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Full virulence is restored by reintroduction of two virulence loci into the live vaccine strain (LVS) of *Francisella tularensis*

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The live vaccine strain, LVS, is one of the most studied strains of *Francisella tularensis*. It has previously been used as a vaccine and found to protect staff from laboratory acquired tularemia. The genetic events leading to the attenuation are unknown and this has hampered licensing of LVS as a vaccine. Interestingly, LVS lacks two regions linked to virulence, RD18 and RD19. The latter encodes a putative type IV pilin (PilA), which contributes to virulence in type B strains, while RD18, encoding the outer membrane protein FTT0918 has been verified to be required for virulence of the highly pathogenic type A strains.

Aim: The aim was to determine to what extent these gene deletions contributed to the attenuation of LVS.

Methods: Each of the deleted regions was restored by genetic complementation *in cis* and the complemented strains were assessed for virulence in a mouse infection model.

Results: Complementation of both RD18 (FTT0918) and RD19 (*pilA*) fully restored virulence of LVS to a level indistinguishable from virulent clinical isolates of type B strains. Reintroduction of a functional *pilA* gene partially restored the ability of LVS to cause infection by the peripheral subcutaneous route in mice, while reintroduction of FTT0918 had a major impact on virulence of LVS in mice both by subcutaneous and intraperitoneal route of infection.

Conclusions: Our work support that the major genetic events resulting in the attenuation of LVS was deletion of the two direct repeat flanked regions RD18 and RD19. As the two attenuating deletion events are irreversible, our work supports that LVS can not revert to become more virulent which could facilitate the licensing of LVS for use in humans.