O5-2 Improving Rev.1 vaccine safety in pregnant ewes

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Abstract

Lack of safety in pregnant animals hinders Rev.1 mass vaccination campaigns, the most cost-effective strategy to control *B. melitensis* in endemic and low-income countries. In addition to its abortifacient effect, Rev.1 is virulent to humans and streptomycin (Strp)-resistant, an antibiotic of choice for treating human brucellosis. Our goal was to develop a Rev.1 vaccine of improved safety for pregnant sheep. Three Rev.1 mutants were studied: Rev.1ΔwadC, lacking the lipopolysaccharide core lateral branch, that enhances immune system recognition; Rev.1Δery1, unable to catabolize erythritol, involved in *Brucella* genital tropism; and Rev.1StrpS, carrying the *B. melitensis* 16M rsmG instead of Rev.1 rsmG (Rev.1ΔrsmG::Tn7BmersmG) and dispaying increased Strp sensitivity. In mice, the mutants protected similarly than Rev. 1 against *B. melitensis* showing the same (Rev.1Δery1) orless (Rev.1ΔwadC and Rev.1StrpS)residual virulence. Two similar but independently conducted safety experiments were carried out in sheep (one for Rev.1ΔwadC and one for Rev.1Δery1 and Rev.1StrpS). Coetaneous 17-month- old *Brucella*-free ewes were synchronized reproductively and mated. Pregnant ewes were randomly allotted into groups of 12-13 animals. At the middle of pregnancy (73-78 days), each group was vaccinated conjunctively with 1-2 × 109 CFU of the corresponding mutant while a control group received the same conjunctival dose of the commercial Rev.1 vaccine (Ocurev®, CZV, Spain). Fever, anorexia, apathy, abortion, perinatal death or lesions were recorded daily. Also, bacterial excretion in vaginal fluids and milk or presence in fetuses was monitored weekly and immediately after abortion/delivery by culturing samples on duplicate plates of both CITA and Farrell selective media. After deliveries, ewes were necropsied and main target organs (spleen, uterus, and mammary, crural, prescapular, iliac and cranial lymph nodes) submitted to bacteriological assessment. The antibody response was assessed weekly by Rose Bengal, Complement Fixation, Agar Gel Immunodiffusion and iELISA tests. Similarly to Rev.1, all mutants induced *Brucella* antibodies 2 weeks after vaccination. However, whereas 61-75% of Rev.1 controls excreted the vaccine and 46-58% suffered undesirable reproductive symptoms, Rev.1ΔwadC, Rev.1Δery1 and Rev.1StrpS did not induce excretion, abortion or perinatal death. Thus, these three safe Rev.1 mutants are suitable candidates for further efficacy experiments in sheep.

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