



Title	Peliosis Hepatis with Chylous Ascites in a Dog
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1 **Short communication**

2 **SPONTANEOUSLY ARISING DISEASE**

3 **Short Title:** Peliosis Hepatis with Chylous Ascites in a Dog

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5 **Peliosis Hepatis with Chylous Ascites in a Dog**

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18 **Summary**

19 A 6-year-old spayed female Toy Poodle dog was referred to the Hokkaido University Veterinary
20 Teaching Hospital for abdominal distension. Abdominocentesis yielded ascitic fluid that had a
21 mildly increased total protein concentration and a 2.7-fold higher triglyceride concentration than
22 plasma, and was interpreted as chylous ascites. The patient had enlarged liver which contained
23 multiple, small, nodular masses and cyst-like structures. Microscopically, these lesions were
24 multifocal dilated spaces containing lymphocytes, endothelial cells, fibrin and islands of
25 hepatocytes. Increased α SMA-positive cells were observed in hepatic sinusoids. Based on these
26 findings, we diagnosed peliosis hepatis with chylous ascites, which is likely to have been due to

27 lymphangiectasia and disrupted hepatic sinusoids. Neither *Bartonella* sp DNA nor mutations in
28 *ACVRL1* and *MTMI* genes were detected although there was a 47-fold increase in hepatic
29 *ACVRL1* expression compared to age-matched control liver. To the authors' knowledge, this is
30 the first report of chylous ascites resulting from peliosis hepatitis in any species.

31

32 *Keywords:* *ACVRL1*; chylous ascites; dog; peliosis hepatitis

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34

35 Peliosis hepatitis (PH) is a rare vascular disease characterized by numerous, randomly dispersed,
36 blood-filled cavities in the liver. The diameter of the cavities typically varies from a few mm to
37 3 cm. PH has been reported in dogs, cats, rats, cattle and humans (Degott *et al*, 1978; Zafrani *et*
38 *al*, 1984; Maxie, 2007), and may occur at any age but is more common in middle-aged or old
39 humans and dogs. Male predominance was documented in humans, but PH has been suggested
40 to be more common in female dogs (Degott *et al*, 1978; Inoue *et al*, 1988; Izumi *et al*, 1994).

41 In humans, PH has been associated with activin receptor-like kinase 1 (*ACVRL1*),
42 myotubularin 1 (*MTMI*) and endoglin (*ENG*) mutations or repression (Laporte *et al*, 2000;
43 Kjeldsen *et al*, 2005). In dogs, *Bartonella henselae*, the causative agent of cat-scratch disease,
44 was reported to be a possible cause of PH (Kitchell *et al*, 2000). Interestingly, in cats, *B.*
45 *henselae* infection is not associated with the disease (Buchmann *et al*, 2010) and no association
46 has been made in other species. Diagnosis of PH in humans is challenging (Daniele Crocetti *et*
47 *al*, 2015; Dai *et al*, 2017) and computed tomography (CT) and magnetic resonance imaging
48 (MRI) do not give conclusive results (Perkocha *et al*, 1990). In addition, PH grossly resembles,
49 and can be misdiagnosed as, a metastatic tumour or liver abscess (Jensen *et al*, 2000).

50 Chylous ascites is characterized by the accumulation of triglyceride-rich peritoneal
51 fluid with a milky appearance (Al-Busafi *et al*, 2014). In humans, chylous ascites has been
52 documented in congenital diseases such as lymphatic hypoplasia, intestinal lymphangiectasia,

53 neoplastic diseases such as lymphoma and Kaposi sarcoma, and in infectious diseases such as
54 tuberculosis and filariasis (Bhardwaj *et al*, 2018). Chylous ascites is rare in animals and has
55 been reported in dogs and cats with malignant neoplastic disease, lymphangiectasia, lymphatic
56 obstruction and lymphatic rupture (Fossum *et al*, 1992; Lott *et al*, 2015; Hatch *et al*, 2018). PH
57 has not been reported as a cause of chylous ascites in any species.

58 A 6-year-old spayed female Toy Poodle dog was referred to the Hokkaido University
59 Veterinary Teaching Hospital for abdominal distension (Supplementary Fig. 1A).
60 Abdominocentesis yielded reddish, opaque abdominal fluid. Fluid analysis revealed nucleated
61 cell counts of 1,300 cells/ μ L, composed mainly of small lymphocytes, along with traces of
62 neutrophils and macrophages with a few red blood cells (8,000 cells/ μ L) (Supplementary Fig.
63 1B). The centrifuged fluid was whitish yellow with a slightly increased total protein
64 concentration (2.6 g/dL). Triglyceride concentration in the abdominal fluid (149 mg/dL) was 2.7
65 times higher than in plasma (55 mg/dL). Based on these findings, chylous ascites was
66 diagnosed. Ultrasonography revealed that the liver was larger than normal and contained
67 multiple, small, nodular masses, and cyst-like structures (Supplementary Figs. 1C and D). The
68 patient was treated continuously with spironolactone and prednisone to manage the ascites, but
69 the clinical condition worsened and the dog died 3 weeks after the initial presentation.

