Project No. and Title: NE1201 Mycobacterial Diseases of Animals
Period Covered: 12-2012 to 12-2013
Date of Report: 15-Jan-2014
Annual Meeting Dates: 19-Oct-2013 to 19-Oct-2013

I. Participants

Last Name	First Name	Affiliation
Angulo	Carlos	CIBNOR
Bannantine	John	NADC-USDA-ARS
Bauman	Cathy	OVC, Guelph
Bermodez	Maria	UABC (Universidad AutÑnoma de Baja California)
Bermudez	luiz	Oregon State
Castro	Andres	National University of Columbia
Chamberlin	William	St. Vinvent's Healthcare System, Billings, Montana
Chang	Yung Fu	Cornell University
Coussens	Paul	Michigan State University
De Buck	Jeroen	University of Calgary
Delamora	Alfonso	UABC (Universidad Autonoma de Baja California)
Desautels	Louis	Canwan
Everman	Jamie	Oregon State Univ.
Fecteau	Marie-Eve	University of Pennsylvania, School of Veterinary Medicine
Gardner	Ian	Univ. Prince Edward Island
Grohn	Yrjo	Cornell University (via Adobe Connect)
Hartman	Bill	MN board of animal health
Hines	Murray	University of Georgia
Hovingh	Ernest	Penn State
Kapur	Vivek	Penn State
Lamont	Elise	University of Minnesota
Lein	Don	Cornell, chair of External Advisory Board
Loy	J. Dustin	Univ. of Nebraska
Magombedze	Gesham	University of Tennessee, Knoxville
Matto	Carolita	UABC (Universidad Autonoma de Baja California)
Olson	Ken	AAMD
Patton	Elizabeth	Wisconsin Department of Ag
Ponce	Elisama	UABC (Universidad Autonoma de Baja California)
Robbe-Austerman	Suelee	USDA-APHIS-NVSL (via Adobe Connect)
Sawakao	Hori-Oshima	UABC (Universidad Autonoma de Baja California)
Schukken	Ynte	Cornell University (via Adobe Connect)
Schumaker	Brant	AVMA, UN, WSVL
Sherman	Gary	USDA-NIFA
Shigetoshi	Eda	University of Tennessee Knoxville

Smith	Rebecca	Cornell University (via Adobe Connect)
Sobecki	Brian	Zoetis
Sreevatsan	Srinand	Univ. Minnesota (via Adobe Connect)
Talaat	Adel	Univ. Wisconsin (via Adobe Connect)
Thompson	Gary	Penn State (via Adobe Connect)
Wu	Ching Ching	NTU

The Second Annual Mycobacterial Diseases of Animals Multistate Initiative (MDA) was held in conjunction with the United States Animal Health Association (USAHA) and The American Association of Veterinary Laboratory Diagnosticians (AAVLD) conference on Saturday, October 19, 2013 (7:00 AM to 6:00 PM) in the Sunset Room at the Town and Country Hotel, San Diego, CA. Besides registered participants, the conference drew close to a total of 70 interested researchers, students, and industry participants.

II. Welcoming Remarks:

Dr. Vivek Kapur (Chair; Penn State); Dr. Paul Coussens (Co-Chair; Michigan State); Dr. Gary Thompson (Admin. Advisor; Penn State); Dr. Don Lein (Chair, External Advisory Board; Cornell) each welcomed the participants, reviewed the objectives for the MDA establishment, and emphasized on the role of the annual MDA conference gatherings in highlighting progress and helping define key scientific issues relating to Mycobacterial diseases (primarily Johne's Diease and Tuberculosis) in animals. These diseases have significant impact on US animal biosecurity and food safety, and the conference helps highlight progress in various areas including epidemiology, diagnostics, pathogen biology, immunology vaccine development, microbial genome diversity and evolution, pathogenomics and comparative genomics, functional genomics, host-pathogen interactions, approaches for disease prevention, and the development and delivery of educational and extension programming.

III. Plenary sessions:

The conference was organized as 5 plenary sessions and a poster session.

