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Optimization of aerosol generation techniques for *Francisella tularensis* LVS and SchuS4 strainsR. L. Sherwood¹, T. Brasel¹, E. Barr¹¹Lovelace Respiratory Research Institute, Albuquerque, United States

Aims: Testing was performed to develop methods for aerosolization of viable *Francisella tularensis* strains LVS and SchuS4 to allow future animal inhalation exposure studies. Aerosolization of viable microbes is a challenging process because of microbial susceptibilities to drying, temperature, and shear forces exerted during the aerosolization process. This process is even more challenging when using vegetative microbes such as *Francisella tularensis*.

Methods: Aerosolization methods were developed for both *F. tularensis* LVS (LVS) and *F. tularensis* SchuS4 (SchuS4) using a 3-jet Collision generator that allowed non-human primates to be challenged with doses ranging from 1 to 10⁶ cfu. Bacteria were grown in a defined medium (Chamberlain's broth) and harvested in log phase for aerosol challenge.

Results: Collision-generated bioaerosols have consistent particle sizes, but the microbes are subjected to considerable shear forces. Initial bioaerosol characterization was performed with LVS with the assumption that SchuS4 would exhibit similar properties. Stability, spray factor, and virulence were determined. LVS spray titers dropped approximately 0.17 log during sprays while SchuS4 spray titers dropped approximately 0.34 logs. Average spray factors for LVS were -6.52 (log₁₀) while spray factors for SchuS4 were -6.97. 30% of mice exposed to 6.95x10³ cfu LVS or 4.37x10² cfu SchuS4 survived. Mice exposed to higher doses of SchuS4 had 0% survival by Day 5.

Conclusions: LVS and SchuS4 have different susceptibilities to aerosolization with LVS having higher spray factor values and greater stability, but lower virulence than SchuS4 in mice. This project has been funded in whole or in part with Federal funds from the National Institute of Allergies and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN266200500040C.