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Liver Abscess Associated with *Streptococcus suis* Serotype 4 in a Duroc Boar

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Abstract

A 7-month-old Duroc boar exhibited anorexia and dark-bloody diarrhea. Despite antimicrobial therapy including enrofloxacin (fluoroquinolones) and tylosin (macrolides), the boar died. Gross examination showed hepatic abscesses. Histological examination showed chronic multifocal necrotizing and suppurative hepatitis with colonies of Gram-positive cocci. Necrosis was observed in the center of affected areas. The lesions were composed of numerous neutrophils, macrophages, a few lymphocytes, and fibroblasts. Dense fibrous connective tissue surrounded these necrotizing and suppurative lesions. Several cocci were also detected in the multifocal necrotic foci in the liver. The bacteria isolated from the hepatic abscesses were confirmed to be *Streptococcus suis* serotype 4 based on the results of 16S rRNA gene sequencing and agglutination tests with antisera. Immunohistochemically, the cocci observed in the hematoxylin and eosin and Gram-stained sections of the liver abscess were strongly positive for *S. suis* serotype 4. Antimicrobial susceptibility testing showed that the isolate was resistant to third generation cephalosporins. Thus, a diagnosis of unique streptococcosis caused by *S. suis* serotype 4 was made. The typical clinical manifestation of *S. suis* infection involves meninges, endocardium, joints, and the lungs. The present boar is the first natural case of porcine liver abscess caused by *S. suis* serotype 4.

Discipline: Animal health
Additional key words: antimicrobial susceptibility testing, gene sequencing, immunohistochemistry, pig, streptococcosis

Introduction

Streptococcus suis, an encapsulated Gram-positive bacterium, is an emerging zoonotic pathogen that causes invasive infections in pigs and humans (Okura et al. 2016). It is composed of phenotypically and genetically diverse strains (Okura et al. 2016). *S. suis* serotypes 2, 3, 7, and 9 are the most frequently isolated from diseased pigs; however, the distribution of serotypes from clinical cases differs depending on geographic location (Goyette-Desjardins et al. 2014). Serotypes 2 and 3 are the most prevalent serotypes in North America, while serotype 9 is the most frequently isolated serotype in Europe (Goyette-Desjardins et al. 2014). In pigs, cases involving other serotypes have rarely been reported.

S. suis can cause a variety of conditions, including meningitis, sepsis, endocarditis, arthritis, and pneumonia in young pigs (Gottschalk et al. 2010, Goyette-Desjardins

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et al. 2014, Staats et al. 1997), and asymptomatic pigs frequently carry the bacterium in their upper respiratory tract and tonsils (Han et al. 2001). To date, there have been no natural cases of liver abscess associated with *S. suis* serotype 4 reported in boars in Japan or anywhere else in the world.

The purpose of this case study was to describe the clinical and histopathological features of atypical *Streptococcus* infection characterized by liver abscess in a boar. 16S rRNA gene sequence data were used to clarify the taxonomic classification of *Streptococcus*-like organisms isolated from the animal. Antimicrobial susceptibility was examined in an attempt to identify effective therapy.

Materials and methods

1. Animal

In December 2015, a 64-day-old male Duroc piglet was moved as a boar candidate from a farm in another area to a farm with 40 sows, 55 suckling pigs, 100 weaned pigs, 120 growing pigs, and 115 finishing pigs in Gunma Prefecture, located in the central part of Honshu Island (Japan's main island). At the age of 71 days, the piglet was vaccinated against Aujeszky's disease. No history of disease or medical/surgical treatment was identified at that time. At the age of 7 months, on the 2nd of June 2016, the boar suddenly exhibited anorexia and abnormal stools, including dark-bloody diarrhea. Despite antimicrobial therapy that included enrofloxacin (fluoroquinolones) and tylosin (macrolides), the boar died on the 6th of June 2016. No clinical abnormalities were observed in any other pigs on the farm.