Plenary session 1: Epidemiology and transmission of mycobacterial diseases in animals
Session Chair: Dr. Ian Gardner (Univ. of Prince Edward Island)

1.1 Dr. Ynte Schukken (Cornell University): Molecular Epidemiology of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in a cohort of dairy cows

Dr. Schukken started by giving an overview of the challenges and opportunities existing in the field of Epidemiology. He then reviewed an ongoing data collection from the longitudinal study done in three states (NY, PA, VT) for approximately 10 years using the mathematical model of infection established by his group. In summary, biannual sera, whole blood DNA, and fecal sampling of +600 cows were collected starting at the first lactation age. Following slaughtering, intestinal samples (Lymph nodes, ileum, ileo-cecal valve) and a fecal sample were also collected. Dr. Schukken's lab used MLSSR and SSR on five loci within and

between hosts to estimate the rate of mutations and to construct Minimum Spanning Trees and Minimum Spanning Networks in cohorts of strains. They found 14 haplotypes, but a very limited set of haplotypes was associated with infection progression to high shedder. The results were further investigated at the NIMBioS, and the models were related to the observed shedding data within the host models of MAP infection. Lastly, the investigation team concluded that the models would need to accommodate multiple shedding patters for both progressors and non-progressors. Dr. Schukken concluded that his team would continue their investigation and research on the JDIP cohort molecular epidemiology of MAP in endemically infected dairy herds. They will also work on developing individual based and within and between host infection dynamics models.

1.2 Dr. Rebecca Smith (Cornell University): Optimizing control of bovine tuberculosis on cattle farms, an economic approach to on-farm outbreak management

Dr. Smith first reviewed the current stochastic model for epidemic disease control of the bTB in cattle herds. She also analyzed the two current testing strategies of animal culling and quarantine length for economic optimization. She concluded that "depopulation" in dairy cattle is expensive, with exceptions if it compared with either the statewide quarantine costs or the cost of spread to other farms. Her research has focused on a faster test-and-removal plan with a 2-month testing interval (although up to 12 is reasonable) and two negative whole herd tests to declare a herd to be bTB-free. Dr. Smith also evaluated the cost for cow-calf herds, and concluded that currently, there is regional variation in preference for depopulation over test and removal. However, longer testing intervals and early detection are the keys needed to ensure clearance of infection in cow-calf herds.

1.3 Dr. Yrjo Grohn (Cornell University): Estimating the effectiveness of vaccination against infectious diseases in food animal populations: A Bayesian modeling and simulation approach

Dr. Grohn's research concentrates on the study design of clinical trial of vaccine and the assessment of vaccination effectiveness in farm animal populations against endemic infectious diseases. The initial study faced issues like change in the proportion of the control and vaccinated animal groups, no comparison herd, and inability to estimate the indirect (herd immunity), total and overall effectiveness of a vaccination program. In order to resolve the issue, Dr. Grohn's research group has designed a Bayesian modeling and simulation approach. The 3-step framework of a Bayesian modeling and simulation approach consists of Step 1 – building a conceptual transmission model with a vaccination program, Step 2 – estimating key epidemiological parameters using Bayesian statistics from a vaccinated herd (population A), and Step 3 – generating a simulated comparison herd (control or placebo population B) with the same herd management and calculating the direct, indirect, total, and overall effectiveness of vaccination. The model was experimented in a simulated case study for a killed whole-cell vaccine against paratuberculosis in dairy herds. Dr. Grohn was able to use the approximate Bayesian computation (ABC with an efficient sequential Monte Carlo method) to estimate key model parameters from the longitudinal prevalence data of the whole herd (adult animals) and the control and vaccinated groups in a vaccinated herd. Finally, they were able to calculate the four types of vaccination effectiveness. For future

studies, Dr. Grohn's research team will be working on adding a number of candidate models to perform model selection, applying stochastic models to allow variability from random structure of data, and using available field data to estimate the indirect, total, and overall effectiveness of a vaccination program for JD research projects.

Plenary Session 2: Extension/Outreach/Human Disease related to mycobacterial diseases in animals
Session Chair: Dr. Ken Olson (Am. Assoc. Mycobacterial Dis.)

2.1 Dr. Ernest Hovingh (Penn State): Johne's & TB Extension & Outreach Challenges & Opportunities

Dr. Hovingh reviewed Penn State extension and outreach goals. The goals are set as development of education materials and delivery plan 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 related to mycobacterial diseases of animals. The program is intended for veterinarians, producers of potentially impacted species, state and federal policy makers, and other stakeholders to provide accurate, high quality, up to date, and easy to access information. Dr. Hovingh stated that this goal might be achieved using websites, resource libraries, etc. remotely, or personal contacts by attending meetings like AABP, AAEV, AVMA, USAHA.

