

Animal Health-Johne's Disease (JDIP): Epidemiology and Transmission

275 Cost-effectiveness of diagnostic strategies to identify *Mycobacterium avium* ssp. *paratuberculosis* super-shedder cows in a large dairy herd. S. S. Aly*¹, R. J. Anderson², R. H. Whitlock³, T. L. Fyock³, S. McAdams³, T. M. Byrem⁴, J. Jiang⁵, J. M. Adaska⁶, and I. A. Gardner¹, ¹Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, ²California Department of Food and Agriculture, Animal Health Branch, Sacramento, ³Johne's Research Laboratory, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, ⁴Antel BioSystems, Inc, Lansing, MI, ⁵Department of Statistics, University of California, Davis, ⁶California Animal Health and Food Safety Laboratory, Tulare.

Paratuberculosis-infected cows that shed *Mycobacterium avium* ssp. *paratuberculosis* (MAP) in excess of 10,000 CFU/g of feces have been termed super-shedders and contribute the most to MAP bioburden on a dairy. Identification of super-shedders in a large dairy herd is challenging given their low prevalence, cost of MAP diagnostic tests, and hence, the large sample size needed. Several diagnostic strategies to detect super-shedders are possible given the different MAP organism- and antibody-detection tests and specimens available to test. The objective of this cross-sectional study was to compare the cost-effectiveness of diagnostic strategies to detect MAP super-shedders in a large dairy herd. The study herd of 3577 Jersey cows had a MAP seroprevalence of 3.5% based on routine testing at dry-off. Cows were housed in 14 freestall pens and the herd manager requested not to move cows between pens during the study. A whole herd survey (reference) and 14 other diagnostic strategies were evaluated and their cost-effectiveness calculated. The reference strategy included quantitative real-time PCR (qrt-PCR) on fecal pools, followed by qrt-PCR of the individual cow fecal samples from the positive pools and a random sample of individual cow fecal samples from suspect and negative pools. The remaining strategies included combinations of serum ELISA, milk ELISA and environmental and pooled fecal samples tested using qrt-PCR. Twenty super-shedders (0.5% of the herd; 2% of qrt-PCR MAP positive cows) were identified and had a mean of 82,040 colony-forming units (CFU)/g of feces. The whole herd survey using qrt-PCR was the most sensitive strategy but sensitivity was $\leq 80\%$ for all 15 strategies. The most cost-effective strategy was to rank lactating cow pens by MAP bioburden, milk ELISA test cows in pens ≤ 32 CT followed by qrt-PCR testing of fecal samples from milk ELISA positive cows. Environmental samples collected using a standardized protocol as part of a diagnostic strategy can improve the cost-effectiveness of detecting super-shedders compared to a whole herd survey by qrt-PCR.

Key Words: cost-effectiveness, MAP super-shedder

276 Correlation between culture and quantitative real-time PCR results for *Mycobacterium avium* subspecies *paratuberculosis* in pooled fecal and environmental samples. S. S. Aly*¹, B. L. Mangold², R. H. Whitlock³, R. W. Sweeney³, R. J. Anderson⁴, J. Jiang⁵, Y. H. Shukken⁶, E. P. Hovingh⁷, D. R. Wolfgang⁷, J. S. Van Kessel⁸, J. S. Karns⁸, J. E. Lombard⁹, J. M. Smith¹⁰, and I. A. Gardner¹, ¹Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, ²Tetracore, Inc., Rockville MD, ³Department of Clinical Studies-New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, ⁴California Department of Food and Agriculture, Animal Health Branch, Sacramento, ⁵Department of Statistics, University of California, Davis, ⁶Section of Epidemiology and Quality Milk Production Services, Department of Population Medi-

cine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY ⁷Department of Veterinary and Biomedical Sciences, Pennsylvania State University, University Park, ⁸Environmental Microbial and Food Safety Laboratory, ANRI, USDA-ARS, Beltsville, MD, ⁹Centers for Epidemiology and Animal Health, Animal and Plant Health Inspection Service, USDA, Fort Collins, CO, ¹⁰Department of Animal Science, University of Vermont, Burlington.

