Animal Health: Johne's Disease

182 Bayesian analysis of longitudinal Johne's disease diagnostic data without a gold standard test. C. Wang*[1], B. Turnbull[2], S. Nielsen[3], and Y. Gröhn[2], *[1]Iowa State University, Ames, [2]Cornell University, Ithaca, NY, [3]University of Copenhagen, Frederiksberg, Denmark.*

A Bayesian methodology was developed based on a latent change-point model to evaluate the performance of milk ELISA and fecal culture tests for longitudinal Johne's Disease diagnostic data. The situation where there is no perfect reference test was considered, i.e., no "gold standard." A change-point process with a Weibull survival hazard function was used to model the progression of the hidden disease status. The model adjusted for the fixed effects of covariate variables and random effects of subject on the diagnostic testing procedure. Markov chain Monte Carlo methods were used to compute the posterior estimates of the model parameters that provide the basis for inference concerning the accuracy of the diagnostic procedure. Based on the Bayesian approach, the posterior probability distribution of the change-point onset time can be obtained and used as a criterion for infection diagnosis. An application is presented to an analysis of ELISA and fecal culture test outcomes in the diagnostic testing of paratuberculosis (Johne's disease) for a Danish longitudinal study from January 2000 to March 2003. The posterior probability criterion based on the Bayesian model with 4 repeated observations has an area under the receiver operating characteristic curve (AUC) of 0.984, and is superior to the raw ELISA (AUC = 0.911) and fecal culture (sensitivity = 0.358, specificity = 0.980) tests for Johne's disease diagnosis.

Key words: Johne's disease, longitudinal, no gold standard

183 Environmental contamination with *Mycobacterium avium* ssp. *paratuberculosis* in endemically infected dairy herds. R. L. Smith*[1], Y. H. Schukken[1], A. K. Pradhan[1], J. M. Smith[2], R. H. Whitlock[3], J. S. Van Kessel[4], D. R. Wolfgang[5], and Y. T. Grohn[1], *[1]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, [2]Department of Animal Science, University of Vermont, Burlington, [3]Department of Clinical Studies, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, [4]Environmental Microbial and Food Safety Laboratory, ANRI, USDA-ARS, Beltsville, MD, [5]Department of Veterinary and Biomedical Science, Penn State University, University Park.*

Environmental contamination with *Mycobacterium avium* ssp. *paratuberculosis* (MAP) is thought to be one of the primary sources of infection for dairy cattle. The exact link between fecal shedding of MAP by individual cows and environmental contamination at the herd level was explored with a cross-sectional analysis of longitudinally collected samples on 3 dairy farms. Composite samples from multiple environmental sites in 3 commercial dairy herds in the Northeast US were cultured quarterly for MAP, providing 898 samples (113 (12.6%) were culture-positive), and all adult animals in the herds were tested biannually by fecal culture (FC), for 6 years. Of the environmental sites sampled, manure storage areas and shared alleyways were most likely to be culture-positive. Environmental sample results were compared with FC results from either the concurrent or previous sampling date at both the herd and the pen level. At the herd level, a 1 log unit increase in average fecal shedding increased the odds of a positive environmental sample by 3.5 and increased the average amount of MAP in the sample by 2.1 cfu/g. At the pen level, the odds were increased by a factor of 3 and the average amount of MAP was increased by 1.1 cfu/g. There was no significant relationship between environmental site status and the distance between shedding animals and the site, and neighboring pens did not affect the results of the pen-level analysis. The amount of MAP in pen-level samples was positively correlated with the number of animals in the pen shedding >30 cfu/g of MAP. At least 6 environmental samples met the criteria for the US Voluntary Bovine Johne's Disease Control Program on 45 of the 65 testing dates; of these, 16 of the 42 FC-positive testing dates were positive by the 6-sample environmental testing method, resulting in a herd sensitivity of 0.38 (95% CI: 0.23 to 0.52), and 0 of the 3 FC-negative testing dates were positive by this method. Although environmental sampling can be used as a tool in understanding the level of MAP infection in a herd or pen, it does not appear to be a sensitive diagnostic method for herd positivity and should be used with caution.

