Francisella tularensis, a gram-negative coccobacillus is the causative agent of tularemia, a potentially fatal zoonotic disease. Under laboratory conditions, F. tularensis enters a viable but non-culturable (VBNC) state. VBNC is a state of dormancy bacteria enter during stressful conditions and can have implications for persistence and pathogenicity.

Our first aim is to use molecular cloning to create recombinant fluorescent proteins for cell wall and division proteins in F. tularensis. Fusions to FtsZ, MreB and RodA will be generated and used to analyse the localization of these proteins during the transition into the VBNC state.

Our second aim is to investigate the effects of transition into the VBNC state on pathogenicity. To test this, we will use microscopy to analyze fluorescently labelled F. tularensis during infection of THP-1 cells. THP-1 cells are a spontaneously immortalized monocyte-like cell line, derived from the peripheral blood of a childhood case of acute monocytic leukemia. THP-1 cells are a model for mammalian blood monocytes that provide protection against infection by gram negative bacteria. Our hypothesis is that VBNC F. tularensis will be able to interact with THP-1 cells in an in vitro infection assay. Further, the ability of VBNC F. tularensis to replicate within THP-1 cells will be analyzed. (Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence).