**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: | 12-2015 to 12-2016 |
| Date of Report: |  |
| Annual Meeting Dates: | Sunday, December 04, 2016 |

**I. Participants**

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| --- | --- | --- |
| **Last name** | **First name** | **Organization** |
| Bannantine | John | USDA-ARS |
| Barletta | Raul | University of Nebraska–Lincoln |
| Bermudez | Luiz  | Oregon State University |
| Coussens | Paul  | Michigan State University |
| DeKuiper | Justin | Michigan State University |
| Frie | Meredith | Michigan State University |
| Grohn | Yrjo | Cornell |
| Holland | Margo | NIFA-USDA |
| Johnson | Peter | NIFA-USDA |
| Kapur | Vivek | Pennsylvania State University |
| Mohamed | Asmaa | Michigan State University |
| Olson | Ken | KEO Consulting |
| Quinn | Fred | University of Georgia |
| Rathnaiah | Govardhan | University of Nebraska–Lincoln |
| Smith | Becky | University of Illinois at Urbana-Champaign |
| Sporer | Kelly | Michigan State University |
| Talaat | Adel | University of Wisconsin |
| Wells | Scott | University of Minnesota |

Report and Brief Summary of Annual Meeting

The 4th Mycobacterial Diseases in Animals (MDA) multi-state annual meeting was held in association with the 2016 Conference of Research Workers in Animal Diseases (CRWAD) Annual Meeting at the Chicago Marriott Downtown Magnificent Mile, Chicago, Illinois on Sunday, December 04, 2016. The meeting drew close to interested 40 individuals from different disciplinary including research scientists, industry leaders, government agencies, and stakeholders.

The conference focused on the two most important mycobacterial diseases of animals – Johne’s disease and bovine tuberculosis, building on the previously held MDA annual conferences.

**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); Dr. Peter Johnson (USDA-NIFA) and Dr. Margo Holland (USDA-NIFA) 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 Disease and Tuberculosis) in animals.

**III. Plenary sessions:**

The conference was organized as 5 sessions.

Session 1: Understanding the biology and pathogenesis of Mycobacterial diseases, as well as the host response to infection.

**1.1 Dr. Luiz Bermudez (Oregon State)**

Dr. Bermudez reviewed the difficulties of studying Johne’s Disease (JD), as the disease has silent periods, takes many years to manifest clinically, and currently is difficult to get significant information about the many phase of the disease using current *in-vivo* models. He proposed establishment of model systems that can be used to obtain crucial information that would unveil key aspects of MAP pathogenesis, and would enable the researchers to compare the different phases of the disease between in vitro and in vivo systems. Dr. Bermudez emphasized the importance of establishing system models in vitro which represent in vivo stages of JD to help dissect the mechanism used by MAP to survive in the host, which could lead to the development of novel approaches for prevention and treatment.

Dr. Bermudez’s lab has developed a novel cell culture passage model that mimics the course of infection in vivo. The developed model simulates the interaction of *Mycobacterium avium subspecies paratuberculosis* (MAP) with the intestinal epithelial cells (MDBK), followed by infection of macrophages and return to the intestinal epithelium. Dr. Bermudez described that the model developed in his lab represents the interaction of MAP with the intestinal epithelial cells, followed by infection of macrophages and return to the intestinal epithelium. His research shows that MAP alters its phenotype during intracellular infection, and the lipid composition of the bacterium changes. The data analysis leads to the hypothesis that these changes may contribute to the shift in the inflammatory response caused by the infected host epithelial cells during the disease progression process.

Another project that is currently being investigated at Dr. Bermudez’s lab is determining the role of luxR homolog gene in invasion of MAP into epithelial cells using *Mycobacterium smegmatis* as a model of infection. The group has shown that the overexpression of LuxR and its dependent genes causes the mycobacterium invade the epithelial cells with greater efficiency than a wild-type invasion.

Moreover, Dr. Bermudez’s lab is currently performing research on the evaluation of prevention of infection by stimulating innate response using *Mycobacterium bovis* as the model of infection. C57 Black mice were immunized with seven recombinant proteins and the mice were challenged with *M. bovis* BCG. The preliminary results indicated that airway immunization protected 90% of the mice, while systemic immunization only protects 15%, furthermore the airway immunization increases IgA in the airway mucus, while the systemic immunization does not.

