NOTE *Pathology*

Systemic *Streptococcus dysgalactiae* Subspecies *equisimilis* Infection in a Yorkshire Pig with Severe Disseminated Suppurative Meningoencephalomyelitis

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(Received 21 October 2013/Accepted 25 December 2013/Published online in J-STAGE 10 January 2014)

ABSTRACT. An 18-week-old female breeding Yorkshire pig displayed symptoms of astasia and subsequently died. Histologically, severe disseminated suppurative meningoencephalomyelitis was detected, as were numerous myocardial microabscesses. Gram-positive cocci were detected in these suppurative lesions, and these cocci reacted with an antibody against *Streptococcus* C group species. Gram-positive cocci were isolated from the liver, spleen, kidney, heart, lungs, pleural abscess and articular fluid of the right tarsal joint. The isolates were β-hemolytic, categorized into Lancefield group C and were identified as *Streptococcus dysgalactiae* subspecies *equisimilis* by analysis of the 16S ribosomal DNA sequence. This is the first report of systemic *S. equisimilis* infection in a pig with severe disseminated suppurative meningoencephalomyelitis.

KEY WORDS: *Streptococcus dysgalactiae* subspecies *equisimilis*, swine, systemic infection.

doi: 10.1292/jvms.13-0526; *J. Vet. Med. Sci.* 76(5): 715–718, 2014

Swine streptococcosis is an important infectious disease caused by *Streptococcus* species. *Streptococcus suis*, *Streptococcus dysgalactiae* and *Streptococcus porcinus* are indigenous bacteria isolated from the tonsils, intestinal tracts and genital organs of clinically healthy pigs. However, they are also opportunistic pathogens capable of causing swine streptococcosis [3, 7, 10, 11, 15], the symptoms of which include sepsis, meningitis, arthritis, endocarditis, cervical abscess, pneumonia, abortion and lymphadenitis [3, 10, 15]. Recently, endocarditis-associated brain lesions were detected in slaughter pigs with left-sided valvular endocarditis, and most cases were caused by *S. suis* [8].

S. dysgalactiae can be divided into two subspecies based on whole-cell protein profiles and biochemical properties [15]. The first is *S. dysgalactiae* subsp. *dysgalactiae*(*S. dysgalactiae*), which includes α-, β- and non-hemolytic strains of animal origin, belonging to Lancefield group C or L. The second is *S. dysgalactiae* subsp. *equisimilis*(*S. equisimilis*), which includes β-hemolytic strains of human origin, belonging to Lancefield serogroup C or G.

S. equisimilis is usually isolated from pigs with verrucous endocarditis showing no other clinical signs of disease, and lesions are usually diagnosed at the time of inspection at the slaughterhouses [7, 11]. *S. equisimilis*-associated human streptococcosis, including severe invasive infections, has also been reported [1]. However, its virulence is not completely understood, because of insufficient bacterial analysis. Additional research should be carried out into these bacteria as they are important in public health and also in the field of veterinary medicine.

The purpose of this study was to describe systemic *S. equisimilis* infection accompanied by suppurative meningoencephalomyelitis in a Yorkshire pig.

Sixty-eight 9–18-week-old female breeding Yorkshire pigs were imported from Canada into Japan by airplane. At the time of arrival in Japan, a 15-week-old pig was unable to stand, and the right tarsal joint was swollen and warm. The white cell count revealed leukocytosis (WBC: 26,900/μl). The pig gradually weakened and died at 18 weeks of age in quarantine. No clinical abnormalities were observed in the remaining 67 pigs.

The pig was vaccinated against swine erysipelas, porcine circovirus type 2 (PCV-2) and Glasser's disease during the departure quarantine period in Canada. In addition, in compliance with animal health requirements, the animal had been treated with oxytetracycline (Liquamycin LA-200; Pfizer, New York, NY, U.S.A.) for leptospirosis.

A necropsy was performed, and tissue samples from major organs were fixed in 10% neutral-buffered formalin and embedded in paraffin wax. Tissue sections (approximately 3 μm thick) were stained with hematoxylin and eosin (HE) and Gram stained for histological examination.

For immunohistochemistry analysis, rabbit polyclonal

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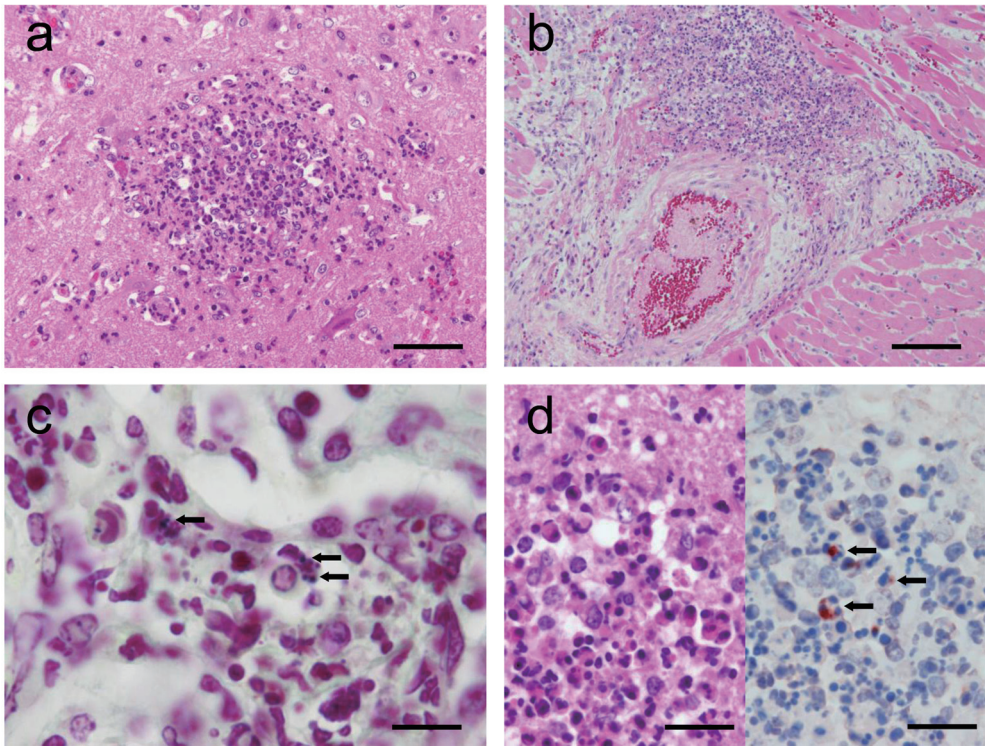

Fig. 1. a. Microabscesses in the cerebrum. HE. Bar=50 μm. b. Moderate to severe neutrophilic infiltrations are detected around the blood vessel in the heart. HE. Bar=100 μm. c. A few Gram-positive cocci (arrows) are detected in the neutrophilic cytoplasm in the cerebrum. Gram stain. Bar=10 μm. d. The cocci in the microabscess in the cerebrum react with the antibody against *Streptococcus* C group bacteria (arrows). HE (left). Immunohistochemistry for *Streptococcus* C group bacteria counterstained with hematoxylin (right). Bar=20 μm.

antibodies against *Streptococcus* C group bacteria (Denka Seiken, Tokyo, Japan) and PCV-2 [12] were used with a commercial kit (*N*-Histofine Simple Stain MAX PO (R); Nichirei Bioscience Inc., Tokyo, Japan).

The liver, spleen, kidney, heart, lungs, articular fluid of the right tarsal joint and pleural abscess were used for bacterial isolation. A simple identification kit (API 20 STREP; bioMerieux SA, Marcy-l'Étoile, France) and a Strept LA "SEIKEN" *Streptococcus* grouping kit (Denka Seiken) were used to identify the isolates.

For genetic tests, genomic DNA was extracted from bacterial colonies using a DNA extraction kit (InstaGene Matrix; Bio-Rad Laboratories, Hercules, CA, U.S.A.). Polymerase chain reaction (PCR) was employed to detect the genes encoding streptolysin O (*slo*) [4], streptolysin S (*sagA*) [6] and streptokinase (pSTKP8) [2].

To test antibiotic susceptibility, the disk diffusion method was performed on *S. equisimilis* isolated from the heart, using antibiotic disks (SN disk; Nissui Pharmaceutical Co., Ltd., Tokyo, Japan). The antibiotics tested were ampicillin, erythromycin, clindamycin, tetracycline and chloramphenicol.

An ~500 bp region of the 16S ribosomal RNA gene (16S rDNA) region was amplified and sequenced using a MicroSeq 500 16S rDNA PCR/Sequencing Kit (Applied Biosystems Life Technologies, Carlsbad, CA, U.S.A.) [14].

Grossly, the right tarsal joint was swollen, and cloudy effusion had accumulated in the articular cavity. An abscess (approximately 2 cm in diameter) was found in the pleura of the 6th costovertebral joint. No gross abnormalities were found in other organs, including the brain and spinal cord.

