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# Tracing outbreaks of *Streptococcus equi* infection (strangles) in horses using sequence variation in the *seM* gene and pulsed-field gel electrophoresis

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ABSTRACT

Strangles is a serious respiratory disease in horses caused by *Streptococcus equi* subspecies *equi* (*S. equi*). Transmission of the disease occurs by direct contact with an infected horse or contaminated equipment. Genetically, *S. equi* strains are highly homogenous and differentiation of strains has proven difficult. However, the *S. equi* M-protein SeM contains a variable N-terminal region and has been proposed as a target gene to distinguish between different strains of *S. equi* and determine the source of an outbreak. In this study, strains of *S. equi* ($n = 60$) from 32 strangles outbreaks in Sweden during 1998–2003 and 2008–2009 were genetically characterized by sequencing the SeM protein gene (*seM*), and by pulsed-field gel electrophoresis (PFGE). Swedish strains belonged to 10 different *seM* types, of which five have not previously been described. Most were identical or highly similar to allele types from strangles outbreaks in the UK. Outbreaks in 2008/2009 sharing the same *seM* type were associated by geographic location and/or type of usage of the horses (racing stables). Sequencing of the *seM* gene generally agreed with pulsed-field gel electrophoresis profiles. Our data suggest that *seM* sequencing as a epidemiological tool is supported by the agreement between *seM* and PFGE and that sequencing of the SeM protein gene is more sensitive than PFGE in discriminating strains of *S. equi*.



## 1. Introduction

*Streptococcus equi* subspecies *equi* (*S. equi*) is a pathogenic bacterium that causes the serious and highly contagious respiratory disease strangles in horses. Clinical signs include fever, swollen lymph nodes of the head and neck, coughing, purulent nasal discharge and depression (Piché, 1984; Dalgleish et al., 1993; Sweeney et al., 2005). Infected horses display mild to severe disease and, although the mortality rate is low, life threatening illnesses such as metastatic abscessation, purpura hemorrhagica and myositis can also occur (Sweeney et al., 2005). The duration of clinical disease can be several months and is usually also of substantial economic importance to horse owners. Development of persistent carriers after an outbreak is now considered an important factor in disease maintenance within a group of horses and spread of the disease to immunologically naïve horses (Sweeney et al., 1989; Newton et al., 1997, 1998).

*S. equi* are β-hemolytic streptococci, belonging to Lancefield group C, and can be identified by conventional biochemical testing (Quinn et al., 1994) or by PCR

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(Artiushin and Timoney, 1997; Alber et al., 2004). To control or eventually even eliminate strangles, verification of the clinical diagnosis by detection of *S. equi* is important. Improved detection of *S. equi* in horses can be achieved by performing real-time PCR on DNA extracted from bacterial cultures (Båverud et al., 2007), or on DNA extracted directly from nasopharyngeal washes or nasal swab samples (Lindahl et al., unpublished data, 2010).

Genetically, different strains of *S. equi* are highly homogenous and thus differentiation has been difficult when using multilocus typing methods (Jorm et al., 1994; Webb et al., 2008).

However, the antiphagocytic M-protein SeM, a major virulence factor found only in *S. equi*, was shown to have a variable N-terminal region by Anzai et al. (2005) that could be used to differentiate strains. The *seM* variability described by Anzai et al. (2005) has been suggested as a tool for epidemiological studies of *S. equi* and determination of the source of strangles outbreaks (Anzai et al., 2005; Kelly et al., 2006). Previously described *seM* types can be accessed in the international database at the *Streptococcus zooepidemicus* MLST website http://pubmlst.org/szooepidemicus/ developed by Jolley et al. (2004).

The widely accepted method of pulsed-field gel electrophoresis (PFGE) is a highly discriminatory DNA-based typing technique that has been used in several epidemiological investigations of *S. equi* subsp. *zooepidemicus* outbreaks, for instance in clinical mastitis in dairy sheep (Las Heras et al., 2002) and in cheese-related human outbreaks (Bordes-Benítez et al., 2006; Kuusi et al., 2006). In contrast to sequencing specific genes, PFGE detects similarities and differences over the whole bacterial genome.

In Sweden strangles is a reportable disease based on clinical signs and/or on confirmed bacteriological diagnosis. This enhances the possibilities of tracing the source of disease in outbreaks and further aids in efforts to reduce the incidence of, or even eradicate, strangles in the Swedish horse population. Thus, we determined the *seM* sequences of *S. equi* strains isolated from Swedish outbreaks during the years 1998–2003 and 2008–2009, and compared findings to the genomic profiles obtained with pulsed-field gel electrophoresis (PFGE) for all strains. The overall aims of this study were to evaluate molecular typing of the *seM* gene as a tool for tracing the source of strangles outbreaks, to determine the *seM* types of outbreaks in Sweden, and to evaluate the correlation between *seM* typing and PFGE for all outbreaks.

