Detection of Marker-Specific Immune Responses in Calves Against a Marked Johne's Disease Vaccine Strain

Lucy Luo, Jeroen De Buck

Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary. 3330 Hospital Drive NW, Calgary AB T2N 4N1. Email: yiyi.luo@ucalgary.ca

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the causative agent of Johne's disease (JD), a chronic progressive enteritis in ruminants that poses a large economic burden on the dairy industry. Control efforts have been hindered by poor diagnostic sensitivity and ineffectiveness of current vaccines in preventing infection. There are also no MAP vaccines approved in Canada due to their inability to differentiate between vaccinated and naturally infected animals (DIVA) with JD or *M. bovis*. With these limiting factors in mind, we aimed to develop a MAP marker vaccine strain capable of eliciting specific immune responses as the first step to creating an effective DIVA vaccine.

Methods: We replaced an immunogenic MAP gene with a non-MAP immunogenic peptide to create a positive and negative marked MAP strain, where specific immune responses against the markers will demonstrate vaccination. The markers were assessed *in vivo* using a calf infection model, in which calves were inoculated with either the marked strain (n=6), a WT field strain (n=6), or uninfected controls (n=4). Whole blood was collected at 3-week intervals until 4.5 months post-inoculation. Cellular immunity was analyzed by detecting marker-specific interferon-gamma release after stimulating whole blood with the marker peptides or their scrambled (a permutation of the peptide sequence) versions, followed by a sandwich interferon-gamma ELISA.

Results: The positive marker was expressed and secreted in the MAP strain, indicating its potential to successfully elicit marker-specific immune responses. To demonstrate calf infection, all WT- and marker-infected calves were confirmed tissue culture-positive for MAP, while uninfected controls remained negative. Surprisingly, the scrambled peptide-stimulated samples showed a significant IFN-g response in marker-infected calves compared to WT-infected ($p=0.016$) and uninfected ($p=0.019$) groups at 4.5 months post-inoculation.

Conclusions: Using a positive immune marker in a MAP vaccine strain, coupled with peptide stimulation in an IFN-g release assay may provide valuable diagnostic tool as part of a DIVA JD vaccine. By overcoming regulatory restrictions on JD vaccines, this will reduce dairy-related economic loss and increase the economic competitiveness of Alberta's dairy industry.