Histologically, severe (cerebrum and brainstem) to moderate (cerebellum and spinal cord) disseminated suppurative meningoencephalomyelitis was detected. The lesions were also characterized by focal microabscesses in the parenchyma of the central nervous system (Fig. 1a). Small numbers of neutrophils had infiltrated into the subarachnoid cavity and cranial pia mater and around the blood vessels of the cerebrum. Vasculitis, with moderate to severe neutrophilic infiltrations, was detected in the heart (Fig. 1b). Numerous microabscesses were also detected in the myocardium. Moderate lymphoplasmacytic and slight neutrophilic infiltrations were detected in the stroma of the kidney. Slight neutrophilic infiltrations were also detected in the hepatic lobular connective tissue, in the splenic pulp and around the bronchus of the lung. A few Gram-positive cocci were detected in the suppurative lesions of the cerebrum (Fig. 1c), brainstem, heart, liver, spleen, kidney and pleural abscess, and some cocci were also detected in the blood vessels. The cocci reacted with an antibody against *Streptococcus* C group bacteria (Fig. 1d), but no positive reactions to an antibody against

PCV-2 were observed in tissue sections.

Neutralization tests for classical swine fever and Aujeszky's disease, indirect enzyme-linked immunosorbent assay for porcine reproductive and respiratory syndrome, a tube agglutination test for brucellosis and viral isolation for swine influenza virus from a nasal swab were conducted as a routine serological and virological examination for imported pigs, and the results were all negative.

Gram-positive cocci were isolated from the liver, spleen, kidney, heart, lungs, articular fluid of the right tarsal joint and pleural abscess. The isolates were β-hemolytic, catalase-negative and oxidase-negative and were identified as *S. equisimilis* by the simple identification kit (profile number 11132041100, 57.2% identity). The bacteria were categorized into Lancefield group C by the *Streptococcus* grouping kit. The *sagA* and pSTKP8 genes, but not the *slo* gene, were positively identified in the isolates, which also showed resistance to erythromycin, clindamycin and tetracycline. Sequencing of the amplified 16S rDNA region confirmed the Gram-positive cocci as *S. equisimilis* (ATCC 35666, 99.2% identity).

S. equisimilis was isolated from the liver, spleen, kidney, heart, lungs and pleural abscess. Furthermore, because Gram-positive cocci reacting with the antibody against *Streptococcus* C group bacteria were detected in the suppurative lesions, the present case was diagnosed as systemic streptococcosis caused by *S. equisimilis* infection. Streptococci are regularly found in the tonsils of pigs, and immunosuppressed pigs are more likely to develop streptococcosis [7, 11]. In this case, the stress of long-distance transportation and environmental changes associated with importation were considered to be relevant to the pathogenesis. The present findings suggested that sepsis occurred and that the causative cocci were transferred hematogenously from the tonsils to the systemic organs, including the central nervous system and joints. Next, suppurative lesions formed in the systemic organs. Suppurative encephalomyelitis in pigs is common [8], but this is the first report of severe systemic *S. equisimilis* infection with disseminated suppurative meningoencephalomyelitis in pigs.

Although the present isolates were identified as *S. equisimilis* by the simple identification kit, the percent identity was not high (57.2%). This low identity may have been caused by the negative result for alkaline phosphatase activity. The biochemical properties used to identify *Streptococcus* species by the simple identification kit are difficult to reproduce consistently [13]. In this case, species identification by 16S rDNA sequence was beneficial.

The *S. equisimilis* strain isolated in this case was categorized into Lancefield group C, which is consistent with a report that *S. equisimilis* isolated from pigs with endocarditis and arthritis is frequently categorized into group C [5, 9]. In addition, the pattern of virulence genes was *slo*(−), *sagA*(+) and pSTKP8 (+). *S. equisimilis* of human origin has been reported to contain all three genes [13], indicating that the pig isolate identified in the current case differs from those of human origin. Furthermore, the isolates were susceptible to ampicillin, indicating that penicillin antibiotics would still be effective, but were resistant to the macrolide erythromycin and tetracycline. This result was consistent with a report that the susceptibility of *S. equisimilis* to macrolide and tetracycline antibiotics was lower than that to penicillin antibiotics [5, 13].

ACKNOWLEDGMENTS. The authors thank Mr M. Kobayashi and Ms M. Shimada for histopathological assistance.

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