Surface antigens of *Mycobacterium avium* subsp. *paratuberculosis* (MAP): Implications for Johne's disease (JD) diagnosis and pathogenesis

Ashutosh Wadhwa, Ph. D. Candidate
Center for Wildlife Health,
Department of Forestry, Wildlife and Fisheries,
The University of Tennessee, Knoxville
awadhwa@utk.edu

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Johne’s disease (JD) - pronounced as “YO-nees”, is also called paratuberculosis, caused by *Mycobacterium avium paratuberculosis* (MAP).

It is a chronic infection, causing reduction in milk production, malnutrition, weight loss and eventually death.

The natural hosts for MAP are wild and domesticated ruminants, including dairy and beef cattle, sheep, goats, red deer, cervids, and camelids.
Introduction

• In the United States, JD has been found in 68% of the dairy herd and causes an estimated annual loss of $220 million to the US dairy industry.

• No treatment available. Early screening and culling is the best practice to control.

• Crohn’s disease (CD), a chronic inflammatory disease, also called as inflammatory bowel disease. Recent researches have shown that not all but at least some cases of CD may be caused by MAP.
MAP – The Bug

- Obligate intracellular bacteria
- Small (0.5 x 1.5 micron)
- Rod shaped bacteria
- MAP has a single circular chromosome.
- It is - slow-growing, gram-positive, acid-fast positive and aerobic.
- The cell wall is made up of lipids and polysaccharides.
- MAP – Unique since it does not produce mycobactin and depend on the host cell to provide iron.
- MAP share 95% of its genes and exhibit homologies of more than 99% between these genes with MAA.
Pathogenesis of JD
Progression of JD/Clinical Signs

JD can be classified in 4 stages:

1. *Silent infection*: Young stock up to 2 years of age. No clinical or measurable clinical signs.

2. *Subclinical infection*: No clinical signs; but could be detected.

3. *Clinical infection*: I/P of 2 – 10 years - gradual weight loss, persistent or intermittent diarrhea and decreased milk production.

4. *Advanced clinical infection*: It arise if animals with clinical signs are not culled. More lethargic, weak and emaciated characterizing “pipestream” diarrhea, hypoproteinemia and intermandibular edema.
Diagnosis

- Fecal / Tissue culturing for MAP – takes 6-8 weeks.

- Intradermal test - low sensitivity and specificity.

- Polymerase chain reaction (PCR) - IS900 insertion sequence, F57 – Costly, lengthy procedure and specialized equipments.

- Complement Fixation test and agar gel immunodiffusion test can also be done but it suffers poor sensitivity.
Sero-Diagnosis and various antigens

- Recent reports suggest that Enzyme linked immunosorbant assays (ELISA) should be used for controlling the disease.

- ELISA’s detect an optical density in milk or serum that correlates to antibody response to MAP.

- Various antigens – internal proteins (MAP p35 K), alkyl hydroperoxide reductases C and D (AhpC and AhpD), recominant polypeptides. – All suffer low sensitivity.

- Ethanol vortex ELISA (EVELISA)- Uses a major cell wall lipopeptide as an antigen- Sensitivity 95%.
The antigen

200 ml of MAP culture

Treatment with 70% ethanol and vortexing

Ethanol Extract antigen
Recent research from our group

New Method of Serological Testing for *Mycobacterium avium* subsp. *paratuberculosis* (Johne’s Disease) by Flow Cytometry


A Highly Sensitive and Subspecies-Specific Surface Antigen Enzyme-Linked Immunosorbent Assay for Diagnosis of Johne’s Disease

Shigetoshi Eda, John P. Bannantine, W. R. Waters, Yasuyuki Mori, Robert H. Whitlock, M. Cathy Scott, and C. A. Speer

Absorbed EVELISA: A Diagnostic Test with Improved Specificity for Johne’s Disease in Cattle

Mary C. Scott, John P. Bannantine, Yumiko Kaneko, Adam J. Branscum, Robert H. Whitlock, Yasuyuki Mori, Clarence A. Speer, and Shigetoshi Eda
Optimization and evaluation of serological tests for JD.

• Need for a rapid and relatively cheap screening test with highest diagnostic sensitivity.

• Need of a Lab-on-a-chip technology for onsite detection of JD.
Proposed Method

i. Testing field serum samples provided by collaborators.

ii. Testing milk samples provided by collaborators.

iii. Development of a microfluidic diagnostic device for Johne’s diagnosis.
Isolation and characterization of molecules in the ethanol extract of MAP.

- Need to identify sub-species specific molecules to explain why MAP occupies a specific biological niche.

- Host immune response to the surface associated lipopeptides of MAP.
Proposed Method

i. Analysis of lipids in MAP ethanol extract using TLC

ii. Purification of MAP-specific lipids using Cyclograph

iii. Structural analysis of MAP-specific lipids using NMR and mass spectrometry

Initial mass spectrometry results
Research Objective III

*In vivo* studies on possible roles of MAP-specific lipids in pathogenesis of JD.

- Which response dominates first – Cell mediated or humoral immune response?

- Effect of MAP specific surface molecules on cytokine production and gene expression.
i. Effects of MAP-specific lipids on cell (macrophage, dendritic cell, epithelial cell) functions

ii. Cytokine productions (BioPlex at Genomics hub)

iii. Gene expression (Microarray)
Statistical Analysis

• All experiments will be conducted in duplicates or triplicates and repeated twice.

• The statistical difference of antibody binding among various sets of conditions and between negative and positive samples will be evaluated by using a Mann-Whitney U test.

• Statistical analysis and depiction of box plots will be conducted using a statistical software, R.

Preliminary result from EV MILK ELISA


References..cont


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Questions ???
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