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Extra- and intracellular proliferation of *Francisella tularensis* (LVS) in presence of amoebae

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Aims: The purpose of the study was to investigate whether *Francisella tularensis (F.t.)* is able to use amoebae as a reservoir for survival under environmental conditions.
Methods: Cultures of *Acanthamoeba castellanii* in ATCC medium no. 712 (PYG) were infected with *F.t. ssp. holarctica* Live Vaccine Strain (LVS). The co-cultures were incubated at different temperatures in medium, PBS, or natural river water and subsequently analysed by means of immunofluorescence, electron microscopy, determination of colony forming units (CFU), and RTq- PCR over different time periods up to several weeks.
Results: CFU determination in co-cultures with amoebae over five days showed a rapid proliferation of LVS from an initial concentration of approximately 10^2 CFU/ml on day 0 up to 10^9 CFU/ml on day 5. In culture medium alone without amoebae, no bacteria could be re-cultured from an inoculum of 10^3 and 10^4 CFU/ml after 3 days.Analysis of immune-stained samples showed an accumulation of *F.t.* around and on the surface of the amoebae. Preliminary results from electron microscopy could support these observations for cultures after 4 days. Association between bacteria and amoebae was tight at focal points of the contact area suggesting the formation of particular contact structures.
Conclusions: Proliferation rates of *F.t.* in presence of amoebae and the finding that the bacteria are mainly located extracellularly indicate that *F.t.* might be able to use metabolic products of the amoebae to facilitate proliferation and survival. The close structural contact between the cells might be used for nutrition of bacteria and could be important for an eventual fast entry of *F.t.* as reaction to negative changes of environmental conditions. Further investigations are carried out in order to determine how long *F.t.* can survive under different conditions in presence of amoebae and whether the localisation of the bacteria changes from mainly extra- to intracellular compartment under sub-optimal environmental conditions.