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NOTE

Pathology

Bovine vegetative endocarditis caused by *Streptococcus suis*

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ABSTRACT. A 5-month-old crossbred beef steer died after exhibiting astasia. A postmortem examination revealed verrucous endocarditis and numerous renal hemorrhages. Gram-positive bacteria were identified in the necrotic lesions of the verruca and mitral valve via histopathological analysis. Multifocal necrosis and hemorrhage were detected in the renal cortex. Gram-positive cocci isolated from the verruca were identified via biochemical tests and 16S rRNA gene sequence analysis as *Streptococcus suis*. Serotyping indicated that the *S. suis* isolates were untypable, following which these isolates were classified as a new sequence type (ST1000) via multi-locus sequence typing. *S. suis* is an important pathogen of pigs. However, clinical cases in cattle are rare. This report is intended to provide information that may be useful in the diagnosis of streptococcal disease in cattle.

KEY WORDS: cattle, endocarditis, multi-locus sequence typing, *Streptococcus suis*

Streptococcus suis is a gram-positive bacterium responsible for causing heavy financial losses to the pork industry worldwide. *S. suis* may cause various diseases such as endocarditis, meningitis and arthritis in swine [1, 14, 17]. *S. suis* has also been recognized as a zoonotic agent responsible for meningitis, endocarditis, and septicemia in humans [3, 20]. Based on their capsular polysaccharide (CPS) antigens, *S. suis* strains have been classified into more than 30 serotypes [11]. A serotype which is most frequently isolated from infected humans and pigs is serotype 2 [4]. Multi-locus sequence typing (MLST) is used worldwide for genotyping *S. suis* [8]. Most *S. suis* isolates from pigs and humans with severe disease conditions are typed as specific clonal complexes (CCs), such as CC1, CC20, CC25, CC28, and CC225 [4].

In animals other than pigs and humans, *S. suis* infection occurs only sporadically [4]. In cattle, *S. suis* has been isolated on several instances from animals suffering from various diseases, including pneumonia, arthritis, meningitis, and septicemia [5, 6, 12]. Recently, the *S. suis* serotype 33 reference strain, isolated from sheep with arthritis, was reclassified as a novel species, *Streptococcus ruminantium* [18]. Several *S. ruminantium* isolates have been identified in heart vegetations of cattle with infective endocarditis [18]. However, it is difficult to distinguish between *S. suis* and *S. ruminantium* using only biochemical-based evaluations [10]. Thus, it is possible that some isolates from diseased cattle that had been identified as *S. suis* were actually *S. ruminantium.* In fact, 16S rRNA gene sequences of 16 streptococcal isolates from diseased cattle showed a similarity of over 99% to that of *S. suis* serotype 33 reference strain [12]. However, these isolates were identified as being *S. suis,* since the reclassification of *S. ruminantium* had not yet been proposed. This indicates that these 16 isolates were likely *S. ruminantium.* Under these circumstances, authentic *S. suis* infections in cattle may go unrecognized. Therefore, accurate identification of streptococcal isolates may be essential for proper diagnosis of streptococcal diseases in cattle.

In the current study, we encountered an authentic case of *S. suis* infection in a bovine calf that presented with vegetative endocarditis. The purpose of the study was to provide information related to the isolated *S. suis* strain as well as the clinical and pathological characteristics of the infected calf.

A 5-month-old crossbred beef steer at a farm breeding 350 head of crossbred beef cattle and 200 head of Holstein cattle presented with fever and astasia in December 2017. The farm was in Aichi Prefecture, located on the Pacific coast on central

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Honshu island (main island), Japan. The symptoms improved slightly on treatment with an antibiotic (cefazolin), thiamine, and steroids. However, even with treatment, the calf died after exhibiting astasia again. The carcass of this calf was moved to Aichi Prefectural Chuo Livestock Hygiene Service Center and a postmortem necropsy was performed. No clinical symptoms, including astasia, were detected in any of the other cattle on the farm.

The necropsy grossly revealed raised, yellow verrucous endocarditis attached to the mitral valve (Fig. 1a). Dark reddish wedge-shaped lesions and petechial hemorrhages were present in a scattered pattern on the kidney surface (Fig. 1b). The amount of pleural effusion was slightly increased and fibrin was found adhered to the surface of lungs. No visible lesions, including abscesses, were found in any of the other organs.

