P3-08 Suppression of *B. melitensis* Rev.1 erythritol catabolism as a strategy to avoid genital tropism to develop a safe brucellosis vaccine

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Abstract

Small ruminant brucellosis by *Brucella melitensis* is manifested mostly as abortion and infertility, symptoms that result from the marked tropism and intense multiplication of these bacteria within the cells of the genital organs and placenta. Animal mass vaccination is critical to control brucellosis and lessen human infection in countries with high prevalence. However, Rev.1, the only available vaccine for small ruminants, keeps genital tropism and thus induces abortion in pregnant females. An obvious strategy for minimizing the abortifacient effects is the suppression of the tropism for the placenta, a phenomenon that has been postulated to be connected to the abundance of erythritol in this organ and the preferential use of this polyol by *B. melitensis*. Based on this hypothesis, we obtained non-polar deletion mutants in ery1 and ery2, genes of the recently unraveled erythritol catabolic pathway. Rev.1Δery1 was unable to metabolize erythritol and its growth was not affected by the presence of this polyol in rich medium. Rev.1Δery2 was also unable to use erythritol but its growth was inhibited by this polyol. Studies in THP-1 monocyte-derived-macrophages and BeWo trophoblasts showed that while both mutants multiplied in macrophages like Rev.1, Rev.1Δery2 multiplication in trophoblasts was significantly lower. In our pregnant mouse model, the deletion of both genes resulted in a decrease in abortions and reduced bacterial replication in the placenta and vertical transmission to the fetuses. The virulence (splenic multiplication curves) and protection assays in mice confirmed the attenuated profile of Rev.1Δery2. In contrast, Rev.1Δery1 showed a multiplication profile similar to Rev.1 and optimum protection against the *B. melitensis* H38 challenge. These results led us to assess the safety of mutant Rev.1Δery1 in pregnant sheep (work O5-2 presented by Muñoz, P. M. et al in this Conference). Presenting author: azuniga@unav.es

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