

Animal Health-Johne's Disease (JDIP): Johne's Disease

M57 Results from the US National Johne's Disease Demonstration Herd Project: Most important areas from the Johne's risk assessment. C. Fossler* and J. Lombard, *USDA:APHIS:VS, Fort Collins, CO.*

The National Johne's Demonstration Herd Project (NJDDHP) in the United States was initiated to evaluate the long-term feasibility and effectiveness of management-related practices designed to control Johne's disease on dairy and beef cattle operations. The NJDDHP began in 2003 and includes 62 dairy herds and 20 beef herds in 17 states. All herds began with culture-confirmed *Mycobacterium avium* subspecies *paratuberculosis* (MAP) on the operation, and all herd owners agreed to make efforts to control Johne's disease on the operation. Risk assessments, management plans, and testing of herds were completed on an annual basis. Results to date indicate that, for both beef and dairy herds, prevalence of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in the third, fourth, and fifth years of participation was significantly lower than prevalence during the first year of participation. An analysis using Poisson regression was undertaken to identify areas from the risk assessment most important with regard to MAP prevalence. Among the main areas from the risk assessment (which included calving area, preweaned heifers, postweaned heifers, bred heifers, cows and bulls, and additions/replacements), preliminary results indicate that for dairy and beef herds, the calving and preweaned heifer areas appeared to be most important with regard to risk of cattle being MAP-positive. Specific factors related to the calving and preweaned heifer areas will be further assessed to identify practices associated with a greater risk for cattle to be MAP-positive. For dairy herds, preliminary results indicate that making sure udders and legs of cows in the calving area are clean, using individual animal calving areas (or allowing fewer animals in the calving area), and preventing Johne's disease clinical or suspect animals from entering the calving area were most important with regard to control of Johne's disease on dairy operations.

Key Words: Johne's, control, demonstration herds

M58 Evaluation of the next-generation Parachek ELISA for high-throughput detection of Johne's disease in milk and serum samples. P. Schacher¹, A. Zurfluh¹, D. Zwald¹, T. Byrem², and A. J. Raeber*¹, ¹Prionics AG, Schlieren, Switzerland, ²AntelBioSystems Inc, Lansing, MI.

Frequent testing of milk samples for antibodies to *Mycobacterium avium* ssp. *paratuberculosis* (MAP) by ELISA has become an important tool in managing control of Johne's disease in dairy cattle operations. Originally developed for serum samples, Parachek ELISA (also marketed as AntelBio Johne's Milk ELISA) has been validated with bovine milk samples for which it received approval from the USDA in 2006 and is currently marketed in the US by AntelBio. In the last 10 years, Parachek, also known as the AntelBio Johne's Milk ELISA, has been used successfully on more than 750,000 samples within the laboratory network of the Dairy Herd Improvement Association (DHIA). To simplify the use and increase throughput, we have further improved Parachek to make it more user-friendly and allow full automation. The performance of the improved Parachek2 was evaluated on a set of 368 bovine milk samples of which 16 were fecal culture positive, 351 were

fecal culture negative or derived from free herds and from 1 sample no culture result was available. Fecal culture for *M. paratuberculosis* was performed by the TREK ESP para-JEM Culture System II. Parachek2 has a sensitivity of 56% (95% CI of 32% - 81%) and a specificity of 98% (95% CI of 92% - 100%). A comparison of the improved Parachek 2 with the currently used Parachek2 showed an almost perfect agreement with a Cohen's kappa value of 0.93. To enhance throughput Parachek2 was automated on a Beckman Coulter Biomek FXP. The Laboratory Automation Workstation was equipped with a 96-well plate washer and a plate reader which allows for a throughput of up to 16 plates in one working day (8.5 h) starting from serum or milk samples. Comparison of the fully automated system with manual processing showed almost perfect agreement with a kappa value of 0.96. These results demonstrate that Parachek2 can be easily run on an automated system with the same excellent performance as with manual processing of the samples using the Parachek and thus enabling laboratories to save time and freeing staff for other work.