Key words: Johne's disease

184 *Mycobacterium avium* ssp. *paratuberculosis* promotes rapid IL-1β release and macropahge transepithelial migration. E. Lamont*[1], S. O'Grady[1], W. Davis[2], T. Eckstein[3], and S. Sreevatsan[1], *[1]University of Minnesota, [2]Washington State University, [3]Colorado State University.*

Pathogen processing by the intestinal epithelium involves a dynamic innate immune response initiated by pathogen-epithelial cell cross-talk, which may be augmented by interactions between host pathways and/or cell types. Studies investigating *Mycobacterium avium* ssp. paratuberculosis, the causative agent of chronic enteritis in ruminants, focus solely on the macrophage and largely neglect responses within the epithelium. We show that *M. avium* ssp. *paratuberculosis* induces phagosome acidification within bovine epithelial (MAC-T) cells as early as 10 min, resulting in upregulation of IL-1β at transcript and protein levels. Previous studies report that IL-1β is a potent macrophage chemoattractant. These initial host-pathogen interactions may dictate a form of cooperative self-destruction in which the host is deceived into reacting to the benefit of *M. avium* ssp. *paratuberculosis*; thereby, setting the tone for the ensuing infection. We hypothesized that *M. avium* ssp. *paratuberculosis* harnesses host responses to recruit macrophages to the site of infection to ensure its survival and dissemination. We investigated macrophage recruitment in response to *M. avium* ssp. *paratuberculosis* using a MAC-T-bovine macrophage coculture system. Within 10 min of infection, macrophages were recruited to the apical side of MAC-T cells. Inhibition of phagosome acidification and IL-1β abrogated this response, while MCP-1/CCL2 blocking had no effect. IL-1β processing was dependent upon Ca^{2+}uptake from the extracellular media and intracellular Ca^{2+}oscillations as determined by EGTA and BAPTA-AM treatments. Thus, *M. avium* ssp. *paratuberculosis* guidance of phagosome-acidification enlists IL-1β processing in an extracellular calcium dependent manner to efficiently transverse the epithelium and into its niche—the macrophage.

Key words: *Mycobacterium avium* ssp. *paratuberculosis*, interleukin 1 beta, macrophage recruitment

185 Real-time estimation of the lacto-presence of *Mycobacterium avium* subspecies *paratuberculosis* in milk and milk products originating from goat and cattle herds endemic for Johne's disease. S. V. Singh*[1], T. Raghuvanshi[1], R. B. Sharma[1], B. Singh[1], A. V.

Singh[1], P. K. Singh[1], A. Kumar[1], and A. Srivastav[2], *[1]Central Institute for Research on Goats, Mathura, Uttar Pradesh, India, [2]College of Veterinary Sciences, Mathura, Uttar Pradesh, India.*

Mycobacterium avium subspecies paratuberculosis (MAP) is the cause of Johne's disease in ruminants and is endemic in countries where investigated including India. Live MAP has been recovered from raw and pasteurized milk and milk products. Information on the status of MAP in the milk and milk products is extremely limited. Real time estimate of lacto-presence of MAP were studied in milk and milk products originating from herds endemic for Johne's disease. Forty eight and 23 individual milk samples were collected from two goat farms (Farm A. and Farm B) and a cattle farm (Farm C) in Mathura region of North India. Eighteen pooled goat milk samples from the Farm A. and B. and 24 paneer (raw cheese from pooled milk) samples were collected. Samples were screened using microscopy, indigenous ELISA kit and IS900 PCR to estimate lacto-presence of MAP. Tests showed variable lacto-presence of MAP in milk and milk products. Using microscopy, 25, 4.2, 5.6 and 12.5%, of milk samples of Farm A. and B, pooled milk and paneer samples were positive, respectively. In milk-ELISA, 25% of individual milk samples of Farm A. and B. were positive, whereas, none of the pooled milk samples were positive for MAP. Screening of 9 and 3 individual milk samples of Farm A. and B, 3 pooled milk samples and 2 samples of paneer by IS900 PCR, one milk sample of Farm A. was positive. Lacto-presence of MAP was higher in milk samples originating from cattle herds and of 23 milk samples, 56.5, 86.9 and 30.4% were positive in microscopy, ELISA and PCR, respectively. Lacto-presence of MAP in the two goat farms was low to moderate and was high in the cattle herd. Load of MAP in livestock herds had significant correlation with presence of MAP in milk and milk products originating from herds endemic for JD, thereby posing serious health risk to human population.

