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**Monitoring the survival of fish *Francisella* pathogens in aquatic microcosms**S. Duodu<sup>1</sup>, D. Colquhoun<sup>1</sup><sup>1</sup>National Veterinary Institute, Fish Health, Oslo, Norway

**Aims:** To investigate the survival or persistence behavior of fish *Francisella* in aquatic environments under different temperature conditions.

**Methods:** Two fish *Francisella* isolates (*F. philomiragia* subsp. *noatunensis*) were inoculated into non-sterile (natural) and sterile freshwater and seawater microcosms and monitored under different temperatures (4 °C, 8 °C, 12 °C) over a period of 60 days. No nutrients were added to the microcosms, simulating conditions in their natural ecological niches where available nutrients may be limiting. Plate culturing, DNA-based real time PCR and in-situ fluorescence hybridisation (FISH) techniques were used to identify and quantify the inoculated strains recovered from the water samples.

**Results:** The persistence of culturable cells was greater in sterile than in natural water samples. In the non-sterile microcosms, *Francisella* remained culturable no longer than 5-12 days on plates. On other hand, culturability of the bacterium was prolonged (>20 days) in sterile microcosms, especially under low temperature conditions. These results were confirmed by quantitative PCR analysis targeting the succinate dehydrogenase (sdhA) gene. Regardless, of the water sample used, PCR quantification of cell numbers (estimated from gene copy numbers) remained at the same level (ca. 10<sup>6</sup> CFU) throughout the experiment. A cell viability assay coupled with FISH analyses showed that the bacterium enters into Viable but not Culturable (VBNC) state after a period in water.

**Conclusions:** Fish *Francisella* may have low survivability in the natural water environments. The data contribute to a better understanding of the behaviour of *F. philomiragia* subsp. *noatunensis* in natural seawater and freshwater environments and suggest that the aquatic bacterial population play an important role in the survival of this fish pathogen. The ability to enter into VBNC state may have some ecological relevance for its spread, after disease outbreaks.