Key Words: Johne's disease, ELISA, milk

M59 Analysis of the immune response to a major membrane protein of *Mycobacterium avium* ssp. *paratuberculosis* in experimentally and naturally infected cattle. G. S. Abdellrazeq*¹, H. M. Rihan², M. J. Hamilton³, A. J. Allen³, K. T. Park³, J. P. Bannantine⁴, J. R. Stabel⁴, and W. C. Davis³, ¹Faculty of Vet Med, Alexandria Univ, Edfina, Rosetta-line, Behera Province, Alexandria University, Egypt, ²Faculty of Vet Med, Mansoura Univ, El Mansoura, Egypt, ³Wash State Univ, Pullman, ⁴USDA-ARS National Animal Disease Center, Ames IA.

The 35 kDa major membrane protein (MMP) of *Mycobacterium avium* ssp. *paratuberculosis* was studied to determine the potential of using MMP to develop a subunit vaccine. A flow cytometric (FC) assay was developed to conduct the study. Comparison of the immune responses to MMP, purified protein derivative (PPD) and soluble antigen (SAg) in experimentally ($n = 5$) infected calves revealed the CD4 response was stronger than the CD8 response to MMP at 3 mo ($P < 0.05$). No significant differences were observed in the CD4 response to MMP compared with PPD and SAg. A significant difference was observed between CD8 response to MMP compared with PPD and SAg ($P < 0.05$). At 12 mo the CD4 response to MMP was still stronger than the CD8 response. The CD4 response to PPD and SAg was significantly stronger than the response to MMP ($P < 0.05$). No significant difference was observed in the CD8 response to MMP compared with PPD and SAg. The CD4 and CD8 response to MMP, PPD, and SAg was significantly higher in naturally infected cows compared with the response in experimentally infected calves, at the preclinical ($n = 3$) and clinical ($n = 5$) stage of disease ($P < 0.05$). The findings show the cell-mediated immune response to MMP develops slowly, as detected by FC, and is not diminished at any stage of infection. These findings indicate that MMP does not elicit a protective immune response in its native form. A strategy is needed to modify MMP so that it elicits a strong early response, essential for blocking the capacity of Map to dysregulate the immune response and cause disease.

Key Words: *Mycobacterium avium* ssp. *paratuberculosis*, MMP, immune response

M60 Flow cytometric and in-house ELISA methods of milk testing for Johne's disease diagnosis. A. Wadhwa*¹, J. P. Bannantine², B. A. Elliot¹, M. C. Scott¹, and S. Eda¹, ¹University of Tennessee, Knoxville, ²United States Department of Agriculture, Ames, IA.

Use of enzyme-linked immunosorbent assay (ELISA) in dairy herds is recommended as a Johne's disease (JD) control measure; however, current ELISA tests suffer low sensitivity. The long-term goal of this project is to develop a sensitive ELISA method of testing milk samples for JD diagnosis. By using a flow cytometric method (FCM), we demonstrated previously that *Mycobacterium avium* ssp. *paratuberculosis* (MAP)-infected cattle produced serum antibodies against surface antigens of MAP. Also, an in-house ELISA test developed using surface antigens of MAP showed a higher level of sensitivity in detecting MAP infections than that of current ELISA tests. The objective of this study is to demonstrate a "proof-of-concept" that surface antigens of MAP can be used for detection of anti-MAP antibodies in milk as well as serum samples. FCM-Intact MAP bacilli were incubated with 48 bovine serum and milk samples and subsequently with fluorescein-labeled anti-bovine IgG secondary antibody. Antibody binding to the surface of MAP was detected by using a flow cytometer. In-house ELISA-Surface antigens of MAP were extracted by a brief treatment of the bacteria with 80% ethanol, coated on a microtiter plate, and reacted with bovine milk samples. Antibody binding to the immobilized MAP surface antigens was detected in an ELISA format by using a horseradish peroxidase-labeled secondary antibody and its substrate. The following conditions were optimized: concentrations of antigen, milk, and secondary antibody for better differentiation of milk samples obtained from JD-positive and JD-negative herds and 6 different mycobacteria were tested as absorbent of cross reactive antibodies. In FCM, a high level of correlation ($r^2 = 0.79$) between antibody binding levels of serum and milk was observed. In the optimized in-house ELISA test, 9 out of 10 JD-positive milk samples showed positive antibody reactions. These results suggest that MAP-infected cattle produce milk antibodies against surface antigens of MAP. Further study may reveal whether a sensitive ELISA test can be developed based on the surface antigens of MAP.

