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Modulation of inter-vaccination interval to avoid antigenic competition in multivalent footrot (*Dichelobacter nodosus*) vaccines in sheep

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ABSTRACT

Virulent footrot is a significant disease of sheep in most sheep farming countries; a strain/serogroup of the anaerobic bacterium *Dichelobacter nodosus* is the essential transmitting agent. Commercial multivalent footrot vaccines containing nine fimbrial serogroups (A through I) of *D. nodosus* produce relatively low and short term antibody responses due to antigenic competition, in contrast to higher and longer responses provided by monovalent or bivalent vaccines. The latter were important components of successful eradication programs for endemic footrot caused by either one or two serogroups of *D. nodosus* in Nepal, Bhutan, and several flocks in Australia. However, the presence of up to six serogroups in some Australian flocks and the use of an annual bivalent vaccination regime to progressively eradicate serogroups would require a long term program. In this study we report the results of a sequential vaccination trial testing different time intervals between different bivalent vaccinations. Intervals of 12, 9, 6, 3 and 0 months were tested. The 1st vaccination was with recombinant fimbrial antigens for serogroups A and B while the 2nd vaccination was with D and E. There were no significant differences between the antibody responses for time intervals of 3, 6, 9 and 12 months whereas there was a reduced response when sheep were vaccinated with two bivalent vaccines (four antigens) concurrently, indicating antigenic competition. Therefore an inter-vaccination interval of 3 months can be applied between two different bivalent vaccines without detrimental impact on the humoral immune responses to the various fimbrial antigens of *D. nodosus*. These results could have wider applications in vaccination against diseases caused by multivalent or multistrain microbes.

1. Introduction

Footrot is a contagious disease of sheep and goats in which strains of the anaerobic bacterium *Dichelobacter nodosus* are essential causative agents. Strains differ in virulence, which is assessed by tests for protease activity. Virulent footrot is a significant disease of sheep in most sheep farming countries. The estimated annual cost of virulent footrot in 2005–2006 for Australia was $18.4 M [1] and for Great Britain was £24 M [2]. Sheep and goats can be immunised against footrot using vaccine containing either whole bacterial cells or native or recombinant fimbriae. The first footrot vaccines produced in 1969 consisted of adjuvanted monovalent whole *D. nodosus* cells [3,4].

By 1974 it was recognised that many antigenically distinguishable strains of *D. nodosus* existed and that whole cell vaccines were rarely protective against heterologous serogroups [5,6]. Studies conducted later indicated that there were eight major serogroups (A, B, C, D, E, F, G and H) of *D. nodosus* in the Australian environment [7,8]. This grouping was later extended to nine serogroups with the incorporation of serogroup I [9]. An additional serogroup 'M' has been identified in New Zealand and Australia [10] and in Nepal [11]. Using a slightly different classification system in the UK, a total of 17 serotypes (A through H and J through R were identified) [12]. In the USA, a total of 21 serotypes (I through XXI) have been identified [13]. The main reason for the different number of antigenic groups in different countries is likely to be the degree to which researchers "lump or split" minor antigenic variants. Multiple serogroups have been reported from individual flocks from different parts of the world and up to six serogroups have been identified from one flock in Australia [9].

Commercial vaccines containing nine serogroups (A–I) protect sheep only for up to 10 weeks [14–16]. Under severe challenge multivalent vaccines only partially protected sheep for a short period [17]. In contrast, at least 16 weeks protection against homologous challenge was provided by specific monovalent or bivalent vaccines [18,19]. Reduced antibody production against individual components of a multivalent vaccine is believed to be due to the phenomenon of antigenic competition [15–17]. This is due to the presence of a family of immunologically related antigens rather than interference by extraneous proteins. However,

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the actual mechanism of antigenic competition is still not fully understood.

Specific footrot vaccination was first tested in Nepal, and here as well as in Bhutan, was applied successfully together with stringent flock inspection and culling of sheep which did not respond to any treatment, to eradicate virulent footrot [20–22].

Unlike traditional control measures such as footbathing in disinfectant or parenteral antibiotic treatment, specific vaccination is not dependent on seasonal or climatic variations in disease expression and can be applied irrespective of the season. However, where multiple (>2) serogroups are present, sequential rounds of bivalent vaccination are required to avoid antigenic competition. From a practical standpoint, the inter-vaccination interval ideally should be short. In current field trials in Australia, an interval of 1 year has been used (unpublished data) as the minimum interval between successive efficacious bivalent vaccines is unknown; 3 months may be an ideal inter-vaccination interval to fit in with sheep management practices on most farms.

1.1. Aim of this study

To evaluate intervals as short as 3 months between successive doses of different bivalent recombinant *D. nodosus* fimbrial vaccines to avoid antigenic competition.

2. Materials and methods

2.1. Animals and treatment groups

Fifty four footrot free Merino hoggets (aged 6–12 months) were randomly allocated into 6 groups of 9 and were identified individually by ear tags.