2. Histological and immunohistochemical examination

At necropsy, tissue samples of the liver, spleen, kidney, heart, lung, stomach, intestines (duodenum, jejunum, ileum, cecum, colon, and rectum), lymph nodes (hepatic, splenic, renal, parotid, submandibular, hilar, inguinal, subiliac, superficial cervical, and mesenteric), tonsil, gallbladder, adrenal gland, trachea, pancreas, central nervous system (cerebrum, middle brain, cerebellum, pons, and medulla oblongata) were fixed in 10% neutral-buffered formalin. Fixed tissues were embedded in paraffin wax, sectioned (approximately 3-μm thick), and then stained for histological examination with hematoxylin and eosin (H&E) and Gram stain.

Immunohistochemistry was performed to detect the *S. suis* serotype 4 antigen. Formalin-fixed, paraffin-embedded tissues (liver, kidney, tonsil, stomach, and intestines) were cut into 3-μm-thick sections, treated with 3% hydrogen peroxide in methanol (to suppress endogenous peroxidase activity), and then treated with 0.1% actinase E solution and incubated at 37°C for 20 min for antigen retrieval. The tissues were then incubated with rabbit anti-*S. suis* serotype 4 primary antibody at a dilution of 1 in 8192 (Statens Serum Institut, Copenhagen, Denmark) for 30 min at room temperature, and subsequently reacted with a secondary antibody (Histofine Simple Stain MAX-PO MULTI; Nichirei Bioscience Inc., Tokyo, Japan). After rinsing with phosphate buffered saline, the specimens were incubated with aminoethyl carbazole (AEC) substrate solution (Histofine Simple Stain AEC solution; Nichirei Bioscience Inc., Tokyo, Japan) at room temperature for 5 min, and then counterstained with hematoxylin. Simultaneously, sections of hepatic tissues into which *S. suis* serotype 1 (strain NCTC 10237), 2 (strain NCTC 10234), 1/2 (strain NCTC 2651), 4 (present isolate), 7 (strain 8074), 9 (strain 22083), or 14 (strain 13730) had been injected were immunolabeled as positive controls. Negative controls were obtained by skipping the primary antibody.

For the detection of the porcine circovirus (PCV-2) antigen, formalin-fixed, paraffin-embedded tissues including the liver, spleen, kidney, lungs, stomach, ileum, tonsils, and lymph nodes (hepatic, splenic, renal, subiliac, superficial cervical, parotid, mandibular, inguinal, mesenteric, and tracheobronchial) were also cut in 3-μm-thick sections. Mouse monoclonal antibodies against PCV-2 (kindly provided by Dr. T. Suzuki, National Institute of Animal Health, NARO, Japan) were used with the commercial kit as described.

3. Bacteriological examination

For bacterial culture, tissue samples of the liver abscess, liver, spleen, kidney, heart, lung, and brain were inoculated onto 5% sheep blood agar and deoxycholate-hydrogen sulfide-lactose (DHL) agar, then incubated at 37°C under 5% CO_2, and aerobic or anaerobic conditions for 18 h.

A simple identification kit (rapid ID 32 STREP V4.0; bioMerieux SA, Marcy-l'Étoile, France) was used to identify the isolates. To confirm the species of the isolates, we extracted the genomic DNA from bacterial colonies using a DNA extraction kit (InstaGene Matrix; Bio-Rad Laboratories, Hercules, CA, USA) in accordance with the manufacturer's instructions. 16S rRNA genes derived from the isolates were analyzed to identify the bacteria (Therese et al. 2009). We also determined the serotype of the strain via agglutination tests using antisera (Gottschalk et al. 1989, Han et al. 2001).