Key Words: *Mycobacterium paratuberculosis*, antigen, diagnosis

M61 Induction of B cell responses upon experimental infection of neonatal calves with *Mycobacterium avium* ssp. *paratuberculosis*. J. R. Stabel*¹, J. P. Bannantine¹, S. Eda², and S. Robbe-Austerman³, ¹USDA-ARS-NADC, Ames, IA, ²University of Tennessee, Knoxville, ³USDA-APHIS-NVSL, Ames, IA.

Animal models are useful for studying host responses to infection and aid in the development of diagnostic tools and vaccines. The current study was designed to compare the effects of different methods of experimental infection: Oral (*Mycobacterium avium* ssp. *paratuberculosis* (MAP) strain K-10; Oral/DXM (pretreatment with dexamethasone before oral inoculation with strain K-10); IP (intraperitoneal inoculation with strain K-10); and Oral/M (oral inoculation with mucosal scrapings from a clinical cow) in neonatal calves. The objective of this study was to determine if infection with MAP over 12-mo period would invoke changes in the percentages of total B cells in the peripheral blood mononuclear cell population and in subpopulations of B cells as determined by CD5, CD25, and CD45RO markers. Over the course of the study, the percentage of total B cells in nonstimulated and antigen-stimulated cell cultures increased ($P < 0.01$) for orally and intraperitoneally infected calves, with the highest percentages noted at 3 and 6 mo post-infection. Oral infection of calves with a clinical strain of MAP (Oral/M) resulted in increased ($P < 0.05$) percentages of CD5^{dim} and CD5^{bright} B cells, regardless of in vitro stimulation, by 9 and 12 mo post-infection. Experimental

infection by all methods resulted in increased ($P < 0.05$) expression of CD25+B cells and CD45RO+ B cells early in the study but the most significant results were observed at 12 mo for calves pre-treated with dexamethasone before oral inoculation with strain K-10 (Oral/DXM) and Oral/M calves. Immunoblot analyses demonstrated the greatest reactivity to a whole-cell sonicate of MAP in sera from IP calves and the lowest was observed in calves orally inoculated with strain K-10. Further evidence of strong MAP-specific antibody responses in the IP calves was demonstrated using the EvELISA method. In summary, the method of experimental inoculation with MAP did affect the induction of B cell subpopulations and the appearance of MAP-specific antibody during the 12-mo study period.

Key Words: *Mycobacterium avium* ssp. *paratuberculosis*, cattle, B cells

M62 Deletion of *relA* attenuates in vivo survival of *Mycobacterium avium* ssp. *paratuberculosis*. K. T. Park*¹, A. J. Allen², M. J. Hamilton¹, A. Grimm¹, H. M. Rihan³, G. S. Abdellrazeq⁴, and W. C. Davis¹, ¹Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, ²Department of Veterinary Clinical Sciences, Washington State University, Pullman, ³Department of Bacteriology, Mycology and Immunology, Mansoura University, Egypt, ⁴Department of Microbiology, Alexandria University, Egypt.

Extensive effort is underway to develop a mutant *Mycobacterium avium* ssp. *paratuberculosis* that can be used as a vaccine to control paratuberculosis in cattle. Studies in mice have shown that deletion of *relA* in *M. tuberculosis* attenuates survival in vivo. Mice infected with the *relA* mutant survived longer with a reduction in bacterial burden and pathology. The objective of the present study was to test the hypothesis that deletion of *relA* also attenuates survival of *Map* in vitro and in vivo. To test the hypothesis we generated a deletion mutant (*relA*) in the K10 strain of *Map*, tagged with the green fluorescent protein gene (*gfp*) and infected calves ($n = 5$). Wild type K10-*gfp* was used as a control ($n = 3$). Analysis in an in vitro assay revealed deletion of *relA* reduced survival in monocyte derived macrophages compared with survival of wild type K10 at 6 d post infection ($12.5\% \pm 6.3$ SD vs. $29.4\% \pm 3.6$ SD) ($P < 0.05$ by Kruskal-Wallis test). Analysis in vivo using a calf cannulated ileum model showed that deletion attenuated survival without altering the immune response to *Map* 3 mo PI. At necropsy, 9 different tissue sites were processed for *Map* culture. No bacteria were detected in any tissue sample ($n = 45$) from calves infected with the mutant at 3 mo PI. In contrast, bacteria were detected in 13 of 27 tissue sites obtained from calves infected with wild type K10 (all 3 animals had at least 4 positive tissues) ($P < 0.05$ by chi-squared test). The findings show that further studies are warranted to determine if a *relA* deletion mutant can be used as a vaccine.

