

Identifying reservoirs of bacteria involved in digital dermatitis in dairy cows and farms with different disease status

Angelica Dias^{1*}, Karin Orsel¹, Jeroen De Buck^{1,1} Faculty of Veterinary Medicine, University of Calgary, AB, Canada
Email: *angelica.petersendias@ucalgary.ca

Despite ongoing investigations, the source of infection and routes of transmission of digital dermatitis (DD), a polybacterial hoof disease, are still under debate. Recent progress in molecular techniques now enables a more in-depth investigation of the bacteria most associated with DD and what their reservoirs are in cattle and the environment. This study aimed to investigate the presence and quantity of DD-associated bacteria in dairy cattle hoof skin, saliva, urine, feces, and slurry. Environmental samples were collected from the free-stall barns, and swabs of lesion or healthy hoof skin were taken from 103 milking cows in addition to saliva, urine, and feces. Animals were classified as DD-free (M0, n=58), with active (M2, n=16) or chronic lesions (M4, n=29). Farms were categorized as free (DD-free, n=2), with only chronic lesions (M4-only, n=2), and with active/chronic lesions (M2/M4, n=2). On DD-free farms, treponemes were only found in saliva, while on M2/M4 and M4-only farms, they were present in saliva, healthy hoof skin, and in slurry. All fecal and urine samples were negative; thus, the presence of treponemes in the slurry suggests transient contamination from active and chronic lesions. Non-treponeme anaerobes were absent in feces but present in urine, healthy hoof skin, saliva, and slurry regardless of disease status. Unlike treponemes, the other anaerobes seem to be ubiquitous, suggesting they are secondary pathogens. Transmission is unlikely through feces and urine as those sources do not seem to be reservoirs for bacteria involved in DD, whereas saliva may serve as a potential reservoir; however, longitudinal follow-up studies are needed to support bacterial shedding and sites of persistence.

Take home message: Insights from this study on potential reservoirs of bacteria involved in DD will guide future investigations focusing on management practices to minimize or eliminate bacterial excretion and DD transmission.

Clearance of a genetically modified *Mycobacterium avium* subsp. *paratuberculosis* strains from calf tissue and partial protection against infection

Razieh Eshraghisamani¹, Jeroen De Buck¹.

¹Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta, Canada. Emails: razieh.eshraghisaman@ucalgary.ca, jdebuck@ucalgary.ca

Commercially available Johne's disease vaccines reduce *Mycobacterium avium* subsp. *paratuberculosis* (MAP) fecal shedding and postpone the clinical symptoms without eliminating the infection and its spread. Our study aimed to develop a vaccine that overcomes the shortcomings of previous vaccines. Essential genes for MAP survival in the host's body were identified. Two essential genes, which play roles in iron acquisition (*BacA*) and fatty acid metabolism (*IcL*), were chosen to be knocked out of MAP genome. To evaluate the persistence of modified strains in tissue and the protection against infection, two calf infection trials were conducted. Twenty-three calves were randomized over four groups including *BacA*, *IcL*, uninfected and wild-type (WT) controls. Calves were inoculated with 10⁹ CFU of each MAP strains at two weeks old. Blood samples were collected every two weeks to study immune responses. Tissue samples were collected 4 months after inoculation. Both modified strains were cleared from tissue without losing their immunogenicity. By inducing stronger immune responses, only *BacA* showed potential capability to protect animals against infection and proceeded to calf challenge trial. Next, a calf challenge trial was conducted to evaluate the efficacy of preventing MAP infection by vaccinating with the *BacA* strain. Twenty-four calves were randomized over four groups including uninfected control, vaccinated, vaccinated/challenged, and infected control. Vaccinated groups got inoculated with 10⁹ CFU of *BacA* strain at 2 weeks old. Challenged groups got inoculated with 2×10⁹ CFU of MAP at 5 weeks old. Tissue samples were collected 4 months after inoculation. The *BacA* strain could only partially reduce MAP persistence in intestinal tissue.

Take home message: Studying MAP vaccine candidates is a significant step in progressing towards a better JD control. This newly developed live attenuated vaccine partially protected animals against MAP infection.