

An abdominal cavity abscess associated with *Salmonella enterica* serovar Typhimurium phage type DT2 in a dog: a case report

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ABSTRACT: Most *Salmonella enterica* serovar Typhimurium strains are capable of infecting multiple hosts. In this report, an abdominal cavity abscess associated with the *Salmonella enterica* serovar Typhimurium phage type DT2 is described in a dog. A dead male dog was admitted to the Department of Pathology at the Faculty of Veterinary Medicine at Erciyes University for necropsy. Anorexia, weight loss and lethargy were the clinical symptoms that were reported by the owner of the dog. The diagnosis was made by histopathological and bacteriological examinations of the lungs, spleen, liver and heart. In addition, the content of the abdominal cavity mass was evaluated in bacteriological analysis. The serotyping, phage typing and antibiotic susceptibility of the isolated bacteria were performed at the Danish Institute for Food and Veterinary Research. To the best of the authors' knowledge, this is the first study reporting an abscess associated with *S. enterica* serovar Typhimurium phage type DT2 in a dog.

Keywords: abdominal abscess; dog; *Salmonella enterica* serovar Typhimurium variant DT2; minimum inhibitory concentration; resistance profile

The host range of *S. enterica* subsp. *enterica* serotype Typhimurium (*S. Typhimurium*) is extremely broad and includes humans, ruminants, domestic fowl, rodents and birds. However, some serotype Typhimurium variants have an only very narrow host range whereas other species may transmit infections to multiple hosts (Baumler et al. 1998; Rabsch et al. 2002). Although the majority of *S. Typhimurium* strains affect several animal species, the phage types (DT) 2 and 99 of *Salmonella Typhimurium* have been associated with systemic diseases particularly in pigeons (Pasmans et al. 2003; Helm et al. 2004). Serotype Typhimurium can be differentiated into numerous distinct variants by phage typing and other epidemiological typing methods (Pang et al. 2013). There are several studies reporting the isolation of medically important serotypes for humans such as *S. Typhimurium*, Corvallis, Dusseldorf, Enteritidis and others in domestic dogs around the world (Kocabiyik et al. 2006; Tsai et al. 2007; Hoelzer et al. 2011). *Salmonella* infections in dogs are generally clinically asymptomatic; infections in the gastrointestinal tract may manifest as enterocolitis and endotoxaemia, the common symptoms

of which are fever, vomiting, anorexia, dehydration and depression (Hoelzer et al. 2011). The formation of abscesses due to *Salmonella* infections was attributed to *Salmonella* serotypes in several cases in humans (Su et al. 2003; Lin et al. 2006; Singh et al. 2011). In addition, *Salmonella* serotypes, which cause soft tissue abscesses in guinea pigs, hamsters (Iijima et al. 1987), horses (Blikslager et al. 1991), columbiform birds (doves and pigeons) (Pasmans et al. 2003) and a grey collie (Moazed et al. 1990), have been previously reported. Clinical symptoms such as systemic arthritis and osteomyelitis in horses (Platt 1973), and enteritis, metritis and abortion in rabbits (Agnoletti et al. 2010), have been observed in outbreaks associated with *Salmonella Typhimurium* serotypes.

Pigeon *Salmonella enterica* subsp. *enterica* serovar Typhimurium variant Copenhagen is recognised to be a host-adapted strain (Rabsch 2002; Pasmans et al. 2003). Host-adapted *Salmonella* isolates are extremely lethal for pigeons (Uzzau et al. 2000; Pasmans et al. 2003).

The most striking examples of this apparent adaptation to one particular host reservoir are the

doi: 10.17221/8883-VETMED

serotype Typhimurium phage types DT2 and DT99 which have been cultured from pigeons (Rabsch et al. 2002). It has been demonstrated that pigeon isolates of serotype Typhimurium are different from the ribotypes and biotypes of isolates from people or other animals, and do not represent a source of infection for other hosts (Scholtens and Caroli 1971).

Thus, the Typhimurium serotype prevailing in pigeons (pigeon type) is rarely transmitted to other hosts (Wuthe et al. 1975; Van Oye and Borghijs 1979; Brandis et al. 1980; Rabsch et al. 2002). Epidemiological evidence has shown that serotype Typhimurium has a specific phage type and a narrow host range; it is usually isolated from a specific host, and is rarely isolated from other mammals and birds (Rabsch et al. 2002). These strains cause gastroenteritis, arthritis, orchitis, oophoritis or granulomatous inflammation in all organs, and are likely to be a major cause of bacterial diseases with high mortality in pigeons (Faddoul and Fellows 1965).

