STUART CANTLAY*, NICOLE GARRISON*, RACHELLE PATTERSON*, DONALD PRIMERANO†, JUN FAN† AND JOSEPH HORZEMPA*, *Department of Biological Sciences, West Liberty University, West Liberty, W.V. † Genomics and Bioinformatics Core Facility, Marshall University, Huntington, W.V. Applying RNA-Seq to investigate the transition into a Viable But Non Culturable State (VBNC) for the intracellular pathogen, *Francisella tularensis* LVS.

Many species of bacteria, under conditions of stress or nutrient limitation, enter a state of dormancy referred to as viable but non-culturable (VBNC). VBNC bacteria persist in the environment, are difficult to detect and identify by many standard laboratory methods and can be altered in their susceptibility to antibiotics. Entry into the VBNC state is often accompanied by morphological changes; however, the mechanisms underlying this are poorly understood. Francisella transitions rapidly and spontaneously to a VBNC state, and therefore has the potential to be an excellent model organism for the study of this phenomenon. To investigate the transcriptome of VBNC F. tularensis LVS we extracted RNA from culturable and VBNC cells and carried out an RNA-Seq analysis using both Long-read Nanopore and Illumina Sequencing. Differentially expressed genes (DEGs) were identified using a DE-Seq pipeline. Over 300 genes were significantly upregulated and ~100 genes were down regulated in VBNC cells. Amongst the upregulated genes were some involved in the transport of metals or small molecules, and we have also identified putative transcriptional regulators that may be master controllers of the VBNC process. Our data represents the first transcriptomic analysis of F. tularensis LVS as it transitions into the VBNC state. Identifying genes that are involved in this transition is a critical first step in understanding the mechanisms that drive F. tularensis LVS into the VBNC state and will help us identify factors that allow the resuscitation of these bacteria. This will have important implications for understanding both environmental persistence and pathogenicity of Francisella species. (Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)