Animal Health: Johne's Disease

144 Johne's Disease integrated program – An overview. V. Kapur*, *University of Minnesota, Minneapolis*.

The Johne's Disease Integrated Program (JDIP) is a consortium of scientists from leading academic and government institutions. This consortium includes the diverse disciplines of microbiology, immunology, pathology, molecular and cellular biology, genomics, proteomics, epidemiology, clinical veterinary medicine, public health, extension, and public policy. All of the researchers are committed to finding solutions to better diagnose, treat, prevent and control Johne's Disease (JD). JDIP's mission is to promote animal biosecurity by developing and supporting projects to enhance knowledge, promote education, develop real-world solutions and mitigate losses associated with JD. JDIP promotes efficiencies through collaborative research and by sharing the intellectual and physical resources that are critical to overall success.

JDIP's strategic objectives include:

• Support and facilitate investigator-directed research on JD;

• Create and maintain scientific core facilities to support JD research and training activities;

• Establish translational research capacity for developing and validating diagnostic tests, vaccines, and disease management concepts for JD; and,

• Provide scientific information and support for the development of JD education, prevention, and control programs.

JDIP has four major research thematic areas, each led by a team of scientists working towards a common goal. Themes are organized as four interacting projects: JD epidemiology and disease transmission; diagnostic testing and strain differentiation; *Map* biology and pathogenesis; and, *Map* immunology and vaccine development.

Key Words: Johne's disease, JDIP

145 JEI – Producer focused Johne's information. K. E. Olson*, *National Institute for Animal Agriculture, Bowling Green, KY*.

Johne's disease is a wasting disease that affects cattle and other ruminants in almost every nation. It is estimated to cost the US dairy industry in excess of $200m per year. Beef costs have not been as completely quantified. In addition to producer costs, there is a lingering concern over possible links between Johne's and human health. A substantial portion of the beef and dairy herds in the US are infected with Johne's and the proportion appears to be increasing. Surveys indicate that producer awareness of Johne's disease has increased in recent years; however, participation in state and national Johne's programs is limited. The Johne's Education Initiative (JEI) is part of a cooperative agreement between USDA-APHIS-VS and the National Institute for Animal Agriculture (NIAA) to make 'producer-friendly' information on Johne's disease easily available to producers and those who work with them. Objectives include providing information to help producers:

1. Reduce the likelihood of introduction of M. paratuberculosis into currently uninfected herds or flocks;

2. Minimize impact of the disease in herds or flocks where it is already present, and

3. Control existing cases of the disease and while working to reduce the incidence in susceptible species.

An initial step in this effort has been development of www.johnesdisease.org. The website provides specific information as well as links to programs and people working with Johne's in each state. The JEI is working with other groups such as the National Johne's Work Group (NJWG), the Johne's Disease Integrated Program (JDIP), state program coordinators, extension and USDA agencies to gather and develop information for use with producers that can be made available through the web and by other means. Other nations including Canada, Australia, New Zealand, Japan and the EU are also increasing efforts to educateprodcuers and may offer future collaboration opportunities. Partnerships are being developed with milk marketing organization, livestock markets and other producer groups to define information needs and deliver information directly to producers that will help them address the disease in the future.

Key Words: Johne's, JEI

146 Johne's demonstration project in Texas. M. A. Villarino[1], H. M. Scott[2], and E. R. Jordan*[1], [1]*Texas Cooperative Extension, Texas A & M University, Dallas*, [2]*Texas A & M University, College Station*.

