**Project No. and Title: NE1201 Mycobacterial Diseases of Animals**

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| Project No. and Title: | [NE1201](http://lgu.umd.edu/lgu_v2/pages/showInfo.cfm?trackID=14736) Mycobacterial Diseases of Animals  |
| Period Covered: | 12-2013 to 12-2015 |
| Date of Report: |  |
| Annual Meeting Dates: | 18-Oct-2014 to 18-Oct-2014 |

**I. Participants**

Last Name First Name Affiliation

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| --- | --- | --- |
| anbalgam | priya | newpot labs |
| Bani | Majid | UMKC |
| Bannantine | John | USDA-ARS\_NADC |
| Barletta | Raul | UNL |
| Bermudez | Luiz | Oregon state |
| Bull | Tim | George's Medical School in London, UK |
| Canjan | Irene | missouri state |
| Carter | Michael | usda aphis |
| Coussens | Paul | MSU |
| Djuranovic | Nejena | Qiagen inc. |
| Eckstein | Torsten | CSU |
| Griffin | Frank | otago |
| Grohn | Yrjo | Cornell |
| Hines | Murray | UGA |
| Jing | Cui | Ohio State Agr. |
| Johnson | Peter | USDA-NIFA |
| KAO | Rowland | U of Glasgow |
| Kapur | Vivek | PSU |
| Kiser | Jennifer | Washington State |
| McDonald | Jeannette | Wisc |
| Neiberg | Holly | WSU |
| Nelson | Jeffrey | USDA-APHIS-NVSL |
| Nelson | Cheryl | private practitioner Nelson reproductive |
| Olson | Ken | KEO |
| Palmer | Mitchell | USDA-ARS\_NADC |
| Patton | Elisabeth | Wisconsin Dept of Ag |
| Quinn | Fred | UGA |
| Robbe-Austermann | Suelee | USDA-APHIS-NVSL |
| Sherman | Gary | USDA-NIFA |
| Smith | Rebecca | Univ. of Illinoise |
| Smith | David | NY AG |
| Smith | Julie | U of Vermont |
| Sreevatsan | Sri | UMN |
| Sweeney | Ray | U Penn |
| Talaat | Adel | Wisconsin madison |
| Thomson | Gary | PSU |
| Wells | Scott | UMN |
| Wojeik | Laura | biocsl |

Brief Summary of Minutes of Annual Meeting

The 3rd Mycobacterial Diseases in Animals (MDA) multi-state annual meeting was held this year in association with the annual meeting of the US Animal Health Association (USAHA) and the American Association of Veterinary Laboratory Diagnosticians (AAVLD) at the Sheraton Crown Center Hotel in Kansas City, MO on Saturday October 18, 2014. The meeting drew close to interested 80 individuals from different disciplinary including research scientists, industry leaders, government agencies, and stakeholders in person and via Adobe Connect Website.

The conference focused on the two most important mycobacterial diseases of animals – Johne’s disease and bovine tuberculosis, building on last two year’s very successful conference held in Chicago, IL and San Diego, CA.

**II. Welcoming Remarks:**

Dr. Vivek Kapur (Chair; Penn State); Dr. Paul Coussens (Co-Chair; Michigan State); Dr. Gary Thompson (Admin. Advisor; Penn State, via Adobe Connect); and Dr. Peter Johnson (USDA-NIFA, via Adobe Connect) each welcomed the participants, and highlighted the importance of the annual MDA gatherings. They also reviewed the progress and the contributions made by the MDA research community in defining the key scientific issues relating to Mycobacterial diseases (primarily Johne’s Diease and Tuberculosis) in animals.

**III. Plenary sessions:**

The conference was organized as 6 plenary sessions and a poster session.

