STUART CANTLAY, KRISTEN HAGGERTY & JOSEPH HORZEMPA, Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV, 26074. OpiA, a Type Six Secretion System Substrate, localizes to the cell pole and plays a role in bacterial growth and viability in *Francisella tularensis* LVS.

Francisella tularensis is an intracellular pathogen and the causative agent of tularemia. The *F. tularensis* type six secretion system (T6SS) is required for a number of host-pathogen interactions including phagolysosomal escape and invasion of erythrocytes. One known effector of the T6SS, OpiA, has recently been shown to be a phosphatidylinositol-3 kinase. To investigate the role of OpiA in erythrocyte invasion, we constructed an *opiA*-null mutant in the live vaccine strain, *F. tularensis* LVS. OpiA was not required for erythrocyte invasion, however, deletion of *opiA* affected growth of *F. tularensis* LVS in broth cultures in a medium-dependent manner. We also found that *opiA* influenced cell size, gentamicin sensitivity, bacterial viability, and the lipid content of *F. tularensis*. A fluorescently tagged OpiA (OpiA-EmGFP) accumulated at the cell poles of *F. tularensis* which is consistent with the location of the T6SS. However, OpiA-EmGFP also exhibited a highly dynamic localization and this fusion protein was detected in erythrocytes and THP-1 cells *in vitro* further supporting that OpiA is secreted. Similar to previous reports using *F. novicida*, our data demonstrated that *opiA* had a minimal effect on intracellular replication of *F. tularensis* in host immune cells *in vitro*. However, THP-1 cells infected with the *opiA* mutant produced modestly (but significantly) higher levels of the pro-inflammatory cytokine TNF-α compared to these host cells infected with wild-type bacteria. Together, our results support previous observations that implicate *opiA* in infection and virulence, but also reveal an additional role for *opiA* central to the biology of *F. tularensis* bacteria. *(This research was made possible by NASA West Virginia Space Grant Consortium Training Grant #NNX15A101H and by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence).*