Key words: milk and milk products, lacto-prevalence, *Mycobacterium avium* subspecies *paratuberculosis*

186 Association of Bsa I polymorphism of MHC Class II DRB gene with *Mycobacterium avium* ssp.*paratuberculosis* bacterimia in Jamunapari breed of goats. S.V. Singh, P. Rai, P. K. Singh*, A. V. Singh, M. K. Singh, and J. S. Sohal, *Central Institute for Research on Goats, Mathura, Uttar Pradesh, India.*

Johne's disease (JD) caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is responsible for huge economic losses in livestock productivity worldwide. Failure to detect early infection and lack of effective vaccine hamper control of disease. Studies on variations in host genetics that contribute to resistance or susceptibility to disease may potentially help to control disease effectively. Polymorphism in exon-2 of caprine MHC Class II DRB region further elucidated the effect of genetic variation in this region on MAP bacteremia in Jamunapari goats. Blood samples from 38 adult goats of Jamunapari farm (Central Institute for Research on Goats in Mathura) endemic for JD were analyzed to detect MAP infection using IS900 PCR and characterize MHC class II DRB region using Bsa I. PCR-RFLP. Of 38 goats 26 (68.4%) were positive for presence of MAP in blood. Three genotypes for Bsa I, namely BB, Bb and bb (with genotypic frequency of 0.711, 0.025 and 0.265, respectively) and two alleles B. and b (with allelic frequency of 0.843 and 0.157, respectively) were observed in the DRB second exon region of Jamunapari goats. Non-significant effect of Bsa I. MHC class II alleles with MAP bacteremia was found ($\chi^2 = 1.950$, df = 2, $P = 0.005$). There was no significant difference in distribution of all three genotypes (BB, Bb and bb) between goats positive or negative for MAP DNA in blood. Distribution of homozygous BB alleles was approximately significant ($\chi^2 = 3.779$, $P = 0.052$) in MAP positive group of goats. Genetic variation of MHC Class II DRB region may regulate MAP infection; therefore, a comprehensive study on substantial goat population is needed to get some conclusive impact of BsaI based single nucleotide polymorphism and others of DRB region alleles on JD susceptibility and / or resistance.

Key words: paratuberculosis, MHC class II DRB gene, polymorphism

187 Johne′s program—Impact on education and outreach activities. K. E. Olson*, *KEO Consulting, Schaumburg, IL.*

Federal investment in the Voluntary Bovine Johne′s Disease Control Program declined from $21m in 2003 to $6.8m in FY09. Primary metrics have included number of herds in the program and samples tested in approved labs; however, it is anticipated that program investments have produced other activities that will enhance efforts to address the disease. State Designated Johne′s Coordinators (DJCs), Dairy Herd Improvement Association (DHIA) service units and, dairy and beef producer organizations were surveyed to document program participation changes and identify current activities that will affect the producer′s ability to deal with the disease. Responses were received from 32 DJCs, 16 beef organizations, 5 dairy cooperatives, 12 DHIA organizations and 3 dairy records processing centers. The following program impacts resulting from funding reductions were reported by DJCs: 25 ran fewer individual samples; 25 had fewer Risk Assessments and Management Plans (RAMPs) completed; 24 certified or re-certified fewer veterinarians; 22 conducted fewer educational events; 19 had fewer committee meetings. The DJC survey quantified activities not measured in program metrics that affect producer knowledge and ability to deal with the disease including: 1,434 Johne′s certified veterinarians available to work with producers; 56 meetings with 9.721 participants; 10,720 copies of 27 publications distributed; 13 internet sites devoted to Johne′s information delivery; 12 reported articles in trade publications reaching 9,656 readers. Producer organizations reported:1 beef meeting with 250 producers; 20 dairy meetings with 975 producers; 6 DHIAs trained technicians and 4 met with producers; 207,033 milk ELISA samples run by DHIA, a 10% increase. Research is currently funded by one breed association and JDIP is providing competitive grants that have leveraged additional outside funds. A concern identified is the need for additional communication between program leadership and producers relative to program operation with reduced funding. Program resources have developed a strong infrastructure to assist producer efforts, but additional planning is needed to find ways to maintain needed resource and make them available to producers.