Plenary session 1: Epidemiology and transmission of mycobacterial diseases in animals

Session Chair: Dr. Scott Wells (University of Minnesota)

1.1 Dr. Rowland Kao ((Univ. of Glasgow, UK)):

Keynote speaker for the plenary session 1: Dr. Kao from the Institute of Biodiversity Animal Health, University of Glasgow, Glasgow

Title: Thinking backwards and forwards - evolutionary and epidemiological approaches to modeling high density, high-resolution M. bovis sequence data in cattle and badgers

Dr. Kao covered the importance of the whole genome sequencing as an exciting new tool in the epidemiological toolbox, with the advent of large scale, high density sampling of particular interest to the forensic epidemiologist. He also discussed two different approaches in interpreting these data, one based on the evolutionary paradigm, and the other on epidemiological modeling. Dr. Kao also discussed how each is a powerful tool for elucidating basic principles about M bovis transmission dynamics, but must be interpreted at the appropriate epidemiological scale.

Plenary Session 2: Develop and implement new generations of diagnostic tests for JD and TB.

Session Chair: Dr. John Bannantine (USDA-NADC)

Invited speakers: Dr. Tim Bull (St. George, UK)

 Dr. Suelee Robbe-Austerman (APHIS)

2.1 Dr. Tim Bull title: Improving Rapid Detection and Culture in Mycobacterial Diseases

Dr. Bull stated that despite 100 years of experimentation and development,

culture is still GOLD STANDARD for infective status Mycobacterial media is essentially 7H9 since 1958, and cculture positive can be detected by 42 weeks in clinically evaluated subjects, and environment can drive a loss of culturability. Dr. Bull then indicated that MAP isolated from humans with Crohn’s Disease have an altered genomic arrangement, and sigH regulon (involved in survival, virulence and dormancy) is highly upregulated. He then questioned if we target sigH and sigE regulon expression- would it make MAP grow better? After series of screening and testing conditions, Dr. Bull concluded that addition of TiKa supplements will; help stimulate growth of MTB, M. bovis and MAP, promotes sigH and early re-aeration responses, limits lag phase, delays stationary phase, increases division rate of slow growing mycobacteria at least 2 fold, and finally decrease time to visible colony formation.

2.2 Dr. Suelee Robbe-Austerman: Mycobacterial Diseases of Livestock Current techniques and advances in diagnostics for mycobacterial diseases (organism detection)

Dr. Robbe-Austerman reviewed existing Mycobacterial organism detection assays, and reviewed challenges and advances for each. She then reviewed the advances in genotyping and whole genome sequencing currently used in NVSL to detect mycobacteria in different samples (tissue extraction, fecal/environmental extraction, and culture extraction). The new program uses common Unix programs to output easy to interpret SNP comparisons from multiple VCFs as both SNP tables and fasta files to generate phylogenetic trees. The analysis is done using reference based and reference independent criteria. Finally, defining SNP positions will filter isolates into groups, subgroups and clades.

3. Plenary Session 3: Extension/Outreach/Human Disease: Develop and deliver education and outreach material related to JD and TBc in electronic and print form for use by various stakeholders.

Session Chair: Dr. Ken Olson (KEO consulting)

Invited speakers: Dr. Julie Smith (Univ. of Vermont

 Dr. Jeannette McDonald (Univ. of Wisconsin–Madison)

3.1 Dr. Julie Smith: MDA outreach priorities

Dr. Smith first, shared the result of the survey conducted among producers asking for feedback regarding knowledge and information on JD and bTB. Among other results, she found out that not many producers have knowledge of ways to motivate action to control both diseases. Dr. Smith argued that dairy and beef farmers should know about TBc, and farmers should know about TB in humans as well. More importantly Dairy and beef farmers want to know about JD and bTB. Importantly, producers want to know which animals are truly infected (latent or subclinical). Dr. Smith emphasized that producers need understanding of the basics, and the fact that we need a more holistic approach, as extension efforts will not be successful by trotting out more science.

3.2 Dr. Jeannette McDonald: An Italian risk assessment and management planning tool plus “just-in-time” learning within one practical application

Dr. McDonald has received funding from the US-Italian Fulbright Commission and Italian Ministry of Health to establish a program with goals to create a convenient risk assessment tool, provide education as needed “just-in-time”, simplify the management and testing plan, make it easy to collect data, and establish accountability. They developed a website <http://iramp.izsler.it/>, and the data was linked to national database along with automatic calculations and analysis, and printable summaries for producers. Dr. McDonald mentioned that she is working on several other projects as well.

Plenary Session 4: Improving our understanding of the biology and pathogenesis of Mycobacterial diseases, as well as the host response to infection.