Key Words: live vaccine, paratuberculosis

M63 Microfluidic system for serodiagnosis of Johne's disease. S. Eda*¹, A. Wadhwa¹, J. P. Bannantine², M. C. Scott¹, R. W. Shaw³, and R. S. Foote³, ¹University of Tennessee, Knoxville, ²United States Department of Agriculture, Ames, IA, ³Oak Ridge National Laboratory, Oak Ridge, TN.

Microfluidics (Lab-on-a-chip) technology has been used in various analytical processes, including electrophoresis, single-cell analysis, biochemical assays, and immune assays. The technology offers opportunities for the development of point-of-care diagnostic devices for various infectious diseases. Diagnosis of Johne's disease (JD) is currently conducted in diagnostic laboratories, creating dairy farmers costly expenses

for veterinary service, sample handling, and shipping. An automated point-of-care diagnostic device for JD would reduce diagnosis-related costs and also may improve the accuracy of testing because it would require a minimum of examiner intervention. The long-term goal of this project is to develop a point-of-care serological diagnostic device for JD. For this report, we tested serum antibodies against *Mycobacterium avium* ssp. *paratuberculosis*, the causative agent of JD, using a microfluidic system. In this project, magnetic micro-beads were used as the solid phase for antibody binding reactions. Magnetic micro-beads were treated with ethanol-extracted antigens of *M. paratuberculosis*, serum sample, and then a fluorescently labeled secondary antibody. Antibody binding was subsequently detected by using a flow cytometer. Assay conditions were optimized for higher analytical sensitivity and specificity of the bead-based flow cytometric test. Using the optimized conditions, we then tested JD-negative and JD-positive serum samples in a microfluidic system. Using a well-classified set of 155 serum samples, diagnostic sensitivity and specificity of the flow cytometric test were estimated to be 60.0% and 98.0%, respectively. Five serum samples were tested using a microfluidic system we designed and the results were compared with those of the flow cytometric test on the same set of samples. As a result, a high level of correlation (linear regression, $r^2 = 0.994$) between the results of flow cytometric and microfluidic tests was observed. Our data demonstrated a 'proof-of-the-concept' that JD can be diagnosed by employing a test based on microfluidics technology.

Key Words: paratuberculosis, diagnosis, microfluidics

M64 Evaluation of *Mycobacterium avium* ssp. *paratuberculosis* strains and a locus associated with tissue infection. H. L. Neibergs^{*1}, Y. Schukken², R. H. Whitlock³, A. Pradhan², J. M. Smith⁴, and E. Hovingh⁵, ¹Washington State University, Pullman, ²Cornell University, Ithaca, NY, ³University of Pennsylvania, Kennett Square, ⁴University of Vermont, Burlington, ⁵Pennsylvania State University, University Park.

