MARIAH CASHBAUGH, SHANIA DAVIS, ELIO DELATORE, & JOSEPH HORZEMPA, Dept. of Biomedical Sciences, West Liberty University, West Liberty, WV, 26074. Screening putative transcriptional regulators in *Francisella tularensis* for attenuation using a multifaceted approach.

Francisella tularensis is a gram-negative intracellular pathogen that produces a severe infection known as Tularemia. RNA-Seq data revealed that in the presence of erythrocytes, several genes encoding putative transcriptional regulators were modulated: FTL_0671, FTL_1199, and FTL_1665. Gene deletion strains were constructed using *Francisella tularensis* LVS. The objective of this project was to screen these gene deletion strains for attenuation. A multifaceted approach was utilized to determine the level of replication within macrophages and overall attenuation in vivo. Transforming the bacteria with green fluorescent protein allowed a plate reader to visualize and quantify intracellular growth in macrophages. However, inconsistencies in the data from these experiments led to the utilization of a gentamicin protection assay. This protocol provided a more accurate and reliable method of determining intracellular replication. The results of this experiment revealed a significant increase in the replication of ΔFTL_1665 within macrophages. We sought to determine if these results would translate to hypervirulence in a live model. A chicken embryo infection model confirmed that ΔFTL_1665 was significantly hypervirulent in vivo. In the future, we plan to experiment with the upregulation of the target gene to produce an attenuated strain. This gene may also serve as a potential drug target.