Session Chair: Dr. Srinand Sreevatsan (Univ. of Minnesota)

Invited speakers: Dr. Luiz Bermudez (Oregon State)

 Dr. Adel Talaat (Univ. of Wisconsin-Madison)

 4.1 Dr. Luiz Bermudez: The Development of in vitro model systems to address important pathogenic mechanisms of MAP associated with the survival in ruminants

Dr. Bermudez reviewed the difficulties of studying Johne’s Disease, as the disease has silent periods and takes many years to manifest clinically. He proposed establishment of model systems that can be used to obtain crucial information that would unveil key aspects of MAP pathogenesis.

 Dr. Bermudez‘s lab has developed a couple models that have provided knowledge that can benefit the study of the disease. In one of the models, they have established a sequential infection system from intestinal epithelial cells (first step in the infection) through macrophages and back to epithelial cells (last step on the disease). The three stages were called stages 1, 2, and 3.To determine whether this model would be reliable, they infected epithelial cells and passed the intracellular bacteria through the sequence of steps representing Johne’s disease. Then, they measured the production of chemokines and cytokines following the infection of the first epithelial cells (stage 1) and following the infection of the second epithelial cells (stage 3). In a reproduction of the disease, while the initial infection did not induce inflammatory response (but induced TGF-beta), the infection of the epithelial cells after passage in macrophages (stage 3) resulted in significant release of IL-18, IL-6, IL-8. CCL5, and decrease of TGF-beta. Transcriptome and lipidome of the third passage strain (inflammatory) versus the first passage strain (non-inflammatory) showed significant change in the proteins and lipids. To determine if those genes are expressed in vivo in the bovine mucosa, bacterial RNA was isolated from the intestines of cows with Johne’s Disease, and PCR was performed using specific primers for lipid metabolism related genes. The results confirmed the expression of the genes in vivo. Dr. Bermudez’s lab then used cell wall from MAP to stimulate lymphocytes and macrophages. While the cell wall of bacteria from the first passage induced only mild inflammatory response, the cell wall lipids of the third stage were associated with significant increase of the inflammatory phenotype of macrophages.

In the second model system, they used Acanthamoeba castellanii as a surrogate of bovine macrophages and Alamar Blue as a measure of metabolic activity, to screen a transposon library of MAP for attenuation. The screen takes one day and the read out is colorimetric. They examine whether 30 of the MAP strains identified in the screen were attenuated in primary bovine macrophages. Since we could confirm that all the strains were in fact attenuated, we have began the examination of the mechanisms of pathogenesis. Dr. Bermuudez concluded that the ability to establish system models in vitro which represent in vivo stages of Johne’s disease can help dissect the mechanism used by MAP to survive in the host and can develop novel approaches for prevention and treatment.

4.2 Dr. Adel Talaat: Lessons Learned from Bovine Tuberculosis In Enzootic/Endemic Regions

Dr. Talaat reviewed the pathogenesis and the prevalence of bTB. With a focus on med-size herds, Dr. Talaat, reviewed the Ecology of BTB in Egypt, and shared the results of field and laboratory testing. His team did multiple farm visits at mid-sized farm participating in their project in Egypt. They conducted single intradermal comparative skin test and compared *M. avium* vs. *M. bovis* PPD. They also performed histology of mainly lungs and lymph nodes, and occasionally, liver lesions. The research team performed genotyping of *M. bovis* on the *isolates* from Egypt (*gyrB).* In brief, the team found out that unlike *M. ap* infection, *M. bovis* is enzootic in Egypt, a high percentage of skin test +ve animals show no lesions. Dr. Talaat asked if could develop a better vaccine than BCG. Finally, he mentioned that more tastings are under way in mice and guinea pigs.

Plenary Session 5: Identify markers for susceptibility, and understanding of host genetics.

Session Chair: Dr. Paul Coussens (Michigan State Univ.)

Invited speakers: Dr. Holly Neibergs (Washington State Univ.)

