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**A partial screen for *Francisella tularensis* virulence determinants using *Drosophila melanogaster***M. Åhlund<sup>1</sup>, P. Rydén<sup>2</sup>, A. Sjöstedt<sup>1</sup>, S. Stöven<sup>1</sup><sup>1</sup>Umeå University, Clinical Microbiology, Umeå, Sweden, <sup>2</sup>Umeå University, Mathematics and Mathematical Statistics, Umeå, Sweden

**Aims:** Identification of new virulence determinants has so far mainly been performed in mammalian systems. But, gene-by-gene *in vivo* testing in mice is expensive and ethically questionable. To enable high-throughput screening of single gene knockouts we developed a protocol using *Drosophila melanogaster* as a screening host and a well-characterized transposon mutant library of *Ft novicida*.

**Methods:** By using fly survival as readout we tested 249 *F. tularensis* genes which had previously been implicated in virulence in mouse infection models. Candidates were further characterized in additional experiments like viable count analysis in flies and in mouse macrophages.

**Results:** We identified 43 transposon insertion mutants that were attenuated also in flies. These include almost all loci in the *Francisella* pathogenicity island plus other genes required for intracellular survival and responses to environmental stress. Additional testing further splits these mutants into four functional groups, of which one seems to be involved in inducing a chronic inflammation-like state. Interestingly, we found seven mutants to be hypervirulent in *Drosophila*.

**Conclusions:** Our screening model probably best resembles the intraperitoneal route of infection in mammals. The overlap of “hits” with conventional negative selection screens in mice was similar to the overlap between such studies. Our results reiterate the importance of intracellular bacterial growth for virulence, but they also demonstrate a role of conserved *in vivo* physiological reactions for the survival of such diverse hosts as flies and mice.