

1 **Report of Acute Hepatic Necrosis caused by *Salmonella* serotype I 4, 5, 12:-:1,2 in a**  
2 **Dog**

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12 **Acute hepatic necrosis was diagnosed in a dog. Gram staining and fluorescence *in situ***  
13 **hybridization identified *Salmonella enterica* in the liver, subsequently confirmed as**  
14 ***Salmonella enterica* serotype I 4, 5, 12:-: 1,2. This is the first report of acute hepatic**  
15 **necrosis with liver failure caused by *Salmonella* in a dog.**

16  
17 **CASE REPORT**

18 A six month old, fully vaccinated female, entire Staffordshire bull terrier was presented for  
19 examination with a history of two days of vomiting, loose stools, lethargy and reduced  
20 appetite. Clinical examination was unremarkable, the dog appeared in good condition and  
21 was not dehydrated, the temperature was normal, and the animal was very bright and  
22 responsive. A decision was made to give standard dose subcutaneous injections of

23 maropitant and ranitidine plus a bland diet of chicken and rice. The dog was always fed with a  
24 commercial dry diet.

25 The dog was re-examined after four days, and the owner reported improvement with  
26 no vomiting. However the dog still had a reduced appetite and loose stools. Mucus membrane  
27 coloration and body temperature were not recorded. Blood work was offered, but the owner  
28 declined for financial reasons.

29 Two days later, during the night, the dog deteriorated very quickly with marked  
30 lethargy, vomiting and diarrhea. The dog was brought to the emergency service. The patient  
31 appeared very lethargic and mildly dehydrated with severe icterus. Biochemistry and  
32 complete blood count showed severely increased liver enzymes: alkaline phosphatase (ALP)  
33 1493u/l (range 23-212), alanine aminotransferase (ALT) 969u/l (10-100), total bilirubin (TBil)  
34 182 umol/l (0-15) and decreased urea 0.9 mmol/l (2.5-9.6). Haematology showed that white  
35 blood cells were mildly increased; white cell count  $17.7 \times 10^9/l$  (6-10.9) with granulocytes  
36  $15.6 \times 10^9/l$  (3.3-12). Symptomatic treatment with standard doses of maropitant and  
37 clavulanate potentiated amoxicillin were given subcutaneously and fluid therapy was started.

38 The following day, physical examination showed continued severe icterus. No  
39 dehydration was reported, temperature was normal, and abdominal palpation and  
40 auscultation were unremarkable. Also, an abdominal ultrasound was largely unremarkable  
41 with the liver reported as normal in size with a slightly hyperechoic appearance. Blood was  
42 taken for leptospira antigen PCR, and was found to be negative. Symptomatic treatment and  
43 fluid therapy were continued.

44 The next day the biochemistry results showed an increase in liver enzymes; ALT  
45 1455u/l(5-60), ALP 1909 u/l( $\leq$ 130), TBil 225.5 umol/l(0-15), cholesterol 9.80 umol/l (3.2-

46 6.2), while the urea remained low 2.1mmol/l (2.5-9.6). Extensive discussion with the owner  
47 was undertaken to rule out any possible cause of intoxications. The owner confirmed that  
48 there was no possibility of mushroom ingestion and the dog was walked daily by a beach. The  
49 possibility of intoxication with green-blue algae (*Microcystic aeruginosa*) was ruled out  
50 because there was no ground water by the beach. Possible ingestion of hepatotoxic drugs  
51 was also ruled out by the owner. Interestingly, the owner reported the accidental ingestion of  
52 a raw rotten egg that was found during the daily walk a few days previously. Unfortunately,  
53 despite aggressive fluid therapy and symptomatic treatment, the dog developed hepatic  
54 encephalopathy with seizures and was euthanized the day after the hospitalization.

55 The owner gave permission for a post mortem to be performed in order to understand  
56 the cause of death. An incision along the *linea alba* from the xiphoid process of the sternum to  
57 the pubic area was performed to expose the abdominal cavity. The liver was abnormal in  
58 color, with a diffuse dark hemorrhagic appearance (Fig. 1). A liver sample for culture was  
59 taken, and another sample was sent for histopathology evaluation. Considering the history of  
60 vomiting, but despite the normal appearance, a sample for histopathology was taken from the  
61 stomach and small intestine as well. The rest of the abdomen appeared normal. The chest  
62 cavity was opened. The lungs and the heart were checked and no signs of abnormality were  
63 seen. Hemorrhage in the thymus was noticed, so a sample for histopathology evaluation was  
64 taken.

