, was the predominant form seen in these dogs. The more usual micro- or macronodular types of cirrhosis were, however, also represented, so that these dogs do not display only 1 specific subtype of chronic hepatic

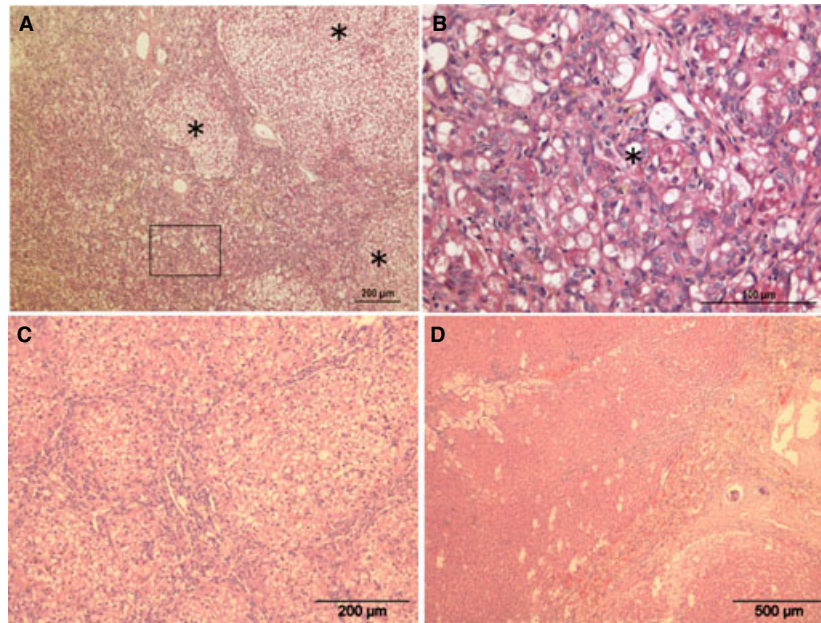


Fig 2. HE staining of representative cases of 3 different types. (A) Lobular dissecting hepatitis (LDH). Low power magnification. Large areas with abnormal architecture attributable to lobular dissection by fibrous tissue and ductular proliferation; in addition some hyperplastic parenchymal nodules are present (*). (B) LDH. Higher power magnification of boxed area in Figure 2a. Dissection of the liver parenchyma by fibrous tissue and ductular proliferation around single and small groups of hepatocytes; presence of some pigmented macrophages and lymphocytes; regularly ballooning of hepatocytes. (C) Micronodular cirrhosis. Low power magnification. Abnormal architecture with nodular transformation of the liver attributable to centrocentral and centro-portal bridging fibrosis with ductular proliferation. Diffuse moderate ballooning of hepatic parenchyma. (D) Macronodular cirrhosis. Low power magnification. Parts of 2 large parenchymal nodules separated by a broad fibrous septum. In the large parenchymal nodule spotty ballooning of hepatocytes.

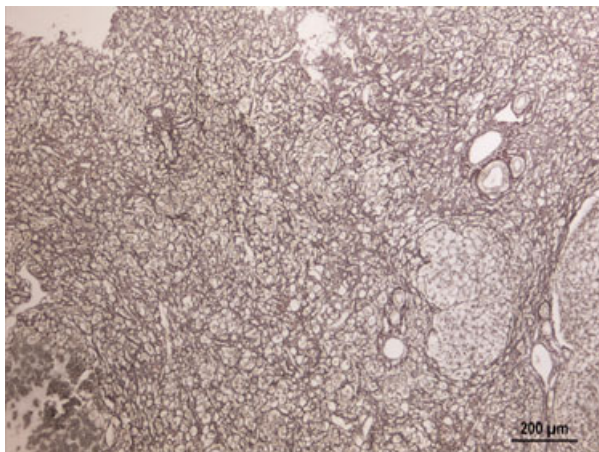


Fig 3. Reticulin stain of a representative case of lobular dissecting hepatitis histology. Low power magnification. Abnormal architecture attributable to dissection of the liver parenchyma by fibrous tissue (reticulin fibers) around individual and small groups of liver cells; within these areas some preexistent portal areas can be recognized. Small hyperplastic parenchymal nodules are also present (lower right).

tis/cirrhosis. A possible relationship between these 3 forms of cirrhosis could only become obvious when in future studies successive biopsies are studied of different stages of the disease.