At necropsy, tissue samples of the liver, spleen, kidney, heart, lung, stomachs, intestines, and brain were fixed in 10% neutral-buffered formalin. Fixed tissues were embedded in paraffin wax, sectioned at a thickness of approximately 3 μm, and stained with hematoxylin and eosin (H&E) and gram staining for histological examination.

Histological examination of the mitral valve revealed accumulated layers of fibrin and numerous gram-positive bacterial colonies lined by infiltrating neutrophils and macrophages (Fig. 1c and 1d). Granulation tissue was present underneath the fibrin layer and infiltrating neutrophils. Neutrophils and gram-positive cocci had also infiltrated into the heart muscle tissues. Remarkable thrombosis in the cortical interlobular artery, as well as coagulation necrosis in renal tubules suggesting infarct, were observed in the kidney (Fig. 1e). Renal interstitium was also necrotic, hemorrhagic, and was edematous with infiltrating neutrophils. Fibrin was detected in the Bowman's capsules with congested glomerular capillaries. Gram-positive cocci were detected in glomerular capillaries (Fig. 1f) and cytoplasm of macrophages infiltrating the renal interstitium (Fig. 1g). Congestion, edema, and hemorrhages were observed in the lungs. No lesions were observed in any of the other tissues or organs, including the brain.

For purposes of bacterial isolation, tissue homogenates prepared from the liver, spleen, kidney, heart, vegetation, lung, and brain were spread on normal blood agar, chocolate agar, and deoxycholate-hydrogen sulfide-lactose agar plates. The inoculated plates were incubated at 37°C under 5% CO_2 in air and anaerobic conditions. Gram-positive cocci were isolated from only the mitral valve vegetation sample. No bacteria were isolated from any of the other samples tested. The three isolates from the valve vegetation were subjected to biochemical testing using the API 20 Strep system (BioMérieux, Inc., Marcy l'Etoile, France), in accordance with the manufacturer's instructions. The three isolates were identified as *S. suis,* with all three displaying an identical profile (4351413).

Identity of the isolates was confirmed using a *recN* PCR assay specific for the detection of authentic *S. suis* [7] as well as via sequence analysis of the 16S ribosomal RNA gene [9]. All three isolates were positively amplified in the *recN* PCR assay. Partial sequence analysis of the 16S rRNA gene of the three isolates (1,504 bp region) indicated that they were identical to each other and that they showed a 98.7% similarity to the sequence of the *S. suis* type strain. These results indicated that these three isolates were authentic *S. suis*. The 16S rRNA gene sequences were deposited to DNA Data Bank of Japan (Accession Number: LC377187).

In addition, a *cps*-typing PCR assay which predicts serotypes of isolates [10] and a MLST analysis of the seven genes *aroA*, *cpn60*, *dpr*, *gki*, *mutS*, *recA*, and *thrA* [8], were performed in an attempt to genotype the isolates. The three isolates were untypable via *cps*-typing, indicating that they may possess a new type of CPS, or had lost their capsule. Therefore, additional analysis of the *cps* gene cluster of these isolates is needed. Results from MLST classified the three isolates into the same sequence type (ST1000), which is a new type within *S. suis* (allele types: *aroA/cpn60/dpr/gki/mutS/recA/thrA*=45/49/175/7/72/45/174). Based on these results, the three strains isolated from the endocarditis lesion were considered to be derived from the same clonal origin. ST1000 did not belong to any previously identified CCs, including CC1, CC20, CC25, CC28, and CC104, which contain many human and swine clinical isolates. This suggested that the novel genotype was uniquely associated with the current bovine case.

To determine the antibiotic susceptibility of the new *S. suis* isolates, the disk diffusion test was performed according to CLSI method [2]. Antibiotic disks included the BD BBL Sensi-Discs (Becton, Dickinson and Co., Franklin Lakes, NJ, U.S.A.) for penicillin (PCG), ampicillin (ABPC), amoxicillin (AMPC), and sulfamethoxazole trimethoprim (ST), KB disks (Eiken Chemical Co., Ltd., Tokyo, Japan) for lincomycin (LCM), and VKB disks (Eiken Chemical Co.) for enrofloxacin (ERFX). The breaking points of these antibiotics were based on the reports by Soares *et al*. [16]. Antibiotic susceptibility test results are shown (Table 1). The isolates were resistant to PCG and LCM and demonstrated intermediate susceptibility to ABPC, AMPC, ST, and ERFX. The isolates were not susceptible to any of the antibiotics used in the tests.