65 The histopathology report of the liver was diagnostic of acute hepatic necrosis. The  
66 pathologist described areas of severe and diffuse liver necrosis, with only some normal  
67 hepatocytes present in the portal areas associated with fatty change. Inflammatory infiltrates  
68 of neutrophils, lymphocytes and plasma cells were present with multifocal areas of

69 cholestasis. Multifocal areas of hemorrhage were also present. The stomach was  
70 histologically normal. The thymus was histologically normal except for multifocal areas of  
71 hemorrhage. The pathologist, after re-evaluation with other pathologists, found no evidence of  
72 infectious canine hepatitis virus as there were no viral inclusions seen. The intestine and  
73 stomach were normal on histopathology and culture was not performed.

74 Toxicology examination was performed on the dog food biscuits eaten by the dog in  
75 the previous couple of weeks and the sample was found to be negative for aflatoxin. The  
76 microbiology report from the liver isolated the presence of Group B *Salmonella enterica*,  
77 sensitive to the 8 most common antibiotics used in veterinary medicine. Bacteria were  
78 subsequently cultured with an enrichment technique and evaluated. The result was the  
79 presence of a monophasic Typhimurium-like *S. enterica* serotype I 4, 5, 12:-: 1,2. Further  
80 Gram staining of the liver tissue showed the presence of Gram negative bacteria diffusely  
81 within the parenchyma (Fig. 2) collocated with the necrosis and Fluorescence *in situ*  
82 Hybridization (FISH) confirmed the bacteria to be *S. enterica* (Fig. 3).

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85 To the best of our knowledge, this is the first case of acute hepatic necrosis in a dog  
86 caused by *S. enterica* serotype I 4, 5, 12:-:1,2. This *Salmonella* is rarely isolated in human  
87 infection, but the real pathogenic and zoonotic risks are unknown. The possibility of another  
88 unknown aetiology for the acute hepatic necrosis in this case was considered but many other  
89 causes were ruled out and the positive culture and FISH make *Salmonella* the most likely the  
90 cause.

91 In human medicine, *Salmonella enterica* serovar Typhi has been reported to cause  
92 hepatic liver enzyme increases with various degrees of liver impairment (1). This is usually  
93 due to typhoid fever, a generalized multisystemic illness with fever. In rare human cases,  
94 jaundice and severe hepatitis have been described as a complication of infection with *S.*  
95 Typhi (1, 2). In this infection the liver histopathology usually shows mononuclear cell  
96 infiltration with focal minimal portal tract infiltration and areas of focal necrosis (1, 2). The  
97 mechanism by which *S. Typhi* causes hepatitis is unclear. Extra-intestinal *Salmonella enterica*  
98 serovar Typhimurium (ESTM) infections have occasionally been reported in children under  
99 five years old and in immunocompromised individuals (3-5). In immunocompromised patients,  
100 arthritis, osteomyelitis and solitary or multiple abscesses in the spleen, liver and brain have  
101 been described (5), while in children, meningoencephalites, pericarditis, cholecistitis and  
102 Hepatitis have been reported (3).

103 In dogs salmonellosis is often asymptomatic, clinical signs of infection are uncommon  
104 with severe clinical signs in young or debilitated patients (6). A previous case report described  
105 three acute cases of *S. Typhimurium* infection in young puppies which resulted in sudden  
106 death (7). The necroscopy of these patients revealed some areas of hepatic necrosis, but the  
107 areas of necrosis were not diffuse and did not cause liver failure, and the dogs likely died of  
108 systemic inflammation due to septicemia rather than liver failure (7). There are two case  
109 reports of young dogs with localized infection with *S. Typhimurium*, in one case the bacteria  
110 were localized in the kidney and in the other case the bacteria were localized in the  
111 gallbladder, the bacteria were not systemic in either case (8, 9). To our knowledge, the  
112 isolation of *Salmonella* Serotype I 4, 5, 12:-: 1,2 with hepatic necrosis and liver failure has  
113 never been described previously.

114 In this case report, it is possible that the *Salmonella* could have caused the mild  
115 symptoms of illness during the first week of infection with very mild signs of vomiting and  
116 diarrhea, later causing septicaemia with a poor host response considering the young age of  
117 the dog. Unfortunately blood culture was not performed due to the previous administration of  
118 antibiotics and the lack of clinical and laboratory findings suggestive of septicaemia (e.g. fever  
119 and disseminated intravascular coagulation). Therefore, septicaemia cannot be confirmed or  
120 ruled out completely. The lack of fever could be due to the prompt injection of antibiotics or it  
121 is possible that there was fever but it was transient and not picked up at the time of the clinical  
122 examination. The culture and sensitivity showed that the *Salmonella* isolated was sensitive to  
123 the previously administered amoxicillin-clavulanic acid with a MIC $\leq$ 2 (Reference range 2-  
124 32). Despite that, considering the isolation of the bacteria in the liver tissue, it is possible that  
125 the administration of the antibiotic could not reach the necessary concentration to affect the  
126 growth of the bacteria. Furthermore antibiotic treatment could have been detrimental rather  
127 than protect from the bacterial infection. For example, it has recently been shown that mice  
128 infected with *S. Typhimurium* and treated with antibiotics not only have increased  
129 concentrations of fecal *salmonella* but also increased morbidity and mortality compared with  
130 non-antibiotic treated mice (10).

