# Animal Health-Johne's Disease (JDIP): Basic Biology/Immunology/ Vaccine Development

**55 A novel approach to evaluate the cost-benefit of use of Johne's disease vaccine while considering effects on the bovine tuberculosis eradication program.** F. J. Zagmutt*[1], L. A. Espejo[2], H Groenendaal[1], J. R. Lima[2], E. Patton[3], I. A. Gardner[4], and S. Wells[2], [1]*Vose Consulting*, *Boulder, CO*, [2]*College of Veterinary Medicine, U. of Minnesota*, *St. Paul,* [3]*Division of Animal Health, Wisconsin DATCP*, *Madison, WI*, [4]*School of Veterinary Medicine, U. of California Davis*, *Davis*.

Johne's disease (JD) vaccination has the potential to reduce losses for individual herds. However, the effectiveness of widespread adoption of JD vaccination on JD control, and the effects of cross-reactivity (CR) between *M. avium* ssp. *paratuberculosis* (MAP) and *M. bovis* on the control and eradication of each disease is unknown. The objective of this study was to assess the cost-benefit of Johne's Disease (JD) vaccination on MAP infected dairy herds, and its effect on the bovine tuberculosis (bTB) eradication program in the USA. The results of 12,957 parallel fecal culture (HEY media) and serum ELISA (IDEXX) from 8 dairy herds enrolled in the MN JD Demonstration Herd Program over 9 years, and 970 fecal culture from 3 JD-vaccinated dairy herds enrolled in the WI JD Demonstration Herd Program for 4 years were used to build a model for the within-herd spread of JD and estimate its parameters. The uncertainty in the parameters was linked to the progression of the disease in each animal based on tests results. Herd and test parameters estimated with a latent-class Bayesian analysis were used to calculate the confidence in the disease status of each animal in time, and then the status of animals in each iteration was grouped to calculate spread parameters dynamically. The parameters were used to simulate disease spread in vaccinated and non-vaccinated herds. The model predictions using different scenarios of vaccine efficacy and disease spread were used in an economic break-even analysis. As bTB animal prevalence in the US may change in time, the threshold of bTB prevalence that would make JD vaccination not economic was calculated for each scenario. Even in scenarios with very high bTB prevalence (1% and higher) and a 10% relative drop in Sp of CFT in vaccinated herds, vaccination provided financial benefits (NPV > 0). However, while vaccination reduced herd-level JD losses at the producer level, the majority of the costs of CR due to JD vaccination are borne by the government. This economic threshold was highly sensitive to the reduction of JD losses resulting from vaccination.

**Key Words:** Johne's, bovine tuberculosis, modeling

**56 Stochastic simulations of a multi-group compartmental model for Johne's disease on US dairy herds with test-based culling intervention.** Z. Lu*, Y. H. Schukken, R. L. Smith, and Y. T. Gröhn, *Cornell University, Ithaca, NY.*

The objective of this study was to evaluate the effectiveness of test-based culling intervention and its impact on fadeout of Johne's disease in dairy herds using a stochastic modeling approach. Infection elimination may be an important goal of control programs; only in stochastic infection models can true infection elimination be observed, as fadeout. To investigate the stochastic dynamics of Mycobacterium avium ssp. paratuberculosis (MAP) infection in US dairy herds with test-based culling intervention, we developed a continuous time Markov chain model with both horizontal and vertical transmission. The stochastic model predicted fadeout and within-herd prevalence to have a large variance. Although test-based culling generally decreased prevalence over time, it took longer than would be desired by producers to eliminate an endemic MAP infection from a herd. Uncertainty analysis showed that, either using annual fecal culture tests and culling only high shedding animals or culling of both low and high shedders while delaying culling of low shedders for 12 mo, MAP infection persisted in many herds beyond 20 years. Using a semi-annual whole-herd fecal culture test and culling of both low and high shedders, with a 6-mo delay in culling of low shedders, MAP infection in many herds would be extinct within 20 years. Sensitivity analysis of the cumulative density function of fadeout suggested that combining test-based culling and reduction of transmission rates, through decreased contact between susceptible calves and shedding animals, may be more effective than either control strategy alone in eliminating endemic MAP infection. We also examined the effects of other factors, such as herd size, heifer replacement, and adult cow infection on the probability of fadeout.