To test antimicrobial susceptibility, we performed

the Kirby-Bauer disk diffusion test on *S. suis* serotype 4 isolated from the liver, using Mueller-Hinton agar with 5% sheep blood and antimicrobial disks (Sensi-disk; Becton, Dickinson and Company, NJ, USA) in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute, 2014). The antimicrobials tested were oxacillin (1 μg), penicillin (10 μg), ampicillin (10 μg), cefotaxime (30 μg), ceftriaxone (30 μg), cefepime (30 μg), vancomycin (30 μg), erythromycin (15 μg), azithromycin (15 μg), clarithromycin (15 μg), tetracycline (30 μg), doxycycline (30 μg), levofloxacin (5 μg), moxifloxacin (5 μg), ofloxacin (5 μg), gatifloxacin (5 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), chloramphenicol (30 μg), rifampin (5 μg), clindamycin (2 μg), and linezolid (30 μg).

Results

1. Gross pathology

For a diagnosis, necropsy was performed on the animal at the Gunma Livestock Health Laboratory. Gross necropsy examination of the animal showed liver abscesses (Fig. 1), enlargement of systemic lymph nodes, pale and firm kidneys, ulceration in the cardiac part of the stomach, and dark-bloody watery contents in the intestine (middle jejunum to colon). Multiple white-yellow foci up to about 20 mm in diameter were evident in the liver. An ulcer of approximately 60 mm in diameter was detected in the cardiac part of the stomach. No gross lesions were found in other organs, including the intestines and central nervous system.

2. Histological and immunohistochemical findings

Histological examination showed multifocal encapsulated abscesses in the parenchyma of the liver (Fig. 2). Necrosis was observed in the center of affected areas. The lesions were composed of numerous neutrophils, macrophages, a few lymphocytes, and fibroblasts. Dense fibrous connective tissue surrounded these abscesses. Numerous Gram-positive cocci were detected in the abscesses and multifocal necrotic foci in the liver.

In the other organs, lymphoid tissues (ileal Peyer's patches and systemic lymph nodes) were characterized by lymphocyte depletion as well as macrophage, epithelioid cell and multinucleated giant cell infiltration. Ulceration in the cardiac part of the stomach was accompanied by several Gram-positive cocci and Gram-negative bacilli. Interstitial nephritis was also detected, with a proliferation of connective tissue. No abnormalities were detected in any of the other organs.

Immunohistochemically, the cocci observed in the H&E and Gram-stained sections of the liver abscess were strongly positive for *S. suis* serotype 4, as were the cocci in the positive control slide containing *S. suis* serotype 4 (Fig. 3). The reactions were detected in the liver, but not in the other organs examined. The rabbit polyclonal antibody to *S. suis* serotype 4 reacted immunohistochemically with *S. suis* serotype 4, but not with *S. suis* serotypes 1, 2, 1/2, 7, 9, or 14. PCV-2 antigen was detected in the cytoplasm of macrophages in the tonsils, ileal Peyer's patches, and lymph nodes (subiliac, superficial cervical, and hepatic), but not in the other organs examined.

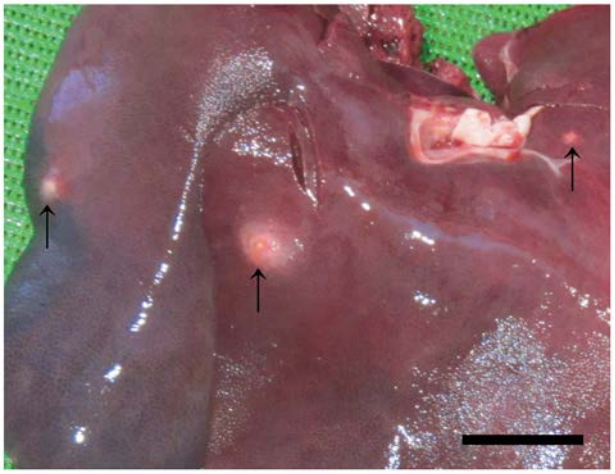

Fig. 1. Gross findings of the liver showing multiple white-yellow foci (arrows).
Bar = 5 cm.