As previously mentioned, a unique aspect of the present study is finding a case of infective endocarditis in cattle, caused by authentic *S. suis*. Bovine *S. suis* infections [5, 6, 12] have been reported previously. However, these reports were published prior to the confirmation of the new species *S. ruminantium* [18], also isolated from diseased cattle, which is very difficult to identify separately from *S. suis,* using only biochemical tests. This suggests that some isolates labeled as *S. suis*, may actually not have been *S. suis*. In the current study, the identification of *S. suis* was confirmed by 16S rRNA sequence analysis and *recN* PCR, which can specifically detect authentic *S. suis* [7]. Thus, to our knowledge, our study is the first to definitively demonstrate the association between authentic *S. suis* and bovine endocarditis.

In addition to vegetative endocarditis, suppurative nephritis, thrombosis in the cortical interlobular artery and coagulation necrosis in the renal tubules were also observed in the calf from which the *S. suis* was isolated. Suppurative nephritis caused by *S. suis* has been reported in mice experimentally infected with serotype 2 strain 10 and in a pig naturally infected with serotype 9 strain [15, 19]. While no bacteria were isolated from the kidney lesions of the calf in the current study, gram-positive cocci were present in the kidney glomeruli and cytoplasm of macrophages in the renal interstitium. These suggested that the suppurative nephritis lesions may have been secondary to renal infarctions caused by endocardial thrombosis due to *S. suis* infection. Although it is unclear why *S. suis* was not isolated from the kidneys in this case, this may be explained by the following three reasons:

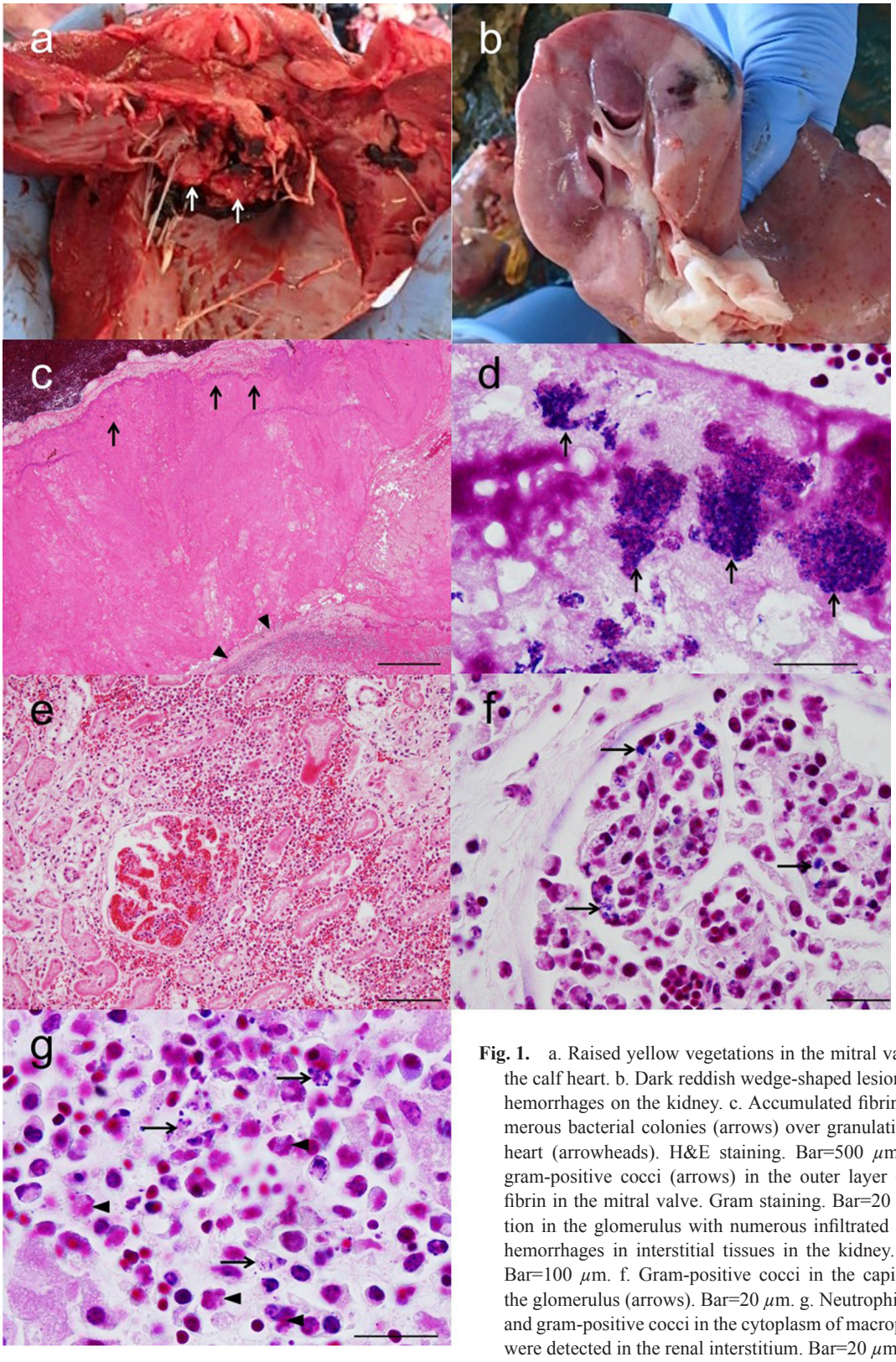

Fig. 1. a. Raised yellow vegetations in the mitral valve (arrows) of the calf heart. b. Dark reddish wedge-shaped lesions and petechial hemorrhages on the kidney. c. Accumulated fibrin layer with numerous bacterial colonies (arrows) over granulation tissue in the heart (arrowheads). H&E staining. Bar=500 μm. d. Numerous gram-positive cocci (arrows) in the outer layer of accumulated fibrin in the mitral valve. Gram staining. Bar=20 μm. e. Congestion in the glomerulus with numerous infiltrated neutrophils and hemorrhages in interstitial tissues in the kidney. H&E staining. Bar=100 μm. f. Gram-positive cocci in the capillary vessels of the glomerulus (arrows). Bar=20 μm. g. Neutrophils (arrowheads) and gram-positive cocci in the cytoplasm of macrophages (arrows) were detected in the renal interstitium. Bar=20 μm.

(i) differences in the source sites of tissue samples used in the bacterial and histological examinations; (ii) the bacteria were eliminated by the antibiotic treatment; (iii) The number of bacteria in the lesion was insufficient for isolation.

MLST and *cps*-typing indicated that the isolates from the calf in the current study were different from isolates from swine and humans with severe diseases, including endocarditis [4, 13]. Thus, it remains unclear whether the new isolates would cause pathogenesis in humans and swine. Interestingly, these isolates exhibited resistance or intermediate susceptibility to PCG, ABPC,

Table 1. Results of antimicrobial susceptibility testing

Antibiotic disk	μg/disc	Zone diameter (mm)			
		Inhibition circle	Resistant	Intermediated	Susceptible
Penicillin (PCG)	10 units	15	19	20–27	28
Ampicillin (ABPC)	10 μg	24	18	19–25	26
Amoxicillin (AMPC)	25 μg	25	18	19–25	26
Lincomycin (LCM)	2 μg	0	15	16–18	19
Sulfamethoxazole trimethoprim (ST)	25 μg	17	16	17–18	19
Enrofloxacin (ERFX)	5 μg	19	16	17–22	23

and AMPC, which are antibiotics currently in use for the empirical treatment of humans and pigs with *S. suis* infections. However, insufficient information is available on sequence types, serotypes or antibiotic susceptibility of authentic *S. suis* isolates from diseased cattle. Further studies on MLST, serotypes, and antibiotic susceptibility using more isolates from diseased cattle are needed.

In conclusion, we reported a case of bovine endocarditis caused by authentic *S. suis*. There are few reports on *S. suis* infection in cattle. Furthermore, some isolates in those reports may not have been *S. suis,* due to the recent reclassification of some these isolates as *S. ruminantium*. In addition, there are no detailed pathological reports on diseases in cattle caused by *S. suis* or by *S. ruminantium*. Additional data on *S. suis* isolates from diseased cattle accurately identified via *recN*-PCR and/or 16S rRNA sequencing will be beneficial in the precise diagnosis of streptococcal diseases in cattle. Furthermore, serotype, MLST, and antibiotic susceptibility data related to *S. suis* isolates from cattle will be helpful in furthering our understanding of their genetic and serological diversity, in addition to contributing towards the development of effective antibiotic treatments.

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