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SHORT COMMUNICATION Clinical Case Report

Severe soft tissue infection with septic shock caused by *Streptococcus canis* sequence type 9 harboring M-like protein allele 1 in a miniature dachshund

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Abstract

We report a case of *Streptococcus canis*-induced severe soft tissue infection (SSTI) in the left hindlimb of a miniature dachshund (male/13-year-old), with septic shock. The dog underwent immediate exploration/debridement of necrotic area in the hindlimb, with imipenem administration after culture sampling. The pathogen was isolated from necrotic tissue and blood, and then identified using mass spectrometry/molecular characterization (sequence type 9 harboring M-like protein allele 1). The dog experienced recurrent soft tissue inflammation in the hindlimb 115 days after onset and received ampicillin treatment, after which the dog was cured without further recurrence. This is the first report of an animal cured of SSTI in Japan. *S. canis* should be considered a causative bacterium when examining/treating animals with SSTI and recurrence.

Key Words: septic shock, severe soft tissue infection, *Streptococcus canis*

Streptococcus canis, first reported in 1986[2], forms large, smooth, gray-white colonies with beta-hemolysis on 5% sheep blood agar plates. According to Lancefield grouping, *S. canis* is classified as a group G streptococcus based on cell wall carbohydrate antigenicity. In healthy dogs, *S. canis* forms part of the resident microflora of the oropharynx, skin, genitourinary tract, and anus[1]. This bacterium is an emerging zoonotic pathogen that causes self-limiting dermatitis. However, in some cases this bacterial infection leads to severe diseases in companion animals, including arthritis, streptococcal toxic shock syndrome (STSS), necrotizing fasciitis, septicemia, and pneumonia[3,9]. The *S. canis* M-like protein (SCM), reported to be a virulent factor, has diverse allele types that are associated with the distribution of sequence types (STs)[5]. Therefore, the relatedness between the SCM allele type/ST and disease severity needs to be clarified. Here, we report severe soft tissue infection (SSTI) caused by *S. canis* (harboring SCM allele type

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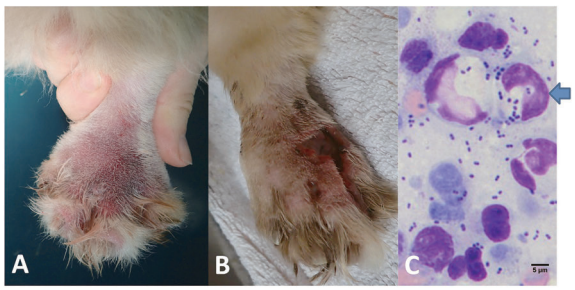

Fig. 1. Macroscopic images of the affected left hindlimb before (A) and after (B) debridement and microscopic images of the Giemsa-stained samples (C) using the fine needle aspirate of the necrotic tissue after debridement. Local inflammatory conditions, partial subcutaneous hemorrhage, and necrosis were observed. Appearance of round-shaped microorganisms was found within a phagocyte (arrow). Scale bar = 5 µm

1 and ST[9)] in the left hindlimb of a miniature dachshund, associated with septic shock.

The affected animal was a miniature dachshund (male, 13 years old): the case had a history of chronic pancreatitis. The dog was emaciated (body weight, 4.5 kg). The dog owner observed sudden inflamed conditions and swelling in the left hindlimb (Fig. 1A) in the morning in 2019, and the dog was immediately brought to the Murata Animal Hospital.

Upon admission, physical examination revealed inflammatory conditions, partial subcutaneous hemorrhage, and necrosis in the left hindlimb. The body temperature was 39.1 °C, heart rate was 124 beats per min, and respiratory rate was 42 breaths per min (panting observed). The dog was in vasoconstricted shock, based on the findings of weak pulse quality, cold extremities temperature, white and pale mucous membranes color, and prolonged capillary refill time (>2 sec). The dog was hydrated and the left hindlimb was explored, debrided, washed, and disinfected with 20% chlorhexidine gluconate (20% Hibitane Gluconate, Sumitomo Dainippon Pharma Co., Ltd., Tokyo, Japan; Fig. 1B). Blood isolation culture was requested to the Sanritsu Zelkova Veterinary Laboratory, followed by intravenous administration of imipenem (dosing of 10 mg/kg/day every 12 hr, for 3 days). The white blood cell count was slightly elevated at 12,710/µl (band neutrophils of 1,652/µl, including morphological changes with toxic granules in part) and the level of C-reactive protein was >10.0 mg/dl. Other blood parameters remained within the normal range. Fine needle aspiration (FNA) specimens of the necrotic tissue after debridement were subjected to Giemsa staining and isolation culture. Microscopic examination of the Giemsa-stained FNA specimens (Fig. 1C) showed appearance of round-shaped microorganisms within a phagocyte. Group G *Streptococcus* was isolated from both the necrotic tissue and blood and was identified as *S. canis* using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for rapid diagnosis without biochemical property data. The dog was diagnosed with SSTI, accompanied by STSS, based on the following similarities to human cases of SSTI and STSS; (i) isolation of *S. canis* from sterile sites of closed tissue and blood, (ii) shock status, and (iii) sudden onset and rapid progression of the pathological conditions. Based on the antimicrobial susceptibility test results, intravenous administration of imipenem was switched to intravenous administration of ampicillin (dosing of 40 mg/kg/day every 12 hr, for 13 days) for de-escalation. Clinical treatment was initially effective. However, there was recurrence of the soft tissue inflammatory conditions at the same site in the left hindlimb 115 days after the first onset. Microscopic findings of the Giemsa-stained FNA specimens from the site of recurrence showed inflammatory cell infiltration without the presence of microorganisms, and the culture result with the FNA specimens was also negative. We did not perform the blood culture since the dog revealed no shock status. Intravenous administration of ampicillin (dosing of 40 mg/kg/day every 12 hr, for 16 days) was initiated, without exploration and debridement. After the second intervention, the dog's condition improved. Following this, there was no recurrence of symptoms (follow-up for a period of one year).

