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Characterisation of mutants within the *Francisella* pathogenicity island

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Francisella tularensis is a gram-negative facultative intracellular bacterial pathogen capable of causing severe disease, tularaemia, in many mammalian species, including humans. The bacterium is able to evade the immune system and, upon escape from phagosomes, multiply within the macrophage cytosol. However, the mechanisms for infection are poorly understood. A 34 kb genomic region known as the *Francisella* pathogenicity island, FPI, plays an important role both in phagosomal escape and in the subsequent replication. Some of the FPI members show homology to proteins thought to be conserved structural components of type VI protein secretion systems (T6SS), including IglA and IglB, while others are unique to *Francisella* spp. In this study, in-frame deletion mutants within individual genes of the FPI were constructed and the obtained mutants were analysed for their ability to grow within murine macrophages, escape from phagosomes and cause cytotoxicity. Their impact on virulence was also analysed *in vivo* using a mouse model of infection. To elucidate the assembly of the putative T6SS, binding assays and protein fractionation techniques were applied to determine the protein-protein interactions and intrabacterial location of the corresponding gene products.

Keywords: *Francisella*, Type VI secretion, pathogenicity island, intracellular replication