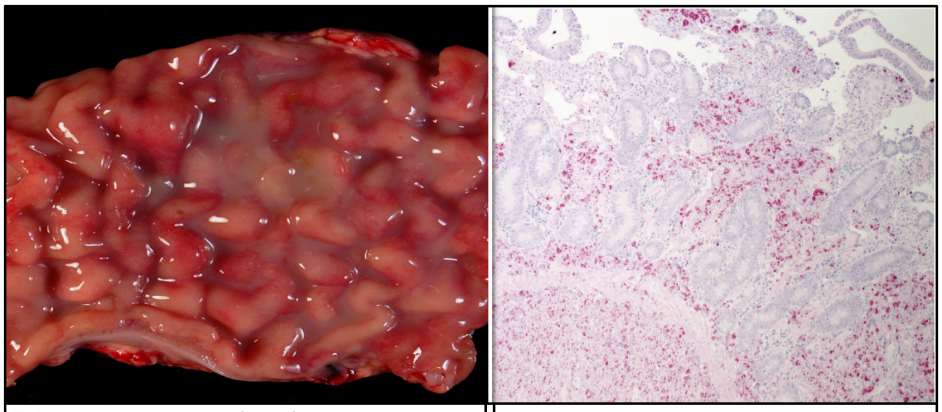
Discovery of Novel Secreted Antigens of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) as Biomarkers for Johne's Disease

Johne's Disease is a chronic enteritis that affects ruminant animals (**Figure 1**) and is an economically significant problem for dairy farmers. Because Johne's disease does not currently have a cure, it is important to quickly and accurately diagnose animals to stop infection spread in the heard. The fact is that currently used diagnostic "biomarkers" (MAP antigens) cannot be recognized by all infected cattle in silent and early subclinical stages of disease, and these animals are not accurately diagnosed. In this project, we aimed to investigate MAP antigens that are produced by bacteria within the cellular environments that are relevant to Johne's disease such as the bovine intestine, mammary gland and milk, and find marker(s) that possibly will help to identify MAP infection.

To achieve the goal, we exposed MAP pathogen with whole cow milk, and infected bovine intestinal epithelial cells and mammary epithelial cells for 24 hours. Then milk and cell culture samples were purified and isolated proteins were submitted for Mass Spectrometric sequencing. Overall, 141, 142 and 10 bacterial proteins were identified in bovine intestinal epithelial cells, mammary epithelial cells and milk, respectively (**Figure 2**). While 61 bacterial proteins were commonly found in the intestinal and mammary gland epithelial cells, interestingly, we found one MAP protein that was present in all experimental groups. Protein analysis identified MAP_0966c/PE_PPE_C domain-containing protein. While the function of MAP_0966c in MAP is uncharacterized, its homologues PPE51 protein in *Mycobacterium tuberculosis* is implicated in immunostimulation and virulence, enhancing bacterial growth in acid environment and reducing drug tolerance. This protein is also related to antigenic variation in *M. tuberculosis*. Through bioinformatics analysis, we found that MAP_0966c is lipoprotein and has significant prediction to be released/secreted in cells (TAT signal peptide p=0.0366).

In summary, we discovered MAP antigen MAP_0966c in biologically relevant environments and it has a highly probability to be a good diagnostic biomarker. The role of MAP_0966c in bacterial pathogenesis and its immunostimulation ability is currently under investigation. The manuscript is in preparation.

Figure 1



Thickened intestinal wall (ileum) with mucosal lesions from an animal with Johne's disease

Histology of the small intestine stained for *M. avium* subsp. *paratuberculosis* (in red)

Pictures are obtained from the CCVM pathology lab

Figure 2