2.2 Ken Olson (AAMD) Johne's Disease Outreach: Education/Outreach
Develop and deliver education and outreach materials to stakeholders

Dr. Olson first reviewed progress made at the extension, education, and outreach components, namely, cooperated with the VBJDCP to address education needs, provided tools for producers, shared information with other scientists, shared information with producers, and shared information with USDA and industry partners. For the VBJDCP Education, his efforts led to Veterinary Certification and Recertification DHIA Modules designed for MAP Screening, MAP Testing, and MAP Basics for Milk Sampling Field and Laboratory Technicians (http://ce.vetmed.wisc.edu/Johnes_Disease_Individual_Courses). Dr. Olson also reviewed the development of education and training tools for producers including Johne's Disease for Beef Producers, Johne's Disease for Goat Producers, Johne's Disease for Dairy Producers in both English and Spanish versions that were developed at the UW-Madison School of Veterinary Medicine. Information regarding JD was shared with attendees at large national and international conferences inculding ADSA/ASAS, AABP, USAHA, Discover Conferences, ICP and World Dairy Expo. The group has worked with WMMB, USDFRC, ADSA, DRPC's, and have held numerous meeting with editors of lay journals and conducted numerous radio interviews. JDIP also has a regular newsletter (e-version on JDIP site), and collaborates with ADSA by providing proceedings for the Searchable Proceedings of Animal Conferences (S-PAC). JDIP representatives also attended meetings with USDA leadership (APHIS, NIFA, ARS,), partner organizations (AVMA, IDFA, NMPF, NASDA, AFBF), and Congressional staff. With the transition of JDIP to MDA, we plan to build on past efforts and include new collaborators in the effort, and continue to identify information needs and seek new ways to provide the information. MDA

objectives are to build awareness of the MDA multistate initiative among stakeholders, share information on what has been and is being done through the multistate initiative with scientists, industry and government leaders, provide information and tools for use by producers.

2.3 Dr. William Chamberlin (St. Vinvent's Healthcare System, Billings, Montana): Evidence continues to accumulate supporting a role for *Mycobacterium avium paratuberculosis* as one of the causes of the Crohn's Disease Syndrome

Dr. Chamberlin began by reviewing evidences that continue to accumulate supporting a role for *Mycobacterium avium paratuberculosis* as one of the causes of the Crohn's Disease Syndrome (CD). CD is associated both with defects in innate immunity and also with MAP. Immune deficiencies predispose to microbial infections. Impaired neutrophil migration seen in CD results from improper cytokine signaling by macrophages. Macrophage function is impaired. MAP infects and perturbs macrophages. Genome Wide Association Studies implicate defective autophagy in Crohn's Disease. Autophagy is an evolutionary conserved cellular mechanism to protect cells from invasive microbes whereby endoplasmic reticulum membranes surround a target, fuse with lysosomes and destroy the invasive microbe. Defective autophagy leads to persistent intracellular infection, dysregulated cytokine signaling, impaired MHC-Ag presentation, dysregulation of the immune network and failure to down-regulate NLP3 inflammasome driven inflammation. Dr. Chamberlin concluded that gene mutations predisposing to Crohn's Disease also predispose to Leprosy and Tuberculosis, and that antibiotic regimens directed against MAP may be dramatically successful. He suggested that future therapies should stimulate autophagy and address whichever microbes are involved. The medical community is mostly unaware of these discoveries. MAP may play a role in other chronic inflammatory diseases currently categorized as being 'autoimmune'.

Plenary Session 3: Diagnosis of mycobacterial diseases of animals
Session Chair: Dr. Vivek Kapur (Penn State)

3.1 Dr. Ian Gardner (UPEI): Diagnosis of Mycobacterial diseases of animals: challenges, successes and expectations

Dr. Gardner started off by describing the Mycobacterial disease diagnosis challenges in animals vs. humans. Testing purposes are more varied in animal populations than human populations, because of the multiple species, diverse matrices, different epidemiological units, and limited resources (incl. funding) to validate tests. In wildlife, there are additional challenges in test validation studies, and there is a need for animal-side rapid tests where timely decisions can be made about interventions e.g. culling. Dr. Gardner also reviewed diagnostic challenges in test evaluation studies for *Mycobacterium paratuberculosis* (MAP), namely, protracted incubation period with latent infection, unpredictable disease progression, low MAP loads in tissues and feces of many animals, and intermittent shedding of MAP in feces. Technical and logistical challenges for Mycobacterial diseases were also reviewed as 1) Many proteins are common to *M. bovis*, MAP, and other mycobacteria isolated from animals, 2) Multiple tests likely still needed to balance sensitivity and specificity for different