Treatment Groups 1, 2, 3 and 4 had "inter-vaccination intervals" of 12, 9, 6, and 3 months respectively between two different bivalent vaccines, Group 5 had 0 month interval i.e. the two bivalent vaccines given simultaneously. The control group (Group 6) did not receive any vaccine. Inter-vaccination interval was calculated from the 1st dose of the first vaccine.

2.2. Vaccines and vaccination

Recombinant fimbrial vaccine antigens and vaccines were prepared as previously described [23]. Two batches of bivalent recombinant *D. nodosus* fimbrial vaccines were used in this trial. Vaccine 1 contained serogroups A and B while vaccine 2 contained serogroups D and E.

All the animals in Groups 1–5 were vaccinated with 1 ml of the first vaccine (A and B) subcutaneously at the neck, below the left ear. A second (booster) dose of the same vaccine was given after a month. The animals were given the second vaccine (D and E) after the appropriate inter-vaccination interval, subcutaneously, below right ear, with a booster after 1 month. The first bivalent vaccine was given on the left side while the second was given on the right side of the neck. Control group animals did not receive any vaccine.

2.3. Serum samples and agglutination test

Blood samples (8 ml) were collected from the jugular vein of all animals before each dose of vaccine was administered and then monthly for 6 months after each course of vaccination. Serum samples were tested for antibody responses to individual homologous antigens in vaccines (A, B, D and E) using a microtitre agglutination test [24].

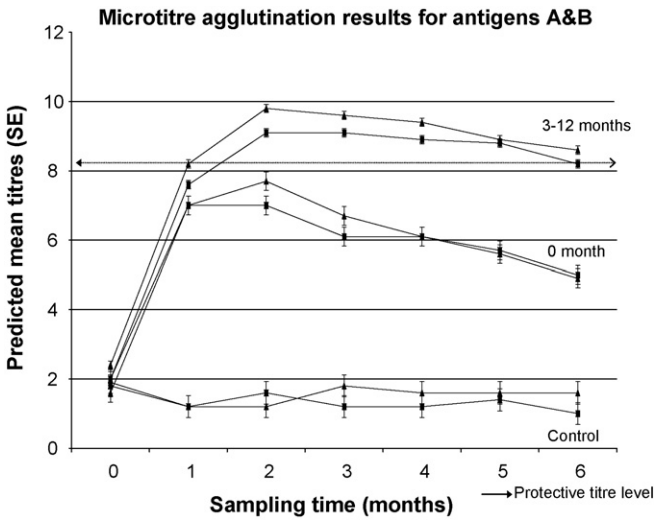

Fig. 1. Microplate agglutination test titre for antigens A and B for 6 months following vaccination. Sheep were given antigens A and B as bivalent vaccine, then revaccinated with antigens D and E after the intervals shown. Data are predicted means and standard error from the REML model (▲, antigen A and ■, antigen B).

2.4. Statistical analysis

The differences in specific agglutination titre between sheep vaccinated with antigens A/B and D/E at intervals of 0, 3, 6, 9 or 12 months were compared at each time point after vaccination (months 0, 1, 2, 3, 4, 5 and 6) using restricted maximum likelihood (REML) in a linear mixed model (GenStat Release 10.1 2007, Lawes Agricultural Trust, Rothamsted Experimental Station, VSN International). Antigen, vaccination interval and time point after vaccination and their interactions were included as fixed effects in the statistical model, while animal was included as a random factor to account for repeated measures. Least squared difference was used to assess the significance of differences between predicted means. Significance was assessed at the 1% level.

3. Results

Serum agglutination test results against homologous vaccine antigens (A, B, D or E) are presented in Figs. 1 and 2 together with

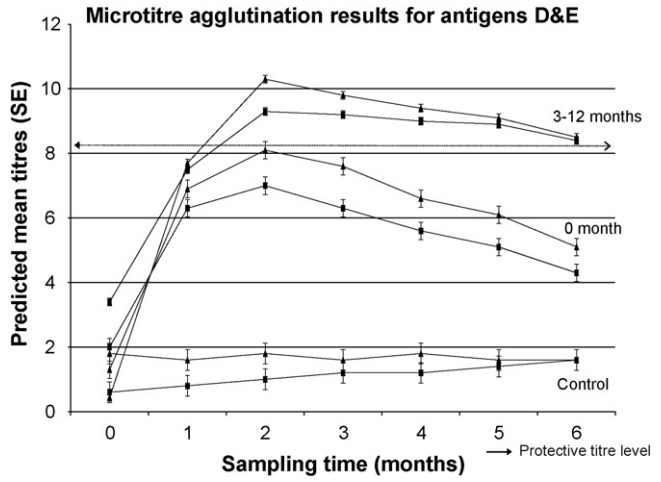

Fig. 2. Microplate agglutination test titre for antigens D and E for 6 months following vaccination. Sheep were given antigens A and B as bivalent vaccine, then revaccinated with antigens D and E after the intervals shown. Data are predicted means and standard error from the REML model (▲, antigen D and ■, antigen E).