**Key Words:** Johne's disease, test-and-cull, stochastic modeling

**57 Unrestricted transmission of highly pathogenic Indian Bison type of *Mycobacterium avium* ssp. *paratuberculosis* in India.** S. V. Singh*, B. Singh, A. Tiwari, A. Kumar, P. K. Singh, and A. V. Singh, *Central Institute for Research on Goats*, *Makhdoom, Farah, Mathura (UP), India, 281 122*.

The aim of present study was to determine the status of MAP infection and its genotype in free ranging wild species, primate, soil, river water, milk and humans to understand the disease transmission for future evaluation of regional and national JD control programs and improvement in diagnostics and vaccine designing. A total of 74 fecal samples from wildlife species [Monkeys (Primate)-25, Hog Deer-20, Wild bison-7, Chinkara deer-16, Blue bull-6], 39 stool samples from humans (with/ without inflammatory bowel disease; IBD), 51 soil samples (from grazing land), 8 water samples from different Yamuna river bank, were collected from North India and screened for the presence of MAP and further its genotyping by IS1311 PCR-REA. In 74 fecal samples from wild animals 50% and 46.9% were positive by microscopy and PCR [40.0, 20.0, 42.8, 100.0 and 66.6% (by microscopy) and 4.0, 15.0, 57.1, 75.0, and 66.7% (by PCR) Monkeys, Hog deer, Wild Bison, Chinkara deer and Blue bull, respectively). Of the 51 soil samples, 52.9 and 29.4% were positive for MAP by microscopy and PCR, respectively. In 8 samples from Yamuna river 12.5 and 37.5% sample were positive by microscopy and PCR. Out of 39 stool samples from humans 28.21 and 20.51% samples were positive in microscopy and PCR. There was significant high occurrence of MAP in samples collected from persons suffering from IBD. All the PCR positive samples in present study were showed restriction profile of 'MAP Bison type' by IS 1311 PCR-REA. High presence of MAP of same genotype i.e. Bison type in different free ranging wild animal species, primate, human, soil and river water indicated host unrestricted and high rate of transmission among different animals species, serious risk to human health and active biohazard for environment.

**Key Words:** *Mycobacterium avium* ssp. *paratuberculosis*, wild ruminants, environmental samples

**58 *Mycobacterium avium* subspecies *paratuberculosis* produces endospores.** E. A. Lamont*[1], J. P. Bannantine[4], A. Armién[1], D. S. Ariyakumar[3], and S. Sreevatsan[1,2], [1]*Veterinary Population Medicine,*

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Achievement of latency represents pathogenic mycobacteria's ultimate stratagem for survival, yet it remains one of the least understood and ill-defined aspects of its lifecycle. Two debated viewpoints concerning latency are that 1) bacilli are in a non-replicating state of chronic persistence (dormancy) and 2) replicating bacilli periodically escape from the granuloma and enter adjacent vessels to infect new sites. We hypothesize and show that MAP is capable of spore production in one year old MB7H9 cultures and AK-sporulation media. Experiments were conducted in triplicate. All MAP endospore samples, including germinated spores, were positive for IS900. All cultures were determined to be free of contamination by absence of growth on BHI plates. In addition to spore visualization, we have identified several mycobacterial candidate genes corresponding to those in the sporulation pathway of several *Bacillus* and *Streptomyces* species. Research investigating Bacillus spp. pathogenicity indicates that the stringent response regulated by carD is essential to robust spore production. Quantitative real-time PCR (Qt-RT-PCR) analysis of dormant MAP cultures show a 15-fold upregulation of carD compared with log-phase controls. MAP cells primed by carD may utilize sporulation as an alternate survival tactic to combat a hostile host environment to achieve latency. The hypothesized critical roles of carD and sporulation in latent infection is expected to aid in novel mitigation strategies to combat MAP infection, which may have far reaching benefits to other mycobacterial infections, including M. tuberculosis.

**59 Transcriptional analysis of MAP genes contributing to invasion and persistence in ileal mucosa of cattle.** S. Khare*[1], K. Drake[2], and L. G. Adams[1], [1]*Department of Veterinary Pathobiology, Texas A&M University*, *College Station*, [2]*Seralogix Inc.*, *Austin, TX*.