Key words: Johne's, JDIP

188 Mathematical modeling of *Mycobacterium avium* subspecies *paratuberculosis* infection transmission in dairy cattle: Current status and future directions. Z. Lu*[1], R. Mitchell[1], R. Smith[1], Y. Schukken[1], Y. Gröhn[1], K. Ahmadizadeh[2], M. Teose[2,3], T. Damoulas[2], and C. Gomes[2], *[1]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, [2]Department of Computer Science, Cornell University, Ithaca, NY, [3]Center for Applied Mathematics, Cornell University, Ithaca, NY.*

The objective of this presentation is to review the current status and to outline the future research directions of mathematical modeling of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infections

in dairy cattle. As one of the most important infectious diseases in dairy cattle, MAP infections cause considerable economic losses in the dairy industry and pose a potential threat to public health. Control of MAP is difficult due to the long incubation period and poor diagnostic tests for early MAP infections. To have a better understanding of MAP transmission dynamics and to evaluate the effectiveness of MAP control strategies in dairy cattle, several within-herd mathematical compartment models on the basis of MAP infection history have been developed using deterministic and stochastic modeling approaches. We reviewed these mathematical models and examined their usefulness and limitations. Subsequently, we identified 4 potential research directions in mathematical modeling of MAP infection at different levels of organization. The first is to investigate how MAP is transmitted between herds in a large spatial region. In the United States, studies showed that at least 70% of dairy herds were infected with MAP. To understand this high herd-level MAP prevalence and find effective control strategies for MAP at herd level, construction of a network structure of animal movements will be necessary. The second research direction is to continue modeling the within-herd level of MAP infection, considering more challenging problems such as MAP strain competition, co-infection, and the importance of environmental transmission. The third research direction is to study the infection process of MAP and host immune response at the individual animal level. Mathematical models at this level will be particularly helpful for understanding the potential mechanism of MAP vaccines. The fourth direction is to build individual-based economic optimization models and to find optimized MAP control strategies within a herd.

Key words: paratuberculosis, mathematical modeling, dairy cattle

189 Vertical transmission or increased susceptibility to MAP? E. Knupfer[1], R. M. Mitchell*[2], A. K. Pradhan[2,3], A. Kramer[1], J. Dieguez[4], R. H. Whitlock[5], T. Fyock[5], and Y. H. Schukken[2], *[1]Utrecht University, Utrecht, the Netherlands, [2]Cornell University, Ithaca, NY, [3]University of Maryland, College Park, [4]Universidade de Santiago de Compostela, Spain, [5]University of Pennsylvania, New Bolton Center.*

MAP-infected dairy cattle are assumed to be a high risk for transmitting infection to their daughters. Alternatively, if both dam and daughter are genetically more susceptible to MAP, they may be both infected but not necessarily due to vertical transmission. Using strain typing techniques including multi locus short sequence repeat (MLSSR) typing allows a potential distinction between vertical transmission and genetic susceptibility. Analyzing strain diversity in longitudinal data sets provides additional insight into within-herd infection dynamics, including the transmission of MAP from dams to daughters. To investigate the importance of vertical transmission, we identified 12 pairs of dams and daughters for which both animals are known MAP infected from the Regional Dairy Quality Management Alliance (RDQMA) study herd in NY. All adult animals were tested for MAP via fecal culture semi-annually for seven years. Tissue samples were available on a subset of cull animals. Animals were considered MAP-infected if they ever cultured positive or if any of their tissues cultured positive at slaughter. Cultures were performed at University of Pennsylvania on HEYM solid media. Positive cultures were substreaked and processed for MLSSR typing. Following genotyping, isolates from each dam-daughter pair were compared to determine whether they shared the same MAP genotype. Environmental MAP burden at birth was assessed via typing of MAP-positive environmental samples (collected four times a year) and known MAP-infected animals present on the farm during the high-risk first year of life. Of the 12 infected dam-daughter pairs, 9 had identical strains shared between the dams and daughters. In addition, 2 daughters had the dam's strain as well as another circulating strain. Overall, there were 7 strains represented in the daughters that did not come from dams (2 daughters had multiple strains which did not originate from the dam). These results lend additional importance to the impact of genetics on susceptibility, as 5 of 12 daughters carried different strains of MAP than their dams, even when concurrently infected with the dam's strain.