131 Bile culture has been found more sensitive than liver tissue culture in the detection of  
132 bacteria, but bile culture was not performed in this case. The finding of a positive culture in  
133 liver tissue indicated the presence of *Salmonella* and the positive FISH for the bacteria in the  
134 liver parenchyma confirmed this finding. Culture of faeces was not performed because no  
135 obvious signs of small intestinal pathology could be seen clinically or on histopathology.

136 The possibility of toxin exposure was extensively discussed with the owner after the  
137 histopathology report. The owner described the dog as a fussy eater, with no scavenging  
138 behavior and ruled out any exposure to any fungal toxins or *Microsporium*. The owner did not  
139 have a garden, and living by the sea, the daily walk was beside the beach. Considering the  
140 hostile environment of the beach by the sea for mushroom growth, the presence of toxic  
141 mushroom ingestion was considered very improbable. Furthermore, the absence of a pond  
142 ruled out the possibility of *Microcystis aeruginosa* algae ingestion. The possibility of ingestion  
143 of any kind of drugs was ruled out by the owner. Infectious hepatitis was ruled out by the  
144 pathologist who could not see any inclusion bodies specific of the disease and the dog was  
145 regularly vaccinated.

146 Considering the history, the isolation of *S. enterica* serotype I 4, 5, 12:-:1,2 in acute  
147 liver failure with necrosis, makes this case interesting and highlights the need to consider this  
148 in liver disease in canine patients where no other cause is evident.

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184 **FIGURE LEGENDS**

185 **FIG 1** Necroscopy of the liver showing macroscopic appearance of acute liver necrosis.

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187 **FIG 2** Gram stain of a section of the liver showing a small number of localised areas  
188 highlighting Gram negative bacteria morphologically compatible with *S. enterica* (Scale bar 20  
189  $\mu\text{m}$ , Magnification x600).The photo taken with Philips Digital Image System,

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191 **FIG 3** Section of liver analysed using FISH and showing *S. enterica* in the liver parenchyma.

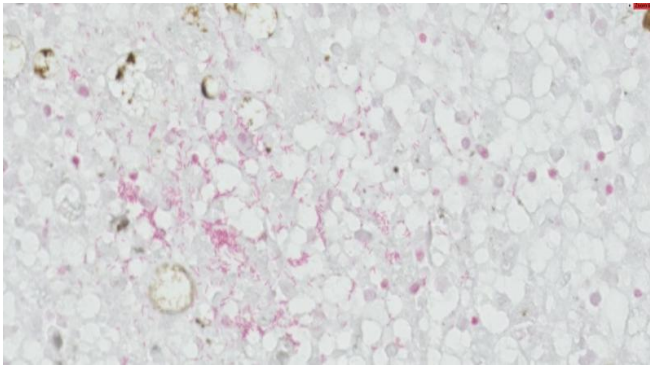
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194 **FIG 1** Necropsy of the liver showing macroscopic appearance of acute liver necrosis.

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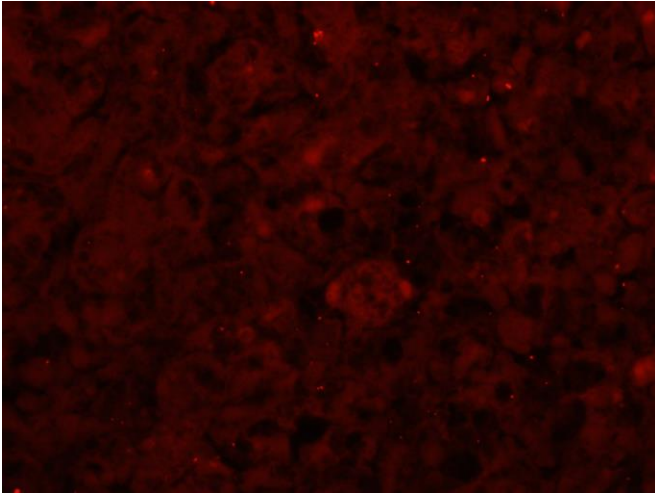


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