Bovine paratuberculosis (Johne's Disease) is a chronic debilitating disease of cattle. Recently, dairy veterinary practitioners and producers have become more aware of the benefits of establishing Johne's Disease control measures. The objective of this investigation was to evaluate the effect on milk production and culling patterns of subclinical manifestation of Johne's Disease. Initially a retrospective cohort study in a commercial dairy located in northwest Texas was conducted. Production data were collected starting in April, 2001 using a paired comparison scheme. The cohort consisted of cows of similar age (+/- 30 days), lactation, and origin (purchased or home raised). Production parameters (lifetime milk production, milk per lactation) and individual performance parameters (days in milk, days in dairy, cull date and reasons for culling) were collected from 120 ELISA positive and 120 ELISA negative for Johne's Disease cows, for as long as the animals were maintained on the premise. Statistical comparison (t-test) and linear regression analysis on the cohort data collected to date indicate a significant reduction in milk production from ELISA positive cows when compared to ELISA negative cows (-4,105 kg lifetime). Currently, 28.33 % of the ELISA positive and 60.34 % of the ELISA negative animals remain in the herd. The reduction of milk production in the ELISA positive cows started in the second lactation and continued for the remainder of the cow's productive life, regardless of when the cow was detected as ELISA positive. Seropositive cases of JD have higher likelihood of developing concurrent conditions and were more apt to be culled earlier.

Key Words: Johne's disease

147 Georgia Johne's Disease demonstration herd. M. Pence*, *University of Georgia, Athens*.

The Georgia Johne's disease demonstration herd project is evaluating the long-term effectiveness and feasibility of management-related disease control on the herd prevalence of Johne's disease infection in Georgia cattle production units. This project is attempting to control Johne's disease through implementation of specific on-farm manage-

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ment and testing practices to reduce direct and indirect transmission of Johne's disease infection by sanitation and/or management protocols. Since the most susceptible animal is the neonatal calf, a large portion of that goal is directed at reducing or eliminating calf contact with infective adult cow manure. To accomplish this goal an infected beef herd was segregated by age of the dam, cattle were tested yearly before calving and test positive cattle were moved to an off farm location. We are also working with an infected dairy herd to alter calf flow and calving location to reduce exposure of neonatal dairy heifers to Johne's disease. Since the incubation period of Johne's disease is 1-10 years, it may be several years before the outcome of altering management to control Johne's disease can be properly evaluated. However, we have seen a reduction in test positive environmental samples and a reduced number of cows that test positive. In 2004 6/1845 cows were culture positive, in 2005 6/2001 cows were culture positive. In 2004 we had 53/2147 cows blood test positive and in 2005 we had 43/1613 cows blood test positive. In 2004 we had 2/12 positive environmental culture samples and in 2005 we had 1/24 positive environmental samples. This presentation will focus on the temporal and spatial relationships of neonatal calves to infective cattle and the environmental contamination with Johne's disease.

Key Words: Johne's disease

148 Results from Minnesota Johne's Disease demonstration herd control program. C. Ferrouillet* and S. Wells, *University of Minnesota*, *Saint Paul*.

Six dairy cattle and 3 beef cattle herds in Minnesota have participated in a Johne's disease (JD) demonstration herd control program for the past 6 years. Objectives of this project were to 1) evaluate the longterm effectiveness and feasibility of management-related disease control on development of JD on dairy and beef cattle operations, 2) provide information and materials for education and training of public and private practice veterinarians and cattle producers, and 3) develop and evaluate management, testing, and monitoring strategies for use in control of JD in cattle herds. Cattle herds involved in this project have been selected based on history of clinical JD in the herd with organism detection confirmation, willingness of the herd owner to keep records including individual animal identification, cattle movements, and health events, and willingness to implement management practices to control JD. Within each herd, baseline and annually renewed information has been collected through completion of a JD risk assessment and herd management plan to define and prioritize risks and recommend management changes to address those risks identified in the written herd plan. In addition to monitoring of clinical JD, fecal and serum samples from all cattle have been tested by bacterial culture and ELISA, respectively, on an annual basis. Results to date from these herds show a reduction in clinical Johne's disease in nearly all dairy and beef cattle herds after implementation of management changes. A reduction in fecal shedding has occurred in most herds, while a reduction in ELISA prevalence has been observed in some herds. Variability in test results (culture and ELISA) from individual cattle has been observed, especially in cattle identified with low positive test results. Despite this variability in individual cow test results, several herds consistently tested positive as evidenced by results from environmental cultures through time. Much potential remains for use of these data and stored biologic samples in future epidemiologic and other studies.

Key Words: Johne's disease, Demonstration herd, Control

149 Preliminary results from the national Johne's Disease demonstration herd project. J. Lombard*, S. Jensen, B. Wagner, and L. Garber, *USDA:APHIS:VS; Centers for Epidemiology and Animal Health, National Center for Animal Health Surveillance, National Animal Health Monitoring System*, *Fort Collins, CO*.

A total of 70 dairy operations are currently enrolled in the National Johne's Disease Demonstration Herd Project (NJDDHP) in the United States. The Project began in 2003 and should continue for 5 to 7 years. Data being collected include herd-level risk assessment and management plans with additional animal-level data. A subset of these operations were selected for inclusion in this study based on the availability of accurate dam-daughter identification and fecal culture and serum ELISA test results for *Mycobacterium paratuberculosis (M. paratb)* infection, as well as herd-level risk assessment information. Two multivariable logistic regression models were developed to determine risk factors for cattle testing either fecal culture or ELISA positive. Mixed model analysis was performed to evaluate milk production among cows in different testing categories. To date, thirty-nine dairy operations comprising 3,602 dam-daughter pairs from eight states were included in fecal culture analysis. Serum ELISA results were available for 40 dairy operations from ten states, and included 3,687 dam-daughter pairs. Preliminary analysis revealed that daughters born to fecal culture positive dams were at 1.8 times the odds of being fecal-culture positive as those daughters born to fecal-culture negative dams. Other significant covariates in the fecal culture analysis included lactation number and the following risk areas: calving area, cow area and additions/replacements. Similarly, ELISA result analysis demonstrated that daughters from serologically positive dams were at 1.7 times the odds of testing positive compared to daughters of test-negative dams. Other significant covariates for the ELISA model included lactation number and cow risk area. Cows that tested fecal culture positive or serum ELISA strong positive had significantly lower mature equivalent 305 day milk production compared to other test categories. Results of the NJDDHP will quantify milk production loss associated with test status and more importantly assist in identifying and prioritizing specific on-farm management practices that reduce *M. paratb* transmission.

Key Words: Johne's, Paratuberculosis, Control

150 Early diagnosis of Johne's Disease: evaluation of methods. J. Stabel*, S. Robbe-Austerman, and K. Kimura, *USDA-ARS-National Animal Disease Center*, *Ames, IA*.

Measurement of secreted interferon-(IFN)-γ has been suggested as a tool for the early detection of animals infected with mycobacterial pathogens, including *Mycobacterium avium* subsp. *paratuberculosis* (MAP), the causative agent of Johne's disease. Removal of infected animals at an early stage will help allay the spread of this disease through the herd. In the present study, the accuracy and reliability of the IFN-γ and the intradermal skin tests were evaluated in calves born to naturally infected cows at different time points after birth. At 13-14 months of age calves were necropsied and tissue culture was correlated with results of the 2 assays. Ten of the 17 calves were tissue culture positive (TPos) for MAP and 7 were negative (TNeg). Within the TPos calves, the skin test identified 5 at 4-6 months of age, 4 at 6-8 months of age and 7 at 12-13 months of age. Comparatively, measurement of IFN-γ identified 2 calves at 4-6 months, 4 at 6-8 months, and 10 at 12-13 months. Of the TNeg animals, the skin test identified 3 calves at 12-13 months of age, whereas the IFN-γ identified 1 at 4-6 months, and 6 at the later time points. In a separate experiment, augmentation of secreted and intracellular IFN-γ responses by intradermal injection of

johnin purified protein derivative was achieved in adult cattle naturally infected with MAP. Intracellular IFN-γ was higher within CD4+ and CD8+ subpopulations for infected cows compared to healthy controls throughout the study. When T cell populations were further defined by CD45RO+ expression, intracellular IFN-γ was higher for CD8+/CD45RO+ lymphocytes compared to CD4+/CD45RO+ cells. These results indicate that intradermal sensitization of cows in the early stage of infection with antigen will enhance the sensitivity of detection by the IFN-? assay.

