M47 Development of a lab-on-a-chip immunoassay system for diagnosis of Johne's disease. A. Wadhwa*[1], K. Yang[1], X. Liu[1], J. Bannantine[2], S. Eda[1], and J. Wu[1], *[1]University of Tennessee Knoxville, Knoxville, [2]United States Department of Agriculture, Ames, IA.*

Johne's disease (JD) is caused by infection of mostly ruminants (including dairy cattle) with *Mycobacterium avium* ssp. *paratuberculosis* and is responsible for a significant economic loss to the US dairy industry. Diagnosis of JD is currently conducted in diagnostic laboratories, creating dairy farmers costly expenses for veterinary service, sample handling, and shipping. An automated on-site diagnostic device for JD would reduce the diagnosis-related costs. Lab-on-a-chip (LOC) technology has been used in various analytical processes and is offering opportunities for the development of on-site diagnostic devices. In this report, we developed and tested a LOC immunoassay system based on AC electrothermal (ACET) effect for detection of JD-specific antibodies in bovine serum samples. The LOC used in this study was composed of poly-dimethylsiloxane microchannels sealed over an ACET electrode chip. The surface of the ACET electrode chip was coated with *M. paratuberculosis* antigen and, after blocking uncoated surface, reacted sequentially with bovine serum sample and fluorescently labeled secondary antibody. Liquid flow was electrically controlled by ACET micropumping effect. The level of antibody binding was then measured by using a LED-induced fluorescence with a low cost mini-spectrometer. JD-positive and JD-negative serum samples were tested with this LOC immunoassay system. Antibody binding in JD-positive serum was detected by this ACET-based system after loading the serum and secondary antibody for 3 min each. Without the ACET effect, the antibody binding was not detectable after the 3-min reactions. The level of antibody binding in the JD-positive serum was greater than that of JD-negative serum. Higher antibody binding was observed at the electrode edges and we assumed that ACET caused a swirl of liquid around the electrode and thereby accelerated the antibody-antigen interaction. This assumption was supported by our theoretical prediction and numerical simulation. This ACET-based LOC immunoassay may form a basis for the development of an on-site JD diagnostic method.

Key words: Johne's disease, diagnosis, lab-on-a-chip

M48 Immune activation after immunization of neonatal calves with a commercial heat-killed vaccine. J. R. Stabel*[1], W. R. Waters[1], J. P. Bannantine[1], and K. Lyashchenko[2], *[1]USDA-ARS-National Animal Disease Center, Ames, IA, [2]Chembio Diagnostic Systems, Medford, NY.*

A major drawback of current whole-cell vaccines for *Mycobacterium avium* ssp. *paratuberculosis* (MAP) is the interference with diagnostic tests for bovine tuberculosis and paratuberculosis. The current study was designed to explore cross-reactivity of the current USDA commercial vaccine for MAP with diagnostic tools for bovine TB and to assess host responses to vaccination. Neonatal dairy calves were assigned to treatment groups consisting of: 1) Control – no vaccine (n = 5); and 2) Vaccinate – Mycopar vaccine (n = 5). Peripheral blood mononuclear cells were isolated before and after vaccination and stimulated in vitro for measurement of interferon-(IFN)-γ, interleukin (IL)-4, IL-10, and IL-12, and to assess differences in lymphocyte populations by flow cytometry. Results from this study demonstrated a rapid initiation of MAP-specific IFN-γ in Vaccinate calves by 7 d, with robust responses continuing throughout the study. Vaccinate calves also had IFN-γ responses to BoPPD, with moderate reactivity to ESAT-6/CFP-10, an *M. bovis* recombinant fusion protein. Interestingly, IL-4 and IL-10 were markedly decreased in Vaccinate calves only on d 7 and 14 of the study and thereafter were similar to Controls. Vaccinate calves began to seroconvert at 4 mo with all calves having detectable MAP antibody by 6 mo. Only one Vaccinate calf had a positive (suspect) skin test response to *M. bovis* PPD and none of these calves reacted in *M. bovis* serologic tests. These results suggest that vaccination with Mycopar will interfere with diagnostic tools for the detection of paratuberculosis but have low interference with *M. bovis* diagnostics.