**Session 2: New generation of diagnostic tests for JD and TB**

**Paul Coussens (Michigan State University), John Bannantine (USDA-NADC)**

2.1 Paul Coussens (Michigan State University)

Dr. Coussens’s lab currently investigates the phenotypic diversity in the immune response against *Mycobacterium avium* subsp. *paratuberculosis* in MAP-infected dairy cows. Dr. Coussens emphasized that with no effective vaccines or treatments for Johne’s disease, characterizing and understanding immune responses to MAP is critical for future control efforts. To gain understanding of immune responses to MAP antigens in vitro, peripheral blood mononuclear cells (PBMCs) from 203 healthy Holstein cows (Johne’s negative, JD-) and 78 ELISA-positive Holstein cows (Johne’s positive, JD+) in eight farms were isolated and PBMCs were cultured in the presence of no stimulation, MAP antigenic stimulation or pokeweed mitogen (PWM) stimulation and immune responses to MAP were characterized using flow cytometry. The study aimed to determine an in-depth immune phenotype of bovine peripheral blood mononuclear cells (PBMCs) and their response to MAP. The study was conducted in eight farms and in Johne’s positive (JD+) and negative (JD-) cows of 2-9 years of age for close to two years.

The results indicated that no B or T cell subsets showed significant activation in response to MAP stimulation when considered in total. Further analysis revealed a large degree of diversity in T cell responsiveness to MAP in culture: some cows demonstrated activation (>5% increase in activated cells), some showed no response (less than a 5% increase or decrease in activated cells) and some demonstrated reduced activation (>5% decrease in activated cells), observed in both in JD- and JD+ cows. However, the proportion of cows with a positive T cell response was significantly higher in JD+ cows and the proportion of non-responding cows was significantly lower. In summary, the data suggested that 1) A large proportion of JD+ cows show no response to MAP stimulation, 2) A large proportion of JD- cows show T cell responses to MAP, 3) Some JD- and JD+ cows show a decrease in reactive T cells to MAP relative to un-stimulated cell culture, and 4) Both positive and negative changes are stable over a 6 month period.

2.2 John Bannantine (USDA-NADC)

Dr. Bannatine discussed the current challenges in reliable diagnostic of *Mycobacterium bovis* infection using traditional serological methods. Dr. Bannatine suggested improvement on the current TB skin test and Interferon-Gamma Release Assays (IGRAs) testes. Another suggestion was to develop a JD test that does not react with TB testing or surveillance, given the close phylogenetic relationship between Map and the human pathogen, *Mycobacterium tuberculosis* (MTB). Based on that, Dr. Bannatine’s lab in collaboration with Dr. Kapur’s lab at Penn State are currently performing research on the application of whole proteome Mtb protein array to identify seroreactive and diagnostic MAP antigens. Since the current serological assays suffer from low sensitivity especially for MAP infected animals during the early stages of infection, using a whole proteome microarray from MTB would enable early detection of MAP. The preliminary results of analyses have identified several candidate MAP proteins of potential utility for the early detection of MAP infection.

**3. Epidemiology and transmission of *Mycobacterial* diseases in animals (JD and bTB).**

3.1 Scott Wells (University of Minnesota)

Dr. Wells started off by questioning if the researchers could find a way to detect pathogens and control pathogen transmission, both within-herd transmission and between-herd transmission. In order to conduct research for within-herd transmission, longitudinal observational studies, dynamic epidemiological models, economic cost models have been evaluated and clinical/test data have been collected. Dr. Wells explained that challenges to continue with this research are improved clinical / test data, human behavior data, and social epidemiologic studies. The other form of transmission is between-herd transmission. Data collected for this form of transmission is
epidemiologic data (location, movements), phylogenetic data (WGS) and the studies focus on social network analysis, integrated within- and between-farm models, and phylo-dynamics. There is a need to improve epidemiologic data, linked phylogenetic data, and methods to analyze/evaluate WGS data. Finally Dr. Wells reviewed the key
questions in this field, listed below:

How to identify source(s) of infection?
How to reduce transmission / control disease?
How to predict / modify human behavior?
How to target surveillance for pathogens?
How to characterize mixed infections within animals?
How to use metagenomics to describe infection in populations?