Key Words: Johne's disease, *Mycobacterium avium* subsp. *paratuberculosis*, Early diagnosis

151 The impact of Mycobacterium avium subsp paratuberculosis fecal shedding and clinical Johne's disease on lactation performance. E. A. Raizman*[1,2], J. Fetrow[2], S. M. Godden[2], and S. J. Wells[2], [1]*Purdue University*, *West Lafayette, IN*, [2]*University of Minnesota*, *St Paul*.

The objective of this study was to evaluate the lactation performance (measures of health, production, reproduction, and survival) of cows shedding Mycobacterium avium subsp paratuberculosis (Map) in feces prior to calving and of cows culled with clinical signs of Johne's disease (JD) during the subsequent lactation. 1,052 cows from two Minnesota dairies were enrolled in the study. Fecal samples were collected immediately prior to calving and tested using bacterial culture to detect Map. Observed signs of clinical disease (milk fever, retained placenta, metritis, ketosis, displaced abomasum, lameness, mastitis, pneumonia, and Johne's disease) and lactation performance data were recorded for each cow. Of the 1,052 study cows, 84 cows (8%) had Map positive fecal samples (46% light, 26% moderate, and 28% heavy shedders). Lactation length of fecal positive cows was on average 106 d less than negative fecal culture cows. Mean milk production for negative fecal culture cows was 9,700 kg and for light, moderate, and heavy shedders was 8,100 kg, 6,300 kg and 2,700 kg, respectively. Fecal culture positive cows were less likely to be bred and conceive than negative cows. Fifty-six cows were culled with clinical signs consistent with JD and remained in the herd an average of 202 days less than cows that completed the study and 49 days less than other cows culled from the herd. Cows culled with clinical signs of JD produced on average 3,280 kg less milk than cows that completed the study. Study results demonstrate the economic impact of fecal shedding and clinical JD on cow lactation performance, information important in motivating dairy producers to implement JD control programs.

Key Words: Johne's disease, Milk production, Reproduction

152 Identification and implications of MAP supershedders. E. Hovingh*[1], R. H. Whitlock[2], R. W. Sweeney[2], T. Fyock[2], D. R. Wolfgang[1], J. Smith[3], Y. H. Schukken[4], and J. S. Van Kessel[5], [1]*Pennsylvania State University*, *University Park*, [2]*University of Pennsylvania*, *Kennett Square*, [3]*University of Vermont*, *Burlington*, [4]*Cornell University*, *Ithaca, NY*, [5]*United States Department of Agriculture*, *Beltsville, MD*.

The objective of this research was to determine the range of colony-forming units (cfu) per gram of Mycobacterium avium paratuberculosis (MAP) in manure from cows previously classified as heavy shedders, and the role of these animals in Johne's Disease (JD) in dairy herds. Cattle are typically classified as light, moderate or heavy shedders based on the number of MAP colonies seen on Herrold's egg yolk media (HEYM). Laboratories routinely report the number of cfu up to 70 per HEYM tube. (Seventy cfu corresponds to an estimated 1,470 MAP cfu per gram of manure.) Counts above 70 are reported as 'too numerous to count' and these cows are classified as heavy shedders. Preliminary investigation found that 1:100 and 1:1,000 dilutions of feces from heavy shedders were appropriate for enumerating the actual concentration of MAP in these samples. Subsequently, more than 200 fecal samples from animals currently identified as heavy shedders were diluted and cultured on HEYM. Supershedders (SS) were defined as animals that had manure with more than 10,000 cfu/gm. Culture results on diluted samples demonstrated that a substantial proportion of heavy shedders were SS, with some animals exceeding 1,000,000 MAP cfu/gm. Whole herd cultures from 4 herds found that 10% to 15% of all culture positives were SS. Not all of these SS cattle demonstrated clinical signs of JD at the time of sampling. It was calculated that SS cattle shedding the highest concentration of MAP were shedding more cfu than 2,000 moderate or 20,000 light shedders. Subsequent culture in one herd after the removal of a SS identified up to 15 animals that were potential false positives on the previous culture. This study has demonstrated the previously undocumented presence of MAP supershedders. The results suggest that SS contribute disproportionately to the herd environmental bioburden and the exposure of uninfected animals to MAP organisms. The data also suggest that the bioburden in herds with a SS present may contribute to herdmates being incorrectly identified as culture positive. Identification and removal of SS animals is likely to be a critical component of a JD control and management program.