Quantitative real-time PCR (qPCR) testing for *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in fecal samples is a rapid alternative to culture on Herrold's egg yolk medium (HEYM), the traditional reference test for MAP. Although the sensitivity and specificity of these 2 tests has been described, the correlation between qPCR cycles-to-threshold (Ct) values and colony-forming units (CFU) on HEYM has not been evaluated. The objective of the present study was to estimate the correlation between qPCR and HEYM culture results in fresh and frozen pooled fecal and environmental samples and model the association between results of both assays. Quantitative HEYM culture results for 1997 pooled fecal samples from cows in 14 herds in 4 states, and 802 environmental samples from 113 dairies nationwide were correlated with their respective qPCR results. The Spearman's rank correlation between assays was good (-0.66) in both fresh and frozen pooled fecal samples and excellent (-0.76) and good (-0.61) in fresh and frozen environmental samples, respectively. Furthermore, the correlation varied from good (-0.53) to excellent (-0.90) depending on size of fecal pools. Truncated linear regression models indicated a significant negative association between CFU and Ct in fecal pools of all sizes and in both individual and pooled environmental samples. The use of qPCR instead of HEYM can yield more timely quantitative MAP detection on a herd basis and allow for incorporation of qPCR in many dairy herd testing strategies to reduce the risk of MAP transmission.

Key Words: culture PCR correlation, pooled and environmental samples, *Mycobacterium avium* ssp. *paratuberculosis*

277 Fecal culture and direct PCR in determining *Mycobacterium avium* ssp. *paratuberculosis* infectivity. C. C. Wu*¹, J. E. Williams², T. L. Lin¹, and G. R. G. Monif³, ¹Purdue University, West Lafayette, IN, ²University of Florida, Gainesville, ³Infectious Diseases Incorporated, Bellevue, NE.

The present study was conducted to evaluate the utility of fecal culture, direct fecal real-time PCR, and direct fecal nested PCR in determining the status of *Mycobacterium avium* ssp. *paratuberculosis* (Map) infectivity in dairy herd. Eight hundred and 27 (827) fecal samples were collected from 2 dairy herds participating in Johne's Disease Demonstration Herd Program. Fecal culture was carried out by using Trek ESP system with IS900 PCR confirmation. Direct fecal Map real-time PCR uses heat shock protein gene (hsp) as the target in PCR (VetAlert TM Johne's Real-Time PCR, Tetracore). Direct fecal Map nested PCR is based on IS1311 (FecaMap, Infectious Diseases Inc.). The percentages of fecal samples positive for Map were 13.5% by culture, 11.6% by real-time PCR, and 21.8% by nested PCR. Using positivity by direct fecal culture as the gold standard, 35 samples were positive for Map by real-time PCR with 83.3% accuracy and 51 positive for Map by nested PCR with 77.3% accuracy. Using positivity by culture or both PCR as the gold standard, 112 samples were positive for Map by culture with 97.3% accuracy, 49 positive for Map by real-time PCR with 85.0% accuracy, and 65 positive for Map by nested PCR with 78.8% accuracy. The results indicated that using positivity for Map by culture or both

PCR as the gold standard is a more accurate tool in determining the status of Johne's disease infectivity in dairy herd.

Key Words: *Mycobacterium paratuberculosis*, fecal culture, direct fecal PCR

278 Estimation of test parameters for fecal culture and serum ELISA for detection of *Mycobacterium avium* ssp. *paratuberculosis* fecal shedding. L. A. Espejo*¹, F. J. Zagmutt², H. Groenendaal², and S. J. Wells¹, ¹University of Minnesota, St. Paul, ²Vose Consulting, Boulder, CO.

