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Experimental infection of New Zealand Merino sheep with a suspension of *Mycobacterium avium* subspecies *paratuberculosis* (*Map*) strain Telford: Kinetics of the immune response, histopathology and *Map* culture

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ABSTRACT

A long-term study was undertaken to monitor immune responses, faecal cultures and clinical disease in sheep experimentally infected with *Mycobacterium avium* subspecies *paratuberculosis* (*Map*) strain Telford. New Zealand Merino lambs (N = 56) were challenged with three oral doses of *Map* suspension. The lambs were weighed and faecal and blood samples obtained at different time-points. At 63 weeks post-challenge, surviving sheep were euthanised and samples of liver, ileo-caecal valve and mesenteric lymph node were collected for histopathology and *Map* culture. High IFN-γ and antibody responses were evident as early as 8 weeks post-C1 which persisted until the end of the trial. Approximately 92% of the sheep shed *Map* in faeces at 36 weeks post-challenge, with the prevalence decreasing to around 40% at the end of the trial. Thirteen sheep progressively lost weight and were euthanised between weeks 32 and 58 post-challenge. Nearly 58% of surviving sheep exhibited histo-pathological lesions in at least one of the three tissues sampled, while 42% showed acid-fast bacilli in at least one tissue. A positive *Map* culture in at least one tissue was obtained from approximately 85% of sheep. These results indicate that the three doses of *Map* challenge were highly effective in establishing Johne's disease in NZ Merino lambs.
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1. Introduction

Johne's disease (JD) or paratuberculosis is a chronic intestinal infection of ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (*Map*). Several experimental sheep infection studies (reviewed by Hines et al., 2007; Begg and Whittington, 2008) have been conducted to determine the pathogenesis and immunological aspects of JD and to evaluate protective efficacy of vaccines. These studies are frequently difficult to reproduce because the infectious material is derived from either intestinal mucosal extracts or *ad hoc* laboratory-adapted cultures of *Map* that are not clearly defined. An experimental model for JD in sheep was developed in Australia (Begg et al., 2010), based on a lyophilised, low passage (level 5), pure culture, seed-stock of *Map* strain, Telford 9.2, isolated from a clinical ovine case. A series of experiments conducted in that study showed authentic experimental reproduction of the natural features of JD in sheep that were less than 2 years of age. The range of histopathological lesions observed ranged from very mild focal lesions to extensive granulomatous inflammation as might be found following natural infection. Faecal shedding was intermittent with 61% of animals classified as infected at necropsy being culture positive at least once. Immunological assays showed that interferon gamma (IFN-γ) assays detected animals in the earlier stages of infection whereas antibody ELISA detected animals more often as the infection progressed. All these features plus the fact that the

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majority of animals did not show signs of clinical disease is consistent with the spectrum of infection and disease that could be expected in sheep that have been naturally infected.

The primary objective of this long-term study was to investigate the kinetics of immune responses and faecal shedding of *Map*, as well as the incidence of clinical disease in New Zealand Merinos infected with three doses of cultured *Map* strain Telford 9.2. The trial was performed at Massey University in the Manawatu region of North Island, New Zealand using Merino wether lambs. Three doses *of Map* organisms were administered over a four week period and immune responses and animal weights were monitored at regular intervals and faecal samples collected for *Map* culture. Antibody responses and purified protein derivative of *Mycobacterium avium* (PPDA) induced IFN-γ responses were measured at regular intervals and the trial terminated after 63 weeks post-infection. Surviving animals were necropsied and tissue samples collected for histopathology and *Map* tissue culture.

2. Materials and methods

2.1. Experimental animals, Map challenge and sampling

Fifty six purebred New Zealand (NZ) Merino wether lambs were procured from a commercial farm with no previous history of JD (no unexplained mortalities or progressive weight loss in adult sheep). Serological screening for *Map* antibodies in 20 randomly selected ewes in the source farm revealed all the ewes (that were over 6 years of age when tested) to be negative for JD. Also, all 56 lambs tested negative for *Map* sensitisation, in a whole blood IFN-γ assay, performed using a Bovigam® kit. The lambs were managed under conventional NZ sheep farming conditions in a specially prepared set of quarantine paddocks and grazed on pasture throughout the trial.