Mycobacterium avium ssp. *paratuberculosis* (*Map*) strains vary by the presence of multiple copies of fatty acid metabolism genes which are thought to increase the virulence of the mycobacterium and potentially increase the incidence of Johne's disease. We previously identified a locus on chromosome 3 (*ss86341066*) that showed that animals with an A allele were 3 times less likely to be tissue infected with *Map* than those with a C allele (Settles et al. 2009, *Animal Genetics*, 40, 655–662). The objective of this study was to determine if there was an interaction between *Map* strains and the genotypes of hosts with *Map*-infected tissue. *Map* strain types were identified by multilocus short sequence repeat analysis from ileo-cecal lymph nodes, ileo-cecal valve, or ileum and fecal samples from 19 Holstein cows representing 3 herds. *Map* strain types coded as 2, 3, 4, 6, 9, 11 and 12 were identified in 10, 1, 4, 4, 1, 1, and 1 sample, respectively. Three animals had 2 different strains isolated from their tissue and feces (2 animals-type 2 and 4; 1 animal-type 3 and 6). *Map* strains 3, 4, 6, 9, 11 and 12 were combined into one group for analysis due to the small number of observations. A Fishers exact test compared the *ss86341066* allele frequencies with the *Map* strain that infected each animal. For animals infected with *Map* strain types 3, 4, 6, 9, 11 and 12 the frequency of the minor protective allele (A) was 0.0 and the frequency was 1.0 for the major allele (C). For animals with *Map* strain type 2, the frequency of the minor allele (A) was 0.25 and the frequency was 0.75 for the major allele (C). The frequency of the *ss86341066* alleles differed ($P < 0.05$) between animals with *Map* strain type 2 and *Map* strains type 3, 4, 6, 9, 11 and 12. These data suggest that *Map* strain type 2 may be more virulent in the Holstein animals tested than *Map* strain types 3, 4, 6, 9, 11 and 12 and that there

is an interaction between *Map* strains and *ss86341066* associated with tissue infection. Further studies with larger sample sizes will be needed to confirm and further characterize this interaction.

Key Words: Johne's, strains, locus

M65 Genome sequence of a *Mycobacterium avium* subspecies *paratuberculosis* isolate from a patient with Crohn's Disease. L. Li^{*1}, A. Amonsin², S. Sreevatsan³, and V. Kapur¹, ¹Penn State University, University Park, ²Chulalongkorn University, Bangkok, Thailand, ³University of Minnesota, St. Paul.

Mycobacterium avium subspecies *paratuberculosis* (*Map*) has been associated with human Crohn's disease, a chronic inflammatory bowel disease. To identify polymorphic sequences with potential relevance to host specificity and evolution, an isolate recovered from a Crohn's disease patient, MAP4 has been sequenced. Second-generation sequencing approaches were used to generate a total of 88.5 million base pairs of finished sequence, representing an 18-fold coverage of the genome. Currently, the genome has been assembled into ~60 contiguous pieces accounting for ~98% of the K10 genome. Whole-genome comparison of MAP4 with K10 revealed a lack of major large-sequence polymorphisms. A total 171 single-nucleotide polymorphisms (SNPs) were identified, with 152 in coding regions, which is ~6-fold lower than SNPs found in genome comparison of 2 *Mycobacterium tuberculosis* strains. Two-thirds of the SNPs in coding regions were non-synonymous and were primarily found in genes encoding metabolic pathways, cell envelope, and virulence genes involved in invading mammalian cells. Fifty-eight synonymous SNPs were identified and the pattern of synonymous nucleotide substitution between 2 genomes at 4,350 putatively orthologous loci showed a synonymous substitution rate of 3.7×10^{-5} , suggesting a relatively recent divergence between MAP4 and K10. Many of the SNPs identified in MAP4 were verified in 10 isolates from human and cattle and the results indicate that these were not specific in isolates from human hosts. Overall, the comparison of 2 genome sequences confirmed restricted allelic variation in *Map*, and clearly showed the considerable similarity in sequences between *Map* isolates recovered from cattle and humans.

Key Words: *Mycobacterium avium* ssp. *paratuberculosis*, genome sequence, single-nucleotide polymorphism

M66 Impact of vaccination against Johne's disease on lactation performance of dairy cows: Milk production, reproduction and overall culling. J. R. Lima^{*1}, E. Patton², B. Knust¹, J. Bohn³, and S. J. Wells¹, ¹University of Minnesota, St. Paul, ²Wisconsin Department of Agriculture, Madison, ³Veterinary Clinic, Amery, WI.