The objective of this study was to estimate the probability of the fecal culture and serum ELISA to correctly identify cattle that shed high, low, and no fecal concentrations of *Mycobacterium avium* ssp. *paratuberculosis* into the environment. The results of 12,957 parallel fecal culture (HEY media) and serum ELISA (IDEXX) from 8 dairy herds enrolled in the Minnesota Johne's Disease Demonstration Herd Program over a 9 year period were used for this study. The conditional probabilities that test results indicate high, low, and no fecal shedding, given the true shedding status of the animal P(test results|true status) were estimated using Bayesian Markov-Chain Monte Carlo methods. The model assumed no gold standard test, independence between both tests and Dirichlet distribution for the priors. The shedding levels using fecal culture were categorized as high with ≥ 50 colonies/slant, low with $0 < \text{colonies/slant} < 50$, and no fecal shedding with no detectable colony growth on the slants. Likewise, levels for serum ELISA were established based on OD values, with ≥ 1.0 (high), ≥ 0.25 and < 1.0 (low), and < 0.25 (negative). Informative prior distributions of the conditional probabilities were given by one of the co-authors (SJW). The probability of the serum ELISA to correctly identify high fecal shedders (P(High|High)) was 69%, while the same probability for fecal culture was 59%. The probability of incorrectly identifying animals that were high fecal shedders as no shedding (P(No shedding|high)) was 10% for serum ELISA and 3% for fecal culture. The probability of correctly identifying animals that were not shedding (P(No shedding|no shedding)) was 99.8% for serum ELISA and 98.9% for fecal culture. These posterior conditional distributions improve understanding of the fecal culture and serum ELISA, and this information can be used to model the transmission of Johne's disease on dairy farms taking into account the uncertainty of these tests.

Key Words: Johne's disease, diagnostic tests, Bayesian inference

279 Effect of delaying exposure to Johne's disease until adulthood on development of new infections in adult dairy cows. S. J. Wells*, N. Kubat, L. A. Espejo, and S. M. Godden, University of Minnesota, St. Paul.

Control programs for Johne's disease (JD) in US dairy cattle are designed with focus on preventing exposure by *Mycobacterium avium* ssp. *paratuberculosis* (MAP) in young replacement cattle, as these cattle are considered to be at highest risk of infection. The objective of this study was to compare rates of subclinical and clinical MAP infection in cattle raised in an environment presumed free of JD to those of cattle raised in an infected environment. Through a survey of uninfected Minnesota dairy herds (Levels 3 or 4 of the Voluntary Johne's Disease Herd Status Program for Cattle), we identified JD infected herds that previously purchased replacement cattle from uninfected herds. Over a 3 year period, blood and fecal samples were collected in infected herds from 78 purchased replacement cattle that were raised in uninfected herds (exposed) and homebred cows of similar age and stage of lactation (non-exposed controls). Serum samples were tested using an

ELISA (IDEXX) for detection of antibodies to MAP and fecal samples were tested for detection of MAP using bacterial culture (HEY media). While results from the first year of testing of cattle across multiple ages indicated that dairy cattle raised in JD low risk herds (Level 3 or 4) and introduced to Johne's infected herds were less likely to test positive for MAP than herd mates raised in infected herds, results over time from survival analyses showed that this difference in risk was reduced later in life. The hazard ratio point estimate from Cox regression for exposed compared with unexposed cattle was 0.70 (95% CI = 0.40,1.23) and 0.64 (95% CI = 0.33,1.24) for fecal culture and ELISA, respectively. These results suggest risk of MAP infection in adult dairy cattle which should be considered in development of comprehensive JD control programs.

Key Words: Johne's disease, susceptibility

280 Importance of latent infected animals in MAP infection dynamics in dairy herds. Y. H. Schukken*¹, A. K. Pradhan¹, R. M. Mitchell¹, Z. Lu¹, R. Smith¹, Y. T. Grohn¹, R. H. Whitlock², E. Hovingh³, J. Smith⁴, J. A. VanKessel⁵, J. Karns⁵, and D. Wolfgang³, ¹Cornell University, Ithaca, NY, ²University of Pennsylvania, Kennett Square, ³Pennsylvania State University, State College, ⁴University of Vermont, Burlington, ⁵ARS-USDA, Beltsville, MD.