Key Words: Johne's disease, Supershedder

153 Use of a Fecal PCR assay on environmental samples: Implications for detection of dairy cattle herds infected with Johne's disease. N Cernicchiaro*[1], S. J. Wells[1], C. Muñoz-Zanzi[1], J. Gaulke[2], and C. Wees[2], [1]*University of Minnesota*, *St. Paul*, [2]*Minnesota Veterinary Diagnostic Laboratory*, *St. Paul, MN*.

Johne's disease is an intestinal disease in ruminants caused by *Mycobacterium avium subsp. paratuberculosis* (Map). The objective of this study was to evaluate the application of a fecal PCR assay for diagnosis of Map in environmental samples and to determine its sensitivity for detection of dairy cattle herds infected with Johne's disease. Two samples were collected from up to five different locations at the farm: cow alleyways, manure storage, calving area, fresh cow area, and sick cow area (with a total of 6 to 10 samples per herd). A total of 409 environmental fecal samples from 49 Minnesota dairy farms known to be infected from previous testing were tested using Taqman PCR (ISMav2) assay and fecal culture (FC, HEYM using the 72-hour sedimentation protocol) for Map. Results were analyzed at the herd and sample level and by location comparing PCR results with those from culture. Herd level analysis showed that of 49 infected farms, 65% were positive by PCR and 90% by FC. From those 44 herds positive by FC, 71% were also positive by PCR. 48 % of herds had at least one high or very high bacterial load environmental sample by FC and PCR was able to detect 71% of those herds. At the sample level, 49% of environmental samples were positive by FC. Of those positive samples by FC, 22% were also positive by PCR. PCR was able to detect 36% of high and 59% of very high bacterial load samples. Within environmental areas, highest detection rates were found in fresh cow area (30%), manure storage (27%) and cow alleyways (21%). Our results demonstrated that the PCR test can be used as a screening test for detection of high bacterial load environmental samples in herds of unknown status due to the lower costs and quicker turn around time.

Key Words: Johne's disease, Environment, PCR

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154 Evaluation of a risk assessment tool in characterizing environmental Salmonella and Mycobacterium paratuberculosis status in dairy herds. D. Collette*, L. Minicucci, and S. J. Wells, *University of Minnesota, St. Paul.*

The objective of this study was to evaluate the validity of an on-farm risk assessment tool in characterizing the status of manure-cycle pathogens on dairy farms from environmental samples. A risk assessment tool developed by study investigators was used to evaluate management practices on Minnesota dairy farms targeting Salmonella and M. paratuberculosis, the causative agent for Johne's disease (JD). Two visits were conducted by two evaluators to Minnesota dairy herds randomly selected from a list of state JD control program herds. Data was collected using the risk assessment tool from 36 herds from January to June 2004 and again from 38 herds from June to August 2004. Environmental samples were collected from high risk areas on the farms, including cow alleyways, fresh cow pens, and manure storage, and bacterial culture was performed to characterize Salmonella and M. paratuberculosis environmental status. The highest mean Salmonella risk scores were from the cow herd, biosecurity, and growing heifer areas and the highest mean Johne's disease risk scores were from the cow herd, biosecurity, and site management areas. At the first visit, 33% of herds had positive environmental samples for Salmonella and 37% of farms were positive at the second visit. Forty-two percent of herds were categorized as high M. paratuberculosis bacterial load herds at each herd visit. The criteria for a high bacterial load herd was defined as having an environmental sample with a shedding score of three or four. At the first herd visit, herds with high environmental M. paratuberculosis bacterial loads were more likely to be Salmonella-positive farms than herds with low M. paratuberculosis bacterial load ($P < 0.05$). Preliminary analysis demonstrated lack of association between mean risk scores overall or within individual management areas and Salmonella or M. paratuberculosis environmental status. This is an indication that an evaluation of herd management on-farm using a risk assessment tool does not predict manure-cycle pathogen environmental status on the day of the herd visit.