Our objective was to evaluate the impact of vaccination against Johne's disease on lactation performance measured by milk production, reproductive outcomes and overall culling rate. Three dairy herds in the state of Wisconsin previously identified with *Mycobacterium avium* ssp. *paratuberculosis* (MAP) infection, by a combination of diagnostic tests (serum ELISA and TREK fecal culture) and presence of clinical disease were enrolled in this study. Within each herd, heifer calves at birth were systematically allocated to a vaccination or control group, until 2 cohorts of 50 animals or 10% of the adult cow herd was formed, establishing an overall sample size across herds of 307 animals (vaccinated = 162 vs. control = 145). Vaccination was performed up to 35 d of age. From each of the study cows a fecal sample was collected at first freshening, at 90 d of pregnancy in the first lactation, at 90 d of pregnancy for all following lactations and at time of culling to assess MAP infection. Milk production, reproductive performance and culling

data, from the year 2005 until 2009, were obtained from the dairy herd records and fecal culture results were collected from the laboratory. Animals were classified positive for MAP if the TREK fecal culture score was ≥ 1 . Mixed modeling was used to analyze differences in total milk production per lactation between the 2 groups. Logistic regression was used to evaluate conception at first breeding and Cox regression was used to estimate days in milk (DIM) to conception and days from birth until culling. Vaccination against Johne's disease had no effect on total milk production across lactations, conception at first breeding and DIM to conception. Time to test positive for Johne's disease was statistically significantly longer among vaccinates but no difference was observed in overall culling rate between groups. In this study, vaccination against Johne's disease had no improvement on overall lactation performance.

Key Words: *Mycobacterium avium* ssp. *paratuberculosis*, Johne's disease, lactation performance

M67 Effect of Tri-Lution (T), a synbiotic, on milk production and shedding of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) in a commercial dairy herd. D. M. Albin*, C. Jones-Anding, D. P. Casper, D. A. Spangler, and G. A. Ayangbile, *Agri-King, Inc., Fulton, IL.*

The effects of feeding T (synbiotic) in a dairy herd with Johne's disease on milk production and shedding of MAP (causative agent of Johne's) was evaluated for 10 mos. Thirty-six lactating Holsteins on a commercial dairy farm were divided into 2 groups, T (n = 19) and control (C, n = 17), based on days in milk and fecal shedding of MAP. Tri-Lution was added to a corn silage, haylage, and high-moisture corn diet. Tri-Lution was included at 56.7 g/cow/d for 22 weeks, and then 113.4 g until the end. Milk production and composition data were obtained monthly via Dairy Herd Improvement Association sampling. Fecal samples were collected every other week. Samples were used to assess MAP shedding via polymerase chain reaction. Fat-corrected milk (3.5%, FCM) production was increased for cows fed 56.7 g T within 5 mos compared with cows fed C (33.2 \pm 4.3 vs 26.8 \pm 2.4 kg/d for T and C, respectively). Cows fed 113.4 g T within 3 mos (8 mos total) had greater FCM production than cows fed C (33.0 \pm 3.9 and 25.1 \pm 4.5 kg/d). In addition, when cows were fed 56.7 g T, the decline in milk production was slower than C (-0.6 vs -1.2 kg FCM/cow/mo) and less linear ($r^2 = 0.56$ for T, 0.81 for C). For cows fed T, milk shedding of MAP was reduced by 39%, and incidence of shedding (number of positive samples) was reduced 16%, with 56.7 g T vs C. When cows were fed 113.4 g T, following 56.7, MAP shedding in milk was further reduced 9%, while incidence was further reduced 1%. For cows fed 56.7 g/d T fecal shedding of MAP was reduced by 18%, while incidence was reduced 5%. With 113.4 g T, fecal shedding was further reduced 3%, while incidence was further reduced 8% vs C. Relative standard error of shedding data ranged from 8.7 to 30%. Tri-Lution improved milk production, and reduced milk and fecal shedding of MAP (Johne's), over a 10-mo period. Tri-Lution can be a beneficial management practice on commercial dairy farms with Johne's disease.