Key words: *Mycobacterium avium* ssp. *paratuberculosis*, cattle, bovine tuberculosis

M49 Phenotype array analysis of *Mycobacterium avium* ssp. *paratuberculosis* K10 phoP mutant and wild-type. J.-W. Chang, J. Scaria, and Y.-F. Chang*, *Cornell University, Ithaca, NY.*

Mycobacterium avium ssp. *paratuberculosis* (MAP) is recognized as a broad host range mycobacterial pathogen with the ability to initiate and maintain systemic infection and chronic inflammation of the intestine of a range of histopathological types in many animal species. Even though MAP can survive in a variety of environments, it is extremely slow growing and fastidious. A better understanding of the complete physiology of MAP can lead to novel preventive strategies and identification new vaccine candidates in MAP genome. Therefore we have compared the complete metabolic parameters of MAP phoP mutant and its wild-type. A phoP mutant was constructed using allelic exchange method in MAP strain K10. Biolog phenotype MicroArray (PM) is a respiration-based assay system that can test up to 2,000 phenotypic traits simultaneously. The complete metabolic profile of the MAP mutant and wild-type was obtained by screening against Biolog phenotype microarray (PM) panels 1 through 8. For each PM panel, 30% increase in signal intensity over negative control was considered positive. The PM analysis revealed that deletion of phoP had a global impact on MAP metabolism. The greatest impact of phoP deletion was on utilization of nitrogen, sulfur and phosphate sources. Likewise phoP deletion severely impaired the ability of MAP to utilize nutritional supplements, such as hematin, thymine, deferoxamine and N-Acetyl-D-Glucosamine. Alteration of utilization of carbon sources such as L-Arabinose, Acetoacetic acid, D-Psicose and Pyruvic acid was also observed. In several bacteria it has been established that PhoPR 2-component system is the master regulator and in *Mycobacterium* tuberculosis PhoPR 2-component system is essential for virulence. Our results in the present study are consistent with these observations and a detailed study of metabolism related genes identified in this analysis can be good candidates for drug intervention or vaccine development.

Key words: *Mycobacterium avium* ssp. *paratuberculosis*, phenotype microarray, phop mutant

M50 Characterization of monoclonal antibodies specific for molecules uniquely expressed on bovine dendritic cells. G. S. Abdellrazeq*[1], S. Tomida[2], and W. C. Davis[2], *[1]Alexandria University, Edfina, Behara Province, Egypt, [2]Washington State University, Pullman.*

Progress in elucidating the function of dendritic cells (DC) in cattle has been limited by the availability of monoclonal antibodies (mAbs) that

identify lineage restricted molecules expressed on DC. In this report, we describe the development and characterization of a set of mAbs (LND25A, LND41A, DC5A, and DC77A, all IgG1 isotype) that react with molecules expressed on DC. Initial analysis using flow cytometry show the mAbs label a population of CD14-MHCII+CD11c+ cells that comprise less than 1% of peripheral blood mononuclear cells. Cross comparison of specificity of the mAbs using Zenon IgG1 s step reagents conjugated with different fluorochromes showed the mAbs recognize 2 different molecules, one recognized by LND25A and LND41A the other recognized by DC5A and DC77A. In vitro studies with cultures of adherent cells derived from peripheral blood mononuclear cells revealed LND41A labeled a population of loosely adherent cells with the phenotypic features of DC5. The cells possessed numerous dendrites similar to those seen on DC. Preliminary use of LND41A to identify DC present in the ileum of cows at the clinical stage of Johne's disease showed that the mAb recognizes a population that is distinct from macrophages. Further studies are in progress to show the mAbs recognize molecules expressed on all DC.