In this case report we describe and discuss the pathological and bacteriological findings of an abdominal cavity abscess associated with *Salmonella enterica* serovar Typhimurium phage type DT2 in an Anatolian Karabash dog.

Case description

A dead male dog was presented for necropsy to the Department of Pathology at the Faculty of Veterinary Medicine at Erciyes University by the humane society of Kayseri within approximately two hours after the occurrence of natural death. There was no detailed information about

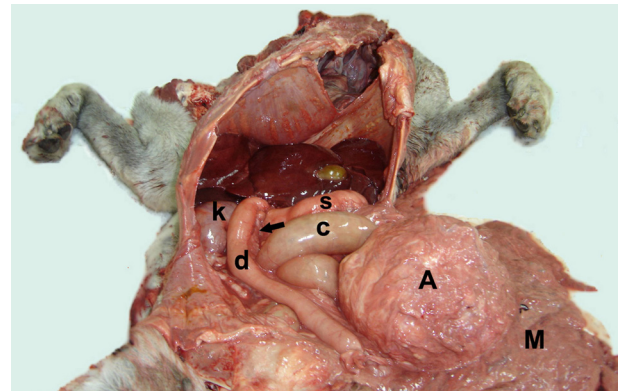


Figure 1. Appearance of abscess in abdominal cavity. A = abscess, M = mesenterium, k = kidney, s = stomach, d = duodenum, c = caecum, arrow = pancreas

the dead dog and thus, no information pertaining to acute/chronic gastrointestinal tract infections or immune and nutritional status. Nevertheless, anorexia, weight loss and lethargy were observed before death. No therapeutic interventions were carried out.

Following gross examination at necropsy, samples of the lungs, spleen, liver, heart and abdominal cavity mass were collected for histopathological and bacteriological examinations. In addition, content from the abdominal mass was taken for culture. For histopathological examinations, tissue samples were fixed in 10% neutral-buffered formalin, embedded in paraffin wax, sectioned at a thickness of 5–6 µm, mounted on glass slides and stained with haematoxylin and eosin (HE), Crossman's triple stain and Brown-Brenn Gram stains. For bacteriological examinations, all of the samples were inoculated onto blood agar (Blood Agar Base, Merck, Germany, supplemented with 7% sheep blood),

Table 1. Susceptibility testing of *Salmonella* Typhimurium

Antimicrobial agent	MIC	RP	Antimicrobial agent	MIC	RP
Amoxicillin + clavulanic	≤ 2	S	Gentamicin	≤ 1	S
Ampicillin	2	S	Nalidixic acid	≤ 4	S
Apramycin	≤ 4	S	Neomycin	≤ 2	S
Cefpodoxime	1000	I	Spectinomycin	32	S
Ceftiofur	1	S	Streptomycin	32	R
Chloramphenicol	4	S	Sulphamethoxazole	≤ 64	S
Ciprofloxacin	≤ 0.03	S	Tetracycline	≤ 2	S
Colistin	≤ 4	S	Trimethoprim	≤ 4	S
Florfenicol	4	S			

Ciprofloxacin represents fluoroquinolones (enrofloxacin). Tetracycline represents doxycycline and other tetracyclines
MIC = minimum inhibitory concentration (µg/ml), RP = resistance profile, S = susceptible, I = intermediate, R = resistant

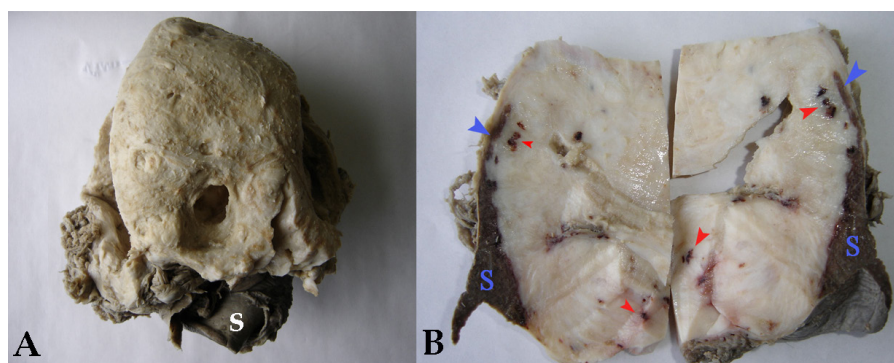


Figure 2. Macroscopical appearance of abscess. **A:** The inner surface of the abscess. **B:** The cut surface of abscess. The abscess shows lobular appearance of the greyish-white cut surface with presence of haemorrhagies (red arrow heads) in the abscess that adhered to the spleen (S, blue arrowhead)