Key Words: synbiotic, Johne's disease, milk production

M68 Survivability of *Mycobacterium avium* subsp *paratuberculosis* in grass silage after fermentation and exposure to low pH and high organic acids. S. A. Flis*¹, K. L. Cook², and C. S. Ballard³, ¹*Bourdeau Bros., Middlebury, VT,* ²*USDA-ARS, Bowling Green, KY,* ³*W. H. Miner Agricultural Research Institute, Chazy, NY.*

Mycobacterium avium ssp. *paratuberculosis* (Map) is a pathogen of concern in dairy production due to its ability to withstand harsh conditions

and cause new infections. Infection is a result of ingesting Map from contaminated feed, water, or manure. The study objective was to evaluate the ability of Map to survive low pH and high organic acid concentrations encountered as part of ensiling. Three experiments were conducted to evaluate survivability and the ability to differentiate live and dead bacteria. Study 1 evaluated survivability in grass silage fermented in vacuum bags. Forage was inoculated with live Map, dead Map, or no Map (control) and incubated for 25, 50, 75, or 100d. Fermented forage averaged 4.7 \pm 0.11 pH, 8.3 \pm 2.1 lactic acid (% DM), 3.5 \pm 0.9 acetic acid (% DM), and 0.05 \pm 0.03 propionic acid (% DM). Study 2 evaluated survivability in buffered citric acid solutions of pH 4, 5, 6, and 7. Live Map bacteria were added to solutions and bacterial concentrations were measured at 0, 5, 15, 20, 30, and 35 d. Study 3 evaluated survivability in exudates from control silage in study 1. Exudates were filter sterilized to eliminate background population interference and pure live Map was exposed to exudates for 0, 5, 10, 15, 20, 25, or 30 d. Study 1 found no changes in concentration of Map regardless of number of fermentation days or viability/presence of MAP inoculated. After exposure to citric acid in study 2, samples were analyzed for Map concentration by PCR (total bacteria) and propidium monoazide (PMA; live bacteria). Study 2 found that live MAP concentration decreased as pH decreased and exposure time increased with a 2-fold log reduction for pH 4 at 37d. In study 3, no change in Map concentration was found when bacteria were exposed to exudates. These results indicate that while Map is sensitive to low pH, this only occurs with concentrations of acid higher than experienced with proper forage fermentation. Map present in manure and applied to forage grasses may survive ensiling process and silage may therefore be a potential route of infection.

Key Words: *Mycobacterium avium* ssp. *paratuberculosis*, silage, quantitative PCR

M69 A membrane associated serine protease of *Mycobacterium avium* subspecies *paratuberculosis* plays a role in resistance to phagosomal acid stress. A. Kugadas*¹, H. K. Janagama¹, E. A. Lamont¹, and S. Sreevatsan^{1,2}, ¹*Department of Veterinary Population Medicine, University of Minnesota, Saint Paul,* ²*Department of Veterinary Biomedical Sciences, University of Minnesota, Saint Paul.*

Pathogenic mycobacteria successfully survive in the acidic micro-environment of the phagosome. We hypothesize that *Mycobacterium avium* ssp. *paratuberculosis* (MAP) expresses a membrane associated serine protease encoded by MAP0403, in response to phagosomal acidification and is vital for the intracellular survival. Expression of serine protease by MAP K-10 was studied at 10, 30, and 120-min. post infection of bovine monocyte derived macrophages treated with or without bafilomycin to block phagosomal acidification. MAP serine protease was significantly upregulated exclusively in the acidified phagosomes. Highest level of MAP0403 expression coincided with the timing of peak phagosome acidification in the macrophages. Inasmuch as *Mycobacterium smegmatis* mc² 155 cannot resist and persist in the acidified phagosome, we cloned the open reading frame of MAP0403 via a pSM417 vector into *M. smegmatis* mc² 155. Compared with controls, *M. smegmatis* mc² 155 transformants carrying the MAP serine protease show a temporal survival advantage during the in vitro acid stress for 30 and 120min. Our studies suggest that MAP serine protease is critical in resisting the phagosomal acid stress by MAP. Further establishment of this mechanism will lead to better understanding of a proximal step in the pathogenesis of mycobacterial infections to establish an intracellular niche.