Key words: *Mycobacterium avium* subspecies *paratuberculosis*, vertical transmission, strain typing

190 MAP co-infection or evolution? R. M. Mitchell*[1], E. Knupfer[2], A. K. Pradhan[1,3], A. Kramer[2], J. Dieguez[4], R. H. Whitlock[5], T. Fyock[5], and Y. H. Schukken[1], *[1]Cornell University, Ithaca, NY, [2]Utrecht University, Utrecht, the Netherlands, [3]University of Maryland, College Park, MD, [4]Universidade de Santiago de Compostela, Spain, [5]University of Pennsylvania, New Bolton Center.*

Co-infection is important in human tuberculosis (Htb), especially in high prevalence environments where multiple exposures increases likelihood of infectious progression. This phenomenon is not often studied in bovine mycobacterial infections, partially due to the lack of differentiation between host-specific strains of MAP. However, as illustrated by recent research on cull animals and longitudinal sampling on dairy farms which includes tissue samples at slaughter, cattle live in a very high prevalence environment. If the many subclinically infected animals which are in close contact with herdmates are intermittently shedding throughout their lives, this will result in multiple exposures. We examined whether animals shed the same MAP strain throughout their lifespan. All animals on the NY farm in the Regional Dairy Quality Management Alliance (RDQMA) were tested for MAP using fecal sampling semi-annually for seven years. Tissue samples were available on a subset of cull animals. Cultures were performed at University of Pennsylvania on HEYM solid media. All positive fecal cultures and one or more positive tissue sample from each animal were evaluated by multilocus short sequence repeat typing (MLSSR) for each animal. Strain types were assigned based on number of repeats at each MLSSR locus selected. In the 96 animals with at least one positive fecal or tissue culture over the course of the longitudinal study, there were 13 strain types identified. Of the 59 animals with only one sample processed, 4 (7%) had mixed infections (clear evidence of two sequences of differing repeat lengths) when analyzed. Of the 37 animals with more than one sample analyzed, 19 (51%) had more than one MAP strain throughout life including 17 with mixed infections. This close study of within-farm dynamics reveals that it is not uncommon for animals to be infected with multiple strains of MAP. The often close relationship between strains in multiple infections brings to mind the possibility that what we are seeing in MAP is really within host evolution of MAP strains rather than (mixed) co-infection in a portion of multiply infected animals.

Key words: *Mycobacterium avium* subspecies *paratuberculosis*, strain typing, multiple infections

191 Towards understanding endemicity of MAP infection in dairy herds. R. M. Mitchell*[1], G. F. Medley[2], and Y. H. Schukken[1], *[1]Cornell University, Ithaca, NY, [2]Warwick University, Coventry, UK.*

Maintenance of MAP infection on dairy farms is non-trivial in terms of infectious dynamics. Indeed a large portion of animals do not shed MAP throughout their lifetimes despite being truly infected when their tissues are cultured at slaughter. In this study we incorporate the

infection biology of MAP into a mathematical model which takes into account age and dose dependent infectious progression. The model incorporates a large proportion of truly infected but never shedding animals which are observed as MAP positive in tissue culture studies but not in fecal shedding. We modified our current models of MAP in Matlab to incorporate a slow-progressing latent category based on previously published data on dose and age dependent infection probabilities. In this model, animals either shed shortly following exposure and enter an early shedding compartment, or do not shed early and enter a slow-progressing latent state- where they remain for an extended period of time. Animals which shed early have a shorter duration of latency following this early compartment and progress to late shedding. Infectious progression is modeled differently for animals which receive high or low initial doses of MAP. Animals which receive high doses of MAP spend a longer period of time in the early shedding compartment and are therefore more likely to come into contact with susceptible young animals during this time of increased infectiousness. Model output indicates that endemic MAP infection is possible at transmission rates that would not allow successful entry of MAP in a fully susceptible population. Indicating that MAP endemicity is driven by different infection dynamics than the initial establishment of MAP in a dairy herd. Our model indicates that there are 2 sustainable MAP infection equilibria at relative contact rates of less than one between adult animals and calves. This structure appears to be unique to dairy cattle, and may explain why endemically MAP infected herds occur frequent. Our findings explain why slaughter samples show a dramatically higher prevalence of MAP infection in tissue samples compared with MAP fecal shedding.

