The use of an optimized protocol to isolate eight novel bacteriophages with the ability to lyse MAP

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Johne's disease (JD) is a chronic infectious enteritis of ruminants and causes losses of $90 million CAD to the Canadian dairy industry annually. This enteritis is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). JD spreads among youngstock through the ingestion of feed and water contaminated with infectious faeces. Currently there is no cure for JD and due to the long incubation time of the disease, test-based culling has proved ineffective at preventing the spread of JD. Mycobacteriophages, viruses with the capacity to kill mycobacteria, have potential as anti-mycobacterial agents. They have been used successfully to control mycobacterial infections such as those caused by *M. avium*. Isolation of new MAP lysing bacteriophages is an important step to using phages to control JD infection. We optimised an isolation protocol by faecal spiking and the testing of different isolation solution compositions. We screened 475 environmental samples for the presence of mycobacteriophages through phage enrichment with both MAP and the fast-growing *M. smegmatis*. These samples were taken from farms with a known JD presence. Samples included soil, manure pits, lactation barn, faeces straight from the cows, milk and drain water. We isolated 14 phages. After fingerprinting these phages by restriction enzyme profiling, we concluded that 11 of those phages isolated were distinct and novel. Further characterisation of their host range shows that 8 are capable of lysing genetically different MAP strains. We have further characterised the cross resistance, lysogeny and effect of pH on these novel phages. Eight novel mycobacteriophages, with a variable capacity to lyse distinct MAP strains, were discovered and characterized.
Take home message: Newly isolated mycobacteriophages have the potential to be used in strategies to prevent the spread of JD on diary farms. We recommend the use of a cocktail of different phages in the preventative strategies given their variable host range.

Assessing herd-level Johne's disease prevalence based on environmental samples

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Detecting Mycobacterium avium subsp. paratuberculosis (MAP) in environmental samples by culturing methods followed by qPCR has adequate sensitivity (Se) and specificity (Sp). Although the Se of the method is acceptable, the culturing protocol is expensive due to specific materials required to stimulate MAP growth and the protocol takes up to 50 days to be completed. Direct MAP detection relying on efficient DNA extraction methods followed by qPCR is an alternative due to its efficiency. Improvements of the Se and Sp of this method might come from changing the MAP target gene, as most single-copy MAP-specific genes are less sensitive. The aim of the study was to evaluate the accuracy of different MAP-specific target genes to detect MAP-positive environmental samples. Environmental samples were collected on 24 Alberta dairy farms. At each farm, 3 sets of samples were collected from the lactating cow barn and manure storage areas. DNA was extracted followed by qPCR targeting the following genes IS900, F57, ISMAP02, hspX, mbtA-MAP217, MAP0865 and 251. Of the 24 farms, 42% of farms were positive by IS900, while 25% were positive by ISMAP02. Additionally, 8% of the farms were identified as MAP positive by the single copy genes F57, mbtA-MAP217, MAP0865, 251, while 4% of farms were identified as positive by hspX. IS900 and ISMAP02 detected a higher percentage of MAP positive farms compared to the other target genes, but a higher sample size is still required to determine which MAP target gene will be the best one to be included into the detection of the herd-level MAP prevalence based on environmental samples and perhaps the development of a multiplex qPCR will improve the Se and Sp of the test.
Take home message: The research will provide a less expensive and more accurate diagnostic test to detect MAP-positive farms based on environmental samples. The IS900 and ISMAP02 target-genes are more sensitive to detect MAP on environmental samples compared to F57, mbtA-MAP217, MAP0865, 251 and hspX.