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Exploration of *Campylobacter jejuni* survival mechanisms in house flies

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Background

Houseflies have been shown to play an important role in the transmission of *Campylobacter jejuni* to poultry in poultry houses. An experimental fly model has been established to investigate the survival dynamics of *C. jejuni* in the housefly.

Objectives

Identification of *C. jejuni* genes required for persistence/survival in the housefly.

Methods

A *mariner* transposon mutant library of ~10,000 unique transposon insertion mutants was constructed in *C. jejuni* M1. Five groups of 10 flies were individually inoculated with 10^6 CFU of the mutant library in a 1 μl volume via their proboscis. After 4 h incubation at 20°C mutants were recovered from flies on mCCDA-chloramphenicol plates. To identify the mutants that were unable or less able to survive in flies, the relative abundance of each mutant (inoculum versus recovered) was determined by massively parallel sequencing of transposon insertion sites (Tn-seq). Directed gene deletion mutants were constructed for validation using an overlap PCR method.

Results

After correction for genes required for *in vitro* growth of *C. jejuni* M1, a total of 48 genes were identified for which mutants showed >2-fold attenuated ($P_{adjusted} < 0.05$) survival in flies. A number of gene deletion mutants have been generated for validation of the Tn-seq data and for further characterization.

Conclusions

Using a combination of high-density transposon mutagenesis and genome-wide targeted sequencing of transposon insertion sites, we have identified a set of genes which may be required for survival/persistence in the house fly, and which could potentially serve as novel targets for intervention strategies.