A consistent clinical feature in the dogs presented here was PH, reflected by the presence of APSCs or ascites²

in all dogs. Despite the reported negative prognostic effect of ascites in dogs with chronic liver disease,^{22,23} the dogs with ascites in this report demonstrated long-term survival (with a 95% lower CI of 913 days). This further supports the idea that the syndrome presented here may be different from previously reported cases.

Corticosteroids were used in all cases. It is uncertain if this may have influenced their survival considerably, although the possible beneficial effect of this drug on Cocker Spaniel hepatitis has been reported.^{24,a} Corticosteroids have been reported to have a direct or indirect (ie, via suppression of the inflammation) anti-fibrotic effect.^{25,26} In addition, corticosteroids were reported to suppress TGF-beta pathway and hence suppress fibrosis.²⁶ In the present cases, severe fibrosis with a large number of activated hepatic stellate cells was present. In addition, macrophages, an important source of cytokines that stimulate the activation of stellate cells and progenitor cells, were also present. Most of the here reported cases responded to the treatment which always included glucocorticoids. However, the actual effect on the liver is uncertain because follow-up biopsies were not taken. Prednisolone may also have various adverse effects such as gastrointestinal bleeding. In fact, 1 case in this study showed rapid deterioration after starting treatment. Because of the retrospective nature of this study, the true efficacy of corticosteroids on ACSH cannot be determined and future prospective research is obviously needed.

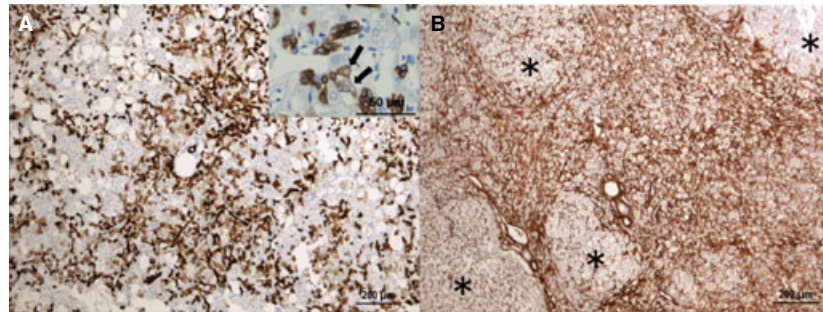


Fig 4. Immunohistochemistry for CK19 and alpha smooth muscle actin. (A) Anti-CK19 antibody staining ($\times 40$ and $\times 100$ [the insert]). Highly positive cells composing epithelial-like structures, suggestive of putative liver progenitor cells (LPCs), are present throughout the lobules. Some small cuboidal cells adjacent to the LPCs showed weaker and membranous positivity for CK19 (putative intermediate hepatocytes, arrows in the insert). (B) Anti-alpha smooth muscle actin antibody. Positive hepatic stellate cells were seen throughout the lobules. Note that the number of positive cells was far less in the regenerative nodules.

In addition to prednisolone, we also used S-adenosyl methionine, vitamin E, and glycyrrhizin as an antioxidant. The necro-inflammatory response was not very severe in these cases, and the clinical effect of these medications or corticosteroids has never been proven in a randomized study. Theoretically, antioxidants could contribute to inhibit the further development of necro-inflammatory lesions and fibrosis.²⁶

American Cocker Spaniels are reported to be predisposed to chronic hepatic disease,¹⁸ and there have been several different reports of American Cocker Spaniels with hepatitis.^{a,18,19,27–32} One report describes clinical and morphological features of 16 cases of American Cocker Spaniel hepatopathy.^a The clinical signs related to hepatic failure, ie, PH and profound ductular reaction, were similar to the present cases, but the histological features (ie, not lobular, but periportal inflammatory lesions) and very poor prognosis (12 of 16 dogs died within a month) were clearly different. Chronic hepatitis and chronic progressive hepatitis were also reported in both American and English Cocker Spaniels. In these cases, the clinical picture including the chronic course, ascites, and hypoalbuminemia was similar to the present cases. Although histological inflammation was the main component in these cases, the described disruption of liver parenchyma by reticulin and fine collagen fibers suggests that the liver disease of these cases may be comparable to the cases described here.

