

STUART CANTLAY, CHRISTIAN KAFTANIC AND JOSEPH HORZEMPA, Department of Biological Sciences, West Liberty University, West Liberty, WV, 26074. PdpC, a Type Six Secretion System Substrate, is Required for Erythrocyte Invasion 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 phagolysosomal escape and invasion of erythrocytes. An effector of the T6SS, PdpC, is required for phagosomal escape and we wanted to test if PdpC was also required for erythrocyte invasion. We constructed a *pdpC*-null mutant in the live vaccine strain, *F. tularensis* LVS. The *pdpC*-null strain is required for invasion of both human and sheep erythrocytes and reintroduction of a copy of *pdpC*, *in trans*, rescues this phenotype. Differential Immuno-Fluorescence Microscopy (DIFM) showed that the *pdpC*-null strain is affected in attachment as well as invasion. Further, a fluorescently labelled *pdpC*-null strain of *F. tularensis* LVS was unable to proliferate in THP-1 human peripheral blood monocyte cells. Finally, we constructed a fluorescent fusion of *pdpC* to *emgfp* and the resulting PdpC-EmGFP fusion protein localizes as discrete foci in a subset of broth cultured *F. tularensis* LVS cells. Our results confirm previous observations that PdpC is required for infection and virulence in phagocytic host cells and are the first description of an effector of the Type Six Secretion System that is required for erythrocyte invasion. The *pdpC-emgfp* strain will be a useful tool to further characterize the role of PdpC in both macrophage infection and red blood cell invasion. (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).