

REPORT OF THE COMMITTEE ON JOHNE'S DISEASE

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The Committee met from 12:30 to 5:30 p.m. on Sunday, October 21, 2007 at John Ascuaga's Nugget Hotel, Reno, Nevada. There were 78 attendees. The Committee recognized Robert Whitlock and John Adams for their years of service to the Committee, including leadership and for serving as Co-Chairs of the National Johne's Working Group (NJWG) since its inception.

Status of 2006 Resolutions and Recommendations

RESOLUTION 11: INDEMNIFICATION TO ELIMINATE CATTLE CONFIRMED POSITIVE FOR MYCOBACTERIUM AVIUM PARATUBERCULOSIS (MAP)

The United States Animal Health Association (USAHA) recommends that the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) request necessary funding to provide limited indemnification of cattle for producers who participate in the National Johne's Control Program, meet all Program Standards and cull to slaughter any animal confirmed positive for *Mycobacterium avium paratuberculosis* (MAP) by an officially recognized test provided further that the indemnification will apply only to animals determined to be clinically normal and a high or moderate MAP shedder.

The USAHA further requests that Congress recognize the importance of funding a Johne's disease indemnification program to augment, and not subtract from, current minimal funding for the National Johne's Control Program. USAHA recommends that this program remain voluntary.

RESPONSE: The United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) appreciates this recommendation and remains committed to improving our Johne's control program. However, we have several concerns regarding the request to provide indemnity for cattle confirmed positive for *Mycobacterium avium paratuberculosis* (MAP).

These include:

- No authorization for indemnity in the statute which establishes the Johne's program (7 USC Sec. 7626). This statute limits USDA to funding requests for conducting research, testing, and evaluation of programs for the control and management of Johne's disease in livestock. In

addition, authorizations of appropriations for the Johne's program only extend through 2007. USDA can not consider acting on this request until the new farm bill updates this restriction.

- Indemnity can only be applied to eradication programs (regardless of whether they are voluntary or mandatory). The Johne's program is a control program. Removal of some infected animals, while leaving others within the herd, will not produce a reduction in the national herd prevalence and can not be considered eradication. Currently, the economic models published show that test and cull programs can not remove the infection from the herds and would not be cost-effective methods to eradicating Johne's disease.
- Any herd owner that would participate in the indemnity program would have to make eradication of the disease the goal of their herd plan which requires the removal of all infected animals. Removal of some infected animals, while leaving others, will not produce a rapid reduction within a producer's herd prevalence levels, thereby prolonging the cleanup efforts.
- Enzyme-linked immunosorbent assay (ELISA) testing is the most cost-effective method of managing the infection on the farm after the presence of MAP has been confirmed in moderate to heavily infected herds. Confirming ELISA positive animals to establish their eligibility for indemnity delays removal of the animal from the herd, in addition to accumulating further costs to the program.
- Producers that are only willing to remove heavily shedding animals after applying for indemnity would not be considered committed to Johne's eradication in their herd. Industry has not provided any information supporting how the inclusion of indemnity would increase participation in the voluntary program, or increase the commitment of producers already enrolled.

As a result of these concerns, VS will not pursue indemnity funds for the Voluntary Bovine Johne's Disease Control Program at this time.

RESOLUTION 12: QUANTITATIVE BULK TANK MILK TESTS FOR DETECTING JOHNE'S DISEASE

The United States Animal Health Association (USAHA) recommends that the United States Department of Agriculture (USDA), Agricultural Research Services (ARS) and the research community have a greater focus on development of quantitative based tests for detecting *Mycobacterium avium paratuberculosis* (MAP) in bulk tank milk.

RESPONSE: United States Department of Agriculture (USDA), Agricultural Research Services (ARS) proactively initiated the development of a quantitative-based test for detecting MAP in bulk tank milk in 2006; this is a quantitative real-time PCR test for Johne's disease in milk and other tissues that uses the unique target sequences, ISMapO2, identified by ARS through the Johne's genome sequence project. ARS has developed a test format that includes a probe enabling the quantification of the amount of MAP DNA present in a test sample. ARS is collaborating with Sandra Godden at University of Minnesota in using this test on colostrums samples obtained from noninfected and infected dairy herds, and to date has evaluated this experimental test on over 350 samples. When completed, the results will be submitted to the University of Minnesota, which will then conduct validation studies by comparing the results to fecal shedding of the bacterium. ARS plans further research on this approach to enabling the quantification of MAP in bulk tank milk.

RECOMMENDATIONS FROM 2006:

1. That USDA continue support of the National Demonstration Herd Project (NDHP) by facilitating meetings with VS providing travel expenses for the NJWG Demonstration Herd Subcommittee to work with Charles Fossler and Jason Lombard and staff at CEAH to analyze the resultant data and prepare manuscripts in a timely manner. Additionally, for CEAH to allocate more funds to assist the Johne's Disease epidemiologists to enhance the efforts of CEAH staff working with the National Johne's Program. Furthermore, that Jason Lombard continues as an active participant in

this process and continues to participate as coordinator of the NDHP with the newly hired John's Epidemiologist Charles Fossler. **Response:** Results from analysis of data from the National Demonstration Herd Project was the focus of a half-day session of the National John's Working Group on October 18, 2007. Preliminary analysis (abstract attached below) shows results are consistent with effectiveness of the control program in reducing incidence of John's disease on cattle operations. An outline of analyses and potential publications was presented.

2. Laboratories that passed the John's organism detection check test outside the normal time sequence (typically February through May each year) should be given "preliminary approval" as an approved laboratory for that specific methodology i.e. solid media, liquid media or PCR testing. Preliminary approval would be given when laboratory results are submitted after NVSL report at the annual USAHA meeting. Additionally, requests for check test kits would be honored from laboratories that are implementing a new test method outside the time when test kits are routinely shipped to participating laboratories. Preliminary approval would be provided following submission of check test results that meet or exceed the test criteria established that year. However, that preliminary approval would not include listing of that laboratory in the approved laboratory list as published in the USAHA proceedings nor would that laboratory be listed on the USDA-APHIS web site of approved laboratories that year. Laboratories that pass the annual organism based proficiency test are officially approved January 1 following the annual USAHA meeting. **Response:** Procedure has been put in place.
3. Laboratories that fail organism detection test and desire a retest should complete the following protocol through NVSL.
 - a. Each laboratory would be required to provide a written self-assessment report outlining possible deficiencies or situations as to what factors lead to an inadequate check test. Included would be a plan to enhance the laboratories proficiency to detect MAP in fecal samples. A template for this report is being developed. If a commercial test kit or test system is being used for organism detection, the company should be contacted to help determine the source of the problem and their findings should be included in the self assessment.
 - b. Each laboratory would be encouraged to seek additional training either from another local laboratory considered proficient in organism detection or at NVSL.
 - c. Letters from NVSL notifying each laboratory about test results will also be sent to the Designated John's Coordinator (DJC) for that state and to the National John's Coordinator (NJC) for their information. Laboratories that do not pass the check test must contact the NJC and their DJC regarding continuation of their opportunity to perform organism detection tests for the Voluntary Bovine John's Disease Control Program.
 - d. Laboratories that fail the organism based check test are encouraged to re-take the check test following submission of their written self-assessment and approval of the National John's Coordinator, if adequate check test kits are available at NVSL.