We defined the present cases as American Cocker Spaniel hepatitis rather than hepatopathy. First, hepatopathy itself tends to describe various histological changes that are not relevant to inflammation, such as “vacuolar hepatopathy” or “drug-induced hepatopathy.”^{33–36} To reflect at least the aspect of the pathophysiological condition ongoing in the present cases (ie, inflammation and subsequent fibrosis), we preferred the term hepatitis here. Furthermore, we feel that the term hepatitis also reflects other clinical aspects of the cases. In fact, more than half of the cases showed increased white blood cells and CRP and most of the cases responded well to the treatment including prednisolone. Although hepatopathy can

refer to broader spectrum of the liver disease including hepatitis, we considered hepatitis a more appropriate term for the present cases. In addition, the use of different terminologies for 1 condition is a continuous cause of misunderstanding and confusion in the veterinary world. In human medicine, this has and is still being resolved by consensus statements of leading specialists in the field.^{37,38} As the diagnosis of canine hepatitis/hepatopathy is only possible by histological examination of liver tissue, it is logical to refer to the preferred terminology for the comparable human disease. On the basis of these reasons, we regarded hepatitis as the suitable term for the condition described herein.

The etiology of this American Cocker Spaniel hepatitis remains unknown. Because of the breed association of the type of hepatitis described here, a genetic component might be possible. Unfortunately, the complete family pedigree was not available and pedigree analysis could not be performed. In 4 cases the available pedigree information showed no relationship within 3 generations. Analysis of larger family groups with confirmed clinical and histological diagnosis will be needed in the future. Autoimmunity or bacterial infection is also less likely because of the low necro-inflammatory activity of the liver histology among all the cases. Another possibility is that quite chronic, persisting (but weak and mild) inflammation leads to the severe fibrosis seen in the present cases. A possible etiology of such a lesion is an unknown virus or the cumulative toxicity to an unknown material. In this hypothesis, breed relation can be explained by genetic susceptibility to the pathogen. One of the most important toxic components in the dog is copper. However, it is unlikely that copper played a major role in the pathogenesis of ACSH because of the absence of increased copper in most cases (11/13) determined by copper staining. Another possibility is alpha-1 antitrypsin deficiency.²⁸ Interestingly, 2 dogs without familial relationship lived in the same house. This could also suggest an infectious or environmental etiology.

Profound ductular reaction is a characteristic histological finding in this study. Hepatocytes have a very

potent regenerative capacity and proliferate when the liver is severely damaged. However, when the damage to the liver transcends the hepatocytic proliferation is inhibited, and progenitor cells within the Canal of Her- ing start to proliferate, giving rise to what is known as ductular reaction.³⁹ In the present cases, this ductular reaction was a prominent finding. The precise nature of these cells remains to be elucidated in future studies.

In conclusion, we describe a special form of chronic hepatitis and cirrhosis in American Cocker Spaniels in Japan. Further studies will be needed to gain insight into its etiology and to examine if the Japanese dogs represent a subtype different from cases reported from the United States and Europe.

Footnotes

- ^a Hardy RM. Chronic hepatitis in Cocker Spaniels — Another syndrome? Proc. 11th ACVIM Forum 1993; 256–258
^b Nemio; Toshiba Medical Systems Corp, Tochigi, Japan
^c EUB-6500; Hitachi Aloka Medical Ltd, Tokyo, Japan
^d Aplio XV; Toshiba Medical Systems Corp
^e DAKO, Glostrup, Denmark
^f Vector Laboratories Inc, Burlingame, CA
^g ROYAL CANIN JAPON, Inc, Tokyo, Japan
^h Hill's-Colgate (JAPAN) Ltd, Tokyo, Japan

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

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Immunohistochemical and immunofluorescence procedure.