

TITLE: USE OF *Caenorhabditis elegans* AND THP-1 MONOCYTES AS MODELS TO STUDY THE VIRULENCE OF PREDOMINANT CLONES OF *Streptococcus dysgalactiae* SUBSP. *equisimilis*

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ABSTRACT:

Among the species of group C *Streptococcus*, *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 a total 115 SDSE isolates, analyzed by the pulsed-field 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 sporadic clones using two *Caenorhabditis elegans* models based on nematode survival and binary choice. In the virulence assay the higher survival rate of the worm was for the sporadic clones at the end of the experiment. In the binary choice assay, nematodes showed a higher preference for strains from sporadic clones or clone A when the choice option was clone A and B. In addition, it was observed by optical microscopy changes in the internal structure of the nematode and a lower number of CFUs/nematodes for the strains representative of the most virulent clones. From these results, the survival of the nematode in medium containing *N-acetylcysteine* (NAC) was evaluated. This is able to inactivate hydrogen peroxide, and may contribute to understanding the role of H₂O₂ as a potential virulence factor. Observed a higher percentage of survival for the nematodes in medium with NAC (Clone A: 36%, Clone B: 39% and sporadic Clones: 60%, $p < 0.05$) when compared to those that did not (A: 15%, B: 17% and sporadic clones: 46%). In parallel we evaluated SDSE phagocytosis by THP-1 monocytes. Preliminary results revealed that all strains had a high rate of phagocytosis, but representatives of clones A and B demonstrated a greater ability to induce monocyte death and in addition a larger number of CFU/mL of SDSE this clones were recovered. In the future, we will evaluate the expression of genes related to the production of reactive oxygen species through real time-PCR of SDSE isolates inside the nematode and monocytes with the objective of confirming the role of ROS as the same factor

responsible for the death of our model study *in vivo* and *in vitro*. In fact, these results are consistent with increased detection and expression of virulence-associated genes between strains of the clones A and B suggesting that they evolved to greater virulence compared to sporadic clones.

Keywords: *Streptococcus dysgalactiae* subspecies *equisimilis*, *Caenorhabditis elegans*, survival test, binary choice, virulence, phagocytosis

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