*Mycobacterium avium* ssp. *paratuberculosis* (MAP) initiate the disease process by invading and passing through the intestinal epithelium. This triggering mechanism is generally very rapid and may lead to a persistent infection. MAP undergoes a complex extracellular and intracellular environment. However, little is known about the bacterium-host interactions that occur at these stages. We hypothesize that MAP meets the challenges of hostile changing environments of ileum as soon as it comes into the contact of the host. We have used the MAP isolated from ligated ileal loop model to test our hypothesis. The objective of the current study was to determine the temporal changes in the MAP gene expression during the early invasion in the ileum. Tissue-associated MAP RNA from MAP infected bovine ligated ileal loop were enriched using "Prokaryote Enrichment kit" that selectively removes the Eukaryotic RNA. This enriched prokaryotic RNA is amplified using MAP-genome derived primers during in vitro transcription. Genomic DNA from in vitro grown MAP was labeled with Cy5 and used as the reference RNA. Enriched and amplified tissue-associated MAP was labeled with Cy3. The Cy5 labeled g-DNA and Cy3 labeled tissue-associated cDNA were co-hybridized on the MAP microarray (obtained from JDIP core facility). After hybridization arrays were washed and dried by centrifugation and were immediately scanned. We will describe in detail the groups of interrelated genes that as a whole represent the activation (perturbation) of pathways or GO biological processes over the time-course of these experiments (1h, 2h, 4h, 8h, 12h). This approach ranks groups of genes/proteins across all time points instead of individual genes in a single time point, to determine differences between experimental conditions. Using this approach, we have identified several mechanistic genes that play key regulatory role during invasion and persistence of MAP in the ileum.

**Key Words:** Johne's disease

**60 The transcriptome of *Mycobacterium avium* subspecies *paratuberculosis* during infection.** C.-W. Wei and A. M. Talaat*, *University of Wisconsin-Madison*, *Madison*.

*Mycobacterium avium* ssp. *paratuberculosis* (M. ap) causes an enteric infection in cattle, with a great impact on the dairy industry in the United States and worldwide. Before contracting a new host, M. ap are known to survive the harsh intracellular microenvironments, especially those inside activated macrophages. To improve our understanding of the pathogenesis of M. ap and help in a better control strategy against Johne's disease, we profiled the transcriptional responses of M. ap mutant or wild type following exposure to variable stress conditions including macrophage microenvironments. Mycobacterial cultures were exposed to heat shock, nitric oxide or H2O2 treatments. Other aliquots were used for infecting J774 cell lines at the 10 MOI (Bacteria: Macrophage). Following each stress, mycobacterial RNA samples were extracted using a Trizol-based protocol. Using DNA microarray analysis, Bayesian statistics revealed the presence of 123 genes that were significantly regulated when in vitro samples were compared with samples collected from M. ap isolated from 2 h post infection of macrophages. This group of genes includes sigH (a global gene regulator) and aceA that were shown before to play a role during infection. Further analysis identified additional 67 genes that were regulated when IFN-γ treated cells were compared with naïve cells before infection including genes involved in iron metabolism (e.g., fdxA). Currently, analysis is underway to generate specific gene mutants to examine their role in M. ap virulence using the mouse model of paratuberculosis. Overall, our analysis indicated a significant change in mycobacterial gene expression once they encounter the macrophage microenvironment. Additionally, the activity status of macrophages seems to play a role in directing the mycobacterial transcriptome to a specific stress-responsive profile.

**Key Words:** M. ap, genomics, pathogenesis

**61 The response of auxotrophic MAP leuD mutant under environment stresses.** J.-W. Chen*, J. Scaria, S. Chandra, and Y. F. Chang, *Cornell University*, *Ithaca, NY*.

*Mycobacterium avium* ssp. *paratuberculosis* (MAP) causes Johne's disease in ruminants. In M. tuberculosis, deletion of leuD gene results in severely attenuated phenotype. To explore the possibility of using leuD mutant strain of MAP as a vaccine candidate for Johne's disease, and to understand the mechanism of attenuation of MAP leuD deletion, a phage-mediated allelic exchange method was used to construct a leuD mutant strain in MAP K10 strain. The leuD deletion was confirmed by both PCR and DNA sequencing. In the absence of leucine supplementation, the growth of leuD mutant is completely inhibited in 7H9 medium. With supplementation of leucine the growth of mutant is restored but grows at a slower rate than that of wild type. To analyze the mechanism of leuD attenuation, mutant and wild type were subjected different environmental stress and following Agilent protocols. Three ug of RNA from mutant and wild types were competitively hybridized against a whole genome Agilent expression array of MAP. Array results were analyzed using Genespring GX 7.3. Arrays were log-transformed and subjected to lowess normalization and fold change analysis of mutant vs. wildtype was performed. Expression levels changes 1.5 fold or more were considered significant. The results of array studies demonstrate that leuD plays an important role in the MAP metabolism and that