3.2 Yrjo Grohn (Cornell University)

Dr. Grohn’s presentation was on the Mycobacterial Transmission Dynamics in Agricultural Systems and the integration of Phylogenetics, Epidemiology, Ecology, and Economics.

The main goal of Dr. Grohn’s lab is to develop a quantitative methodology for incorporating whole genome sequence (WGS) data into bacterial transmission models for infectious diseases incorporating *ecology, economics, molecular biology, and epidemiology.* Furthermore, his lab would apply these methods and models towards better understanding of the principles and dynamics governing transmission of mycobacterial infection.

In collaboration with University of Minnesota and University of Glasgow, the research project focuses on three hypothesis: 1) Wildlife and cattle movement play distinct roles in maintaining bovine tuberculosis in the US and UK, 2) MAP transmission in dairy herds is more complex than originally believed, including crucial contributions from the environment, and 3) Farm economics and cost-benefit based decision making affect transmission dynamics and infection control in agricultural systems. The collaboration seeks to identify evolutionary factors affecting the ecology and transmission of important slow transmission mycobacteria via hybrid MAP transmission & economic model where US-UK combined field data will be used.

The preliminary results of the project were in press and have been presented at the International Symposium for Veterinary Epidemiology and Economics in November 2015.

**4. Vaccines for JD and TBc.**

4.1 Fred Quinn (UGA)

Dr. Quinn focused on development, assessment, and implementation of vaccines for JD and bTB. Dr. Quinn reviewed the existing vaccine methods and tools, and reviewed the current research projects in his lab. For JD, several live-attenuated and subunit vaccines are currently being evaluated in field trials in sheep, deer, goats, boar, and the results are being evaluated using ELISA and fecal culture. For bovine TB, Dr. Quinn’s lab is testing the BCG in field trials and BCG+ booster, and rBCG in laboratory trials. Moving on, Dr. Quinn’s lab will concentrate on live-attenuated JD and bTB vaccines (BCG plus various booster types) that do not cross- react with TST or a novel DIVA, which minimizes transmission efficiency.

**5. Education and outreach for JD and TBc.**

5.1 Ken Olson (KEO)

The underlying mission of our outreach plan is to provide veterinarians, producers of potentially impacted species, state and federal policy makers, and other stakeholders with accurate, high quality, up to date, and easy to access information and education to assist efforts that will effectively address mycobacterial diseases.

Activities of the past year include:

* ICP – Coauthored presentation on JD programs in the U.S.
* 2016 JAM and 2017 ADSA Annual Meeting – MDA interest session, material available in press room and registration
* World Dairy Expo – met with 10 dairy trade publications, material available
* USAHA – Display and presentations to JD Committee, State, extension and Federal vets
* Sample sales and sponsorship through AAMD

The participants attended a working lunch and concluded discussion.

**VI. Impacts**

1. Research on the evaluation of prevention of infection by stimulating innate response using *Mycobacterium bovis* as the model of infection.
2. Establishment of model systems that can be used to obtain crucial information that would unveil key aspects of MAP pathogenesis, and would enable the researchers to compare the different phases of the disease between in vitro and in vivo systems.
3. Determining the role of luxR homolog gene in invasion of MAP into epithelial cells using *Mycobacterium smegmatis* as a model of infection.
4. Investigation of the phenotypic diversity in the immune response against *Mycobacterium avium* subsp. *paratuberculosis* in MAP-infected dairy cows.
5. Identification of several candidate MAP proteins of potential utility for the early detection of MAP infection.
6. Detection of pathogens and control pathogen transmission, both within-herd transmission and between-herd transmission.
7. Development of a quantitative methodology for incorporating whole genome sequence (WGS) data into bacterial transmission models for infectious diseases incorporating *ecology, economics, molecular biology, and epidemiology.*
8. Better understanding of the principles and dynamics governing transmission of mycobacterial infection.
9. Development, assessment, and implementation of vaccines for JD and bTB.
10. Providing veterinarians, producers of potentially impacted species, state and federal policy makers, and other stakeholders with accurate, high quality, up to date, and easy to access information and education to assist efforts that will effectively address mycobacterial diseases.