Response: Procedure has been put in place.
4. Laboratories that fail two sequential organism detection test and desire a retest should complete the following protocol through NVSL.
 - a. Each laboratory would be required to provide a written self-assessment report outlining possible deficiencies or situations as to what factors lead to an inadequate check test. Included would be a plan to enhance the laboratories proficiency to detect MAP in fecal samples. If a commercial test kit or test system is being used for organism detection, the company must be contacted to help determine the source of the problem and their findings should be included in the self assessment.
 - b. Laboratories in this category will be required to send the person responsible for the organism detection testing to NVSL or to another laboratory with the necessary experience and expertise approved by NJC for further training in mycobacterial detection methods.

- c. Laboratory would be required to purchase and submit results from a second check test following mandatory training at NVSL or another laboratory as approved by the NJC.
- d. Letters from NVSL notifying each laboratory about test results will also be sent to the DJC for that state and to the NJC for their information.

Response: Procedure has been put in place.

5. USDA-APHIS-VS signed a cooperative agreement (05-9100-0996-GR) with a team of scientists to develop a consensus recommendation on diagnostic testing for bovine paratuberculosis in the U.S. These recommendations have been developed and were reviewed and approved by the NJWG. The Committee accepts and recommends that USDA adopt the Diagnostic Testing for Bovine Paratuberculosis in the U.S. as developed under cooperative agreement 05-99100-0996-GR. This recommended test regimen for the detection of paratuberculosis in cattle is included in these proceedings following the Committee Report. **Response:** Accomplished.
6. The Committee recommends that USDA-APHIS-VS provide funding to identify target herd sensitivities and the most cost-efficient testing alternatives for detection of *M. paratuberculosis* in dairy and beef cattle herds at different levels of the Johne's Disease Test Negative Program. **Response:** Funding was provided to the University of Minnesota for this project and a preliminary concept paper report was presented to the National Johne's Working Group on Friday, October 19.
7. The Committee recommends that USDA-APHIS-VS-NVSL continue to develop a systematic protocol for the production and characterization of a uniform, quality Johnin purified protein derivative (PPD) and manufacture Johnin PPD. The Johnin PPDs must be of equivalent sensitivity and specificity from batch to batch. These products must be available for distribution to researchers upon request. **Response:** Efforts are underway by NVSL.
8. The Committee recommends that NVSL provide a pilot test panel of ten test samples, consisting of three or more different mycobacterial species, to interested diagnostic laboratories performing confirmatory PCR tests on all acid-fast suspect positive cultures for *M. paratuberculosis*. The laboratories will provide PCR methodologies and results, reported as positive or negative, back to NVSL. **Response:** Accomplished.
9. The Committee acknowledges and appreciates the improvement and speed in which the Center for Veterinary Biologics (CVB) has licensed products important to the NJCP. We recommend that CVB review milk Enzyme-linked immunosorbent assay (ELISA) in an expedient manner. In order for laboratories to qualify to perform the milk ELISA as a 'program' test, a proficiency test panel must be developed for laboratory approval. The Committee recommends that NVSL acquire milk samples from an outside source and not purchase lactating cows for the sole purpose of providing milk for the proficiency panel. **Response:** One ELISA test kit has been approved by CVB for marketing as a milk ELISA test kit and efforts are underway by NVSL to develop an ELISA test kit.
10. The Committee approved a recommendation that NVSL provide and distribute a fecal sample from a low / moderate shedding cow to be used in a pilot study involving approximately 5 – 10 laboratories for each of the three culture methods (HEY, Trek and MGIT) and quantitative direct PCR to evaluate sources of variation in fecal culture shedding levels. Data will be reported to CEAH. **Response:** This effort was not completed last year, but plans are underway for implementation in 2008.
11. The Committee recommends that USDA and livestock producers expedite the implementation of a national animal identification system (NAIS). NAIS would greatly enhance the ability to identify and control movement of infected animals. We also recommend development of an indemnification program, supported in part by producers, to increase the confidence that these animals will not spread disease to other herds. Furthermore we recommend producers consider

the high risk of introducing Johne's disease when purchasing cattle. **Response:** Efforts continue towards implementation of a national animal identification system.

Ken Olson, National Institute for Animal Agriculture (NIAA), provided the Johne's Education Update. The mission of the Education Initiative is to provide producers and those who work with them reliable, useful, easy to access information about Johne's Disease that is based on the best current science. A primary tool for this effort is the website www.johnesdisease.org. In addition at least 12 Johne's related articles have appeared in producer press since the first of the year. A current effort that is underway with the Johne's Disease Integration Program (JDIP) is a national dairy producer survey that seeks to identify barriers to and useful incentives for participation in the Johne's program. It will also provide insights on information and education needs for the future.

Michael Carter, National Johne's Program Coordinator, presented the Fiscal Year (FY) 2007 Johne's Disease Program Updates.

In 1997, USAHA's National Johne's Working Group (NJWG) appointed a Sub Committee to design an affordable and flexible program based on sound scientific knowledge. The result was the Voluntary Johne's Disease Herd Status Program (VJDHSP). Instead of trying to certify herds free of Johne's disease, the VJDHSP provides minimum requirements for a program to identify herds of low risk with *M. paratuberculosis* infection. These guidelines are used as a model for the Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program (VBJDCP) approved by USDA-APHIS-VS in April of 2002. The latest revision to the program standards occurred in June 2006 with inclusion of pooled fecal samples for level three test negative testing and updating the laboratory approval section of the standards.

For FY 2007 through October 10, 2007, 49 States had adopted VBJDCP or had programs that were considered in compliance with these standards. In fiscal year (FY) 2007, the reported activities includes 599,393 cattle tested by enzyme linked immunosorbent assay (ELISA), 120,170 cattle tested by fecal culture, 11,859 cattle tested by polymerase chain reaction (PCR), 8,483 enrolled herds (6,472 dairy and 2,011 beef) of which 1,709 are test negative herds (1,009 dairy and 700 beef). Herds enrolled as test-negative are progressing through to level four. There are 689 Johne's program level one (390 dairy and 306 beef), 595 Johne's program level two (352 dairy and 243 beef), 127 Johne's program level three (70 dairy and 57 beef), and 289 Johne's program level four herds (197 dairy and 92 beef).

In FY 2007 United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Service (VS) received \$12.0 million. Of this, \$5 million was distributed through cooperative agreements with the States for use with the National Johne's Demonstration Project (\$1.8 million to 17 States), and \$3.2 million through State Cooperative Agreements. This is also the third year for funding the Johne's Education Initiative (JEI) coordinator through a cooperative agreement with NIAA. Accomplishments include maintaining the JEI website with the inclusion of a section linking producers to State websites, identifying VBJDCP herds and the coordination of contacts with industry groups for Johne's Disease Integrated Project's Johne's Economics White Paper.

The purpose of the Johne's Economics White Paper, supported by APHIS, was to produce a white paper discussing consumer perception and likely impact to the dairy industry if *Mycobacterium avium paratuberculosis* (MAP) was determined to have a causal role in Crohn's disease. The final draft has been written and the authors would like to publish it in the winter or spring of 2008.

Along a similar vein, APHIS-VS also supported a colloquium organized by the American Academy of Microbiology on the subject of *Mycobacterium avium paratuberculosis*: Incidental Human Pathogen or Public Health Threat. APHIS-VS did this to ensure animal agriculture was represented during the discussions and that VS is aware of the direction that the colloquium was moving in. A final report is expected in the spring of 2008.

