**NOTE**

**Bacteriology**

## Genetic analysis of *Streptococcus equi* subsp. *equi* isolated from horses imported into Japan

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### ABSTRACT.

Strangles is a commonly diagnosed and important infectious disease of equids worldwide, caused by *Streptococcus equi* subsp. *equi*. We determined the SeM genotypes of *S. equi* isolated from imported horses at the Japanese border within the past 8 years, which allowed us to classify 12 strains isolated from these horses from each exporter into four allelic groups. These alleles were different from the alleles of past isolates found in Japan. Furthermore, four strains classified into the same allele were isolated from horses from one exporter over several years. In this study, *S. equi* isolates from different exporters had different SeM alleles. Attention to the hygiene status of farms will be necessary to prevent the incursion of strangles.

**KEY WORDS:** Animal Quarantine Service, genetic analysis, SeM, *Streptococcus equi*

Strangles is a commonly diagnosed and important infectious disease of equids worldwide, caused by *Streptococcus equi* subsp. *equi* [7, 14, 16]. It is characterized by a mucopurulent nasal discharge and acute swelling with subsequent abscess formation in the submandibular and retropharyngeal lymph nodes [4, 14, 16]. *S. equi* infects the horse’s cranial lymph glands and is highly communicable to other horses. Outbreaks of strangles have been reported in many countries, and sporadic outbreaks have occurred in Japan since 1992 [2, 5, 7, 18]. In recent years, the number of draft horses imported into Japan for a feeder has increased, and accounts for approximately 85% of the total volume of imported horses; strangles has occurred only in herds of draft horses during the quarantine period of importation. In the past, the outbreak of strangles in domestic horses was thought to be caused by imported draft horses [2]. Molecular studies have used the gene sequence encoding the N-terminal end of the M-like protein (SeM) for genotyping and identifying the sources of the outbreaks [1, 6, 8–11]. The purpose of this study was to investigate the genetic relationships among *S. equi* strains isolated from imported draft horses within the past 8 years at the Japanese border by the Animal Quarantine Service (AQS) using SeM genotyping, to confirm colonial variants of the strains, and to determine minimum inhibitory concentration (MIC) of antibiotics against the strains for evaluating the treatment and control strategies.

Twelve *S. equi* isolates were collected from imported draft horses from 2010 to 2017 (Table 1). Bacterial isolation from the nasal swab was performed. Swabs were streaked on Columbia agar plate (Columbia Agar; Becton, Dickinson and Co., Franklin Lakes, NJ, U.S.A.) with 5% sheep blood and incubated at 37°C for 24 to 48 hr under 5% CO2 conditions. Two of the 12 isolates were isolated in the AQS Moji branch and 10 were isolated at Kagoshima Airport, Moji sub-branch. Eleven isolates were isolated from clinical cases in which the horses showed fever, nasal discharge, an increase in the white blood cell count, and/or swelling of the submandibular lymph nodes (all except No. 11). The horses were imported by 5 Canadian exporters.

The isolates were cultured on Columbia agar with 5% sheep blood at 37°C for 24 hr. After the incubation, genomic DNA was extracted from the bacterial colony using a DNA extraction kit (InstaGene Matrix; Bio-Rad Laboratories, Hercules, CA, U.S.A.). The isolates were cultured for more than 24 hr to observe the colony morphology.

The entire structural SeM gene was amplified by PCR using the primer pairs; 5’-CAA AAA AGT GTG CCC ATA AC-3’ and
Table 1. Characterization of *Streptococcus equi* isolates in this study