Mycobacterium avium ssp. *paratuberculosis* (MAP) is an important infectious disease of dairy cattle. Prevalence of test positive cows in dairy herds is often low. Prevalences between 0 and 10% are most common. Such low infection prevalences would be expected to result in infection die-out in a proportion of herds due to culling that is unrelated or related to MAP infection status. In reality MAP infection die-out in dairy herds is not observed. At the very least it is not reliably documented. This would imply that complex mechanisms play a role in MAP infection maintenance in dairy herds. The objective of this presentation is to investigate the role of latent infected animals in endemically MAP infected dairy herds. Animals with a latent infection are defined as animals that are not detected to shed the organism while being tested using common testing schemes used in MAP control programs, but that turn out to be tissue positive when sampled and cultured at slaughter. We studied the potential contribution of latent infected animals to the vertical transmission route of infection and the contribution to a low rate of low shedding of these animals while in a high stress period. High stress periods were assumed to occur when an animal was calving and when severe clinical diseases such as mastitis, DA and lameness occurred. The importance of latent infected animals was evaluated in a previously described MAP model. We used simulation modeling to evaluate scenarios with intermittent shedding of latent MAP infected animals, increased vertical transmission in these animals and shedding while in high stress periods. Modeling the potential contribution of latent infected animals resulted in a better description of long-term low test prevalence herds and a more accurate prediction of a very low probability of infection die-out in dairy farms.

Key Words: MAP, modeling, latent infection

281 Impact of Johne's disease vaccines on a dairy herd: a mathematical modeling approach. Z. Lu*, Y. H. Schukken, R. L. Smith, and Y. T. Gröhn, Cornell University, Ithaca, NY.

The objective of this study was to investigate the potential impact of Johne's disease vaccines on the dynamics of MAP infection in a dairy herd using a mathematical modeling approach. To reduce the prevalence of MAP infection, vaccination has been applied as a control measure in some dairy herds. However, Johne's disease vaccines are imperfect and

several types of vaccine efficacy have been observed, i.e., vaccines may provide a partial protection for susceptible calves, reduce infectiousness/shedding level of animals shedding MAP, lengthen the latent period of infected animals, slow the progression from low shedding to high shedding in infectious animals, or reduce clinical disease. To quantitatively study the impacts of Johne's disease vaccines, we developed a deterministic multi-group vaccination model consisting of 18 nonlinear ordinary differential equations. The model was parameterized using data from US dairy herds. An analytical expression of the reproduction ratio (R) incorporating several vaccine efficacies was obtained. Our analytical and numerical results show that Johne's disease vaccines may have a positive, zero, or negative effect in the reduction of prevalence. Some vaccine efficacies are beneficial to individual animals, but may not be useful to a herd-level control plan. We also studied the impact of multiple vaccine efficacies on the dynamics of MAP transmission. This work is helpful to understand various outcomes in field studies of Johne's disease vaccines and to provide a tool to evaluate vaccine efficacies in Johne's disease control.

Key Words: Johne's disease, vaccination, modeling

282 Estimating the efficacy of imperfect paratuberculosis vaccines in dairy cattle from longitudinal field data with Markov Chain Monte Carlo models.

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The purpose of this study was to determine the efficacy of vaccination against paratuberculosis in dairy cattle. For this purpose, a statistical model has been developed to analyze longitudinal field data from dairy herds with endemic paratuberculosis and control programs involving vaccination. Infection with *Mycobacterium avium* ssp. *paratuberculosis* (MAP) is difficult to detect, due to the long latent period and imperfect diagnostics. In commercial dairy herds, many animals could be culled before their infection status can be verified diagnostically. Due to this uncertainty as to true prevalence, it is challenging to obtain an unbiased estimate of vaccine efficacy. In addition, vaccines can do more than just decrease susceptibility to disease; vaccination against MAP may also decrease infectiousness, increase the duration of latency, or slow progression of clinical disease. To overcome the observed complexity of early censoring (culling) and multiple potential vaccine efficacies, we developed a Markov Chain Monte Carlo model. The potential vaccine effects are inter-related, but may have different and even opposite economic impacts. Markov Chain Monte Carlo (MCMC) models have been used extensively to estimate parameters for stochastic models from data sets with missing information. These models allow for simultaneous estimation of several variables in the presence of nonlinear relationships. In this study we developed an MCMC model to estimate 5 different possible vaccine effects (on vertical transmission probability, horizontal transmission rate, duration of latency, duration of subclinical disease, and rate of progression to clinical disease), while correcting for missing information (such as true infection status and time of infection). The 50% confidence intervals of the model's posterior distributions contain the true value of the parameters of interest when simulated over a wide range of vaccine efficacies. This is an effective tool for estimating vaccine efficacy from field data.

