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Short communication

Safety and immunogenicity of a prototype anti-*Chlamydia pecorum* recombinant protein vaccine in lambs and pregnant ewes

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ABSTRACT

Arthritis and kerato-conjunctivitis caused by *Chlamydia pecorum* in lambs are difficult to diagnose and treat. We tested the ability of a prototype *C. pecorum* vaccine (SC-vaccine), comprised of *C. pecorum* major outer membrane protein (MOMP-G) and polymorphic membrane protein G (PmpG), to trigger a *Chlamydia*-specific humoral and cell-mediated immune response in lambs and pregnant ewes. Vaccinations with the SC-vaccine (one and two injections) were very well tolerated by all ewes and lambs. Although the overall immune responses of ewes to SC-vaccination was poor, their lambs showed stronger antigen-specific immune response than lambs from control vaccine ewes. SC-vaccination in lambs triggered production of systemic anti-MOMP-G and anti-PmpG IgG antibodies and secretory IgA in the ocular mucosa. Double vaccination caused statistically significant increases in the height and duration of the humoral response. Antigen-specific IFN-γ was produced in the peripheral blood mononuclear cells of vaccinated lambs.

1. Introduction

Chlamydia pecorum is an obligate intracellular bacterial pathogen that commonly causes asymptomatic gastrointestinal tract infection but is also associated with sporadic abortion, arthritis and kerato-conjunctivitis in sheep and cattle [1]. The most severe form of *C. pecorum*-associated disease is polyarthritis, which causes inflammation and swelling of the joint(s) of the leg(s) resulting in lameness and weight loss in growing lambs. A challenging aspect of the management of this disease is that diagnosis of *C. pecorum*-related diseases is difficult and antimicrobial therapy is only partially effective [2,3].

A vaccine approach to control *C. pecorum* infections in lambs constitutes a highly attractive alternative. Development of an effective anti-chlamydial vaccine has been an ongoing challenge for the *Chlamydia* research community, as identifying efficient immunogenic *Chlamydia* antigen(s) and the full nature of the cellular and humoral immune responses orchestrated by production of the cytokine, interferon-γ (IFN-γ), together with specific and neutralizing anti-chlamydia antibodies, are still under investigation in many hosts [4–6]. Increasing evidence also suggest a critical role for secretory IgA antibodies (sIgA) produced by the mucosal epithelium in the protective immune response of the *Chlamydia* infected organisms [7]. Activation of the immune system in response to chlamydial infections causing arthritis can occur in mucosal tissues [8] and should be considered when developing a vaccine against *Chlamydia*. Recent studies show that vaccination with a combination of several *Chlamydia* membrane proteins including MOMP-G and PmpG can illicit *Chlamydia*-specific cell mediated and humoral immune responses in vaccinated animals [9–11]. In the absence of a current challenge model for this chlamydial disease, in the current study, we assessed the suitability of a divalent chlamydial recombinant protein vaccine for *C. pecorum* infections in sheep. We developed a sheep chlamydia vaccine (SC-vaccine) and examined the safety and immune responses of vaccinated pregnant ewes and their lambs.

2. Material and methods

2.1. Vaccine formulation

The SC-vaccine contained adjuvants mixed with the adhesin domain of sheep *C. pecorum* PmpG (amino acid 27–520) from *C.*

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pecorum ovine IPA (VR629), and 50 μg of recombinant MOMP-G protein from *C. pecorum* koala MC/Marsbar strain [4]. The control (C-) vaccine contained adjuvants mixed with PBS (details in Supplemental Material 1).

2.2. Vaccines safety evaluation

Sixteen synchronized pregnant ewes were randomly assigned into two groups receiving either the SC-vaccine or the C-vaccine (See Supplemental Material 2). Twins from the same mother received different vaccination regimes as delineated in Supplemental Material 2.

For safety evaluations, health assessments were performed on all ewes and lambs every four weeks from the first date of vaccination up to a period of 16 weeks. For immunological assessments, whole blood, serum and plasma, and ocular swabs were collected (Supplemental Material 2).

2.3. Immunological assessments and statistics

ELISAs were performed and End Point Titres (EPT) calculated as previously described [12], using Polysorp plates (Sigma-Aldrich; Supplemental Material 3). Semi-purified elementary bodies (EBs) from *C. pecorum* strain IPA were obtained as previously described [4] and used for ELISA and *in vitro* neutralization assays. Experiments to assess gene expression of sheep IFN-γ were performed as previously described [4,13] with tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta transcript (*YWHAZ*) as reference control (See details in Supplemental Material 3).

