P5-02 Exploring new candidates to develop a *B. ovis* vaccine based on S-LPS devoid mutant H38ΔwbkF and core defective derivates

Nerea López-Fernández,[1] *Aitor Elizalde-Bielsa*,[1] *María-Jesús De Miguel*,[2] *Miriam Salvador-Bescós*,[1] *Amaia Zúñiga-Ripa*,[1] *Maite Loperena-Barber*,[1] *Sara Andrés-Barranco*,[2] *Maite Iriarte*,[1] *Jose María Blasco*,[2] *Ignacio Moriyón*,[1] *María Pilar Muñoz*,[2] *Raquel Conde-Álvarez* [1]

Abstract

Brucella ovis, though non-zoonotic, is a serious cause of reproductive failure in sheep. No country has been declared free from this infection and eradication by test-and-slaughter has been claimed very seldom. Rev.1, the only available vaccine for small ruminants, is effective against *B. melitensis* (zoonotic) and *B. ovis*. However, Rev.1 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 and a *B. ovis* specific vaccine not interfering in the smooth lipopolysaccharide (LPS) based tests used to diagnose *B. melitensis* is needed. In a recent work1, we demonstrated that subcutaneous vaccination of rams with the rough (R) O-polysaccharide (O-PS) mutant H38ΔwbkF confers protection similar to Rev.1 against B. ovis, while not interfering in the Rose Bengal and Complement Fixation tests used for *B. melitensis* diagnosis. However, since H38ΔwbkF interferes in *B. ovis* serodiagnosis, we also tested aB. ovis mutant (Bov::CAΔwadB) defective in the LPS-core lateral branch. While Bov::CAΔwadB did not provide protection, it caused low if any interference in the *B. ovis* agar gel immunodiffusion (AGID) test recommended by WOAH, an observation related to its modified core epitopes. The aim of this work was to explore whether similar LPS-core defects in H38ΔwbkF reduces the interference in *B. ovis* diagnosis while maintaining its efficacy against *B. ovis*. We constructed two mutants (H38ΔwbkFΔwadB and H38ΔwbkFΔwadC) carrying the expected core and O-PS defects, as shown by SDS-PAGE and Western-blot. In mice, both mutants showed marked attenuation with respect to H38ΔwbkF and did not protect against *B. ovis*. Thus, both were discarded for further research in sheep. These results confirmed the important role of the core for *Brucella* virulence and that over attenuation leads to ineffective vaccines. Therefore, we are exploring the use of the conjunctival route with H38ΔwbkF as a strategy to reduce the persistence of vaccinal antibodies and assessing protective efficacy of H38ΔwbkF by this route in rams.

Presenting author: rsantos@vet.ufmg.br

Keywords: *Brucella ovis*, Lipopolysaccharide, Serodiagnostics, Vaccine

1 *Instituto de Salud Tropical- Instituto de Investigación Sanitaria de Navarra- Dpto. Microbiología y Parasitología, Univ. Navarra, Pamplona, Spain*

2 *Dpto. Ciencia Animal, Centro de Investigación y Tecnología Agroalimentaria de Aragón-IA2 (CITA-Univ. Zaragoza), Zaragoza, Spain*