there are more than 500 genes that belong to different pathways that are modulated in different stress conditions. These genes are distributed across Cluster of Orthologous Gene (COG) categories. The major COG categories include energy production and conversion, lipid transport and metabolism, inorganic transport, secondary metabolite production and cell membrane biogenesis. These results indicate that deletion of leuD in MAP results in global changes in lipid transport, cell secretion apparatus and changes in cell membrane biochemistry. Mice studies showed that inoculation of 107 cells of leuD mutant can provide partial protection of mice when challenged after 16 weeks with wild type.

**Key Words:** Johne's disease, LeuD mutant, microarray

**62 A gene specific to *Mycobacterium avium* ssp. *paratuberculosis*, but only at the transcription-translation level.** J. P. Bannantine*[1], R. E. Briggs[1], E. A. Lamont[2], J. R. Stabel[1], and S. Sreevatsan[2], [1]*National Animal Disease Center*, *Ames, Iowa,* [2]*University of Minnesota*, *St. Paul*.

There is no known antibody that detects *M. avium* ssp. *paratuberculosis* and does not cross react with other *M. avium* subspecies. In the present study, a monoclonal antibody was identified from mice immunized with a cell membrane fraction of *M. avium* ssp. *paratuberculosis* strain K-10. This antibody detected a protein in *M. avium* ssp. *paratuberculosis* whole cell extracts, but did not bind to any of the 20 non-paratuberculosis subspecies strains tested in immunoblot assays. The antibody was further tested with 15 strains of *M. avium* ssp. *paratuberculosis* and showed variable expression levels of the target binding protein in select strains. This target binding protein was identified by screening a *M. avium* ssp. paratuberculosis-lambda phage genomic expression library with the monoclonal antibody. The identity of the protein was encoded by a gene that was not annotated in the *M. avium* ssp. *paratuberculosis* K-10 genome sequence and showed no similarity to other proteins in sequence databases. Furthermore, this gene has extensive overlap with an annotated gene on the opposite strand. The epitope detected by the mAb was precisely mapped to 7 amino acids and served as an anchor point in studies that identified the start and stop locations for this unique gene. Similarity searches reveal that the DNA sequence is present in other MAC complex species. However, expression analysis shows that only *M. avium* ssp. *paratuberculosis* makes a transcript and expresses the protein in macrophages as well as when cultured in Middlebrooks 7H9. The protein is not a strong antigen, but it is detected in the context of Johne's disease. These findings have new implications for comparative genomics and gene regulation differences among mycobacteria.

**Key Words:** protein, antibody, Johne's disease

**63 Binding affinity of *Mycobacterium avium* ssp. *paratuberculosis* 85 complex to 40 kDa domain of fibronectin.** C. J. Kuo*[1], J. Bannantine[2], V. Kapur[3], and Y. F. Chang[1], [1]*Cornell University*, *Ithaca, NY*, [2]*NADC*, *Ames, Iowa*, [3]*Pennsylvania State University*, *University Park*.

Antigen 85 complex proteins (Ag85), consisting of members 85A, 85B and 85C, have been shown to be the important secreted antigens and are retained in the cell wall of Mycobacterium avium ssp. paratuberculosis (MAP). Ag85 binds specifically to a host extracellular matrix protein, fibronectin (Fn), which consists of N-terminal domain (NTD), gelatin-binding domain (GBD), cell-binding domain (CBD), and 40kDa domain. Fibronectin-binding proteins are important virulence factors of MAP and Ag85 may contribute to the adherence, invasion, and dissemination of organisms in host tissue. However, the critical residues of Fn involved in Ag85 binding are still unknown. Our objective of this study is to identify the Fn binding domain(s) to antigen 85 complexes. In this study, we constructed and overexpressed antigen 85A of MAP in *E. coli*. Four domains of Fn were tested for Ag85-Fn interaction by using native gel electrophoresis and ELISA. Except the 40 kDa domain (including 12, 13, 14, and v region), no significant binding was observed for other Fn domains. Subsequently, the synthesized peptides based on the binding sequence of Fn were subjected to a competition binding assay. Our data shows that these peptides can partially abolish the interaction between MAP and host cells. This is the first report to identify the Fn binding domain in Ag85.