All statistical analyses were performed using GraphPad Prism version 5 (GraphPad Software, LaJolla, CA, USA) and IBM SBSS statistics 22. To determine main effects and interactions of groups and vaccine, a repeated measures analysis of Variance (ANOVA) was conducted for each of the dependent variables. Following significant main effects, Mann-Whitney t-Tests were conducted with the p values set at $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

3. Results

3.1. Vaccine safety

In the pregnant ewes, clinical assessments revealed no difference between vaccination with the SC-vaccine or the C-vaccine on physical parameters and on the delivery time and circumstances (Supplemental Material 2). In lambs, vaccination with SC-vaccine did not impact on growth or any of the other physical parameters measured when compared to lambs vaccinated with the C-vaccine (Supplemental Material 2). Importantly, no swelling of the joint or gait abnormalities were observed in any vaccine groups.

3.2. Systemic and mucosal antibody responses post-vaccination with SC-vaccine in ewes

The SC-vaccinated ewes showed a modest but significant increase in IgG EPT for MOMP-G up to 16 weeks post-vaccination compared to the control vaccine group (Fig. 1A). Compared to the anti-MOMP IgG antibody responses, vaccinated ewes showed a strong anti-PmpG IgG antibody response at four weeks post vaccination, which remained higher than observed for control

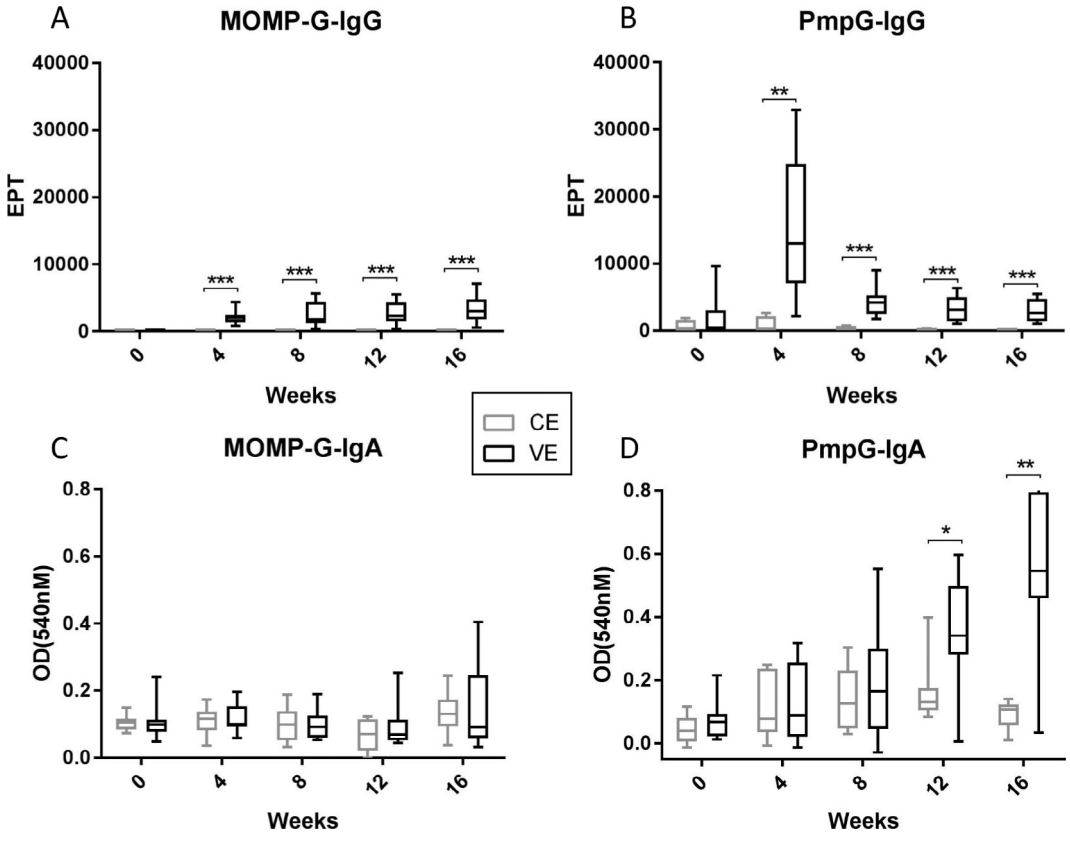

Fig. 1. End point titre of circulating IgG and mucosal sIgA against MOMP-G and PmpG antigens in SC-vaccinated and C-vaccinated ewes. (A) and (B). EPT values of anti-MOMP (A) and anti-PmpG (B) IgGs in sera were determined by ELISA using recombinant MOMP-G and PmpG proteins for each SC-vaccine and Control-vaccine ewe. *(C) and (D).* EPT values obtained for mucosal specific IgA antibody response to MOMP and PmpG antigens in ocular swabs samples following immunization of the ewes with SC- or C-vaccines.

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