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

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

CASE REPORT

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

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

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

Two days later, during the night, the dog deteriorated very quickly with marked lethargy, vomiting and diarrhea. The dog was brought to the emergency service. The patient appeared very lethargic and mildly dehydrated with severe icterus. Biochemistry and complete blood count showed severely increased liver enzymes: alkaline phosphatase (ALP) 1493u/l (range 23-212), alanine aminotransferase (ALT) 969u/l (10-100), total bilirubin (TBil) 182 umol/l (0-15) and decreased urea 0.9 mmol/l (2.5-9.6). Haematology showed that white blood cells were mildly increased; white cell count $17.7x10^9$/l (6-10.9) with granulocytes $15.6x10^9$/l (3.3-12). Symptomatic treatment with standard doses of maropitant and clavulanate potentiated amoxicillin were given subcutaneously and fluid therapy was started.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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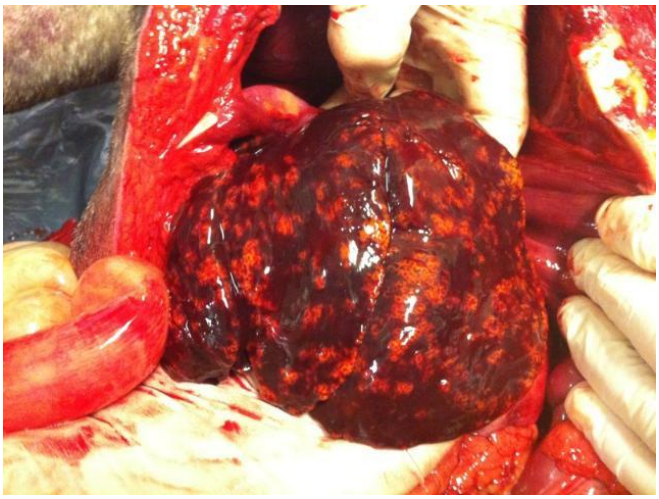
FIGURE LEGENDS

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

FIG 2 Gram stain of a section of the liver showing a small number of localised areas highlighting Gram negative bacteria morphologically compatible with *S. enterica* (Scale bar 20 µm, Magnification x600).The photo taken with Philips Digital Image System,

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



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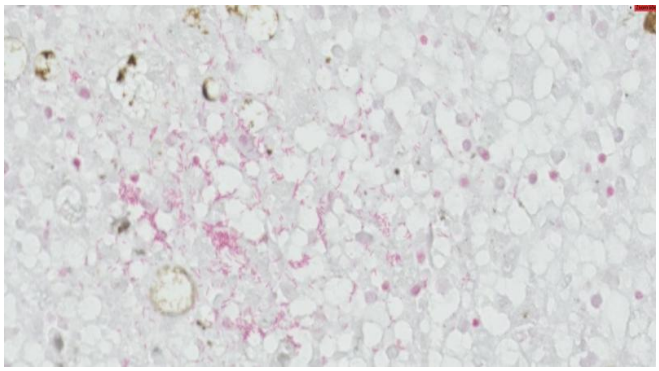

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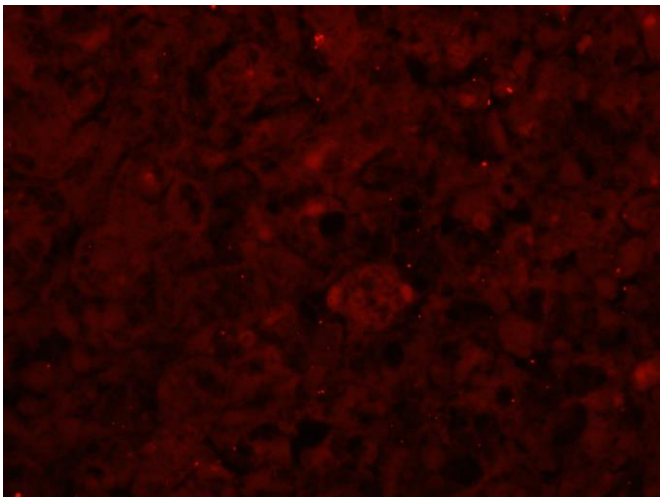

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