Key words: dendritic cells, monoclonal antibodies, Johne's disease

M51 Identification of *Mycobacterium avium* ssp. *paratuberculosis* genotypes on Alberta dairy farms with high-resolution melt analysis of multiallelic short sequence repeats. J. David, R. Mortier, H. Barkema, and J. De Buck*, *Dept. of Production Animal Health, Fac. Veterinary Medicine, Calgary, Alberta, Canada.*

Disease prevention through epidemiology-based management practices might be the best option for Johne's disease (JD) control at the moment. This strategy depends partly on a thorough understanding of the genotypic diversity of MAP in Canadian herds as natural strain variants may possess unique pathogenicities that may require tailored management practices. Strain discrimination is also important for epidemiological investigations to understand origins of infection and identify risk factors that influence transmission. Molecular epidemiology might also allow visualization of how specific genotypes are more successful in spreading. In this study we aimed to develop an accurate and rapid genotyping technique based on known short sequence repeat (SSR) variants and to identify the genetic variability of MAP within Alberta. Methods: Serum, milk and fecals were collected from 1917 individual animals over 3 years of age on 24 dairy herds in Southern Alberta. Fecals were cultured using the liquid culture ParaJem system. Positive cultures were subcultured on solid media to obtain single colonies. Genomic DNA from these pure isolates was prepared for genotyping. Apart from sequencing of 3 SSR loci (G1, G2, GGT), high resolution melt (HRM) assays were developed to identify the allelic diversity. Results: Thirty-five isolates were obtained from 10 of the herds. Respectively 3, 3 and 2 alleles were discovered for the 3 SSR loci, resulting in several different genotypes. HRM proved more reliable than sequencing due to problems to resolve the exact number of guanine repeats in G1 and G2 by the classical approach. Farms with multiple genotypes were discovered. The same herds are currently being resampled. Genotyping of the new isolates will allow us to determine the true genotypic diversity on those farms more accurately. Genotyping using SSR HRM analysis is a useful tool to elucidate the distribution of MAP genotypes in a geographic area.

Key words: paratuberculosis, genotyping, transmission

M52 Complete genome sequence of a *Mycobacterium avium* subspecies *paratuberculosis* Isolate from a patient with Crohn's disease. L. Li*[1], J. P. Bannantine[2], S. Sreevatsan[3], and V. Kapur[1], *[1]Penn State University, University Park, [2]National Animal Disease Center USDA-ARS, Ames, IA, [3]University of Minnesota, St. Paul.*

Mycobacterium avium subspecies *paratuberculosis* (MAP) has been identified in some human patients with Crohn's disease. To identify genetic differences between MAP isolates recovered from humans and those associated with bovine Johne's disease, we characterized the complete genome sequence of strain MAP4 recovered from the breast milk of a Crohn's disease patient. Massively parallel sequencing approaches were used to generate a total of 88.5 million base pairs from a randomly sheared MAP4 genomic DNA library, which were assembled into contiguous sequence fragments with an estimated 60 large (~2kb) and 350 small (<0.5kb) gaps physical or sequence gaps. A primer walking approach with Sanger based sequencing was applied to close all remaining gaps in a iterative manner and areas with low quality sequence re-sequenced to obtain an assembled single high quality genome sequence. Compared with the described previously bovine MAP K10 genome, the size of MAP4 genome is about 3.0kb smaller as a result of several sequence deletions, including in one copy of the insertion sequence element, IS900. Importantly, the analysis revealed no large genome scale rearrangements in MAP4 as compared with strain K10, and ~3kb of deletions and ~300 bp of insertions were distributed across the genome. The results also confirmed the presence of 59 additional SNPs, which together with the 174 SNPs identified in our preliminary studies account for a total of 233 SNPs between these 2 isolates. Interestingly, more than half of the newly identified SNPs were located in 2 genes (MAP1432 and MAP2495), both of which contain repetitive sequences and are orthologs of *Mycobacterium tuberculosis* Rv1173 that encodes a cell wall protein. Taken together, our analysis of the MAP4 and K10 genome sequences confirmed the high similarity between strains from these 2 different mammalian hosts, and suggest a relative paucity of genetic variation among strains recovered from humans and cows.

