Genomic variation of uterine isolates of *Streptococcus equi* subspecies *zooepidemicus*

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**Introduction**

The commensal *Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*), the organism most common isolated from uterine infections in mares, displays genetic and antigenic variation which may play a role in pathogenesis and complicate vaccine development. Such variation also challenges researchers to differentiate between experimentally introduced organisms and normal flora. A study was performed to determine the extent of genomic and antigenic variation of uterine isolates of *S. zooepidemicus*, and if isolates collected after experimental infection could be shown to be identical to the inoculated isolate.

**Materials and methods**

Uterine isolates of *S. zooepidemicus* (n=21) were analyzed by pulsed-field gel electrophoresis (PFGE), and by bactericidal testing in blood of ten horses of varying immunological history. In addition, 13 isolates of *S. zooepidemicus* from fetal and maternal tissues of experimentally infected mares were compared to the inoculated isolate by PFGE. Extraction of genomic DNA followed by restriction endonuclease digestion and PFGE of the DNA fragments was performed as previously described, with the modification that genomes of all isolates were first digested with *Sma* I, but those with identical *Sma* I restriction patterns (pulsotypes) were also digested with *Apa* I. For bactericidal testing, aliquotted suspensions (250 µL) of each isolate at varying concentrations were prepared, frozen at -80 °C and post-thaw colony counts determined. Suspensions yielding post-thaw counts of 7 to 50 c.f.u per 100 µL were used for bactericidal testing. A 100 µL inoculum of each isolate was incubated in 1.5 mL of fresh blood of a single horse for 48 hours rotated end-over-end at 37 °C. After 48 hours, tubes of blood were either bacteriologically sterile (score = 1) or had been hemolyzed by streptococcal multiplication (score = 0). The procedure was repeated in 10 horses, yielding a specific sequence of 1’s and 0’s for each isolate across 10 horses. Strain differentiations by bactericidal testing and PFGE were compared.

**Results**

Isolates yielded 16 different pulsotypes and 13 different bactericidal test sequences. Isolates showing identical pulsotypes showed identical bactericidal test sequences, or sequences different at only one horse. The 13 isolates recovered after experimental infection yielded two pulsotypes, 10 isolates identical to the inoculated isolate, and three isolates of a different pulsotype. Total DNA of both pulsotypes was within 0.5% (2079.46 kb versus 2088.58 kb), and the variation consistent with a single large inversion mutation of approximately 300 kb.

**Discussion**

These data indicate that genomic and antigenic variation of *S. zooepidemicus* is widespread among uterine isolates, and is possibly caused, in part, by inversion mutation.

**Keywords:** *Streptococcus zooepidemicus*, PFGE, bactericidal test, uterus, equine

**Reference**