Two new initiatives were started in FY07: (1) a project in conjunction with University of Minnesota to evaluate the current test negative component of the VBJDCP to assess if the testing strategies accomplish the desired level of confidences and a second part of the project is to explore options for a more cost efficient herd classification program; and (2) a proposal as a three-year project to validate Johne's vaccines developed through JDIP.

Bob Whitlock, University of Pennsylvania, provided the NJWG report. The full report is included in these proceedings.

Jason Lombard, CEAH-VS-APHIS-USDA, presented Johne's Disease in U.S. Dairy Herds - Results from the National Animal Health Monitoring System (NAHMS) Dairy 2007 Study.

Preliminary results from the NAHMS Dairy 2007 study suggest that the program is educating producers. About 90 percent of dairy producers knew basic information about the disease and more than 55 percent were knowledgeable about the disease. More than 30 percent of dairy producers had implemented some form of a Johne's disease (JD) control program. Review of important management practices confirms that producers are changing calf management practices to better control the disease. More than 30 percent of producers reported confirming JD on the operation within the past year. The majority of producers used the serum ELISA test to confirm JD. Preliminary culture results from six environmental samples collected per operations suggests that more than half of dairy operations are infected, with increased prevalence as herd size increases. In conclusion, the voluntary control program appears to be providing producers with the tools they need to start controlling JD. With the majority of dairy operations infected, the control of JD becomes even more critical.

Scott Wells, University of Minnesota, and Mike Carter, National Johne's Disease Program Coordinator, presented the Future of the National Johne's Disease Control Program – Strategic Planning Process.

The National Johne's Disease Control Program to date has been developed as a voluntary program supported by USDA-APHIS-VS. Due to recent erosion in federal support, concerns regarding the future of the program were expressed. Central to the discussions was the importance of considerations of program changes to meet the needs of the cattle industry and individual producers. As a result of discussions, consensus was reached for the development of a new national strategic plan for control of Johne's disease.

The Committee approved a recommendation that a Subcommittee be established to initiate and spearhead the formulation of a new, comprehensive strategic plan to guide the future of Johne's disease control and management efforts in the United States. This Subcommittee will present an initial draft of the strategic plan at the Johne's Working Group meeting at the National Institute of Animal Agriculture meeting in 2008, with a final draft to be presented to the Committee for approval at the 2008 USAHA Annual Meeting.

This Subcommittee should include representatives from all pertinent JD groups. Including, but not limited to, the cattle industry, private veterinary practitioners, academia, government and allied industries.

Randy Capsel, National Veterinary Service Laboratories (NVSL) presented the 2007 Johne's Fecal Proficiency Test Summary and Serology Proficiency Test Summary.

The 2007 Johne's fecal organism-based proficiency test had 71 laboratories participating. These laboratories consisted of 65 United States laboratories, five Canadian laboratories, and one laboratory from Sweden. In total 127 kits were shipped, with results submitted for 119 kits. After sample validation, five low shedder samples were removed from Lot 1 kits, four low shedder samples were removed from Lot 2 kits, and no samples were removed for scoring from Lot 3 kits. This indicated a possible issue with testing sensitivity due to the low isolation from low shedder samples. Fecal culture techniques resulted in 68 of 83 kits meeting the passing criteria. Direct fecal PCR techniques resulted in 30 of 36 kits meeting the passing criteria.

The fecal pooling proficiency test had 33 laboratories participating, with 43 kits being shipped. Of these 43 kits, 33 kits received passing scores, seven kits did not pass, and three kits did not have results returned.

Results were mailed in early October for all laboratories. Retesting is available by contacting the National Veterinary Services Laboratory (NVSL) after receipt of results. A listing of the approved laboratories for both standard proficiency kits and pooling kits is included in these proceedings.

A confirmatory PCR testing panel was made available in 2007. The first set of kits resulted in mixed results, thus resulting in a second lot being shipped to requesting laboratories. All laboratories received satisfactory scores for the second lot of kits.

The 2007 serologic proficiency test had 80 United States (U.S.) laboratories and 10 international laboratories participating. Overall, six individuals utilizing the IDEXX Herd Check[®] test kit and seven individuals utilizing the BIOCOR Parachek[™] test kit did not receive passing scores. This is prior to retest performances being submitted. Reports were mailed in mid-September 2007 and retest panels were shipped at the same time final reports were distributed. A brief overview of progress at the NVSL on the Johnin PPD work and milk ELISA implementation was presented.

Judy Stabel, USDA, Agriculture Research Service (ARS) provided the Scientific Advisory Subcommittee on Johne's Disease. The Subcommittee Report was approved by the Committee. The report is included in these proceedings at the end of this report.

Committee Business and Resolutions

Four Resolutions were taken under consideration, amended, and approved and forwarded to the Committee on Nominations and Resolutions.

National Johne's Working Group (NJWG) Report

Bob Whitlock
Co-Chair

The NJWG met on Thursday afternoon October 18 and all day Friday October 19, 2007 during the United States Animal Health Association (USAHA) Annual Meeting, Reno, Nevada. The meeting was opened with self-introduction of all NJWG members and guests in attendance at 1:00 p.m. The meeting was Chaired by Bob Whitlock, Co-Chair NJWG and Scott Wells, Chair of the USAHA Committee on Johne's Disease. More than 100 persons attended the day-and-a-half meeting.

Scott Wells provided an update on Resolutions passed by the Committee last year. Veterinary Services (VS) responses to Resolution 11- Indemnity for animals that are clinically normal but Johne's disease (JD) positive as moderate or high shedders. USDA-APHIS-VS expressed the following concerns: 1). there is no current authorization in the program standards for paying indemnity. this would have to wait until passage of the new farm bill to make the necessary changes; 2) indemnity is only available for eradication programs and Johne's disease is a control program. An owner would need to commit to eradication of all positive animals not just moderate and high shedders; 3) data indicates that test and cull methods are not the most feasible strategies; 4) enzyme linked immunosorbent assay (ELISA) is the most cost effective testing method but confirmation on ELISA positives would delay moving the highest infected animals out of the herd; 5) producers who require indemnity to remove moderate or high shedders lack commitment to the program. APHIS-VS will not pursue funding for indemnity at this time

Resolution 12 – Testing bulk tank milk for *Mycobacterium avium paratuberculosis* (MAP):
Recommends that United States Department of Agriculture (USDA), Agriculture Research Service (ARS) develop a method for quantitative testing of bulk tank milk. Response: USDA-ARS is working with the University of Minnesota. ARS has developed a polymerase chain-reaction (PCR) probe and is evaluating 350 samples comparing fecal shedding to MAP in milk. A USDA, Cooperative State Research, Education and Extension Service (CSREES) National Research Initiative (NRI) grant has supported this initial work. The investigators will need to continue the research and validate the probe on non-infected herds. For exact wording of the resolutions see:<http://www.usaha.org/meetings/proceedings.shtml> Page 490, 2006 USAHA Proceedings and <http://www.usaha.org/committees/jd/jd.shtml> this last site is for Committee on Johne's Resolutions for 2006.

Several recommendations were approved by the Committee on Johne's in 2006:
For more details, consult pages 403 to 406, 2006 USAHA Proceedings.

1) Demo Herd Project – Continued support for the Demo Herd Project.

APHIS-VS is committed to this project and it remains a priority for funding.

2) National Johne's Check Test (NJCT): Organism Based Test- Preliminary approval will be given to laboratories taking and passing the check test at times other than the annual test date. They will be notified but they will not have their laboratory information added to the web site list until the following test cycle.