70 At necropsy, irregularly circumscribed red foci of up to 3 cm \times 2.5 cm in diameter
71 were observed multifocally in all lobes of the liver (Fig. 1). The dark-red foci had a cyst-like
72 appearance and blood-tinged fluid oozed from the cut surface. Microscopic examination
73 revealed that these lesions were multifocal dilated spaces containing lymphocytes, endothelial
74 cells, fibrin and islands of hepatocytes (Fig. 2). Some dilated spaces also contained copious
75 amounts of red blood cells and blood clots. The lymph vessels in portal regions were moderately
76 dilated compared with a normal control liver from an age-matched, female, French Bulldog
77 (Supplementary Figs. 2A and 2B). The number of α SMA-positive cells was higher in the
78 hepatic sinusoids of the patient's liver compared to the control liver (Fig. 3). Jones'

79 methenamine silver staining of liver sections revealed disruption of reticulin fibres and
80 thickening of the basement membrane in the perisinusoidal region compared to the normal age-
81 matched control (Fig. 4).

82 DNA was extracted from frozen liver and an age-matched control using the QIAamp
83 DNA Mini-kit (Qiagen, Valencia, California, USA). Polymerase chain reaction (PCR) was
84 performed for *Bartonella* species as described (Jensen *et al*, 2000; Johnson *et al*, 2003) and was
85 negative (Supplementary Fig. 3A). Total RNA was extracted with Nucleospin® RNA isolation
86 kit (Macherey-Nagel, Düren, Germany) and synthesis of cDNA was performed using the
87 PrimeScript™ Reverse Transcriptase (Takara Bio, Kusatsu, Japan) following the manufacturers'
88 instructions. Canine myotubularin 1 (*MTMI*) and activin receptor-like kinase 1 (*ACVRL1*)
89 coding sequences were cloned using the primers listed in Supplementary Table 1. Mutations in
90 the coding sequences of these genes were not detected by sequential analysis and alignment of
91 the cloned sequences with the canine reference genes on Ensemble database. Reverse
92 transcription quantitative PCR (RT-qPCR) assay was performed to determine the mRNA
93 expression of *ACVRL1* and *MTMI* genes. Results showed that the liver had a 47-fold increased
94 expression of *ACVRL1* compared with the age-matched control liver, while no significant
95 difference was observed in *MTMI* expression (Supplementary Fig. 3B).

96 The definitive diagnosis of PH was made after diagnostic imaging, histopathological
97 examination with special staining for reticulin, and immunohistochemistry for α -SMA. The
98 chylous ascites was likely due to the lymphangiectasia and disrupted hepatic sinusoids in the
99 liver. The space of Disse is the major route of lymph flow in the liver (Ohtani and Ohtani, 2008)
100 and, in this case, the sinusoid basement membrane was disrupted, which likely allowed
101 accumulated lymph to flow into the sinusoid resulting in the formation of cyst-like spaces
102 containing blood and lymph. We hypothesize that the accumulated lymph and blood entered the
103 abdominal cavity due to hepatic rupture, which led to the accumulation of chylous ascites. Other
104 possible causes of chylous ascites such as lymphatic hypoplasia and neoplastia were not

105 supported by physical examination, haematology, blood chemistry, diagnostic imaging,
106 necropsy or histological analyses.

107 Although mutations in *MTM1* and *ACVRL1* coding sequences were not detected in this
108 case, RT-qPCR assay revealed a significant increase in the expression of *ACVRL1* in liver
109 compared with the age-matched control liver. *ACVRL1*, also known as ALK1, is a receptor in
110 the TGF- β and BMP9 signalling pathways (Scharpfenecker *et al*, 2007). Considering most
111 *ACVRL1* mutations in humans are dysfunctional, loss of function of TGF- β or BMP9 signalling
112 is probably responsible for PH development. Since there was no mutation of the coding region
113 in *ACVRL1* in the present case, these downstream signalling pathways of *ACVRL1* might be
114 non-functional (Gu *et al*, 2006; Ricard *et al*, 2010; Ruiz *et al*, 2017). This can result in a
115 compensatory increase in *ACVRL1* expression.