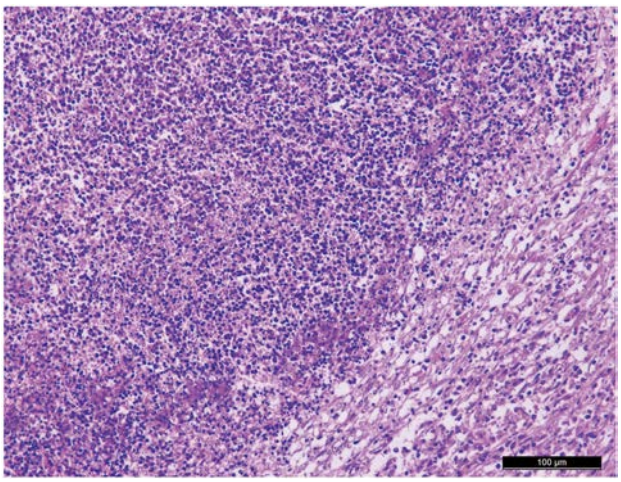

Fig. 2. Hepatic foci demonstrating abscess surrounded by extensive fibrous tissue.
H&E, Bar = 100 μm.

3. Bacteriological findings

The colonies were α-hemolytic, 1 to 2 mm in diameter, grayish white, and spherical in appearance. Gram-positive streptococci were isolated from the liver abscesses. The isolates were identified as *S. suis* II by the simple identification kit (profile number 73073561170, 99.7% identity). Sequences of the amplified 16S rRNA gene regions of all isolates were identical to *S. suis* strains GZ0565 and DN13 (accession numbers CP017142 and CP015557, respectively). The isolates were *S. suis* serotype 4. No bacteria were isolated from any of the other tissue samples.

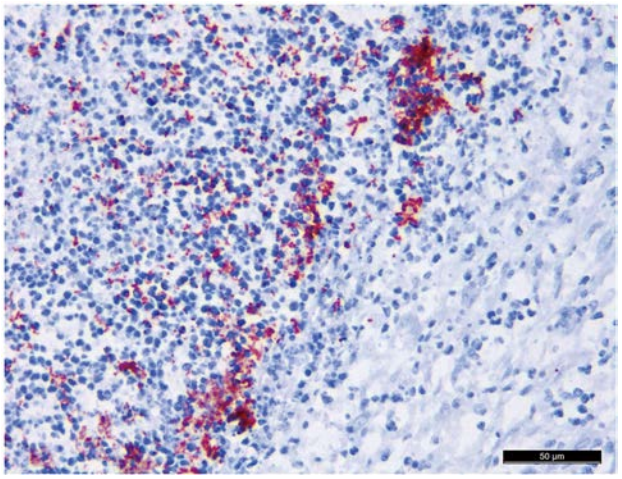

Fig. 3. Immunohistochemistry showing cocci in the hepatic lesion stained with an antibody specific for *S. suis* serotype 4.
Bar = 50 μm.

The strain showed resistance to penicillins (oxacillin and penicillin), the third generation cephalosporins (cefotaxime and ceftriaxone), macrolides (erythromycin and azithromycin), tetracyclines (tetracycline and doxycycline), trimethoprim-sulfamethoxazole, and lincomycin (clindamycin) (Table 1).

Discussion

These results indicated that the liver abscess was associated with *S. suis* serotype 4. The typical clinical manifestation of *S. suis* infection involves meninges, endocardium, joints, and the lungs (Gottschalk et al. 2010, Goyette-Desjardins et al. 2014, Staats et al. 1997). Although *S. suis* serotype 4 has previously been

Table 1. Antibiotic susceptibilities of *Streptococcus suis* isolate

Antimicrobial agent	Disk content (μg)	Inhibition ring (mm)	Breakpoint (sensitive ≧ mm)	Result*
oxacillin	1	10	20	R
penicillin	10	16	24	R
ampicillin	10	27	24	S
cefotaxime	30	22	24	R
ceftriaxone	30	21	24	R
cefepime	30	26	24	S
vancomycin	30	19	17	S
erythromycin	15	15	16	R
azithromycin	15	10	14	R
clarithromycin	15	19	17	S
tetracycline	30	−	25	R
doxycycline	30	14	25	R
levofloxacin	5	22	14	S
moxifloxacin	5	24	15	S
ofloxacin	5	19	13	S
gatifloxacin	5	24	18	S
trimethoprim-sulfamethoxazole	23.75/1.25	−	16	R
chloramphenicol	30	21	21	S
rifampin	5	24	17	S
clindamycin	2	−	16	R
linezolid	30	25	21	S