3) NJCT: Failing laboratories – When a laboratory fails the check test, the Designated Johne's Coordinator (DJC) for that state's laboratory will be notified so that arrangements can be made to test samples from program herds in an approved laboratory. Arrangements can be made to retest and return to approved status.

4) NJCT: Laboratories that fail twice- In the event that a laboratory fails twice then they will need to send personnel to National Veterinary Services Laboratory (NVSL) for training or bring an expert to their laboratory for training. This has not been required yet but things are in place if needed.

5) Best test strategy: The recommendation that USDA accept the Best Test Available Concept.

6) NVSL Provide a source of Johnin PPD – this is an ongoing and laborious task but new batches are in progress.

7) NVSL: Provide a Pilot Panel for PCR Confirmatory Tests. – This was done. 10 samples including three non-MAP mycobacterium was sent out. The first panel included blanks and several laboratories complained because they failed. The second panel was 100 percent pass so no one complained.

8) In anticipation that Center for Veterinary Biologics (CVB) will license the milk ELISA request NVSL is to prepare a Check test. NVSL is working on a panel for Check Test and hope to have it in place by March 2008.

9) NVSL locates low-moderate shedder and sends test panels to various laboratories for repeatability study. This will be done in 2008.

NJWG Treasurer, Ken Olson reported that the NJWG had a balance of \$25,941.42 and income of \$3,456.81 for re-imburement from USDA mini-symposium on vaccination at the 2006 NJWG Meeting. Travel expenses of \$830.40 for Ken Olson and Bob Whitlock to participate in a NJWG planning meeting in Washington, D.C. in June 2007 and checking account expenses of \$53.28 leaving a balance of \$28,514.55 as of September 28, 2007. The Johne's CD project included income of \$93,078.28 and expenses of \$71,140.28. The income from three major sources: \$30,318.28 was the initial balance, sponsors of the CD project, \$27,000 and sales of Johne's CD ROMs for \$35,760.00. To date 895 individual copies of the JD-Disease CD have been sold through the USAHA office and by the agency that produced the CD.

The Strategic Plan for the NJWG and the Committee on Johne's will be updated to meet current guidelines and program implementation standards in 2008. Scott Wells and Mike Carter reminded those present this will be a high priority for 2008 and asked that all present provide input as to the most important factors, goals and objectives that need to be updated. The NJWG and the Committee on Johne's recognized the need for updating of the current Johne's Strategic Plan that was adopted in 2004 and updated in 2005. Resolutions went forward and were adopted by USAHA supporting this activity. They called for strong producer input in development of the plans with support from USDA.

Vivek Kapur provided an update of Johne's Disease Integrated (JDIP) activities with a focus on Phase II. JDIP's mission is to promote, animal bio-security through development and support of projects that enhance knowledge, promote education, develop real world solutions and mitigate losses from Johne's disease. JDIP is a broad-based consortium of more than 140 investigators from academia, industry and government agencies. It has established world-wide collaborations including with a similar program established in 2006 in Europe by the European Union. Strategic objectives include: 1) to support and facilitate investigator-directed research of Johne's disease, 2) to create and maintain comprehensive scientific core facilities to support JD research and training activities, 3) to establish translational research capacity for developing and validating diagnostic tests, vaccines and disease management concepts for Johne's disease, and 4) to provide scientific information and support for the development of JD education, prevention, and control programs.

Primary funding is provided by USDA, Cooperative State Research Education and Extension Service (CSREES), National Research Initiative's (NRI), Coordinated Agriculture Project (CAP) program grants. The initial award was \$4.4 million for 3 years, and then recently JDIP was awarded a continuation grant of \$4.8 million to start in April, 2008. In addition a \$500 thousand grant from APHIS-VS will fund work related to the development and evaluation of vaccines in Phase II. A more comprehensive outline of the program is available at <http://www.jdip.org/>.

Lanny Pace, Liaison with American Veterinary Medical Association (AVMA) Council on Public Health and Regulatory Veterinary Medicine (CPHRVM) reported on changes recommended by the AVMA's CPHRVM for consideration by the AVMA Executive Board. The Executive Board approved the revision to the policy titled Johne's Disease. on November 7, 2007.

Johne's disease is a disease of significant economic importance to cattle and small ruminants. The AVMA will disseminate information and encourage veterinary practitioners to become familiar with ongoing efforts to control and eradicate Johne's disease. The National Academies of Science (NAS) report, Diagnosis and Control of Johne's Disease, indicates that currently available tests and diagnosis management practices are sufficient to control the disease. The AVMA encourages the USDA to review the implementation of the U.S. Voluntary Johne's Disease Herd Status Program for Cattle and to evaluate state programs for their equivalency to the Recommended Standards. In addition, the AVMA supports research in the development of improved diagnostic tests, management practices, vaccines, and their roles in control efforts in herds and flocks. To that end the AVMA supports active

pursuit of maximum and sustained funding to effectively support the USDA Johne's National Control Program. (Proposed by CPHRVM – Mar 2003)

Cost: None

Background: In 1996, the National Animal Health Monitoring System study of U.S. dairy production showed an estimated prevalence of *Mycobacterium paratuberculosis* of 21.6 percent across U.S. dairy herds. Economic analyses at that time also showed that the cost of Johne's disease to individual affected herds can be large and that the national average cost to producers across all herds was \$220 per cow. Premature culling, reduced milk production, and body weight losses in slaughtered cattle are means through which the dairy industry has lost productivity due to Johne's disease.

USDA-VS published its Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program in April of 2002. These national standards include education for producers and veterinarians regarding herd management plans and testing programs for Johne's.

Historically, the USDA-APHIS-VS has provided funding to support this program, in addition to some support provided by states. The highest level of federal funding to support the program was gained in 2005, but since that time, funding levels have diminished.

At its October 10-12, 2007 meeting, the CPHRVM approved a motion to revise the policy titled Johne's Disease. It asserts that the AVMA should actively pursue maximal and sustained federal appropriations for the USDA's Johne's National Control Program, to fully support veterinary and producer education, research, laboratory capabilities, diagnostics, and risk analyses for Johne's disease.

Ernest Hovingh reported that a national survey of dairy producers will assess producer's perception of the importance of Johne's disease from a business perspective compared to other issues facing dairymen today. The program they are being asked to evaluate is the current National Johne's Disease Control Program, as implemented by USDA-APHIS-VS and developed by the National Johne's Working Group, a Sub-committee of the Committee on Johne's. Are producers aware of the program and its potential value? Are producers participating in the program, if so why, if not, why not? What is the knowledge level of producers about Johne's disease? Does the current program meet producer's needs? Are veterinarians providing the services needed as it pertains to the Johne's program to assist herd owners to implement the desired management outcomes? The survey will assess what motivates, engages, deters and frustrates producers about the current Johne's Disease Certification Program.

The survey instrument was developed by a group of 12 experts through an iterative process and made possible by funding by JDIP and is being coordinated through the Pennsylvania State University Survey Center. The final draft is now being beta-tested by dairy producers and when final will be sent to about 6,300 (about 12.5 percent) randomly selected dairy producers across the United States. Distribution to producers is expected to be done during early January, 2008, assuming funding has been provided. The survey center anticipates a 50-60 percent response rate.