MacConkey agar (Merck, Germany) and EMB (eosin methylene blue) agar (Merck, Germany). Plates were incubated at 37 °C for 24–48 h under aerobic, microaerobic (Anaerocult C, Merck, Germany) and anaerobic (Anaerocult A, Merck, Germany) conditions. In addition, mycological examinations were performed by using Sabouraud dextrose agar (Merck, Germany) and media were incubated at 25 and 37 °C. Preliminary identification of the grown colonies was based on phenotypic characteristics such as colony morphology, microscopic morphology and biochemical tests (Quinn 2002). Serotyping and phage typing were performed according to the Kauffmann-White scheme, *Salmonella* phage typing and the Colindale scheme, respectively (Arguello et al. 2014). Antimicrobial susceptibility was established by determining minimal inhibitory concentration values (CLSI 2002).

Gross pathology revealed a fluctuant, lobular, greyish-pink, well-demarcated mass approximately 25 cm in size in the abdominal cavity (Figure 1). The abscess was surrounded by a thick capsule (Figure 2A). The spleen was atrophic, and there was an adhesion between the spleen and the wall of the abscess. The cut surface of the mass was lobular and greyish-white in colour with haemorrhagic foci (Figure 2B). The content of the mass was greyish-white, and exhibited a creamy consistency. No macroscopic lesions were detected in other organs at gross examination.

In histological examinations, slides stained with HE revealed a granulation tissue composed of reactive chronic inflammation cells and fibro-vascular connective tissue in the wall of the abdominal cavity mass. In some areas, a mixture of inflammatory cells was observed. Moreover, both acute and chronic inflammations were detected. The wall of the abscess consisted of ingrowths of capillaries and fibroblasts. Large cells called bizarre cells with nucleomegaly, pleomorphism, and sometimes,

multinucleation, were observed in the abscess wall adjacent to the spleen (Figure 3). In some areas, extensive aggregation of foamy macrophages and many neutrophils and mononuclear cells which had infiltrated the fibrous connective tissue were observed. There was also visible congestion in the connective tissue. Gram staining revealed the presence of Gram-negative bacteria in these areas. The presence of intercellular and capsular collagens was demonstrated using Masson Trichrome staining. Unremarkable inflammatory changes were observed in several sections of the lungs, spleen, liver and heart which were stained with HE.

In bacteriological examination, greyish-white, 1–2 mm diameter, S type colonies were detected on blood agar as a pure culture when incubated under aerobic conditions. Lactose-negative colonies which were observed to grow in both MacConkey and EMB media (at least three) were also identified using phenotypic tests, which included catalase production, oxidase reaction, H_2S production, carbohydrate fermentation and urease activity. Plates incubated under microaerobic and anaerobic conditions were found to be negative for growth. Colonies were identified as *Salmonella* spp. according to the phenotypic characteristics. The serotyping, phage typing and antibiotic susceptibility of this bacterium were performed at the Danish Institute for Food and Veterinary Research at the Department of Microbiological Food Safety in Denmark (Arguello et al. 2014).

The bacterium was identified by serotyping and phage typing as *Salmonella enterica* serovar Typhimurium phage type DT2. The isolate was resistant to streptomycin whereas it was sensitive to amoxicillin + clavulanic acid, ampicillin, apramycin, ceftiofur, chloramphenicol, ciprofloxacin, colistin, florfenicol, gentamicin, nalidixic acid, neomycin, spectinomycin, sulfamethoxazole, tetracycline and trimethoprim.