Breeding and Genetics: Dairy Breeding

155 Genetic impact of utilizing female-sorted semen in commercial and nucleus herds. G. Abdel-Azim* and S. Schnell, *Genex Cooperative Inc., Shawano, WI.*

This study was designed to investigate genetic effects of using sorted semen in a dairy cattle population. Progress was monitored in elite and commercial animals over 20 years of selection. To study the genetic impact of utilizing sorted semen in commercial herds, a scenario in which female-sorted semen was available to commercial herds was evaluated. Second, to study the genetic impact of utilizing sorted semen in nucleus herds, scenarios in which female-sorted semen was used only in a nucleus herd, where multiple ovulation and embryo transfer (MOET) took place, were simulated. Finally, because of the additional advantage of marker-assisted selection when sorted semen is used in nucleus herds, utilization of sorted semen in scenarios that employed marker-assisted selection was simulated. In the scenario where female-sorted semen was used in commercial herds, a large genetic advantage was observed early in commercial cows. Average superiority in first-lactation cows exceeded 30% in year 11 but continued to decrease until it reached 9% in year 20. The increased selection intensity in commercial cows contributed to the genetic merit of future cows (cow→cow contribution) but the contribution of the nucleus grew over time, and gradually marginalized the cow→cow contribution. The genetic advantage of gender control in the MOET scheme was minimal except when marker-assisted selection was also available. Two factors that affected the contribution of marker-assisted selection were studied: within versus across family selection of donors and the number of loci in the QTL component. Schemes that selected donors regardless of their family structure were superior, and more loci in the QTL component increased the effectiveness of sorted semen. Finally a reduced MOET scheme where harvested females were reduced from 42 to 25 per year was studied. It was found that the reduced scheme in combination with female-sorted semen was not genetically inferior to the large scheme in combination with regular semen.

Key Words: Dairy cattle breeding, Marker-assisted selection, Sorted semen

156 Net present value of an artificial insemination: non-sexed versus sexed semen. N. J. Olynk* and C. A. Wolf, *Michigan State University, East Lansing.*

Sexed semen has been a long anticipated tool by dairy farmers to obtain more heifer calves. Using flow cytometry to separate male- and female-bearing sperm decreases sperm numbers per straw and negatively affects sperm viability and longevity leading to decreased conception rates (CR). This decreased CR makes virgin heifers better suited for insemination with sexed semen than lactating dairy cows. Budgets were developed to calculate the net present value (NPV) of an artificial insemination of a virgin heifer using non-sexed and sexed semen. With heat detection rates (HDR) varying widely between farms, and CR varying based on both animal and management factors, multiple scenarios were required to assess differences in NPV with non-sexed versus sexed semen. Bull and heifer calf values were held constant in all scenarios, with heifer calves valued at $500 and bull calves valued at $110. Costs per straw were held constant at $15.00/straw for non-sexed semen and $45.00/straw for sexed semen of approximately the same genetic value. With sex ratios of 49.2% female with non-sexed semen and 90% female for sexed semen, a sample scenario was performed holding heat detection constant at 51%. Base values for CR with non-sexed of 58% and sexed semen of 31% were obtained from K.A. Weigel (2004). With these HDR and CR we observed an approximately $50 decrease in the net present value of an insemination with sexed semen. Holding all other factors constant in this scenario, the CR with sexed semen would need to decrease by no more than 6 percentage points to achieve the NPV obtained with non-sexed semen. In this scenario the value of an average calf increased approximately $150 with sexed semen, although this difference is overwhelmed by the nearly 50% decrease in pregnancy rate. To achieve a positive NPV assuming a 51% HDR, the CR with sexed semen must be at least 20%; assuming a 70% HDR, the CR with sexed semen must be at least 15%. Other considerations include the effect on dystocia, biosecurity, and internal herd growth implications.

Key Words: Sexed semen, Heifer reproduction, Artificial insemination