Mark Kinsel, Designated Johne's Disease Coordinator (DJC), Washington State Department of Agriculture presented information on a summary of more than 400 Risk Assessments and Herd Management Plans (RA/HMP): What can a closer evaluation of 400 RA/HMP tell us? Two major questions seemed to be paramount from producers; a) how much impact does Johne's disease impact my herd and if Johne's disease has a significant impact, what can I do about it?

Study design: A total of 407 Johne's disease risk assessments from Washington (199) and Oregon (208) completed since 2003 were included. They represented 312 herds (160 Washington, 152 Oregon) with 95 follow-ups. There were two Johne's disease status variables: 1) Johne's positive herd defined as at least one clinical cow or one positive test in last year – Results: one clinical cow or one positive test in last year = 52.2 percent of all herds (53.1 percent Washington, 51.3 percent Oregon), 2) Johne's positive cow reported in their herd history-Results: Johne's positive cow reported in the herd history = 72.3 percent of all herds (72.1 percent Washington, 72.7 percent Oregon). Data was entered into RAMP software and imported into database for analysis with Statistix statistical software.

Significant risk factors for being a Johne's positive herd included: herd size (herds > 500 cows had 1.95 times higher risk), adding animals in last year ("open" herds had 1.88 times higher risk), crowding of calving area (crowded areas had 1.23 times more risk), presence of Johne's suspects and/ or clinical cases in calving area (presence of Johne's suspect had 1.22 times more risk), manure soiled udder or legs in calving area (soiled cows had 1.12 times higher risk), and manure contamination of cow feed (contaminated feed had 1.38 times higher risk). Of concern was that only 3 of the 121 herds that

purchased animals in the previous year had biosecurity measures in place to prevent the introduction of Johne's disease. Most herds had no changes in herd scores over time, a source of concern.

Scott Wells presented an overview of the National Johne's Disease Demonstration Herd Project (NJDDHP). This is the fourth year of the project with 17 states participating including 66 dairy herds from 16 different states and 22 beef herds in 10 states. It is an APHIS priority, funded at approximately \$1.5 million per year. It is a long-term project destined to last over five years. Implementation of intervention strategies are to be evaluated, with an emphasis on risk from fecal contamination. Core outcome variables are to be measured, with data shared across states.

Objectives are to:

1. Evaluate the long-term effectiveness and feasibility of management-related disease control on development of Johne's disease and infection on dairy and beef cattle operations.
2. Provide information and materials for education and training of public and private practice veterinarians and cattle producers.
3. Develop and evaluate management, testing, and monitoring strategies for use in control of Johne's disease in cattle herds.
4. Create the opportunity for add-on projects within states to address important research objectives.

Chuck Fossler reported on the current status of the NJDDHP, including preliminary results and publication plans. The primary objective of the project is to evaluate the long-term effectiveness and feasibility of management-related control measures for Johne's disease on dairy and beef operations. Secondary objectives include: providing materials for education and training; evaluation of management, testing, and monitoring strategies; and creating opportunities for additional research. The primary hypothesis is that control of Johne's disease can be achieved through implementation of on-farm management practices to reduce transmission of infection to susceptible cattle. The project, currently in its fourth year, includes 66 dairy herds from 16 states and 22 beef herds from 10 states.

Seven of the beef herds are using whole herd ELISA with fecal culture follow-up as their testing strategy and 15 herds are doing whole herd fecal culture and ELISA. Forty-one of the dairy herds use whole herd fecal and ELISA. Nine herds use whole herd fecal and ELISA some years and whole herd ELISA with subset fecal culture other years. Six herds do whole herd ELISA with a fecal culture follow up on ELISA positive animals. Six herds, all with over 500 cows, do a whole herd or subset or herd ELISA with a fecal culture subset. Four herds do whole herd fecal culture with no ELISA. BioCor ELISA is used by 28 herds use doing 25,197 tests while 58 herds are tested by IDEXX for 85,641 tests and six herds using kinetics ELISA (KELA) for 10,587 tests. Two herds are being tested by a unique ELISA for 1,759 tests. Fecal samples are tested using TREK ESP in 41 herds with 41,873 samples; BACTEC in 19 herds on 15,945 samples; MGIT in seven herds on 4,050 samples; HEY in 49 herds on 28,663; liquid culture in two herds on 349 samples; culture in 31 herds on 5,651 samples and PCR in 14 herds on 4,164 samples. Use of environmental cultures has increased to where it was used by 31 dairy herds and 13 beef herds in 2006. Critical questions being asked include:

1. Has the incidence of clinical cases decreased over the 3 years?
2. What effect does culling high shedders versus keeping high shedders have on herd status?

To determine if the difference is significant they are looking at cohorts the year before versus cohorts the year after. Some changes are not as noticeable because many of the herds were dealing with JD prior to enrollment and some management was already in place. There has been a decrease in environmental positive samples. A quantitative look could suggest a decrease in the bio-burden instead of just negative/positive. Preliminary results of the project indicate that, for those herds with three full years of participation and data submission, there have been reductions in ELISA test prevalence in beef herds and dairy herds from year 1 to year 3 of participation. In addition, for cows tested between 24-48 months of age, there have also been some reductions in incidence as measured by fecal test results in cows born during the first year of study participation compared to cows born two years prior to the beginning of participation. These results suggest that management changes implemented since the beginning of the project have been effective in reducing the incidence of infection. However, these results are preliminary, as not all herds have been followed for a sufficient period to assess changes in incidence in cattle born after the farm's beginning of participation. Two to three more years of following these herds would provide much better evidence of effects of NJDDHP participation because additional cohorts could

be included (i.e., born 1-2 years after project began) and could also include those herds that began project in 2004 and 2005 .

The consensus of those in attendance was that the demonstration herd project is a high priority and that the project should be continued. It was suggested that criteria for continued participation in the project be discussed among the principal investigators and then documented to serve as a guide for investigators. Investigators involved in the project have submitted 38 abstracts/papers and given 68 presentations at scientific meetings, presented the material at 28 workshops and developed an additional 9 add-on projects. More information is available at <http://nahaphis.usda.gov/jddh/index.htm>. A series of publications detailing results from the project herds is planned with the first to be submitted shortly. They include:

- Overview of NJDDHP and of herds at program outset
- Change in prevalence of *M. paratuberculosis* infection after 3 years
- Economic cost of Johne's Disease and Johne's Disease Control Programs
- Changes in incidence of clinical disease in culled cattle and incidence of infection in young adults
- Association between changes in management and prevalence of infection after 3 years
- Associations between environmental and cattle test results
- Effects of vaccination

Bill Shulaw presented data from three demonstration herds in Ohio. One heavily infected dairy herd with 140-159 cows enrolled in July 2004 and were tested two times yearly first with BioCor ELISA and then Trek culture and PCR. They did 1:5 pools in 2006. Udder swabs were done on 20 cows each year through 2006, 62 percent of the swabs were positive. They sold youngstock and bought springing heifers from a dealer three times. In January 2005 calves were moved 12 miles away for rearing. They had one heifer positive at 14 months and no environmental positives. They went from 62 percent to 14 percent and two clinical cases.

The second herd was a small pure bred beef herd. The herd was closed and had 10 percent incident by Herrold's egg yolk medium (HEYM) in 2003. The third herd was a seed stock beef herd with 120 cows. The herd was open and also had a 10 percent incidence on HEYM in 2003. The second herd had three culture positives and went from 10 percent to 5 percent positive. They changed management because heifers were at risk. They do not save heifer replacements but purchased heifers from a negative herd.

