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Phenotypical analysis of the putative 2nd polysaccharide gene cluster of *Francisella tularensis*

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Aims: *Francisella tularensis* has one polysaccharide gene cluster, which encodes the O-antigen, and one putative polysaccharide gene cluster. The function and product of the putative cluster is unclear. The aim of this study is to disrupt the putative polysaccharide gene cluster by deleting a gene and analyse the resultant mutant's phenotype.

Methods: Deletion mutants of the polysaccharide gene cluster were made from subspecies *novicida* and *tularensis* and the virulence of these strains was determined. Sensitivity to guinea pig serum in addition to the uptake and survival in macrophages was compared to that of the wild-type strains.

Results: The *galE* gene was deleted in both subspecies. In the mouse model the subspecies *tularensis* mutant was virulent, whereas in subspecies *novicida* the mutant was attenuated and afforded protection against a *novicida* challenge. Both mutants were impaired in their ability to replicate in vitro and showed no difference to serum sensitivity compared to the wild-type strains.

Conclusions: Our study shows that the putative second polysaccharide gene cluster products for subspecies *novicida* and *tularensis* differ in their role in virulence. © Crown Copyright, Dstl, 2009.