**Key Words:** fibronectin, Johne's disease, adhesin

**64 *MAP* induces calcium-dependent phagosome acidification to enlist IL-1β processing and macrophage recruitment.** E. A. Lamont*[1], S. M. O'Grady[3], T. Eckstein[4], and S. Sreevatsan[1,2], [1]*Veterinary Population Medicine, University of Minnesota*, *Saint Paul*, [2]*Department of Veterinary Biomedical Sciences, University of Minnesota*, *Saint Paul*, [3]*Department of Animal Sciences, University of Minnesota*, *Saint Paul*, [4]*Department of Microbiology, Immunology and Pathology, Colorado State University*, *Fort Collins*.

Processing of *MAP* by the host epithelium involves a dynamic innate immune response initiated by *MAP*-epithelial cell cross-talk, which may also be further augmented by interactions between host pathways and/or cell types (epithelial-macrophage). We show that *MAP* induces phagosome acidification within MacT cells as early as 10 min., resulting in upregulation of IL-1β at transcript and protein levels. Previous studies report that IL-1β is a potent macrophage chemoattractant. We hypothesized that *MAP* harnesses host responses to recruit macrophages to the site of infection to ensure its survival and dissemination. These initial host-pathogen interactions may dictate a form of cooperative self-destruction in which the host is deceived into reacting to the benefit of *MAP*; thereby, setting the tone for the ensuing infection. We investigated macrophage recruitment in response to *MAP* using a MacT-bovine macrophage coculture system. All time points were conducted in triplicate. Within 10 min of *MAP* infection, macrophages were recruited to the apical side of Mac-T cells ($P < 0.05$, ANOVA). Inhibiting phagosome acidification with bafilomycin treatment abrogated this response ($P < 0.05$, ANOVA). Since IL-1β cleavage and subsequent release to the epithelial milieu is dependent upon calcium influx, we next sought to define the role of calcium oscillations in phagosome acidification and macrophage recruitment. Pre-treatment of Mac-T cells with BAPTA-AM, an established intracellular calcium chelator, abolishes phagosome acidification and IL-1β processing to its active form. Thus, *MAP* guidance of phagosome-acidification enlists IL-1β processing in a calcium dependent manner to efficiently transverse the epithelium and into its niche—the macrophage.

**65 Macrophages infected with *Mycobacterium avium* subspecies *paratuberculosis* are highly resistant to apoptosis, while uninfected culture mates are highly apoptotic.** E. Kabara* and P. M. Coussens, *Michigan State University*, *East Lansing*.

Mycobacterial infections have long been implicated in altering the apoptosis status of infected macrophages. Previous research has linked more virulent mycobacteria to an increase in macrophage apoptosis. However, this research often does not take into account that most macrophage culture infections with mycobacteria are mix of infected and uninfected cells, with the infected macrophages comprising less then 50% of the total cell population. More recent studies specifically looking at the differences between infected and uninfected macrophages in a culture

show that most of the infected macrophages are highly resistant to cell death while the uninfected macrophages are more likely to undergo apoptosis. While most of this evidence has arisen from research with Mycobacterium tuberculosis, a large scale microarray project recently performed by our group implicated infection with Mycobacterium avium subspecies paratuberculosis (MAP), the causative agent of Johne's disease in cattle and possibly Crohn's disease in humans, as changing the apoptotic potential of cultures. We hypothesize that MAP-infection prevents apoptosis in infected macrophages while upregulating apoptosis in uninfected macrophages in the same culture to prevent proper immune function while increasing bacterial survival. To study the true apoptotic status of MAP-infected and MAP-uninfected macrophages in the same culture, fluorescent dye was used to label the bacteria before infection and cells were examined individually using flow cytometry. After using the Annexin V/ 7-AAD apoptosis stains, we clearly show that MAP-infected macrophages are much less likely to undergo apoptosis, while uninfected macrophages in the same culture are highly apoptotic. We further demonstrated that MAP-infected macrophages are much more likely to undergo necrosis when stimulated with hydrogen peroxide, TNFa, and FASL. Conversely, uninfected macrophages in a MAP-infected culture are much more likely to undergo apoptosis than control macrophages unexposed to MAP after stimulation with these same agents.

**Key Words:** *Mycobacterium*, paratuberculosis, apoptosis