116 Another hypothesis is that the increased *ACVRL1* is the direct cause of PH. *ACVRL1*
117 expression is induced during angiogenesis but is diminished when vessels mature, which
118 suggests that it has a role in inhibiting angiogenesis and in stabilizing vessel structural integrity
119 (Seki Tsugio *et al*, 2003; Tual-Chalot *et al*, 2014). However, one study reported that *ACVRL1*
120 could stimulate proliferation and migration of normal endothelial cells (Li *et al*, 2008).
121 Overexpression of *ACVRL1* could dysregulate angiogenesis, leading to disorganized hepatic
122 sinusoids. The likely cause of the increased *ACVRL1* expression in this case are mutations in
123 non-coding sequence regions, including the promoter or the introns which could have been
124 missed by the sequential analysis. Another possible cause of increased *ACVRL1* expression is
125 mutations in the genes coding for the transcription factors for the *ACVRL1* gene or for the
126 *ACVRL1* upstream pathway genes. However, the role of *ACVRL1* in canine PH is unclear.

127 To the authors' knowledge, this is the first report of chylous ascites resulting from PH
128 in a dog. This study presents several diagnostic options which are essential for PH diagnosis. As
129 the exact mechanism of PH is unknown, further studies on its pathogenesis and treatment
130 options are warranted.

131

132

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138

139

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141

142

Conflict of Interest Statement

143 The authors declared no potential conflicts of interest with respect to the research, authorship or
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145

146

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219

220

221

222 **Figure legends**

223

224 Fig. 1. Peliosis hepatitis, liver, dog. Numerous, irregularly lined, randomly dispersed, blood-filled
225 cavities throughout cranial aspect of liver. Cyst rupture in caudate lobe (arrow).

226

227 Fig. 2. Peliosis hepatitis, liver, dog. Cyst contains lymphocytes, islands of hepatocytes,
228 endothelial cells and few erythrocytes. HE.

229

230 Fig. 3. Peliosis hepatis, liver, dog. Increased intensity of immunolabelling of α SMA in sinusoids
231 (left) compared with age-matched control liver (right).

232

233 Fig. 4. Peliosis hepatis, liver, dog. Reticulin fibre disruption and thickening of basement
234 membrane in perisinusoidal regions (left) compared with age-matched control liver (right).
235 Jones' methenamine silver.



Fig. 1

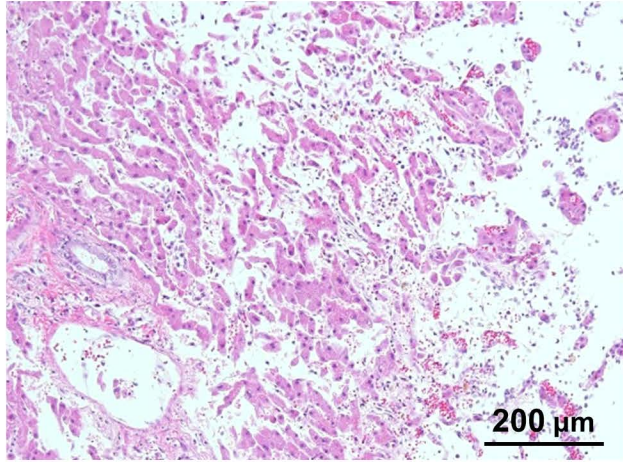


Fig. 2

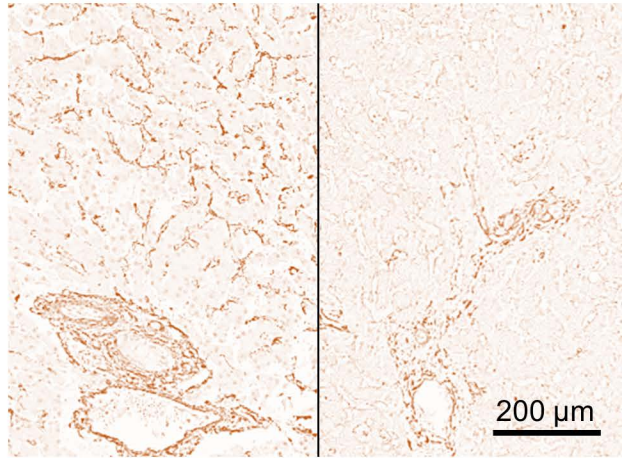


Fig. 3

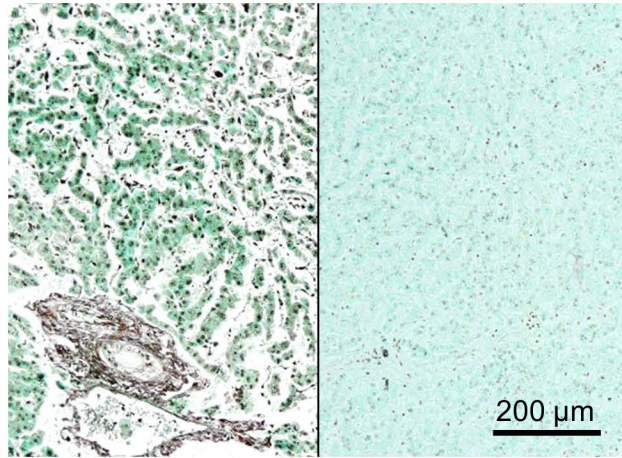


Fig. 4