Key words: *Mycobacterium avium* subspecies *paratuberculosis*, endemicity, models

192 *Mycobacterium avium* subspecies *paratuberculosis*-infected macrophages have different protein and transcriptome profiles than control or uninfected culture mates. E. Kabara* and P. Coussens, *Michigan State University, East Lansing.*

Previously, our group presented evidence that *Mycobacterium avium* subspecies *paratuberculosis* (MAP)-infected macrophages are significantly less apoptotic than uninfected, culture mates (bystander macrophages) found in a MAP-infected culture. This mirrors the apoptotic status of Mycobacterium tuberculosis (TB)-infected macrophages and bystander cells found in TB-infected cultures. Currently, little published work has been done studying apoptotic pathway regulation in TB and MAP-infected macrophages. Therefore, our goal was to investigate the mycobacterial specific mechanism and corresponding host reactions that are employed to prevent apoptosis in mycobacterium-infected macrophages. We hypothesize that MAP-infected macrophages have differential expression of several apoptotic when compared with bystander macrophages. Two distinct methods were undertaken to test this hypothesis. First, we used flow cytometry with fluorescent labeling of MAP bacteria and antibody labeling of host proteins to study the expression and phosphorylation status of host proteins of MAP-infected, bystander, and control macrophages with and without stimulation. Among our many observations, we saw no significant differences in MAPK protein expression and phosphorylation in unstimulated control and MAP-infected macrophages which is in agreement with previous publications on this subject. Second, we isolated RNA from control and MAP-infected macrophages cultures where over 90% of the cells are infected to study a population of MAP-infected macrophages without bystander cells. These samples were used in RT-qPCR testing of apoptosis pathway transcripts. We observed significant upregulation of Caspase 3 and 9 mRNA expression in MAP-infected macrophages as compared with controls while seeing no significant difference in caspase 8 expression between the 2 samples (P-value <0.05).

Key words: Johne's, apoptosis, pathway

193 Effect of changes in management practices on the risk of Johne's disease in Minnesota Johne's disease demonstration dairy herds. L. A. Espejo*, S. Godden, and S. J. Wells, *University of Minnesota, Department of Veterinary Population Medicine, St. Paul.*

Certain management practices have been recommended to minimize transmission of Johne's disease between infected and susceptible cattle. The objective of this study was to evaluate the risk of testing positive and its association with changes in recommended management practices in different birth cohorts. Eight dairy herds and approximately 6,000 cows were enrolled in the Minnesota Johne's Disease Demonstration Herd Program. Herds were monitored for a period between 5 to 10 years. Annual testing for *Mycobacterium avium* ssp. *paratuberculosis* was performed for all cows that calved, using bacterial culture and serum ELISA. Risk assessments were performed annually to measure the level of implementation of the recommended management practices. Eight birth cohorts were defined based on the date of cow enrollment in the program. Birth cohorts −2 and −1 corresponded to cows that were born 2 and 1 year before the beginning of the program, respectively, and cohorts 0 to 5 corresponded to cows that were born 0 to 5 years after the beginning to the program. The annual risk assessment score was used to quantify the level of exposure by birth cohort and herd. A time dependent Cox's regression model was used to model the time to test positive, explained by herd, birth cohort and birth cohort exposure level. Compared with birth cohort −2, there was a reduction of the hazard ratio (95%CI) of bacterial culture positivity of 0.65 (0.49 to 0.85), 0.56 (0.42 to 0.73), 0.66 (0.48 to 0.90), 0.38 (0.26 to 0.58), 0.22 (0.14 to 0.35), 0.22 (0.14 to 0.34), and 0.20 (0.13 to 0.32), for birth cohorts −1, 0, 1, 2, 3, 4, and 5, respectively. Similar results were obtained for serum ELISA. The instantaneous hazard of testing positive for both tests increased with the level of exposure, however, the strength of this association decreased over time. There was a reduction in the transmission of Johne's disease associated with the level of implementation of the recommended management practices.

Key words: Johne's disease, disease control