Key Words: Johne's disease, vaccine, statistics

283 Molecular epidemiology of *Mycobacterium avium* ssp. *paratuberculosis* in three dairy herds in the northeastern United States.

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The objectives of this study were to evaluate (i) whether low shedders of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) were passive shedding animals or whether they were truly infected, (ii) whether these animals could have been infected as adults by contemporary high-shedding animals (super-shedders), and (iii) whether animals in the herds shared the same MAP strains with that were obtained from environmental samples. The MAP isolates were obtained from a longitudinal study of 3 dairy herds in the northeastern United States. Selected isolates from fecal samples and tissues from all animals that were culture-positive at the same time that super-shedders were present in the herds and all environmental isolates were strain typed using a multilocus short sequence repeat technique. We found 15 different MAP strains from a total of 142 isolates from fecal samples and tissues. Eight different strains were found from a total of 102 environmental isolates; 6 of these strains were present in these selected animal isolates. Results indicated herd-specific infection patterns; a clonal infection in herd C with 89% of animals sharing the same strain, whereas herds A and B showed several different strains. Shedding levels and MAP strain typing showed that at least 50% of low shedders have the same strain as that of a contemporary super-shedder. About 57, 50, and 94% of environmental samples shared the same strains as super-shedders on Farms A, B, and C, respectively, which suggests that super-shedders may represent a risk of spreading MAP-infection among adult herd mates. Results suggested that in a dairy herd few cows could be classified as passive shedders whereas more low-shedding cows are truly infected. The sharing of same strain of low shedders with the contemporary super-shedders suggests that low shedders may have been infected as adults by super-shedders. Sharing of same strains of both environmental and animal samples suggests the spread of MAP-infection through environment.

Key Words: *Mycobacterium avium* ssp. *paratuberculosis*, longitudinal study, MLSSR analysis

284 Field evaluation of TG marker IS1311 PCR-REA for rapid differentiation of Indian Bison type *Mycobacterium avium* ssp. *paratuberculosis*.

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The availability of MAP genome in the public database opened various areas including the analysis of native isolates (comparative genomics). Comparative genomics studies at our laboratory have identified some key variations in the genome of native MAP. Studies has highlighted that "Indian Bison type" may represent a new MAP Biotype so far not reported outside India. One important variation in the genome of Indian Bison type isolates is the deletion of TG in the IS1311 element. Taking advantage of this variation on specific PCR-REA based test using BsaJI restriction enzyme was designed to distinguish Bison type isolates of Indian origin from other isolates. Present study analyzed and evaluated the use of this new assay for its practical utility in field for molecular epidemiological investigations. In total 45 previously characterized Bison type DNA samples of Indian origin from different parts of the country were analyzed. Results of the present study showed that all isolates belonging to Bison type genotype from different host species and agro-climatic region of the country had TG deletion in the IS 1311 element. Presence of this variation in all the Indian isolates belonging

to Bison type genotype is indicative of the fact that this variation has been established in all Bison type isolates of Indian origin. Hence this assay can be used in future molecular epidemiological investigations. Also this assay should be used in other parts of the world in order to study the distribution of newly identified MAP biotype.

