ELIO F. DELATORE III, ELLE ROBERTS, STUART CANTLAY, & JOSEPH HORZEMPA. Department of Biomedical Sciences, West Liberty University, WV. Deletion of FTL_1199 to determine the role of this gene in erythrocyte invasion by *Francisella tularensis.*

Francisella tularensis is a bacterium that induces the zoonotic disease tularemia. In the course of infection, *F. tularensis* bacteria invade erythrocytes, a phenomenon that heightens the colonization of ticks after a blood meal. To better understand the mechanism of erythrocyte invasion, we hypothesized that transcription of bacterial genes significant in erythrocyte invasion would be upregulated upon exposure to these host cells. An RNA-seq unveiled that transcription of 7% of *F. tularensis* genes augment when in erythrocyte presence. Of these, we pinpointed three putative transcriptional regulators, namely FTL_0671, FTL_1199, and FTL_1665. The goal was to delete FTL_1199 in *F. tularensis* LVS. Splicing by overlap extension PCR amplified and duplicated the up and downstream (~500 bp each) regions of the target gene in tandem into a shuttle vector that is insecure within *F. tularensis*. This newly generated plasmid, pDEL1199, was mobilized inside of *F. tularensis* by conjugation. Merodiploid strains generated by homologous recombination were isolated and transformed with pGUTS – a stable plasmid that encodes a homing endonuclease (I-SceI) and a kanamycin resistance cassette. Expression of I-SceI within the merodiploid produces a double-stranded break in pDEL1199 that had previously integrated in the chromosome. This breakage resulted in a second recombination that either ensued to wild-type or deletion of FTL_1199 deduced through a PCR. Finally, in ΔFTL_1199 strains, pGUTS was cured by successive cultivation in the absence of selection followed by replica-plating on chocolate II agar ± kanamycin. Gentamicin protection assays involving *F. tularensis* ΔFTL_1199 suggest that FTL_1199 is important in erythrocyte invasion.

(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence, R15HL14735 from NHLBI, and funds from the NASA West Virginia Space Grant Consortium).