Table 1. Phenotypic and genotypic features of *S. canis* strains isolated from a miniature dachshund with severe soft tissue infection in the left hindlimb and septic shock

Strain	FU150	FU149	NCTC 12191(T)
Clinical specimen	Necrotic tissue	Blood	Bovine mastitis
Gross appearance of colonies on a sheep blood agar plate	Non-mucoid, ß-hemolytic large white-colored smooth colonies	Non-mucoid, ß-hemolytic large white-colored smooth colonies	Non-mucoid, ß-hemolytic large gray-white-colored smooth colonies
Carbohydrate group (Lancefield antigen)	Group G	Group G	Group G
Identification using MALDI-TOF MS (score value)	*S. canis* (2.50)	*S. canis* (2.40)	*S. canis* (2.58)
Similarity (%) to the *S. canis* type strain using 16S rRNA sequencing (sequencing size, bp)	100 (742)	100 (740)	
S. canis-specific *cfg* gene encoding the Christie-Atkins-Munch-Peterson reaction factor	Amplified	Amplified	Amplified
S. canis M-like protein (SCM) allele type	SCM allele type 1	SCM allele type 1	SCM allele type 1
Sequence type (ST)	ST9	ST9	ST9
Virulence-associated gene (VAG) profile (*gbp-ap1-fp1-brp*)	*gbp-brp*	*gbp-brp*	*gbp-brp*
Biofilm formation ability (BFA) assessed using crystal violet staining (absorbance, mean + SD of 10 wells)[2)]	0.04 ± 0.020	0.05 ± 0.022	0.18 ± 0.049
Cell invasion ability (CIA) into human keratinocyte HaCaT (colony-forming units of *S. canis*/100 cells, mean + SD of 4 wells)[2)]	0.84 ± 0.26	1.05 ± 0.34	0.87 ± 0.23
Antimicrobial resistance (AMR) phenotype	None	None	None
Gene contributing to resistance to macrolide/lincosamide/tetracycline classes	None	None	None

MALDI-TOF MS, matrix-assisted laser desorption ionization-time of flight mass spectrometry; *gbp*, glucan-binding protein gene; *ap1*, pilus ancillary protein 1 gene; *fp1*, fimbrial protein gene; *brp*, biofilm regulatory protein gene; SD, standard deviation.
[2)] National Collection of Type Cultures (NCTC) 12191(T) of *S. canis* was applied as a quality control to assess the BFA and CIA. The phenotypic features (CIA along without AMR phenotype) and genotypic features (SCM allele type 1, ST9, and VAG profile *gbp-brp*) of FU150 and FU149 included properties (except for BFA) of NCTC 12191(T).

Table 1 shows the phenotypic and genotypic features of *S. canis* strains, FU150 (from necrotic tissue) and FU149 (from blood). Phenotypic analyses included score value using MALDI-TOF MS, biofilm formation ability (BFA) using crystal violet staining and brain heart infusion broth[7)], intracellular invasion ability into human keratinocyte HaCaT[18)], antimicrobial resistance (AMR) phenotypes based on the minimum inhibitory concentrations measured using the broth microdilution method (MICroFAST Panel Type 7J for *Streptococcus* spp., Beckman Coulter Inc., Tokyo, Japan) specified by the Clinical and Laboratory Standards Institute (CLSI) guidelines for beta-hemolytic streptococci (CLSI document M100-S25), and others. Genotypic analyses included percent similarity to the type strain by 16S rRNA sequencing, amplification of *S. canis*-specific *cfg* gene, SCM allele type[8)], ST[5)], virulence-associated gene (VAG) profile (glucan-binding protein gene [*gbp*]-pilus ancillary protein 1 gene-fimbrial protein gene-biofilm regulatory protein gene [*brp*])[7)], and the genotypes contributing to resistance to macrolide/lincosamide/tetracycline antibiotic classes[5)]. The phenotypic features (intracellular invasion ability along without AMR phenotype) and genotypic features (SCM allele type 1, ST9, and VAG profile *gbp-brp*) of FU150 and FU149 included properties (except for BFA) of NCTC 12191(T). In terms of BFA, FU149/FU150 showed the low BFA compared to that of NCTC 12191(T), suggesting the possibility of strain-specific invasiveness rather than local colonization induced by biofilm formation.