Key Words: *Mycobacterium avium* ssp. *paratuberculosis*, TG deletion, IS 1311 PCR-REA

285 Rising incidence of *Mycobacterium avium* ssp. *paratuberculosis* in the North Indian population of animal keepers suspected for IBD/CD. S. V. Singh*, A. Shishodiya, A. Panwar, B. Singh, and A. Kumar, *Central Institute for Research on Goats, Makhdoom, Farah, Mathura (UP), India, 281 122.*

Mycobacterium avium ssp. *paratuberculosis* (MAP) infection is highly endemic in domestic livestock population in India. However, limited information exist on the association of MAP with human's Inflammatory bowel disease (IBD)/Crohn's disease (CD) in the country. Therefore, present study aimed to investigate the association of MAP in animal keepers from peri-urban areas of North India (Ghaziabad and Saharanpur cities) with symptoms of IBD/CD, using multiple diagnostic tests. A total of 131 samples (25 stool, 53 blood and 53 serum) and 108 samples (14 stool, 47 blood and 47 serum) were collected from 54 animal keepers having clinical profiles indistinguishable to IBD (suspected for CD) and from 47 animal keepers without symptoms of IBD (not suspected for CD), respectively. Animal keepers were in contact with animals for variable duration and some of them had habit of raw milk consumption. Stool samples were screened by microscopy and specific IS 1311 PCR-REA. Blood and serum were screened by IS 1311 PCR-REA and indigenous ELISA kit, respectively. Of the animal keeper suspected for CD, 36.0 (9/25), 28.0 (7/25), 12.9 (7/54), and 12.9% (7/54) samples and of animal keepers without symptoms of IBD (not suspected for CD), 14.2 (2/14), 7.1 (1/14), 2.1 (1/47) and 4.2% (2/47) samples were positive for MAP by stool microscopy, stool PCR, blood PCR and indigenous ELISA kit, respectively. According to habits of animal keepers, results showed high occurrence of MAP in humans with habit of raw milk consumption and smoking. All MAP DNA samples were genotyped as 'Indian Bison type' (pre-dominant type of MAP in animals of India). Presence of 'Indian Bison type' genotype and higher prevalence of MAP infection in cases of

suspected animal keepers as compared to not-suspected animal keepers, strongly indicated the role of MAP in causing CD.

Key Words: *Mycobacterium avium* ssp. *paratuberculosis*, Crohn's disease, inflammatory bowel disease

286 Herd-level prevalence of Johne's disease on dairy farms in Utah and the surrounding Intermountain West. D. J. Wilson*¹, K. A. Rood¹, and J. D. Trujillo², ¹Utah State University, Logan, ²Iowa State University, Ames.

The objective was to determine dairy herd-level prevalence of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) infection, the causative agent of Johne's disease (JD) in Utah and some surrounding states. A signed permission slip was required for participation in the study. Two milk samples were collected from each bulk tank on study farms, one month apart. The farms shipped milk to two major milk buyers in Utah, with all milk collected at one of two milk processors. Milk haulers collected an extra bulk tank milk sample for use in the study. Identity of the farms was coded for anonymity by milk plant personnel. An ELISA test for IgG1 antibody against MAP and a PCR to detect MAP, both designed for pooled bulk tank milk were performed on each milk sample. Each bulk tank was to be tested 4 times, twice on each of the 2 samples. The number of tank samples tested per farm ranged from 1 to 24 (most farms had 2 or 4 samples from one or two bulk tanks, respectively). The 2 farms with the most bulk tanks had 6 and 12 tanks, respectively. JD was detected at least once on 67/170 farms tested (39%). The most common positive test results were 1 or 2 positives/4 JD tests (n = 28 farms). The lowest proportions of all tests positive for JD on a given farm were 2/48, 1/8 (n = 3), and 2/12 (n = 2). Highest proportions of positive tests were 8/8, 4/4, 10/12, 9/12, 6/8, 7/12, and 13/24. The finding of 39% of herds positive for JD is similar to other recent estimates of herd prevalence, indicating increased levels of the disease compared with national estimates 15-20 years ago. Some herds were enrolled in a demonstration project to evaluate the effectiveness of using the individual cow JD milk ELISA test and culling to reduce prevalence of the disease. Analysis of data and reporting of results from that study will be completed in the future.

Key Words: Johne's disease, MAP, dairy cattle