testing purposes, 3) Animal-side tests are likely to have greatest utility in many wildlife species for management purposes. There are progresses made in design of validation studies including guidance documents, like OIE Manual Chapter: Principles of Test Validation and cost-effective designs for low prevalence diseases like JDIP community-based test evaluation study. Dr. Gardner proposed modifications to OIE pathway for tests in wildlife species by provisional recognition (national authorities) at stage 2a for an intended purpose(s) for minimum of 30 samples, and by "Sample size credit" for a validated test in a taxonomically related wildlife species. Finally, he reviewed a case study done in the *Mycobacterium bovis* in lions in Kruger National Park, South Africa with an intended purpose of prediction of *M. bovis* infection in clinically affected and healthy lions. STAT-PAK assay was used, as the advantages are fast results (20 minutes), high sensitivity and specificity (Laboratory colleagues), and PPV and NPV (Field colleagues). There are also progresses in reporting in peer-reviewed journals. For instance, developed originally for human health in January 2003, STAndards for Reporting of Diagnostic (STARD) accuracy studies (www.stard-statement.org), and STandards for Reporting of Animal Diagnostic Accuracy Studies – paratuberculosis (STRADAS). STARD is a set of guidelines for transparent and complete reporting, but it is not prescriptive about how a study is designed or analyzed. Dr. Gardner concluded that it is expected that STARD will be adapted to other important animal infectious diseases, and standards for reporting of latent class analyses will be developed for general guidance.

3.2 Dr. Suelee Robbe-Austerman (USDA-DBL-NVSL): Diagnostics for Johne's Disease and Tuberculosis

Discoveries impacting diagnostics since 1882 was first reviewed by Dr. Robbe-Austerman. Next, the existing diagnostic assays for the Antemortem, like cell mediated immune assays, antibody detection assays, and organism detection assays, and for the Postmortem, including necropsy, slaughter surveillance, and finally fingerprint, or genotyping assays were evaluated, and a question was raised as what our Gold standards are. For instance, CMI-skin test, IGRA has ~ 97% specificity and ~ 85% sensitivity for the TB, and ~ 97% specificity and ~ 70-75% sensitivity for the JD. However, the performance of these tests varies widely among species, and in i*n vivo* vs. *in vitro* assays. In contrast, antibody tests are higher in sensitivity/specificity, but the performance varies widely between species. The sensitivity is also low in early stages of infection, and there are many different platforms, CF, AGID, ELISA, lateral flow, arrays/microchips. Dr. Robbe-Austerman concluded the talk by stating the research opportunities to improve diagnostic tests, antigens, extraction, DIVA vaccines, and the importance of developing evidence based consensus statement on diagnostic test selection and usage.

3.3 Dr. John Bannantine (USDA-ARS): New developments and opportunities in JD diagnostics

Dr. John Bannantine talked about new opportunities for Johne's disease diagnostics. He emphasized the need to capitalize on the foundations laid by other members of JDIP for standardized test reporting. The JDIP sample repository was discussed in terms of what it contains, its value to the research community and beyond, as well as how it should best be

used. This talk was concluded with an examination of four of the newest, most exciting developments in Johne's disease diagnostics based on the scientific literature.

Plenary Session 4: Biology and pathogenesis of mycobacterial diseases in animals
Session chair: Dr. Luiz Bermudez (Oregon State)

4.1 Dr. Luiz Bermudez (Oregon State): Biology and Pathogenesis of Mycobacterial Disease in Animals: Overview: Challenges and Opportunities

Dr. Bermudez started off by reviewing the biology of infection in animals. Mycobacterial infections in animals are acquired from the environment or from infected hosts. *Mycobacterium avium* subsp *paratuberculosis* (MAP), *M. avium* subsp *avium* and *M. avium* subsp *hominissuis* are the most common environment-related pathogens, while *Mycobacterium bovis* is the most frequently observed mycobacteria transmitted from host to host. All of them represent challenges in term of diagnosis, treatment and prevention. The improvement in knowledge about the mechanisms of pathogenesis and host response will likely allow for the development of alternative forms of prevention and treatment. Dr. Bermudez then stated that the efficient transmission of *M. bovis* relay on the phenotype acquired in the lung granuloma. The acquisition of MAP depends on a presence of a phenotype developed in the infected host milk and/or intestines. The conclusion would be that because there is a change in bacterial structure, antigens and phenotypes during host infection. Thus, one can begin to understand the enormous difficulty to create reliable diagnostic test as well as effective vaccines. Although attempts have been made, the products are sub-optimal. In conclusion, new technology has allowed generating extraordinary amount of information that needs to be analyzed. Disease models cannot only help us to answer very difficult questions, but also create future research paths.