The third herd is a show herd. They test at sale time selling only test negative cattle. They went from 20 percent to 0 percent JD. In 2005 they had one positive and 2006 one positive and 2007 no positives. Cull any positive right away. Fecal samples from 1,417 cows were combined to make 286 pools. There were 43 false negative pools and four false positive pools (no positive cows). Positive pools had TTP four to five days longer than that of the highest cow in the pool.

Mario Villarino and E.R. Jordan, presented evaluation of disease-control strategies for Johne's disease in a Texas dairy. The Texas Demonstration Project for the control of Johne's disease is currently implementing and evaluating a Johne's disease control strategy based on testing milking cows at the time of dry-off using ELISA, implementation of bio-security measures against the disease (colostrum management and calf management strategies) in two commercial herds. Comparative studies between ELISA positive and negative cows indicate a significant milk production reduction in ELISA positive animals (-8,927 lb) and reduction of days in lactation (-130 days). The direct cost of the disease, evaluated as cost of cow replacement due to premature culling was estimated at \$205 per cow. After six years of implementation of the program, a significant reduction of sero-positive prevalence was found, with a project benefit/cost analysis of \$254, 071 during the project evaluation (2001-2006) (one dairy). Our results demonstrate that using ELISA test results to implement colostrum management protocols, when accompanied with removal of heifer calves to a segregated facility, decreases the sero-prevalence of JD over time as the implementation of the control program progresses.

Beth Patton presented a summary of the Wisconsin Department of Agriculture, Trade and Consumer Protection demonstration herd project. Three herds are participating in a vaccine study in which every other calf was vaccinated until age matched cohorts of 50 calves or 10 percent of the adult

herd were established for each farm. The herds have been participating in the project since September, 2003. Although all herds have experienced significant reductions in fecal culture prevalence, when the infection prevalence was compared between the cohorts, the vaccinated animals had 68 percent lower infection prevalence than unvaccinated controls. In these groups, there was a trend toward higher fecal shedding and increased clinical disease in the non-vaccinated controls (not statistically significant at this stage of the project). Vaccinations are done with a 22 gauge needle and this change in addition to good restraint of the calves while vaccinating has seemed to result in smaller subcutaneous granulomas at the vaccination site.

Bob Whitlock presented information about a Pennsylvania demonstration herd that did ELISA, individual fecal cultures and environmental sampling. Although a risk assessment/herd management plan (RA/HMP) was in place, it appeared the herd owner retained culture positive cattle until they showed clinical signs. Additionally one cow with clinical signs of Johne's disease was located in a pen next to the maternity pen and easily contaminating newborn calves. Additionally water samples for the waterers for adult cows was so contaminated with MAP, that a ten gallons of water was equivalent to a heavy shedder in terms of MAP contamination (an estimated 200,000 colony forming units of MAP). A dairy herd in Ohio used for DJC training had annual fecal and ELISA testing done and made many of the changes recommended by the RA/HMP but still did not cull heavy shedders until they started to show evidence of weight loss and diarrhea, the typical clinical signs of Johne's disease. Retention of heavy shedders for prolonged periods of time only prolongs the reduction in new infections and lessens the impact of funds spent for herd testing.

A plea was made for veterinarians working on JD infected herds when the herd uses individual fecal cultures that culture positive cows need to be culled prior to the onset of clinical disease.

NJWG Reorganization – Retirements are generating a change of leadership for the NJWG. John Adams and Bob Whitlock will be stepping down as Co-Chairs of the NJWG. Scott Wells, University of Minnesota, Jamie Jonker, National Milk Producers Federation (NMPF), and Elizabeth Parker, National Cattlemen's Beef Association (NCBA), will become Co-Chairs of the NJWG beginning January 1, 2008.

Jamie Jonker has been appointed Co-Chair of the National Johne's Working Group by Scott Wells, Chair Committee to replace John Adams who retired from NMPF effective July 1, 2007. Jamie is a Cornell graduate with a PhD in cattle nutrition from the University of Maryland. The report was given by Jamie Jonker:

NMPF is a farm commodity organization representing most of the dairy marketing cooperatives serving this nation. NMPF members market the majority of the milk produced in the U.S., making the NMPF the principal voice on national issues for dairy cooperatives and their dairy farmer members.

NMPF, Animal Disease Prevention and Eradication Policy Statement: Animal diseases continue to reduce profitability for dairy producers and may impede exports and international market development. Diseases such as tuberculosis, brucellosis, Johne's disease and others can significantly increase costs to dairy producers in terms of decreased milk production, loss of animals, and replacement of animals. Preventing any animal disease outbreak in the U.S. remains a primary focus of dairy producers. Any occurrence of an animal disease outbreak or introduction of diseased animals into the U.S. from foreign sources must be addressed promptly to prevent further spreading.

Specifically concerning Johne's disease, NMPF will continue their efforts to secure funding for Johne's disease and it remains a priority.

Specifically, members of NMPF want to prioritize:

- (1) Rapid & accurate testing
- (2) Vaccine development
- (3) Vaccine strategic plan and best management practices
- (4) Education and participation

The NMPF supports:

- adopting programs and securing adequate funding to prevent and/or eradicate animal diseases, including proactive programs that encourage the responsible use of animal drugs by dairy producers;
- expanded indemnity programs for herds infected with brucellosis, tuberculosis, and other pertinent diseases;

- a ban on importing animals, semen, embryos, or other animal derived materials from regions of the world which are not free of animal diseases which may cause the transfer of agents that are pathogenic for animals or humans;
- maintaining effective import inspection and surveillance programs for animals and animal by-products; and,
- developing and implementing appropriate response programs and mechanisms for government and industry in the event of an animal disease outbreak; the development of improved methods for detecting animal diseases; and
- close coordination among federal and state animal disease prevention and eradication programs.

Elizabeth Parker has been appointed Co-chair of the NJWG to replace Gary Weber who is no longer with National Cattlemen's Beef Association (NCBA). Elizabeth began working with NCBA in January 2007. She hails from Abilene, Texas and is a graduate of Texas A and M, College of Veterinary Medicine. She worked approximately seven years in mixed and small animal practices in Texas then went to Washington, DC as an AVMA Science Congressional Fellow, serving on the House Agriculture Committee (HAC) for Ranking Member Congressman Charlie Stenholm. Following the fellowship she remained on the HAC, working for Chairman Larry Combest and Chairman Bob Goodlatte. Prior to joining NCBA as Chief Veterinarian, she was an international consultant for the Food and Agriculture Organization (FAO) of the United Nations in Rome, Italy, working on highly pathogenic avian influenza.

Elizabeth's main responsibilities at NCBA are animal health, animal welfare and homeland security regulatory issues. NCBA has two policies on Johne's disease - one as part of Integrated Disease Research and another on Johne's Disease Program Quality. Elizabeth's talk on Johne's can be summed up with focus on four areas: disease management by producers (including utilizing testing procedures to help in herd management decisions), research, education and outreach to help producers, and realistic expectations of federal funding. For example, lessons learned from the Johne's disease demonstration projects can be utilized to improve programs and herd management. NCBA wants to collaborate with USAHA-APHIS and NMPF, among others, and strongly supports a multidisciplinary, integrated Johne's Disease Program that provides quality to the producer. NCBA urges the Secretary of Agriculture to continue to place Johne's disease as a high priority for significant levels of intramural and extramural research funding and will also continue to work with coalitions to maintain Congressional awareness and support to adequately fund Johne's disease control and research programs. Congressional awareness is a challenge as there is an even greater continual need to educate new staff and new Members who are not aware of Johne's disease nor are they aware of agriculture in general.