| Isolate No. | SeM allele | Exporter | Year of isolation | Clinical or subclinical | Minimum inhibitory concentration of antibiotics\(^{(a)}\) |
|-------------|------------|----------|------------------|-------------------------|----------------------------------|
| 1           | SeM-39     | B        | 2010             | Clinical                | ≤1 × 10⁻¹ ≤1 ≤0.5 16 2 ≤0.5 2 1 |
| 2           | SeM-43     | A        | 2010             | Clinical                | ≤1 × 10⁻¹ ≤1 ≤0.5 8 ≤0.5 ≤0.5 2 1 |
| 3           | SeM-43     | A        | 2010             | Clinical                | ≤1 × 10⁻¹ ≤1 ≤0.5 16 2 ≤0.5 4 1 |
| 4           | SeM-28     | D        | 2011             | Clinical                | ≤1 × 10⁻¹ ≤1 ≤0.5 4 2 ≤0.5 4 1 |
| 5           | SeM-28     | D        | 2011             | Clinical                | ≤1 × 10⁻¹ ≤1 ≤0.5 8 2 ≤0.5 2 1 |
| 6           | SeM-39     | B        | 2013             | Clinical                | ≤1 × 10⁻¹ ≤1 ≤0.5 8 2 ≤0.5 4 1 |
| 7           | SeM-30     | C        | 2015             | Clinical                | ≤1 × 10⁻¹ ≤1 ≤0.5 16 2 ≤0.5 2 1 |
| 8           | SeM-30     | C        | 2015             | Clinical                | ≤1 × 10⁻¹ ≤1 ≤0.5 8 1 ≤0.5 2 1 |
| 9           | SeM-28     | E        | 2011             | Clinical                | ≤1 × 10⁻¹ ≤1 ≤0.5 16 1 ≤0.5 2 1 |
| 10          | SeM-28     | E        | 2011             | Clinical                | ≤1 × 10⁻¹ ≤1 ≤0.5 2 ≤0.5 ≤0.5 2 1 |
| 11          | SeM-39     | B        | 2017             | Subclinical             | ≤1 × 10⁻¹ ≤1 ≤0.5 4 1 ≤0.5 2 2 |
| 12          | SeM-39     | B        | 2017             | Clinical                | ≤1 × 10⁻¹ ≤1 ≤0.5 16 1 ≤0.5 2 1 |

\(^{(a)}\) Antibiotics: ABPC=ampicillin, CEZ=cefazolin, CTX=cefotaxime, KM=kanamycin, GM=gentamycin, TC=tetracycline, CP=chloramphenicol, CPFX=ciprofloxacin.
Fig. 1. Neighbor-joining phylogenetic tree constructed on the basis of the nucleotide sequences of SeM alleles. Bootstrap values are shown in the tree branches (1,000 replicates were performed). Letter in parentheses is the marks of exporters.

Fig. 2. *Streptococcus equi* showing matt-type colony on Columbia agar with 5% sheep blood at 37°C for 48 hr.
on the pathogenicity in horses have been evaluated [3]. Mucoid colonies express large capsules and have been isolated from cases of classical strangles. Matt strains are lysogenized with bacteriophages encoding hyaluronidase, which digests the capsule within 8–10 hr [13, 17], and have been associated with mild atypical strangles [12]. In foreign countries, approximately 80% of S. equi isolates form mucoid colonies, but all strains isolated from imported horses in this study formed matt-type colonies. This suggests that there were subclinical carriers or horses with clinically mild onset of strangles due to matt-type S. equi in the herds. These horses then contracted typical strangles after arriving in Japan due to the stress associated with the long duration of air transportation.

In general, S. equi is susceptible to beta-lactam antimicrobials, such as penicillin and cepham antibiotics, which have been used to treat strangles [14]. None of the strains isolated from imported horses in this study had antimicrobial resistance to these antibiotics. Therefore, the above antibiotics should be effective for the treatment of horses with strangles imported from Canada.

In conclusion, the SeM alleles of strains isolated from imported draft horses were different from those found in domestic race and riding horses in Japan. SeM sequencing analysis revealed that the S. equi isolates from each exporter had different SeM alleles. Continuous investigations using this method at Japan’s border might enable us to evaluate the hygiene status of the farms of the exporters. More attention will be necessary to prevent the incursion of strangles via imported horses in the future.

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