TITLE: COMPARISON OF THE VIRULENCE POTENTIAL OF DIFFERENT *STREPTOCOCCUS DYSGALACTIAE* SUBSPECIES *EQUISIMILIS* CLONES CIRCULATING IN BRAZIL USING *CAENORHABDITIS ELEGANS* MODELS

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ABSTRACT:

Group C streptococci can cause infections in both humans and other animals. Among these, *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE) are the most frequently reported cause of human diseases. In a study carried out in our laboratory it was observed that of the total 115 SDSE isolates—analyzed by pulsed-field gel electrophoresis—57.5% belonged to clone A, 26.1% to B and 16.4% to sporadic (rare) clones. In order to better understand why some SDSE clones (A and B) are prevailing, we evaluated the virulence potential of representatives of these clones in comparison with the sporadic ones using PCR-based gene detection, and two *Caenorhabditis elegans* models based on nematode survival and binary choice. For the survival assay, we transferred approximately 20 L4 larvae into each of the 24-wells of a microtiter plate, where the worms were exposed to the different bacterial clones. The data were analyzed by the Kaplan-Meier survival rate method using GraphPad Prism 6 software. After 3 days, the worm survival rate was 4.44±1.27% for clone B, 10.11±2.24% for clone A and 21.50±3.30% for sporadic clones (p<0.0001). The binary choice assay—where the worms were seeded between equidistant growths of representatives of two different SDSE clones—the nematodes showed a higher preference for representatives of the sporadic clones when confronted with A or B representatives. Comparing clone B isolates from equine and human origins, a significant difference (26.25±9.49% and 3.74±3.69%; respectively; p<0.0001) in the worm survival rates were observed in the first day. All together, these results indicate a higher virulence potential for A and B clones compared with the sporadic ones, being clone B representatives more virulent than those of the clone A. Indeed, these results are coherent with an increased detection of virulence-associated genes among isolates of the predominant clones A and B suggesting they evolved for higher virulence compared with the sporadic ones. Finally, our results demonstrate that SDSE isolates displaying the same pulsed-field pattern B and ST type 129 from different hosts may show variations in the virulence profile in *C. elegans* models.

Keywords: *Streptococcus dysgalactiae* subspecies *equisimilis, Caenorhabditis elegans model*, virulence, predominant clones

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