4.2 Jeroen De Buck (University of Calgary): Paratuberculosis Pathogenesis

Dr. De Buck reviwed the 4 stages that animals infected with *Mycobacterium avium* subsp. *paratuberculosis* slowly go through. During all of these stages MAP interacts with its host at the molecular level. These interactions, together called the interactome, are believed to modulate the immune response, while the specific interactions evolve during the progression of the infection. Soon after invasion of the intestinal tissues, the host's immune cells come under the influence of the pathogen. The more efficient this subversion of the immune system is, the more the immune response will falter and become inadequate to eliminate the pathogen. However, as of yet, it still seems impossible to clearly separate the host immune responses directed against the pathogen from the pathogen's influence on this response. Through several survival mechanisms MAP avoids the generation of a protective immune response. These mechanisms include inhibition of phagolysosome fusion, inhibition of T cell signaling, inhibition of antigen presentation, down- regulation of leukocyte trafficking, modulation of apoptosis, together leading to a dampening of the immune response and eventually to a tolerance to the pathogen in the intestinal and lymphoid tissues. This host-pathogen interactome has recently been studied in great detail at the cell, tissue and entire animal level by transcriptomic and kinomic analyses by several research groups. Complex analyses, including System Bayesian network modeling have been applied to longitudinal gene expression data. As a result, several signaling pathways and important mechanistic

genes have been identified that play a central role in the response against MAP infections. It is attractive to speculate that exactly these identified regulatory mechanistic genes are the (in) direct target of MAP's deliberate interaction with the host's Pathogen Recognition Receptors and other molecules. Dr. De Buck suggested elevating our understanding of paratuberculosis pathogenesis from the descriptive level to the mechanistic-explanatory level, including MAP's virulence factors, and regulatory mechanisms. A high level overview is given of some of these interactions and the resulting dampening immune responses. Emphasis is given to the variable efficiencies of different MAP isolates to subvert the immune system and to the modulating effect of differences in inoculum dose in experimental infection models. Finally, some unexplored opportunities and approaches were highlighted to address the many remaining questions in the pathogenesis of paratuberculosis.

4.3 Srinand Sreevatsan (Univ. Minnesota): Population Genomics of *Mycobacterium bovis*

Dr. Sreevatsan stated the Mycobacterium tuberculosis Complex are 99.9% identical at nucleotide sequence level and have identical 16S rRNA, however, exhibit a spectra of phenotypic characters including host range & virulence. Given the threat of bovine tuberculosis a rapid method to study genetic relationships among strains is needed. A study was conducted to show that genotyping based on SNPs discovered in the *M. bovis* genome is sufficient to resolve strain phylogeny and establish trait-allele association. Two sets of experiments were performed, all isolates previously characterized using standard typing techniques, and SNP Genotyping: iPLEXTM chemistry –SEQUENOM MASSARRAY platform. Phylogenetic analysis was performed. The results indicated that unique SNPs and LSPs (large sequence polymorphisms) in the genomes of *M. bovis* cattle and elk strains drive their host adaptation. Next, whole genome sequencing of two virulent *M. bovis* isolates from the USA were performed along with comparative and functional genomics of *Mycobacterium bovis*. Dr. Sreevatsan concluded with the results of his study as 1) Genomes of 2 USA strains, MBO Corsentino and MBO NE Elk have been sequenced and draft- assembled, 2) 1139 and 1184 SNPs identified in the cow and elk *M. bovis* genomes respectively, 3) No unique LSPs / genes were identified, 4) Genomes are highly clonal and share over 99% sequence identity with reference strain and other members of MTC group.