Key words: *Mycobacterium avium* subspecies *paratuberculosis*, genome sequence, Crohn's disease

M53 *Salmonella* delivery system to develop an efficient vaccine against *Mycobacterium avium* ssp. *paratuberculosis*. S. Chandra, J.-W. Chen, S. M. Faisal, S. P. McDonough, M. A. S. Moreira, C.-F. Chang, and Y.-F. Chang*, *College of Veterinary Medicine, Cornell University, Ithaca, NY.*

Salmonella antigen delivery system is an efficient tool to develop an effective and low-cost vaccine. Since *Salmonella* has the ability to enter inside macrophage cells which are very versatile antigen presenting cell, and can deliver the antigen into cytosol using its type III secretion system. Hence, type III secretion system of *Salmonella* presents an efficient antigen delivery to elicit protective immunity especially cytotoxic T lymphocyte (CTL) response. As *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is an intracellular pathogen, type III secretion system of attenuated *Salmonella* could be a efficient vehicle to deliver MAP's immunogenic antigens into cytosol to induce CTL response for better protection in cattle against MAP infection. To induce better protection, we constructed fusion of immunogenic fragment of Ag85A, Ag85 B, and SOD, and 74F using an efficient delivery component of *Salmonella* (sopE promoter and sopE104) in pSU39 expression vector and tested the delivery efficiency of *Salmonella* expressing pSU39-constructs to deliver MAP antigens(Ag85A Ag85 B, SOD, and 74F) into culture medium via type III secretion system. After that we carried out vaccination experiment

in moue model using C57BL/6 mice. Our results show that *Salmonella* Typhimurium (Δ aroA ; Δ yej) a genetically attenuated strain can deliver MAP antigen via type III secretion system. Further, the animal experiment data proved that *Salmonella* expressing MAP antigen can elicit CTL response to induce protective immunity against MAP infection. Immunological and histopathological analysis show that protective immunity mounted by *Salmonella* delivery system against MAP is equivalent to positive controls. Hence, our data indicate that *Salmonella* antigen delivery system could be proven a better tool to develop an effective vaccine to management of Johne's disease in cattle.

Key words: *Mycobacterium avium* ssp. *paratuberculosis*, delivery system, vaccine

M54 Exploring *M. paratuberculosis* pathogenesis using an in vitro cell culture passage model. J. L. Everman*[1] and L. E. Bermudez[2], [1]*Department of Microbiology, College of Science, Oregon State University, Corvallis,* [2]*Department of Biomedical Science, College of Veterinary Medicine, Oregon State University, Corvallis.*

Mycobacterium avium ssp. *paratuberculosis* (MAP) is the etiological agent of Johne′s disease, a chronic intestinal inflammatory disease that affects ruminants worldwide. Once infected, cattle remain in the subclinical stage of infection for many years before the disease progresses to clinical symptoms. The transition from the subclinical to the clinical stage of the disease has been described as a shift from a T_H1 type response to an antibody-dominated type response; however, the cause for the shift and the onset of the infectious process remains to be determined. We describe an in vitro cell culture passage model in an attempt to gain a further understanding of the changes that occur during the host immune response as well as the bacterial changes that occur during the progression of the disease. By passing MAP through MDBK epithelial cells, RAW 264.7 macrophages, and MDBK epithelial cells sequentially, and utilizing real-time PCR to determine transcript levels of immune signals we have observed that cytokine and chemokine levels do not change after 3 passages through RAW 264.7 macrophages. However, a lower passage number results in a higher level of immune signal transcripts of IL-6 and IL-8. These data, as well as previous findings that demonstrate an increase of an invasive phenotype of MAP after intracellular growth in macrophages, suggest that the serial passage of MAP between macrophage populations may select for a population of bacteria that optimizes infection. A more invasive bacterial population allows for increased survival, while the population also appears to be less inflammatory, resulting in less host damage. To fully understand the mechanisms behind these observations, MAP phenotypes have been obtained and RNA will be extracted for DNA microarray analysis at 1 d and 3 d after infection. This hypothesis could potentially explain why the subclinical phase of the disease persists for so many years and our work begins to decipher the dynamics of antigen expression and host response that occur during the progression of Johne′s disease.

Key words: Johne's disease, paratuberculosis, immune response