When the lambs were approximately 4 months of age, they were challenged orally with three doses of *Map* suspension (from cultures seeded with the reconstituted lyophilised stock described in Begg et al., 2010). The intervals between successive doses were 1 and 3 weeks, respectively (Fig. 1). The lyophilised seed stock was originally prepared from a clonal culture at final passage level 5 (including its primary isolation from sheep faeces) of Telford 9.2, an IS1311S *Map* strain (IS900 RFLP type S1), isolated from a clinical ovine case in New South Wales, Australia. Details of culturing from the seed stock, bacterial enumeration and preparation of inoculum were as described in Begg et al. (2010). *Map* suspensions (10X concentration in phosphate buffered saline, PBS) prepared from the cultures were shipped overnight at refrigeration temperature (2–8 °C) from Australia, diluted (to 1X concentration) with sterile PBS and 10 ml was administered per animal through a syringe to the back of the throat. Viable counts of *Map* were determined at the University of Sydney by end point titration in BACTEC medium, using a three tube most probable number method (Reddacliff et al., 2003). The viable *Map* counts in the three challenge (C) doses were 2.1×10^7 (C1), 9.3×10^6 (C2) and 2.3×10^8 (C3).

The lambs were weighed and faecal samples obtained on the day of C1 and subsequently at monthly intervals. Body weights recorded at 36 and 41 weeks post-C1 were excluded from analyses as the sheep body coat at those time-points was wet and thus inflated the weights. Faecal samples were either cultured (for *Map*) immediately or stored at −80 °C until processed for culturing. Jugular blood samples (to measure immune responses) were obtained at 2 weeks prior to C1, as well as at 4, 8, 16, 32, 45, 49, 54, 58 and 62 weeks post-C1. The study protocol was approved by Massey University Animal Ethics Committee.

2.2. Monitoring post-challenge

Post-challenge, the sheep were monitored daily by a farm technician. Also, the sheep were inspected by an experienced sheep veterinarian on at least a fortnightly basis to monitor their general health and body condition. The sheep were fed pasture throughout the study and at times when this became scarce, supplemented with barley. They received an effective anthelmintic treatment at 3–4 weekly intervals. Animals exhibiting >10% decline in monthly bodyweight and or rapid decline in body condition were euthanized, and necropsied by a veterinary pathologist. Tissue samples (liver, ileum and mesenteric lymph

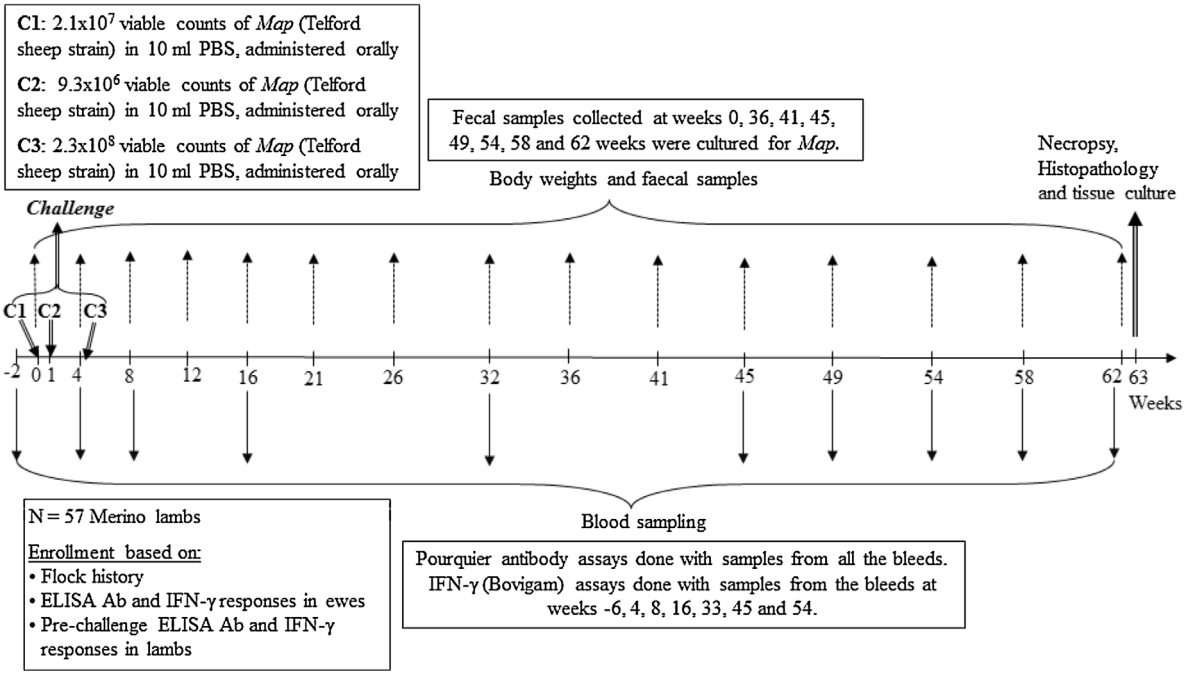

Fig. 1. Illustration of *Map* challenge study design and timeline.

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