Plenary Session 5: New generations of vaccines for mycobacterial diseases in animals
Session Chair: Murray Hines (UGA)

5.1 Paul Coussens (Michigan State): *Mycobacterium paratuberculosis* and the bovine immune system

Dr. Coussens started off by reviewing the *Mycobacterium paratuberculosis* infection subsections, as early infection, subclinical, and clinical infection. Dr. Coussens's research is based on the macrophages in the early infection stage, as the macrophages pre-activated by IFNγ are resistant to persistent infection. Proper macrophage-T cell communication depends upon a complex set of interactions between cell surface receptors and a network of intracellular signals. The goal of most vaccines is to mimic this process with a non-pathogenic stimulant and create immunological memory. MAP infected macrophages produce immune suppressive *IL-10*, and have critical defects in CD40 signaling resulting in Th1-like immunity, and large production of inflammatory *IL-1*, and the symptoms of *IL-1*

toxicity are very similar to clinical Johne's disease. Moreover, Affymetrix microarray data shows that MAP has a profound effect on macrophage gene expression at early times post-infection. Dr. Coussens showed that bioactive *IL-10* inhibits IFNγ production in PBMCs stimulated with MAP, and that neutralization of *IL-10* enhances MAP antigen specific inflammatory responses. Dr. Coussens concluded that, apoptosis of MAP infected macrophages are critical for proper immune responses, as the intracellular pathogen clearance is tied up to cell death pathways. In final, Dr. Coussens presented the results of his research on MAP infection related to vaccines production. He emphasized that any vaccine against MAP should allow infected macrophages to rapidly undergo apoptosis, increasing efferocytosis and proper antigen presentation by other phagocytic cells. Also MAP vaccines should not enhance *IL-10* production or CD40 signaling, and should not induce *IL-1* production in macrophages, or induce *Treg* development.

5.2 Adel Talaat (Univ. Wisconsin): Human and Bovine TB Vaccines

Dr. Talaat presented statistics on the prevalence of Bovine TB (BTB) as a global problem, and presented some information on the host and the risk factors associated with the BTB. As control measures, it is important to follow USDA recommendations and enzootic areas. The key questions are the pathogenesis and strain evolution of human TB, epizoology/epidemiology of BTB, and understanding the best approach to control human TB using drugs vs. vaccines. The obstacles of the BTB vaccines are delivery, efficacy, DIVA, and risk to human. There are current TB vaccines in clinical trials. Dr. Talaat concluded his talk by emphasizing that for the vaccines against Mycobacteria, it is necessary to have better models for vaccine testing and a better delivery for BTB vaccines, and the option of the therapeutic vaccines.

5.3 Dr. Murray E. Hines II (University of Georgia): Johne's disease vaccine efficacy study and validation of a caprine vaccination and challenge model

Dr. Hines emphasized on the importance of development of a *Mycobacterium avium subspecies paratuberculosis* (MAP) vaccine that reduces the incidence of clinical disease and/or fecal shedding of MAP to control Johne's disease (JD). He stated that the objectives of this study were 1) to evaluate the efficacy of 5 attenuated strains of MAP as vaccine candidates alongside one commercially available MAP vaccine (Silirum®, Pfizer) using the protocols and endpoints proposed by the Johne's Disease Integrated Program (JDIP) Animal Model Standardization Committee (AMSC), and 2) to validate the AMSC Johne's disease goat challenge model (see Hines et al., 2007b). Eighty goat kids were vaccinated orally twice at 8 and 10 weeks of age with one of the experimental vaccines or once subcutaneously at 8 weeks with Silirum®, or an oral sham control vaccine consisting of goat milk. Kids were challenged orally with a total of approximately 1.44 X 109 CFU divided in 2 consecutive daily doses using a bovine MAP K10-like isolate (ATCC-700535). Immunological tests performed included Agar Gel Immunodiffusion (AGID), ELISA, and cell mediated response by comparative purified protein derivative (PPD) skin testing (*M. avium*, Johnin and *M. bovis* PPD's). Kids within each group were euthanized and necropsied at 13 months post challenge. The study's results indicated all challenged kids had gross and/or microscopic lesions compatible with JD suggesting none of the vaccines prevented infection. However, there

was a marked reduction in fecal CFU/g and necropsy lesion score in the group given the Silirum® vaccine and a lesser reduction in the 329 vaccine group. A marked reduction in MAP CFU/g and PCR percent positivity was also detected in necropsy tissues from kids given the Silirum® vaccine, and increased CFU/g were detected in tissues from kids given the 315 and 319 vaccines vs. the positive control group. Vaccination also resulted in false-positive PPD skin test reactions for *M. avium* PPD and Johnin. These data show Silirum® was the best performing vaccine followed by attenuated vaccine strain 329. Furthermore, the goat challenge model for Johne's disease has been validated.