 Dr. Frank Griffin (Univ. of Otago, New Zealand)

5.1 Dr. Holly Neibergs: A functional variant in the promoter region of *EDN2* associated with map tissue infection up-regulates EDN2 expression

Dr. Neibergs started by reviewing that Johne’s disease is a fatal transmissible bacterial disease, caused by *Mycobacterium avium* subspecies *paratuberculosis* (*Map*). She then, reviewed a genome-wide association study done previously by her research group that led to the identification of a 70 kb region on Bos taurus (BTA) 3 as being associated with *Map* tissue infection. The objective of the study was to identify possible causal variants for *Map* tissue infection in cattle. Re-sequencing, comparative and fine mapping of the 70 kb region on BTA3 in both Holstein and Jersey cattle was undertaken, identifying 16 SNP positional candidates that were associated with *Map* tissue infection (P <0.01). One of the SNP candidates (SNP 272) was found to overlap two microRNA binding sites. A microRNA interference assay and a mRNA stability assay showed no effect when tested for the two microRNAs (miR-1197 and miR-2339) that were bound to the SNP 272 site*.* Screening of other SNP variants by electrophoretic mobility shift assay indicated that two SNPs (SNPs 105 and 208) displayed different binding affinity to nuclear proteins between the alleles. A luciferase reporter assay confirmed that the transcriptional activity was significantly increased with the susceptible allele of SNP 208 and that it had a synergistic effect with the susceptible allele of SNP 105. Dr. Neibergs said that additional studies are ongoing to determine if other genes are also regulated by the alteration in the transcription factor binding site. These results suggest that at least one functional variant located within a 70 kb region of BTA3may be a causal variant that results in susceptibility to *Map* tissue infection leading to Johne’s disease.

5.2 Frank Griffin: Selection for Heritable Resistance to Control Johne’s Disease in Farmed Deer!

Dr. Griffin said that different breeds of ruminants may display heritable resistance (R) or susceptibility (S) to Johne’s disease (JD). Nonetheless, little progress has been made to identify genetic markers associated with either R or S traits in domestic livestock.

Dr. Griffin current findings are derived a decade long study of 7 disparate breeds of European red deer (*Cervus elaphus*), in a herd that had been chronically infected with *M. ptb* for more than 5 years*.* Sires with a confirmed Resistant (R) or Susceptible (S) phenotype were used in an AI programme with crossbred females, and the phenotype of more than 50 progeny was confirmed immunologically, microbiologically and histopathologically, following experimental infection with virulent *M. ptb*. In a two year breeding programme, 78% of progeny from R sires displayed the R phenotype, while 84% of the S progeny expressed a S phenotype. Detailed RNA-seq and transcriptome analysis of 2 R and 2S animals was used to determine the relative expression levels of immune genes, measured by qPCR. Multiple genes involved in functional pathways of innate and adaptive immunity have been evaluated using a ‘systems biology’ approach to target overall cell function. Dr. Griffin’s research group found out that animals with a S genotype expressed significantly higher levels of genes associated with pro-inflammatory reactions and genes related to chemotaxis and Type 1 interferons. Higher levels of adaptive immune markers and increased levels of Apoptosis was seen in R animals. Additionally, animals with S phenotypes appear express dysfunctional innate immunity and are incapable of clearing mycobacterial pathogens. By contrast, R animals express markers of adaptive immunity, which when combined with adequate levels of innate immunity, promote protection and containment of infection. Disparate breeds of wild deer that have evolved in geographic isolation and have undergone domestication through selective breeding, display polarized phenotypes. Dr. Griffin findings from the study of Johne’s disease in NZ farmed will be contrasted with recent studies in cattle and sheep.

Plenary Session 6: Develop programs to evaluate and develop new generations of vaccines for JD and TBc.

Session Chair Dr. Murray Hines (Univ. of Georgia)

Invited speakers: Dr. Fred Quinn (Univ. of Georgia)

 Dr. Gregers Jungersen (Tech. Univ. Of Denmark)