* S: sensitive, R: resistant

implicated in septicemia and bronchopneumonia (Baig et al. 2015, Chaturvedi et al. 1999, Perch et al. 1983, Wang et al. 2014) in diseased piglets, the present case is the first to demonstrate *S. suis* serotype 4 as a cause of liver abscess in an adult boar.

In the case reported herein, tuberculosis, *Trueperella pyogenes* infection, and *Actinobacillus pleuropneumoniae* serotype 2 infection (Ohba et al. 2008) were also suspected as differential diagnoses. However, the results of bacterial isolation, identification, genetic sequencing of the isolate, and immunohistochemical analysis ruled out these pathogens and confirmed a diagnosis of liver abscess due to *S. suis.* The relationship between the organisms and diarrhea was unclear in the present case.

Young piglets are at risk of *S. suis* infection and may develop the characteristic lesions within a few days (Gottschalk et al. 2010, Goyette-Desjardins et al. 2014). In our case, the lymphoid depletion and immunosuppression associated with PCV-2 (Meng 2013) were likely predisposing factors for the liver abscess associated with *S. suis* serotype 4.

Streptococcus sp. are usually found in the tonsils of pigs and immunosuppressed piglets are more likely to develop streptococcosis (Jensen et al. 2010, Maxie & Robinson 2007). The most interesting finding of this case was the presence of unique hepatic lesions associated with *S. suis* serotype 4. In a previous experimental study, the intravenous inoculation of piglets with *S. suis* serotype 4 proved fatal and histopathological examination revealed suppurative reaction in various organs, including the liver, lungs and spleen (Chaturvedi et al. 1999). The present findings support that *S. suis* serotype 4 causes suppurative reaction in various organs in natural cases. On the basis of these findings, we speculate that the abscess was formed when the causative cocci were transferred hematogenously from the upper respiratory tract and tonsils, which may have been the site of the original lesion, to the liver.

In this study, no *S. suis* serotype 4 antigen was detected in other organs, including the tonsils, and the source of infection could not be identified. Perhaps the liver abscess in this study developed as a consequence of bacteremia associated with *S. suis* serotype 4.

The *S. suis* identified in our study was resistant to penicillins, third generation cephalosporins, macrolides, tetracyclines, trimethoprim-sulfamethoxazole, and the lincomycins. Varela et al. reviewed the antimicrobial resistance patterns of *S. suis* isolates from various countries and reported variations thereof by country (Varela et al. 2013). They reported that the rates of resistance to penicillins (0-27%) and cephalosporins (0-23%) tended to be lower than the rates of resistance to tetracyclines (8-100%), macrolides (0-91%), lincomycins (0-100%), and trimethoprim-sulfamethoxazole (0-60%). The resistance of this strain to third generation cephalosporins is particularly important as cephalosporins are used in both humans and animals. Resistance to penicillin is also significant to veterinary practices. Although this strain was susceptible to fourth generation cephalosporin cefepime, particular attention should be paid to the further evolution of cephalosporin resistance in *S. suis* serotype 4.

To the authors' knowledge, this is the first reported natural case of porcine liver abscess due to *S. suis* serotype 4. *S. suis* is composed of phenotypically and genetically diverse strains (Okura et al. 2016). Moreover, *S. suis* is important in public health and also in the field of veterinary medicine (Gottschalk et al. 2010, Goyette-Desjardins et al. 2014, Staats et al. 1997, Wertheim et al. 2009). Therefore, additional studies are necessary to confirm the differences in pathogenicity among the conventional strains of *S. suis* and the strain isolated in this study.

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