IV. Poster presentations (Golden Pacific Ballroom):

There were also 13 posters presented at the morning and afternoon poster sessions:

1. The New Mycobacterial Diseases of Animals (MDA) Multistate Initiative

   K.E. Olson[1], V. Kapur[2], P. M. Coussens[3] and D. H. Lein[4]

   1 Am. Assoc. Mycobacterial Dis.2 Pennsylvania State University3 Michigan State University4 Cornell University

2. Comparison of Prionics® PC-HP and Prionics® PC-HP Peptide Cocktails and Purified Protein Derivatives for Use in the BOVIGAM® and BOVIGAM® 2G Assays.

   B. Schroeder[1], R. Hardegger[1], K. E. Bass[2, 3], B. J. Nonnecke[3], M. V. Palmer[3], T. C. Thacker[3], W. R. Waters[3] J.-L. Moyen[4], Marcus Doherr[5], E. Gormley[6] M. Vordermeier[7], and A. J. Raeber[1]

   [1]Prionics AG, Wagistr. 27A, 8952 Schlieren, Switzerlan[2]Iowa State University, Ames, IA, USA[3]United States Department of Agriculture, Agricultural Research Service, National Animal Disease Cen ter, Ames, IA, USA[4] Laboratoire Départemental d'Analyse et de Recherche, Coulounieix-Chamiers, France,[5]Vetsuisse Faculty, University of Bern, Switzerland[6]University College Dublin, Ireland, 4Martin Vordermeier, AHVLA Weybridge UK,[7]AHVLA, Weybridge; UK

3. *In silico* epitope analysis of unique and membrane associated proteins from *Mycobacterium avium* subsp. *paratuberculosis* for immunogenicity and vaccine Evaluation

   Perla Carlos-García1, Kris Huygen2, Felipe Ascencio1, Virgine Roupie2, Gracia Gómez-Anduro1, **Carlos E. Angulo-Valadez1**

   1Centro de Investigaciones Biológicas del Noroeste, SC. Instituto Politécnico Nacional 195, Playa Palo de Santa Rita Sur, La Paz, B.C.S. C.P. 23096, México
   2Scientific Service Immunology, Scientific Institute of Public Health WIV-ISP (Site Ukkel), 642 Engelandstraat, 1180 Brussels, Belgium

4. Biomarker discovery by gene expression profiling of *Mycobacterium avium* subsp. *paratuberculosis* infected calves at 3, 6 and 9 months after infection

   Jeroen De Buck
   Veterinary Medicine, University of Calgary

5. Pharmacokinetic of gallium nitrate and gallium maltolate after oral administration in neonatal calves.

   Marie-Eve Fecteau, Caroline S. Monk, Lawrence R. Bernstein, and Raymond W. Sweeney.
   University of Pennsylvania, School of Veterinary Medicine

6. Agent-based model for Johne's disease dynamics in a dairy herd

   Shigetoshi Eda, Jessica Robins, Sarah Bogen, Annet Westhoek, Auldon Francis, Andrew Kanarek and Suzanne Lenhart
   University of Tennessee Knoxville

7. ‘Bio-load’ and bio-type profiles of *Mycobacterium avium* subspecies *paratuberculosis* infection in the farm and farmer’s herds / flocks of domestic livestock of the country: A 28 years study (1985–2013)

S.V. Singh[1], P. K. Singh[2], A.V. Singh[2], J. S. Sohal[3], N. Kumar[1], K. K. Chaubey[1], S. Gupta[1], A. Kumar[4], A.K. Bhatia[5], A.K. Srivastav[5], K. Dhama[6]
[1]Microbiology Lab, Animal Health Division, Central Institute for Research on Goats, India[2] National JALMA Institute for Leprosy and Other Mycobacterial Diseases, India[3] Canadian Food Inspection Agency, St. Hyacinthe, Quebec, Canada[4]School of Life sciences, Khandari, Agra[5]Dept of Microbiology and Immunology, College of Veterinary Science & A.H., U.P[6]Division of Pathology, Indian Veterinary Research Institute, India

8. First mass screening of the human population to estimate the bio-load of *Mycobacterium avium* subspecies *paratuberculosi*s in North India

