"Applying recombinant technology to define the secretome of Mycobacterium avium subsp. paratuberculosis for the discovery of novel immunogens and diagnostic reagents"

IMPROVED UNDERSTANDING OF JOHNE'S DISEASE

PROJECT NO.: 0008-010
RESEARCH INSTITUTION: University of Guelph
LEAD RESEARCHER: Dr. Lucy Mutharia

Background: Johne's disease affects all ruminant animals such as cattle, sheep, goats and deer. Animals become infected when they ingest milk, water or feed on pasture contaminated with feces from infected animals. Once infected, animals may appear healthy for many years, although they could be shedding the bacterium in feces and milk. The bacterium that causes Johne's disease (Mycobacterium avium subspecies paratuberculosis, or MAP) is difficult to diagnose accurately. Attempts to culture MAP from manure are often unsuccessful, and blood and milk tests often miss animals that are in the early stages of the disease. This results in a high rate of "false negatives," which means that the diagnostic test says the animal is not infected even though it is actually infected.

Objectives: To identify and characterize the MAP exoproteome (the entire set of proteins secreted to the environment by the organism) and to identify specific proteins that can be used to increase the specificity and sensitivity of diagnostic tests to decrease false negative and false positive test results.

What they did: Blood samples and fecal shedding status were obtained from 25 MAP infected cows (as identified by the current ELISA test). Cows were split into four groups:

ELISA and fecal high, ELISA high and fecal low, ELISA low and fecal high, and ELISA low and fecal low. Ten control samples were obtained from ELISA and fecal negative cows and calves. These samples were used to screen the MAP exoproteome in order to identify those proteins that trigger an antibody response during infection.

An expression library (a technique which allows for the isolation of genetic elements of interest) was constructed and screened against the blood samples from cows with Johne's disease. This allows for the identification of proteins that react with the antibodies present in the infected animals.

Using ultra-filtration (a method of separating proteins), the researchers were able to split the MAP exoproteome into two fractions. Using this method allowed for the recovery of proteins in their natural state. Further analysis of these fractions with gel-electrophoresis and western blotting let the researchers compare the protein profiles of MAP with other closely related species, and pinpoint the proteins which react with antibodies from blood samples of infected animals.

What they learned: The two MAP exoproteome fractions contained about 76 proteins that had never been discovered before. In addition, when a group of 15-20 of these proteins was screened using blood samples from infected cattle, uninfected cattle, and rats immunized with proteins from related Mycobacterium,

these proteins reacted strongly with the infected cattle samples, but not with the uninfected cattle or the rat samples.

What it means: When compared to another closely related Mycobacterium species, the MAP exoproteome fractions contained very different protein profiles. This implies that the exoproteome is a good target for discovery of novel proteins that have species-specific biological or virulence functions. Since cross reactions can occur with the current diagnostic tests, the fact that these proteins only reacted with samples from infected animals and not the control samples makes them potential targets for a MAP specific diagnostic test. In addition, the strong reactions of some of the proteins indicates that they may be useful indicators in predicting animals with the highest risk of shedding fecal MAP. Future research will determine the function of these new proteins, as well as test a larger sample size to confirm whether one or more of the proteins can be used to differentiate MAP infected from MAP exposed animals, and to identify infected animals prior to shedding and in the early pre-clinical disease stage.