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The Viable but Non-Culturable State of *Francisella tularensis*

Tularemia is a zoonotic infection caused by the Category A biodefense agent *Francisella tularensis*. This bacterium is maintained in small mammals such as rabbits but has also been isolated from the soil, natural water systems, and domestic water supplies. Previous data has shown that *F. tularensis* can enter into a viable but non-culturable (VBNC) state where the bacterium cannot be cultivated by traditional methods, yet the bacterium is alive and maintains a measurable level of metabolic activity. Since most conventional diagnostic tests depend upon cultivation of the bacteria, VBNC bacteria are a serious threat to public health. Therefore, understanding how VBNC *F. tularensis* survive and persist in the environment and how they can be resuscitated back to an easily-detected, culturable form is of utmost clinical importance. The focus of our project is determining culture conditions that cause *F. tularensis* to enter into the VBNC state in the laboratory setting. We have tested various parameters including pH, temperature, and starvation as potential VBNC inducers. Culturability was measured by growth of *F. tularensis* on chocolate agar while viability was measured using a LIVE/DEAD fluorescent staining kit. To date, all conditions tested that have reduced the culturability of *F. tularensis* have also been associated with decreased viability. Current experimentation has focused on whether prolonged incubation of *F. tularensis* at mammalian body temperature will stimulate the bacteria to enter the VBNC state. In the future, we plan to utilize laboratory-generated VBNC *F.tularensis* to determine which genes are expressed during this state that mediate survival.