Isolation of blood-origin *S. canis* from companion animals is rare. As such, our

description of the phenotypic and genotypic features of the invasive strain FU149 is novel. To the best of our knowledge, this is the first report of BFA and the first VAG profile of dog blood-origin strain. In addition, these microbiological analyses suggest that the strains with genetic characteristic (SCM allele type 1 and ST9) are capable of causing severe infection and bacteremia, since the same clone has been recovered from two Japanese patients with *S. canis* bacteremia who were in close contact with or bitten by companion dogs[6,13,15]. Furthermore, the draft genome sequence (GenBank accession number BLRR00000000.1) of FU149 was determined using an Illumina MiSeq benchtop sequencer (Illumina, Inc., San Diego, CA, USA) and was mapped to the complete genome sequence of NCTC 12191(T) (accession number LR134293)[4]. The *de novo* assembly was conducted using the remaining unmapped reads, and 35 contigs were obtained. These contigs were uploaded to PathogenFinder version 1.1 (https://cge.cbs.dtu.dk/services/PathogenFinder/) and DDBJ Fast Annotation and Submission Tool (https://dfast.nig.ac.jp), to identify any pathogenic gene families that were not present in NCTC 12191(T). Seven coding DNA sequences (CDSs) (1,164 bp to 207 bp) were identified concurrently by both application techniques. These CDSs were found to encode DNA integration and phage-associated proteins identical to those in human-origin streptococci (i.e., *S. dysgalactiae* subsp. *equisimilis* strain GGS_124 causing STSS and virulent *S. pyogenes* strain MGAS5005). This suggests genetic transmission between animal-origin *S. canis* and other human-origin virulent streptococci that may induce expression of novel virulent phenotypes. The similar genetic transmission between animal pathogen *S. canis* and human pathogen *S. pyogenes* was confirmed on the basis of the draft genome sequence (accession number NZ_BEWZ00000000.1)[19].

We performed a literature review of case reports of severe infections caused by *S. canis* in four animals (a raccoon dog, a mongrel cat, a mongrel dog, and the current miniature dachshund) in Japan[10,11,14]. These invasive infections included pleuropneumonia, myocarditis, infective endocarditis, and SSTI. With the exception of the current case, all cases proved fatal. In the current case, the owner observed inflamed conditions in the left hindlimb and visited the animal hospital on day one of the illness. The dog received early intervention (exploration and debridement), leading to both the accurate identification of the pathogenic bacterium and drainage of the infectious site. *S. canis*-related SSTI (necrotizing fasciitis) in a domestic shorthair cat was reported from Germany[12]: the cat receiving debridement and reconstruction along with negative pressure wound therapy showed no functional impairment of the limb. Therefore, it is critical that owners detect signs in their companion animals early, and that the animals receive immediate veterinary care at an animal hospital or clinic.

The possibility of septic shock in this case meant imipenem was administered as a first-line treatment, as administration of imipenem is performed as an empiric therapy for companion animals with bacteremia[16]. After confirming the antimicrobial susceptibility test results of no AMP phenotype, administration of ampicillin was started for de-escalation in this case.

Recurrence of the soft tissue inflammatory conditions was observed at the same site in the left hindlimb 115 days after the first onset, although the culture result with the FNA specimens was negative. A recent article reported early surgery for limb preservation in *S. pyogenes*-induced SSTI with subsequent recurrence at the same site approximately 2 months after surgical intervention in a human case[17]. Therefore, veterinarians should be aware of the possibility of streptococcus-associated recurrence for several months after intervention.

To the best of our knowledge, this is the first report of an animal cured of SSTI in Japan. To clarify the efficacy of exploration and debridement, it would be necessary to compare

these results with those of a similar case with a different approach that yielded poor results. In conclusion, the Lancefield group G *S. canis* should be considered a causative bacterium when examining and treating animals with SSTI and recurrence, since the major streptococcal species isolated was *S. canis* (88 cases, 22.4%)[9].

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Conflict of interest

None to declare.

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