

Identifying Essential Genes Necessary for *Mycobacterium avium* subsp *paratuberculosis* to Persist in Dairy Calves

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Background: Johne's Disease (JD) is caused by *Mycobacterium avium* spp. *paratuberculosis* (MAP). Dairy cattle with clinical JD exhibit chronic diarrhea and weight loss. This causes a financial burden to dairy farmers due to milk production losses and early culling of infected cattle. Currently, no safe, effective vaccines are available in Canada to protect cattle from developing JD and prevent MAP from infecting the gastrointestinal tract. To develop a vaccine that prevents MAP from infecting the tissue, essential pathogenic genes need to be identified. These essential genes can then be deleted to develop a weakened vaccine strain that generates a protective immune response.

Methods and Results: 12 Holstein dairy calves were inoculated with a collection of mutated MAP bacteria created from a strain that is common to Alberta. These mutants were created by disrupting a single gene in each bacterial cell in an attempt to create mutants for every single MAP gene. To recover enough MAP from the intestinal tissues calves were orally inoculated with an exceptionally high dose. Calves were euthanized at two and four months post inoculation. The specific mutant bacteria in the tissues at both end points were compared to those in the initial inoculum. The disrupted genes will be identified and compared to the inoculated strain genome to identify the genes that are essential for pathogenicity. Ideally, a virulent gene will be selected that can temporarily infect the tissue at two months and disappears by four months. Four calves were orally inoculated with the parent wildtype strain of MAP to ensure that the mutants were not weakened by the mutation process and still capable of infection. Preliminary results suggest that the mutant MAP performed comparable to the wildtype strain. High numbers of MAP were successfully isolated from ileal and jejunal intestinal tissue. Lower numbers of MAP were found in the ileal lymph nodes and jejunal lymph nodes.

Implications: Identifying virulent genes in MAP that are essential for survival in calf tissue will provide necessary information to develop an attenuated vaccine strain. Knocking out these virulent genes will create a safer candidate vaccine strain for the prevention of JD.