Conjunctival administration of H38Δ*wbkF* rough vaccine as an effective strategy to protect against *Brucella ovis* infection while reducing the diagnostic interference of vaccine antibodies

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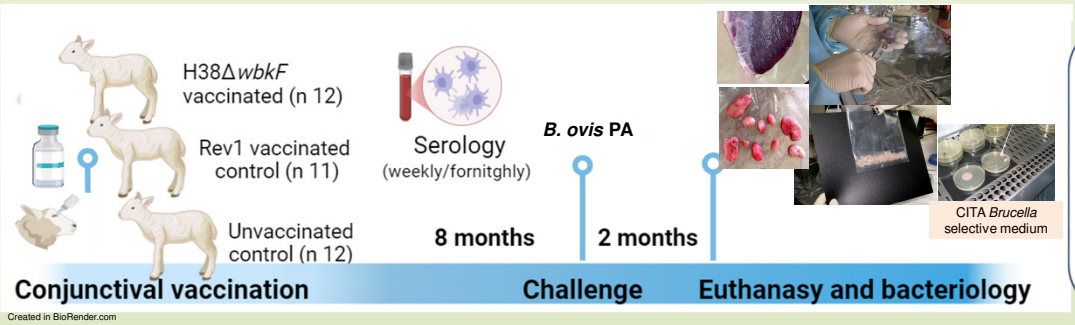
NEED FOR A SPECIFIC *B. OVIS* VACCINE

Brucella ovis, though non-zoonotic, is a serious cause of reproductive failure in sheep. Rev1, the sole vaccine available against brucellosis in small ruminants, effectively protects against both *B. melitensis* (a zoonotic *Brucella* species carrying smooth lipopolysaccharide –LPS-) and *B. ovis* (which exposes rough LPS). However, Rev1 is banned in those regions where *B. melitensis* is eradicated to avoid interferences in *B. melitensis* serosurveillance. Consequently, *B. ovis* is re-emerging in *B. melitensis*-free countries. A *B. ovis* specific vaccine not interfering in the smooth LPS based tests used to diagnose *B. melitensis* is needed. In a recent work, we demonstrated that subcutaneous vaccination of rams with the rough (R) mutant H38Δ*wbkF* confers similar protection to Rev1 against *B. ovis,* while not interfering in the Rose Bengal and Complement Fixation tests used for *B. melitensis* diagnosis. However, H38Δ*wbkF* still interferes in *B. ovis* serodiagnosis by R-LPS based tests.

AIM OF THIS WORK

To evaluate the use of the conjunctival route as a strategy to reduce the persistence of H38Δ*wbkF* vaccine antibodies while maintaining protection against *B. ovis.*

VACCINE EFFICACY ASSAY



A group of rams were vaccinated by conjunctival (CJ) route with 1.5 x 10^9 CFU of H38Δ*wbkF*, another group received similar CJ dose of the commercial Rev1 vaccine (Ocurev ®) and a third one remained unvaccinated. Eight months later, rams were challenged with *B. ovis* PA (4 x 10^9 CFU/ram) and 8 weeks after challenge, all them were euthanized and necropsied for thorough bacteriological examination. Throughout the assay, the serological response was monitored using different routine tests.

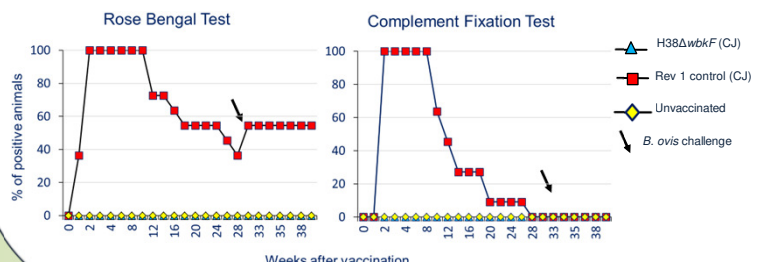
RESULTS

The rough attenuated vaccine **H38Δ*wbkF* administered by conjuctival route protected** against *B. ovis* in rams at the same level as Rev1 reference vaccine.

Vaccine	No. infected animals / total (%) [1]	No. infected organs / total (%) [2]
H38Δ*wbkF*	1 / 12 (8.3) [3, 4]	4 / 96 (4.2) [3, 4]
Rev 1	1 / 11 (9.1) [3]	3 / 88 (3.4) [3]
Unvaccinated	10 / 12 (83.3)	43 / 96 (44.8)

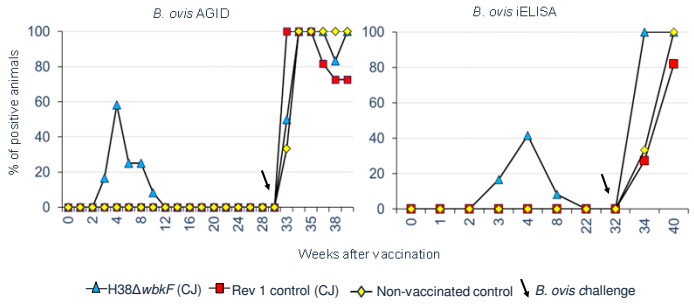
1, Statistical comparisons by Chi-square test (with Fisher-Yates correction when required).
2 Statistical comparisons by STEPBOOT MULTTEST (SAS); **3**, Significant difference ($P < 0.001$) *versus* unvaccinated control; **4**, no significant ($P > 0.05$) *versus* Rev1 vaccinated group

Vaccination with **H38Δ*wbkF* did not interfere in** S-LPS based serological tests routinely used for ***B. melitensis* diagnostics:** Rose Bengal agglutination and complement fixation tests.

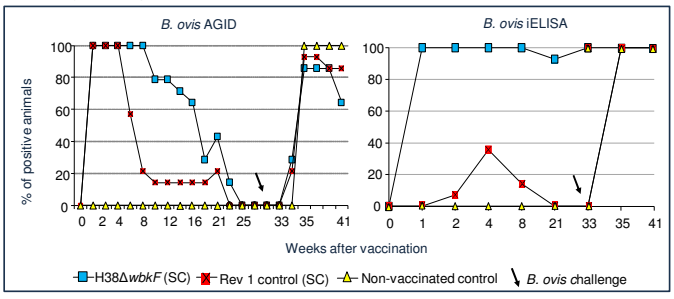


Conjunctival (CJ) administration of **H38Δ*wbkF*** vaccine **reduced** significantly the **persistence of vaccine antibodies** interfering in ***B. ovis*** serological tests with respect to the subcutaneous (SC) administration.

Serological response of rams in *B. ovis* tests after **conjunctival** vaccination



Serological response of rams in *B. ovis* tests after **subcutaneous** vaccination



Serological data after subcutaneous vaccination extracted from **Muñoz *et al.* Veterinary Research (2022) 53:16**
AGID = Agar Gel Immunodiffusion Test

CONCLUSION: our results confirmed the protective efficacy of H38Δ*wbkF* against *B. ovis* infection and demonstrated the usefulness of the conjunctival route in reducing the interference in *B. ovis* serological tests.

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