## 2. Materials and methods

### 2.1. Clinical samples

Clinical isolates ($n = 24$), each representing a randomly selected outbreak of strangles in Sweden from the years 1998–2004, were obtained from the Institute's strain collection (National Veterinary Institute [SVA]). These isolates had been recovered from nasal swabs, nasopharyngeal swabs, pus/abscesses, guttural pouch samples or tracheal washes. In addition, samples of *S. equi* were collected from 36 horses with clinical signs of strangles in eight outbreaks observed in two distinct geographic areas in Sweden during the years 2008 and 2009. Stables selected for sampling (2008/2009) were geographically adjacent and/or their outbreaks coincided in time (Table 1 and Fig. 1). Sampled horses had one or more clinical signs of strangles; swollen or abscessed lymph nodes, serous to purulent nasal discharge, fever, anorexia, coughing, or depression. Samples were obtained by one of the following methods for each horse: nasal swab, nasopharyngeal swab or nasopharyngeal wash. In addition the following reference strains were included in the study: *S. equi* subsp. *equi* CCUG 27367 and *S. equi* subsp. *equi* ATCC 33398/CCUG 23255$^T$ obtained from the Culture Collection, University of Göteborg (CCUG), Sweden.

### 2.2. Culture and biochemical identification

Isolates from the Institute's strain collection were recovered from frozen storage (−70 °C) and subcultured

**Table 1**
Comparisons of *seM* type and pulsed-field gel electrophoresis (PFGE) results for clinical strains from the National Veterinary Institute's strain collection 1998–2003, clinical strains from eight strangles outbreaks (A–H) in 2008/2009 and reference strains of *Streptococcus equi* subsp. *equi*.

| Clinical strain/year | *seM* type | PFGE *Sma*I | PFGE *Apa*I |
|---|---|---|---|
| Bd 30/98 | SeM-9 | II | II |
| Bd 3393/98 | SeM-9 | II | II |
| Bd 8062/98 | SeM-76 | II | II |
| Bd 10521/98 | SeM-9 | II | II |
| Bd 446/99 | SeM-77 | II | II |
| Bd 1611/99 | SeM-78 | II | II |
| Bd 3800/99 | SeM-9 | II | II |
| Bd 5895/99 | SeM-9 | II | II |
| Bd 717/00 | SeM-9 | II | II |
| Bd 2447/00 | SeM-9 | II | II |
| Bd 5430/00 | SeM-9 | II | II |
| Bd 9786/00 | SeM-43 | III | III |
| Bd 387/01 | SeM-9 | II | IIB |
| Bd 3975/01 | truncated | II | IIB |
| Bd 6332/01 | SeM-9 | II | IIC |
| Bd 10623/01 | SeM-6 | IV | IV |
| Bd 43/02 | SeM-9 | II | II |
| Bd 4436/02 | SeM-9 | II | II |
| Bd 7625/02 | SeM-39 | V | V |
| Bd 11870/02 | SeM-9 | II | IIB |
| Bd 16164/02 | SeM-6 | IV | IV |
| Bd 659/03 | SeM-9 | II | II |
| Bd 724/03 | SeM-6 | IV | IV |
| Bd 20514/03 | SeM-6 | IV | IV |

| Outbreak/month/year | No. of strains | Type of stable | *seM* type | PFGE *Sma*I | PFGE *Apa*I |
|---|---|---|---|---|---|
| Outbreak A Nov/08 | 12 | Stud farm | SeM-9 | II | II |
| Outbreak B Nov/08 | 3 | Racing | SeM-1 | IV | IVB |
| Outbreak C Dec/08 | 2 | Riding | SeM-6 | IV | IV |
| Outbreak D Feb/09 | 1 | Racing | SeM-72 | II | II |
| Outbreak E Mar/09 | 3 | Riding | SeM-1 | VI | VI |
| Outbreak F Nov/09 | 5 | Riding | SeM-71 | IV | IV |
| Outbreak G Dec/09 | 4 | Racing | SeM-72 | II | II |
| Outbreak H Dec/09 | 6 | Stud farm | SeM-72 | II | II |
| *S. equi* CCUG 27367 | | | SeM-86 | I | I |
| *S. equi* ATCC 33398 | | | SeM-87 | I | I |