M70 Quantifying Johne's disease infectivity in Indiana dairy herds. C. C. Wu*, T. L. Lin, A. Storm, C. A. Alinovi, and M. P. Ward, *Purdue University, West Lafayette, IN.*

Analysis of fecal culture and ELISA serology for Johne's disease (JD) was conducted on 5 dairy herds to quantify infectivity of JD from 2004 to 2009. Various positive management practices and risk assessment have been employed in these herds. Spearman's coefficient was calculated for the relationship between fecal shedding level and ELISA score. In a large open Holstein herd (900 milking) prevalence of JD by semiquantitative fecal culture was 36.1% in 2004, 18.5% in 2005, and 18.7% in 2006. In a small closed herd (50 milking) none of the tested cows were positive for JD by fecal culture in 2004. This herd did have one cow that was fecal culture positive in 2005 (0.9%), though all cows were negative for JD when tested in 2006. Two small open herds were studied. In one herd (80 milking) 19% of cows were positive for JD by fecal culture in 2004, 9.4% in 2005, and only 3.3% of cows were positive in 2006. The herd prevalence for JD went up to 24.6% in 2007 with new unvaccinated cows added to the herd, but reduced to 4.2% in 2008. In the other small herd (60 milking), 4.2% of the cows were positive for JD by fecal culture in 2005, 4.5% in 2006, and all tested animals were negative for JD in 2007 (0.0%). The fifth herd is a medium-sized (300 milking) open dairy herd where 10% of cows were fecal positive for JD in 2003 and herd prevalence had risen to 31.7% when tested in 2005. In subsequent years, the herd prevalence of JD decreased to 14.8% in 2006 and 9.8% in 2007. Vaccination against JD was practiced in all herds except the small closed herd (50 milking). Between 2003 and 2007, mean ELISA values increased in herds practicing vaccination with little change in the non-vaccinated herds. In conclusion, ELISA testing alone cannot be used to identify positive and shedding animals for culling program. ELISA is best used to screen the status of JD in herd and fecal culture remains the most accurate ante-mortem method to identify animals that are mycobacterial shedders.

Key Words: Johne's disease, infectivity, culture

M71 Preliminary observation of an indigenous Johne's disease vaccine study in infected cattle herd in India. S. V. Singh*, A. Srivastva, B. Singh, A. Kumar, and A. V. Singh, and P. K. Singh, *Central Institute for Research on Goats, Makhdoom, Farah, Mathura (UP), India.*

Ineffective control strategies of Johne's disease (JD) by 'test and cull' method, banned on culling of cows due to social issue, high presence of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) in livestock, humans and in environment, difficulties in early diagnosis etc. were major challenges to successful JD control program in India. An indigenous JD vaccine has been developed using a native MAP strain of 'Indian Bison type' of goat origin and found very effective in preventing and also curing of JD in goats and sheep. In present study the status of MAP infection and the efficacy of indigenous vaccine for the control of JD in a naturally infected cattle herd (based on preliminary data) were described. Fecal, blood and serum samples from 135 cows (100 adult and 35 calves), showing moderate to advanced symptoms of JD were processed for assessing the status of MAP infection before vaccination by microscopy, PCR and ELISA test, respectively. A total of 60 and 31.85% cows were positive by ELISA and blood PCR. Positive samples by PCR were further genotyped as MAP Bison type by IS 1311 PCR-REA. Out of 32 calves and 51 adult cows, 65.6 and 84.3% were positive in microscopy of fecal smear, respectively. Necropsy of few died cows showed advanced gross lesions of JD. All the cows were vaccinated, sampled (20%) and monitored for improvement in physical condition, reduction in MAP shedding, morbidity, mortality and immune response. Sample results and other data recorded before and 2 mo post vaccination showed reduction in number of MAP shedder/positive cows as well as shedding level, reduced morbidity and mortality despite of extreme winter condition, accelerated sero-conversion, checked diarrhea, increased appetite, no untoward reaction etc. were recorded. Conclusively, developed indigenous vaccine may perform equally (as in previous study on goat and sheep) in controlling the clinical cases of JD in cattle.

Key Words: indigenous vaccine, Johne's disease, *Mycobacterium avium* ssp. *paratuberculosis*