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Vaccination of sheep against *M. paratuberculosis*: immune parameters and protective efficacy

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Abstract

Johne's disease in ruminants is caused by the pathogenic bacterium *Mycobacterium avium* subspecies *paratuberculosis* (*Map*). Currently available *Map* commercial vaccines protect against clinical disease but not infection. In this study, the proprietary Johne's vaccine *Neoparasec*™ and an aqueous formulation of *Map* 316F (*AquaVax*) were tested in sheep. Detailed immunological examination of blood and gut-associated lymphoid tissues was carried out on animals after vaccination and challenge with virulent *Map* to identify markers of protective immunity. *Neoparasec*™ vaccination provided significant protection against disease while *AquaVax* did not. Immune animals had stronger cell-mediated responses and altered proportions of $CD4^+$, $CD8^+$, $CD25^+$ and B cells in blood, spleen and the gut lymphatics, than diseased animals.

Keywords: Johne's disease; Protective immunity; Vaccination

1. Introduction

Vaccination has been used since 1926 [1] to control Johne's disease. The types of vaccines used have included live attenuated strains of *Mycobacterium avium* subspecies *paratuberculosis* (*Map*) and heat killed or sonicated preparations. Mineral oil adjuvants are routinely included in the vaccine formulation. Vaccines are normally administered subcutaneously to sheep from 2 weeks to 4 months of age. A limitation for the widespread use of Johne's vaccination is the development of abscesses at the injection site [2]. Protective efficacy studies show that while clinical disease may develop in some vaccinated animals, there is a significant reduction in the prevalence of diseased animals [3,4]. While vaccination reduces the total number of animals excreting organisms, it does not result in a decrease in the overall prevalence of infection [2,5].

Another complication following vaccination is that sensitisation with vaccines causes interference with immunological tests used for diagnosis of natural *Map* infection and tuberculosis (TB) due to *M. bovis* infection. This is a real concern for the use of *Map* vaccines [6] in animals such as cattle and deer that are naturally susceptible to tuberculosis. National Tuberculosis Eradication programmes used worldwide for cattle and deer are based on skin testing protocols that rely on immunodiagnostic testing. *Map* vaccination can interfere with TB surveillance schemes involving skin testing as a herd screening test. This is due to the high degree of antigenic cross reactivity between antigens in the vaccine strain of *Map* and mycobacterial pathogens such as *M. bovis* [6].

Protective immune responses to *Map* have not been looked at in detail previously in ruminants; however, as with other mycobacterial diseases, it is hypothesised that a vigorous CMI (Type 1) response is important for protection against *Map* infections [7–9]. Empirical approaches used historically for vaccines to control Johne's disease use mineral oil adjuvants to evoke an aggressive immune response, on the premise that strong immune reactions are likely to be optimal. Recent advances in our understanding of the fundamental mechanisms of immunity require that prophylactic

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protocols be designed to evoke the most appropriate, rather than the most vigorous immune responses [10,11]. Vaccination of deer with an aqueous formulation of BCG, in a prime-boost protocol, has been shown to provide significant protection against *M. bovis* infection [12] involving predominantly a Type 1 pathway of immune reactivity. By contrast, vaccines using mineral oil adjuvants were not protective [12].

The first objective of this study was to monitor the immune responses in animals vaccinated with a commercial vaccine *Neoparasec*™, compared with an aqueous suspension of *Map* strain 316F (*AquaVax*). The second, was to compare retrospectively, immune responses in unvaccinated animals that were uninfected following experimental challenge with *Map*, with those that developed disease. Finally, the immune responses seen in vaccinated animals and unvaccinated sheep that survived infection were compared to determine if there were definitive immune profiles typical for protection. Cell-mediated immunity was monitored using T cell lymphocyte transformation (LT) and Interferon-γ assays. Antibody assays (ELISA) were used to determine if there were qualitative differences between animals that were resistant to infection from those that developed disease. Comparative changes in the immune responsiveness and lymphocyte subpopulations of gut and peripheral tissues were examined to determine whether markers of systemic or localised immunity could be used as a signature for a protective response.

2. Methods

2.1. Animal ethics

The animal experiments carried out in this study were carried out under ethics approval licences numbered: P453, P499, P518 and P594, approved by the Invermay AgResearch Animal Ethics Committee.

2.2. Experimental animals

The experimental sheep comprised 130 Merinos, all of which were castrated males. The lambs were selected from flocks in which no Johne's disease had been previously observed and were held with ewes until weaning at 2.5–3 months of age. After weaning, lambs were randomly allocated to experimental treatment groups as required. The lambs were kept on pasture under standard New Zealand sheep farming conditions, with supplementary feeding during winter.

2.3. Immune profiles in lambs vaccinated with oil adjuvanted versus aqueous live formulations

A group of 30 merino lambs were assigned randomly into three groups. One group was vaccinated with a commercial oil adjuvanted live (316F) vaccine (*Neoparasec*™-Merial NZ Ltd.) and another group with a live aqueous (316F) vaccine (*AquaVax*). The third group was left as unvaccinated controls. The lambs were moved to the experimental facility after weaning (11 weeks old) and were vaccinated at 3 months of age. The *Neoparasec*™ vaccine was administered according to proprietary recommendations; 1 ml dose given subcutaneously. The *AquaVax* formulation consisted of live *Map* (316F) in a buffered saline solution at a concentration of 1×10^8 cfu/mL given as a 1 mL dose subcutaneously into the neck. The animals vaccinated with *AquaVax* were given a booster inoculum of the vaccine 1 month later.

2.4. Protective efficacy of vaccines

Ninety merino lambs were assigned randomly into three groups each containing 30 animals. At docking (2 weeks of age), the lambs were vaccinated using the protocol outlined in Table 1. The *Neoparasec*™ vaccine was used according to the manufacturers specification in the first group of 30 lambs. *AquaVax* formulation (*Map* 316F at 1×10^8 cfu/mL in buffered saline) was given in 1 mL doses to the second group. One month after the primary vaccination a booster dose of *AquaVax* was given. The final group of 30 lambs were left as unvaccinated controls. The lambs were weaned at 2.5 months of age. At 3 months of age the lambs were challenged orally with virulent *Map*, given three times at weekly intervals as 1 mL doses (5×10^8 cfu/mL) of a gut tissue homogenate of *Map*, isolated from a sheep with clinical Johne's disease (JD3). This experimental infection regime produces gut histopathology within 9 months and the onset of clinical disease within 11 months post-challenge [13]. A group of 10 sentinel unchallenged animals were included in the experiment to provide background immune parameters. Animals were slaughtered between 10 and 22 months post-

Table 1
Vaccination and infection protocols used in the second sheep study

Vaccination protocols				
Treatment group[a]	Vaccine formulation	Vaccine dose (cfu)	Booster vaccination	Infectious challenge dose[b]
Control	N/A	N/A	N/A	N/A
Neoparasec™	316F in oil	6×10^8	None	5×10^8
Aqueous vaccine	316F in saline	1×10^8	1 month later	5×10^8
Unvaccinated	N/A	N/A	N/A	5×10^8

[a] The lambs were vaccinated at 2–4 weeks of age and infected at 3 months of age.
[b] Animals were challenged three times at weekly intervals.