6.1 Dr. Fred Quinn: Programs to develop and evaluate new generations of vaccines for bovine TB

Dr. Quinn reviewed the importance of improved understanding of the mechanisms of pathogenesis and response of the natural host to infection by Mycobacterium bovis in developing the next generation of more efficacious vaccines. Dr. Quinn mentioned that unfortunately, the practical and logistical challenges and overall high costs associated with appropriate studies in the relevant host (the calf or cow) have forced investigators to use less than optimal model systems. Even when investigators can use the bovine infection model, these same limitations rarely allow for the incorporation of all relevant natural infection parameters into studies, including oral vaccination, repeat oral dosing with small numbers of infecting bacilli, assessments of effects on animal-to-animal transmission, and disease outcome from long-duration controlled studies. Rodent models are not useful for studying most of these parameters, and thus the utilization of ferret and larger animals may be necessary. Dr. Quinn suggested using the bovine long-duration transmission model and other medium and large models that more accurately mimic aspects of the natural course of disease, the next generation of vaccine candidates, including mucosal vaccines, could be more accurately examined for disease prevention, and perhaps of more relevance, prevention of disease transmission.

6.2 Dr. Gregers Jungersen: Development of a recombinant multi-stage DIVA vaccine against Johne’s disease

By taking advantage of results from vaccine development against *Mycobacterium tuberculosis*, Dr. Jungersen’s research group developed a new (FET11) vaccine against *M. avium* subsp. *paratuberculosis* (Map) based on recombinant antigens from acute and latent stages of Map infection. A hall-mark in the development of the vaccine was a requirement not to interfere with diagnostic tests for bovine TB and Johne’s disease allowing a continued diagnosis of Map infection in vaccinated animals (DIVA vaccine).

In two post-exposure vaccination trials with 28 calves and 15 goats, respectively, animals were orally inoculated with live Map in their third week of life and post-exposure vaccinated at different times after inoculation or with different vaccine constructs. In response to vaccination animals developed vaccine-specific antibody and cell-mediated immune responses, but no measurable antibody responses by ID Screen® ELISA, PPDj-specific IFN-γ responses or positive PPDa or PPDb skin tests. At termination 8 or 12 months of age, relative Map burden was determined in a number of gut tissues by quantitative IS900 PCR and revealed significantly reduced levels of Map and reduced histopathology. Diagnostic tests for antibody responses and cell-mediated immune responses corroborated the observed vaccine efficacy: Five of seven non‐vaccinated calves seroconverted in ID Screen® ELISA indicating the progression of infection, while only four of 14 FET11 vaccinated calves seroconverted and a later time point after inoculation. Similarly, increased PPDj-induced IFN‐γ responses over time in non-vaccinated calves, while FET11 vaccinated calves had significantly reduced responses in PPDj IFN-γ assay from 40 to 52 weeks compared to non-vaccinated calves. Dr. Jungersen concluded that these results indicate the FET11 vaccine can be used to accelerate eradication of paratuberculosis while surveillance or test-and-manage control programs for TB and JD remain in place.

**VI. Poster sessions:** There were also 9 posters presented at the morning and afternoon poster sessions:

1. The Mycobacterial Diseases of Animals (MDA) Multistate Initiative - a cooperative effort addressing animal diseases

K.E. Olson1, V. Kapur2, P. M. Coussens3 and D. H. Lein4

1 KEO Consulting; 2 The Pennsylvania State University; 3 Michigan State University; 4 Cornell University

2. Catalyzing Innovation and Scientific Discoveries in Mycobacterial Diseases of Animals.

K.E. Olson1, V. Kapur2, P. M. Coussens3 and D. H. Lein4

1 KEO Consulting; 2 The Pennsylvania State University; 3 Michigan State University; 4 Cornell University

3. The Genome of a *Mycobacterium avium* subspecies *paratuberculosis* Isolate Recovered from a Crohn’s Disease Patient reveals Close Genetic Association with Isolates Recovered from Cattle

Lingling Li1, Michael Mwangi1, Rebecca Cote1, John P. Bannantine2, Juan Antonio Raygoza Garay1, Srinand Sreevatsan3, and Vivek Kapur1

1 Department of Veterinary and Biomedical Science, The Pennsylvania State University, University Park, PA; 2 National Animal Disease Center USDA-ARS, Ames, IA; 3 Veterinary Population Medicine, University of Minnesota, St. Paul, MN

## 4. Comparison of 3 fecal culture, 2 fecal PCR, 2 serum ELISA, and milk ELISA for diagnosis of paratuberculosis in US dairy cattle.