doi: 10.17221/8883-VETMED

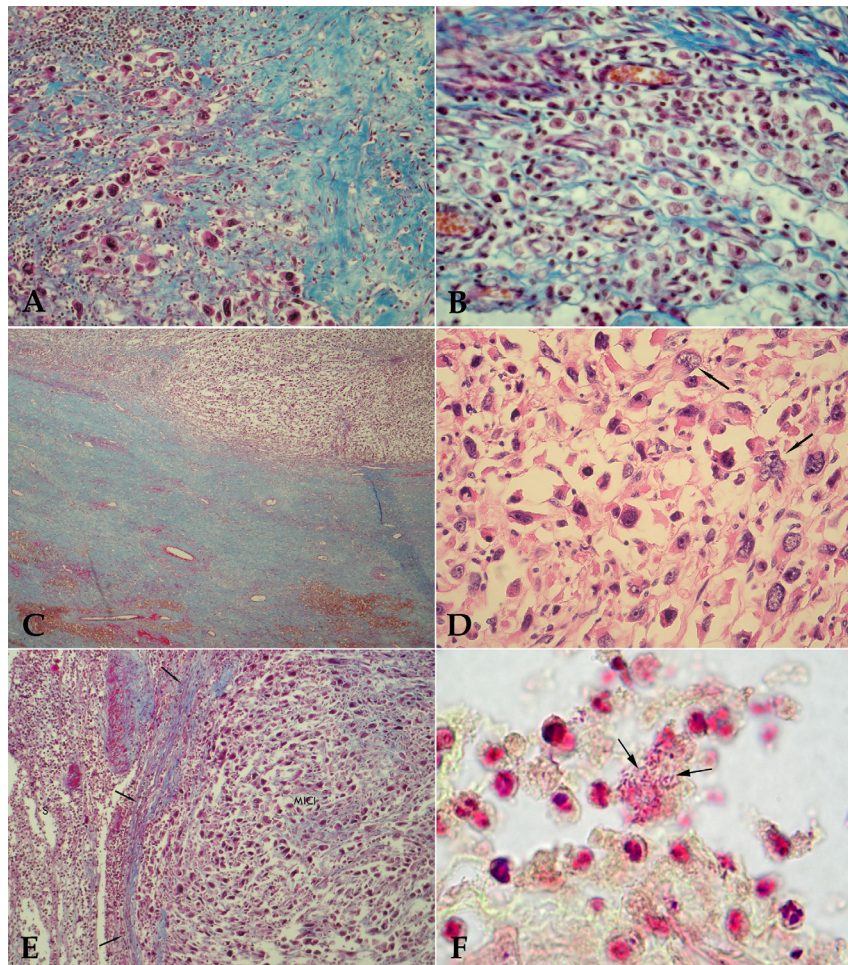


Figure 3. A granulation tissue. **A:** The granulation tissue illustrates bizarre cells, lymphocytes and plasmocyte cell infiltrations in the connective tissue. Masson trichrome; $\times 200$. **B:** The granulation tissue shows accumulation of foamy macrophages. Masson trichrome; $\times 400$. **C:** Low-power magnification of abscess wall exhibiting fibrovascular, haemorrhagic connective tissue and acute and chronic inflammatory cell infiltration. Masson trichrome; $\times 20$. **D:** Reactive chronic inflammatory cells with multinucleated giant cells (arrows). Hematoxylin-eosin; $\times 400$. **E:** Adhesion to the spleen parenchyma. Spleen (s) capsule was surrounded by connective tissue of abscess wall (arrows). MICI: Multinucleated-chronic inflammatory cells. Masson trichrome; $\times 200$. **F:** Gram negative bacilli (arrows) in lumen of vessel. Gram staining; $\times 1000$

DISCUSSION AND CONCLUSIONS

Salmonella strains have been reported to be host-specific (Baumler et al. 1998; Uzzau et al. 2000) and extremely pathogenic to pigeons (Pasmans et al. 2004). The biotype and ribotype of *S. Typhimurium* isolates in pigeons differ greatly from human and animal isolates (Scholtens and Caroli 1971; Van Oye and Borghijs 1979). Rabsch et al. (2002) also reported that the serotype *S. enterica* serovar Typhimurium phage type DT2 has been commonly isolated in pigeons, and emphasised that the serotype Typhimurium, a dominant serotype in pigeons, is hardly ever transferred to other hosts,

which reduces its negative impact on humans. On the other hand, environmental contamination is a crucial risk factor for the spread of infections in doves and pigeons, which may transmit diseases to humans through livestock systems (Hoelzer et al. 2011). *Salmonella* strains with the same genotypic features have been isolated from wild birds, cats and people, especially from babies, demonstrating the direct or indirect transfer of the pathogen to humans from wild birds. Cats which have contact with or that eat contaminated birds can play an important role as a mediator between birds and humans (Hauser et al. 2009). Pet dogs are often in close contact with their owners, including children,

and they can shed salmonellae for weeks without any clinical signs (Salehi et al. 2013).