Scott Wells gave a report on his evaluation of progress made by dairy and beef cattle herds in the Minnesota Johne's Disease Control Program. The objective of this study was to evaluate progress made by Minnesota cattle herds in the control of Johne's disease through participation in the Minnesota Johne's Disease Control Program. Data showed a reduction in the risk of within-herd Johne's disease transmission and seroprevalence through time in dairy and beef cattle herds in the Minnesota JD Management Program, consistent with a positive effect of the program on control of Johne's disease.

Mike Carter presented an overview of International Johne's Control Programs. The review included the basic programs for Australia, Denmark, Japan, and the United Kingdom. Australia's program has been primarily developed and funded by the involved industry for each species. Their program includes a test negative classification component. This test negative component is included into a dairy score used to provide producers with information useful to assess the risk of introduction of MAP when purchasing animals. The beef industry has a similar classification that includes a beef only classification that acknowledges the low prevalence of MAP in the beef industry.

Denmark is also developing an industry driven program based on multiple testing, using milk ELISA. Cows are classified as green, yellow, or red based on the number of positive tests and response of the test. Management decisions and handling of the animals are based on the classification of the animals. Denmark also includes a heavy education component that ties the program to *Salmonella* control as well.

Japan's emphasis is on eradication of Johne's disease through active surveillance and removal of test positive animals. The basic program includes surveillance test every five years. Positive herds enter a

monitoring program where animals are repeatedly test until the herd is tested negative at least twice. Partial indemnity is available for test positive animals.

The United Kingdom was included briefly as an example of a country that is relying completely on industry for the control of Johne's disease and the animal health officials support the industry through guidelines and education.

Scott Wells presented a draft report from an APHIS-VS funded group project with additional information expected at the next NJWG meeting. The concept paper was entitled Herd Testing Strategies to Achieve Classification Levels for U.S. Voluntary Bovine Johne's Disease Control Program. The concept paper essentially places both the status program and the management program in a single herd classification program ranked by presumed herd prevalence of Johne's disease. A second draft of the proposal was presented, discussed and then tabled until further refinements. A new version will be presented at a future meeting.

Jeanette McDonald provided an update and evaluation of Online Johne's Education. The online Johne's education effort consists of modules and certificate programs for both veterinarians and producers. For veterinarians, the Online Johne's Disease Veterinary Certificate Program, was developed consisting of seven modules covering the basics of Johne's disease pathobiology and epidemiology, diagnostics and test interpretations, risk assessment, and management and control in dairy and beef operations. The seventh module is an update module that covers new and emerging topics, management strategies, and diagnostic technology. This module also is used for recertification of Johne's certified veterinarians. For practical application four virtual farm visits (dairy and beef) are created so that veterinarians can practice assessing the risk of Johne's disease occurrence and developing management plans for different types and sizes of operations with varying levels of disease prevalence. We also have modules that address Johne's disease in goats, sheep, cervidae, camelids, and bison.

For producers, the modules have been revised for the certificate program to specifically provide relevant information. These modules are organized by type of operation (dairy or beef) and species (goats, sheep, cervidae, camelids and bison.) In addition, we are developing a series of four modules where producers talk to producers about the economic impact of Johne's disease and control efforts on their businesses.

Currently evaluation studies are underway of both the certificate program and the dairy producer module. The purpose of the studies is to gain further insights into the impact of the respective education programs on veterinarians' and producers' knowledge and practice. In addition, veterinarians' and producers' individual learning preferences, strategies, and activities are being assessed during and after their participation in the online education programs. Veterinarians and producers are being recruited as subjects for these studies and appeal to DJC's and others to help identify eligible candidates. Eligible candidates are those who have not yet started any of the Johne's education courses. They will be given a pre-test and a post-test, asked to fill out a pre-course questionnaire, and post-course questionnaire, and a subset will be asked to participate in a post-course phone interview. They will be compensated for their efforts (\$200 - \$300 depending on the extent of their involvement.) Please contact Jeannette McDonald at mcdonal7@wisc.edu or 608-263-5170 for possible recruits or further questions.

Ken Olson provided the Johne's education update. The mission of the education Initiative is to provide producers and those who work with them reliable, useful, and easy to access information about Johne's disease that is based on the best current science. A primary tool for this effort is the website www.johnesdisease.org. In addition at least 12 Johne's related articles have appeared in producer press since the first of the year and several radio/pod cast interviews have been given. Six industry groups assisted in distribution of Johne's information to the 60,000+ World Dairy Expo attendees in Madison, Wisconsin. A current effort that is underway with JDIP is a national dairy producer survey that seeks to identify barriers to and useful incentives for participation in the Johne's program. It will also provide insights on information and education needs for the future.

Yung-Fu Chang previously reported on the *in-vitro* cellular immune responses to recombinant antigens (rAgs) of *Mycobacterium avium paratuberculosis* (MAP). He reported on the differential immune responses and protective efficacy of four rAgs of MAP (85A, 85B, 85C, and Superoxide dismutase [SOD]) used with adjuvants, monophosphoryl lipid A containing synthetic trehalose dicorynomylate and cell

wall skeleton (MPLA) and bovine IL-12, against MAP challenge in calves. Group I was administered the four rAgs along with MPLA and IL-12. Group II was administered with four rAgs and MPLA. Group III received MPLA and IL-12. Group IV was given MPLA alone. rAgs induced significant lymphoproliferative responses in vaccinated animals (Groups I and II). All the four rAgs induced significant IFN-g production from 11-23 weeks after primary vaccination (APV), except for SOD. Significant increase was noticed in CD3+, CD4+, CD8+, CD21+, CD25+, and gd+ cells against all four rAgs in the vaccinated animals. rAg-specific expression of IL-2, IFN-g and TNF-a was significantly higher in the two vaccinated groups. 4/8 animals in Group I, 3/8 animals in Group II, and 3/4 animals in Groups III and IV were found positive for MAP in one or more tissues. Among the seven positive animals in groups I and II, except for one animal, the others had < 10 CFU. Isolation was confined to one tissue in these animals, except in one, wherein MAP was isolated from two tissues. In the control Groups (III and IV), majority of the positive animals had five tissues positive for MAP, with >300 CFU. Preliminary data from this study indicated that all four rAgs induced a good Th1 response and conferred protection against MAP infection in calves.