R. W. Sweeney1, I. Gardner2, M. Hines3, R. Anderson4, T. Byrem 5, M. Collins 6, A. Glaser 7, E. Hovingh 8, L. Jones3, S. Wells9, B. Whitlock10.

1 U. of Pennsylvania; 2 U. of Prince Edward Island; 3 U. of Georgia; 4 California Dept. of Food and Agriculture; 5 Antel BioSystems; 6 U. of Wisconsin; 7 Cornell University; 8 Penn State University; 9 U. of Minnesota; 10 University of Tennessee

5. Genome wide association study confirms association of *Mycobacterium avium paratuberculosis* tissue infection on BTA16 and BTA22

Jennifer Kiser1, Holly Neibergs1

1 Washington State University

6. Antigenicity of Envelope Protein Complexes of *Mycobacterium avium* subsp. *paratuberculosis*

F. L. L. Leite1, T.A. Reinhardt2, J.P. Bannantine2, J. R. Stabel2

1 Iowa State University, Ames, IA; 2 USDA-ARS, Ames, IA

7. ZAP-70, CTLA-4 and proximal T cell receptor signaling in cows infected with *Mycobacterium avium* subsp. *paratuberculosis*

 Fernando L. Leitea, Livia B. Eslabãob, Bruce Peschc, John P. Bannantinec, Timothy A. Reinhardtc, Judith R. Stabelc

a Department of Veterinary Microbiology and Preventive Medicine, Iowa State Univ., Ames, IA 50011; b Centro de Desenvolvimento Tecnológico, Núcleo Biotecnologia, UFPel, Pelotas, RS, Brazil; c USDA-ARS, National Animal Disease Center, Ames, IA 50010

8. An Outbreak of Bovine Tuberculosis in Beef Cattle and White Tailed Deer in Minnesota

Glaser L.1, Carstensen M.2, Shaw S.3, Wuenschmann A.4, Robbe-Austerman S.3, and Wells SJ.5

1 Minnesota Board of Animal Health; 2 Minnesota Department of Natural Resources; 3 USDA Animal and Plant Health Inspection Service; 4 University of Minnesota, Veterinary Population Medicine; 5 Center for Animal Health and Food Safety, University of Minnesota

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9. Landscape context and deer-cattle interactions: A targeted approach to bovine tuberculosis surveillance

Ribeiro Lima J.1, Forester JD.2, Carstensen M.3, Cornicelli L.3, Wells SJ.1

1 Department of Veterinary Population Medicine, University of Minnesota; 2 Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota; 3 Minnesota Department of Natural Resources

**VI. Impacts**

1. Establishment of a powerful tool for elucidating basic principles about M bovis transmission dynamics, interpreted at the appropriate epidemiological scale.
2. Discovery of TiKa supplements will, which helps stimulate growth of MTB, M. bovis and MAP, promotes sigH and early re-aeration responses, limits lag phase, delays stationary phase, increases division rate of slow growing mycobacteria at least 2 fold, and finally decrease time to visible colony formation.
3. Establishment of a new program that uses common Unix programs to output easy to interpret SNP comparisons from multiple VCFs as both SNP tables and fasta files to generate phylogenetic trees for mycobacterial cultures, using reference based and reference independent criteria.
4. Conduction of a survey among producers asking for feedback regarding knowledge and information on JD and bTB, and setting criteria for the next steps based on the results among milk and beef farmers.
5. Establishing an international relationship with Italy, and creation of a convenient risk assessment tool, provide education as needed “just-in-time”, simplify the management and testing plan, make it easy to collect data, and establish accountability, and development of a website <http://iramp.izsler.it/>.
6. The Development of in vitro model systems to address important pathogenic mechanisms of MAP associated with the survival in ruminants
7. Evaluation of results from a study performed for the Bovine Tuberculosis In Enzootic/Endemic Regions of Egypt.
8. Discovery of a functional variant in the promoter region of *EDN2* associated with map tissue infection up-regulates EDN2 expression
9. Selection for Heritable Resistance to Control Johne’s Disease in Farmed Deer
10. Development of Programs to develop and evaluate new generations of vaccines for bovine TB
11. Development of a recombinant multi-stage DIVA vaccine against Johne’s disease