Previous studies have shown that the most predominant serotypes of salmonellae in dogs were Typhimurium, Heidelberg, Kentucky, Corvallis, Düsseldorf and Enteritidis (Kocabiyik et al. 2006; Tsai et al. 2007; Hoelzer et al. 2011). Although the majority of *S. enterica* serovar Typhimurium strains affect multiple hosts (Helm et al. 2004), there are no reports regarding the existence of serovar Typhimurium phage type DT2 in dogs.

Even though infections in dogs associated with salmonellae serotypes have been reported to be clinically asymptomatic, infections in the gastrointestinal tract may lead to enterocolitis and endotoxaemia. The common symptoms of these conditions include fever, vomiting, anorexia, dehydration and depression (Hoelzer et al. 2011; Cobb and Stavisky 2013). In this case, although the dog had no specific clinical symptoms, anorexia, weight loss and lethargy were observed by the owner of the dog before death (Hoelzer et al. 2011; Cobb and Stavisky 2013). However, no macroscopic lesions were detected in the gastrointestinal system at necropsy. Extra-intestinal occurrence of salmonella usually happens rarely and only in immunocompromised or elderly hosts (Minohara et al. 2002). In this case, there was no information about the age, feeding behaviour or immune status of the animal.

Pennycott et al. (2006), Philbey et al. (2008) and Taylor and Philbey (2010) have claimed that wild birds carry *Salmonella* serovar Typhimurium strains similar to those found in pets and farm animals. Several sporadic salmonellosis outbreaks thought to be associated with *Salmonella* infections in wild birds have been reported in pets (especially cats) (Alley et al. 2002; Refsum et al. 2002). In England, Pennycott et al. (2006) investigated the cause of death of wild birds by evaluating the post-mortem findings of the carcasses. The authors have shown that *Salmonella* Typhimurium DT40 and DT56 in finches and sparrows, DT41 and DT195 in seagulls, and DT2 and DT99 in wild pigeons were isolated as dominant variants, and that these 'wild bird' strains were, at the same time isolated from cattle, sheep, pigs, chickens or turkeys. The results of the present study therefore support the results of Horton et al. (2013) who investigated the hypothesis concerning the association of some sporadic salmonella infections in pets with salmonella infections originating from garden birds. The evidence

obtained in their study showed salmonella infection in domestic livestock to be associated with wild birds, and also supported the hypothesis that wild birds threaten public health directly or indirectly.

Serotype Typhimurium isolates that were isolated from different species were found to be more closely associated with each other compared to other salmonella serotypes. This is explained by the adaptation mechanisms of different host reservoirs. At this point, although there is no direct evidence for this hypothesis, a possible mechanism in the development of such variants could be a phage-mediated transfer of host-specific virulence factors. Andrews-Polymenis et al. (2004) suggested that serotype Typhimurium DT2 and DT99 pigeon isolates are the best model possessing the necessary and sufficient genetic markers for disease in calves (enterocolitis) and for salmonella-induced enterocolitis in humans. The authors explained that the rare occurrence of disease caused by DT2 and DT99 in humans is possibly due to the scarcity of these serotypes in food animal reservoirs (i.e. livestock and domestic fowl) rather than the inability of these phage types to cause enterocolitis.

In previous studies, it was described that the ingestion of contaminated food is a major risk factor for salmonella infection. Further, it was reported that the consumption of carrion and faeces by dogs leads to a high risk of transmission and re-infection (Morse et al. 1976; Weese et al. 2005; Hoelzer et al. 2011). In this case, the isolation of serotype *Salmonella* Typhimurium phage type DT2 from the contents of an abscess formed in the abdominal cavity of a stray dog may be either due to the consumption of an infected pigeon or of wild poultry carrion contaminated with faeces in the animal shelter, as indicated in previous studies (Morse et al. 1976; Hoelzer et al. 2011). Although the serotype Typhimurium "pigeon type" is rarely transmitted to other host species (Rabsch et al. 2002), in this case, the finding of a Typhimurium serotype in the abscess of a dog may suggest that the agent should be considered in epidemiological studies in dogs.

Acknowledgement

We are grateful to the Danish Institute for Food and Veterinary Research at the Department of Microbiological Food Safety for the serotyping,

doi: 10.17221/8883-VETMED

phage typing and antibiotic susceptibility testing of the isolated bacterium.

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Received: 2015–03–02

Accepted after corrections: 2016–03–10

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