Shoor Vir Singh[1], Naveen Kumar[1], Jagdip Singh Sohal[2], Ajay Vir Singh[3], Pravin Kumar Singh[3], Narottam Das Agrawal[1], Saurabh Gupta[1], Kundan Kumar Chaubey[1], Rajib Deb[4], Kuldeep Dhama[5] and Krishna Dutta Rawat[1]
[1]Microbiology Lab, Animal Health Division, Central Institute for Research on Goats (CIRG), India[2] Canadian Food Inspection Agency, St. Hyacinthe, Quebec, Canada[3] National JALMA Institute for Leprosy and Other Mycobacterial Diseases, India[4]Animal Genetics and Breeding, Project Directorate on Cattle, Indian Council of Agricultural Research, India[5]Division of Pathology, Indian Veterinary Research Institute (IVRI), India

9. Johne's disease diagnostics during the first 18 months of infection in a goat model

Torsten Eckstein,
University of Colorado

10. Elastin, a Novel Extracellular Matrix Protein Adhering to Mycobacteria Antigen 85 Complex

Chih-Jung Kuo1, Christopher P. Ptak2, Ching-Lin Hsieh1, Bruce Akey1, and **Yung-Fu Chang**1
1Department of Population Medicine and Diagnostic Sciences, and 2Department of Molecular Medicine, College of Veterinary Medicine, Cornell University

11. Paratuberculosis in the small ruminant dairy industry of Ontario, Canada

Cathy Bauman
Ontario Veterinary College

12. Host-*Mycobacterium avium* subsp. *paratuberculosis* Interactome Reveals a Novel Iron Assimilation Mechanism linked to Nitric Oxide Stress during Early Infection

Elise A. Lamont, Wayne W. Xu, and Srinand Sreevatsan,
University of Minnesota

13. Mathematical modelling of the Th1/Th2 immune response in Mycobacterium avium subspecies paratuberculosis infection in ruminants

Gesham Magombedze, Shigetoshi Eda and Vitaly V. Ganusov,
University of Tennessee

Closing Remarks:

Dr. Kapur thanked all the presenters and the participants and a brief discussion of next steps was held.

The meeting was adjourned at 6:00 pm.

V. Accomplishments:

Investigators have made considerable progress in the following areas:

Dr. Grohn leads the epidemiology research group, as they concentrate on the study design of clinical trial of vaccine and the assessment of vaccination effectiveness in farm animal populations against endemic infectious diseases. His research group has also designed a 3-step framework of a Bayesian modeling and simulation approach, and the model was experimented in a simulated case study for a killed whole-cell vaccine against paratuberculosis in dairy herds. Dr. Grohn was able to estimate key model parameters from the longitudinal prevalence data of the whole herd (adult animals) and the control and vaccinated groups in a vaccinated herd. Extension is led by Dr. Ken Olson, as they continue to collaborate with the VBJDCP to address education, share information, and provide tools for producers, other scientists, industry partners, and USDA. Dr. Olson also attends DC meetings with USDA leadership (APHIS, NIFA, ARS,), partner organizations (AVMA, IDFA, NMPF, NASDA, AFBF), and Congressional staff. Furthermore, his efforts concentrate on the awareness of the MDA MI among stakeholders, industry and government leaders. Dr. John Bannatine leads the diagnosis research group as they continue to research on the newly identified MAP specific monoclonal antibody, 17A2 that can for the first time differentiate MAP from other Mycobacterium. JDIP Vaccine development led by Dr. Murray Hines has reached its final stage, and publications are in the process. The data from the vaccine study will be published in the *Frontiers in Microbiology Journal*.

VI. Impacts

1. Application of an established mathematical model of infection in data collection from the longitudinal study done in three states (NY, PA, VT) for approximately 10 years.

2. Implementation of a faster test-and-removal plan with a 2-month testing interval in epidemic disease control of the bTB in cattle herds.

3. Establishment of a Bayesian computation (ABC with an efficient sequential Monte Carlo method) modeling and simulation approach to estimate the effectiveness of vaccination against infectious diseases in food animal populations.

4. Evaluation of vaccine efficacy and development of standard evaluation in the validation of vaccine candidates

5. Comparison and evaluation of the fully sequenced two USA virulent *M. bovis* isolates.

6. Establishment of a sample repository from JDIP samples for further testing and evaluation.

7. Evaluation of vaccine candidates against MAP in controlled blinded studies.