Judy Stabel with S. Robbe-Austerman and Bill Davis presented infection models useful for studying host responses to infection to aid in the development of diagnostic tools and vaccines. The majority of experimental models for ruminants have utilized an oral inoculation of live MAP in order to establish infection, mimicking the fecal-oral route of transmission generally observed in the field. The current study was designed to compare the effectiveness of oral and intraperitoneal inoculation on the host immune response to MAP infection. Twenty neonatal holstein calves were obtained from status level 4 herds and randomly assigned to 5 treatment groups: 1) control noninfected (C), 2) oral, 3) oral with dexamethasone pretreatment (oral/DXM), 4) intraperitoneal (IP), and 5) oral/mucosal (oral/M). The oral group was fed milk replacer containing 10^{10} cfu of live MAP, strain K-10, 2x per day for 14 consecutive days. The oral/DXM group was inoculated in the same manner as the oral group but the calves were administered 0.25 mg/kg BW dexamethasone IV for 3 consecutive days prior to bacterial challenge, and again on days 28 and 56 post-challenge. Intraperitoneal inoculation of calves with 10^{10} cfu MAP, strain K-10, was performed on days 0, 7, 14, and 21 of the study. The oral/M calves were inoculated by feeding milk replacer containing live MAP obtained by scraping the ileal mucosa from a clinically infected cow on days 0, 7, and 14. All calves were housed in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited BSL-2 facilities during the study. Throughout the study, blood and fecal samples were obtained from calves on days -5 and -4 prior to the first inoculation of MAP, and then on days 7, 14, 21, 28, and monthly thereafter for the 12 month term of the study. Blood samples were processed for isolation of peripheral blood mononuclear cells (PBMC) followed by incubation with medium only (nonstimulated), concanavalin A (ConA), a whole cell sonicate of MAP (MPS), and johnin purified protein derivative Johnin purified protein derivative (JPPD) for 24 and 48 hr for determination of cytokine secretion, lymphocyte proliferation, and flow cytometric analyses. Results demonstrated that oral inoculation of calves significantly increased lymphocyte proliferative responses to K-10 MPS at 12 months. Secretion of antigen-stimulated iNOS by Princeton BioMedtech Corporation (PBMC) was higher for oral infection groups at both 6 and 12 months post-infection compared to control calves. IP calves had the earliest antigen-specific IFN-g responses at 7 d post-infection, preceding responses noted for other infection groups that followed between 90 and 120 d. Average IL-10 responses to ConA and MPS were higher at 1 and 6 months and declined significantly by 12 months post-infection. At 1 month, oral and oral/M calves had higher MPS-stimulated IL-10 than other treatment groups. By 12 months only the oral/M calves had higher IL-10 secretion than control calves. Intracellular IFN- γ and IL-10 levels were measured for CD4+, CD8+, and gd T cell subpopulations. At 3 months post-infection, there was significantly higher IFN- γ in CD4+ cells stimulated with MPS in the oral treatment. Intracellular IL-10 was higher in CD4+ and CD8+ T cells in oral and IP calves compared to the other treatments. These results demonstrate that exposure and infection to MAP will invoke early immunologic responses characterized by IFN-g, IL-10, and iNOS secretion.

Todd Byrem, Antel BioSystems, presented an overview of activities by Dairy Herd Improvement (DHI) organizations in providing Johnes testing by milk ELISA to their membership. There are currently eight DHI milk testing laboratories, with direct access to over 2 million cows in the US, that offer the Johnes milk ELISA on samples routinely collected by DHI technicians and submitted for traditional component analysis. Convenience and lower cost to practitioners and producers underlie continued growth in testing volume anticipated to exceed 150,000 units in 2008. Guidelines and procedures for

ELISA testing have been drafted by a task force appointed by National DHI to provide quality assurance standards for participating laboratories. Standards have been developed for both the Field Service (sample collection and submission) and Laboratory (testing) components of DHI and compliance will be audited annually. Participating DHI organizations are encouraged to implement these guidelines and procedures, and to coordinate their activities with state designated Johne's coordinators and herd veterinarians as they provide testing services to dairy producers.

Ken Olson presented an introduction to the Johne's Roundup, a producer/industry-driven initiative to develop a strong, on-going grassroots base to support continuation of the National Johne's Disease Control Program. The concept is still in the developmental stages, but is seen as an important effort to help maintain federal funding for the program that has declined from the original \$21 million authorized in the 2002 Farm Bill to approximately \$12 million received currently. The effort would work to help identify program components that are critical to program success, document "cost share" components that currently or could exist and communicate producer/industry activity and funding needs to Congress. Interested parties were invited to a special session following the Friday afternoon to discuss the concept in greater detail and plan next steps forward.

REPORT OF THE SCIENTIFIC ADVISORY SUBCOMMITTEE ON JOHNE'S DISEASE

Judy Stabel,
Agriculture Research Service
USDA

The Scientific Advisory Subcommittee on Johne's Disease met on October 17, 2007 from 9:00 a.m. to 12:00 p.m. The major discussion point during the meeting was the consideration of incorporation of the milk ELISA test into the Program Standards as a herd screening tool for paratuberculosis. The Subcommittee discussed the merits of the test with presentations by Scott Wells, Todd Byrem, and Jason Lombard. A comprehensive assessment of the test suggest that the sensitivity and specificity of the milk ELISA test is comparable to levels attained with serum ELISA test, a key test in the herd certification program. The milk ELISA assay has been offered as a service by AntelBio (Michigan) on Dairy Herd Improvement Association (DHIA) samples submitted to their laboratory. Other DHIA laboratories are acquiring training to engender the ability to run the milk ELISA test. The internal quality control of these laboratories will be overseen by Quality Certification Services and include both the field and laboratory components of this testing. The ParaChek test (Prionics) is the only test currently licensed in the US for the detection of *Mycobacterium avium paratuberculosis* antibodies in milk. The cost of the test (\$5-\$6), combined with the rapid availability of results, is attractive to the producer. Further reduction in cost projections may be available if the milk ELISA test is used on bulk tank milk samples rather than individual cow samples. However, a comparative analysis of herd level detection using a subsample of individual cows with bulk tank milk should be undertaken before this is recommended. The Subcommittee recommended that the milk ELISA test be incorporated into the voluntary control program as an official screening test for Johne's disease. The Subcommittee also suggests that DHIA field technicians should be certified for collection of samples and that laboratories running these samples should run a milk proficiency test annually that is administered by NVSL.

Prevalence and Incidence to Date of *Mycobacterium avium paratuberculosis* Infection in Herds Participating in the U.S. National Johne's Disease Demonstration Herd Project

Charles P Fossler
Veterinary Services

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 was started in 2003, but final herd enrollment numbers were not reached until 2005. The NJDDHP includes 66 dairy herds and 22 beef herds in 17 states. In the subset of herds with three years of participation, preliminary estimates indicate a significant reduction in MAP prevalence in the third year of participation compared to the first year by ELISA testing in beef herds and in dairy herds by ELISA testing and fecal culture testing for cattle shedding at moderate to high levels. These results suggest that herd prevalence has decreased since the beginning of the project. For the subset of herds with four years of participation, Cox proportional hazards methods were used to examine incidence of MAP in cattle born after beginning participation in the project compared to cattle born prior to participation. Cows were divided into 3 cohorts: -2 = cows born 13-24 months prior to program participation, -1 = cows born 1-12 months prior to program participation, and 0 = cows born 0-11 months after beginning the program. Preliminary estimates indicate that dairy cattle born since the beginning of the project had a significantly decreased risk of being fecal culture positive and of fecal shedding at moderate to high levels compared to cattle born 2 years prior to the start of the project (Fecal-culture positive: Cohort -1: HR 0.64, p=0.03; Cohort 0: HR 0.53, p=0.02; Fecal shedding at moderate-to-high levels: Cohort -1: HR 0.68, p=0.07; Cohort 0: HR 0.54, p=0.04). These results suggest that management efforts initiated since the beginning of the project were effective in reducing incidence of MAP. However, further analysis is needed to identify those efforts that have the greatest effect on incidence. In addition, a longer follow-up period would provide much better evidence of effects of NJDDHP participation because additional cohorts could be included (i.e., born 1-2 years after the project began) and could also include those herds that began the project in 2004 and 2005.

REPORT OF THE COMMITTEE ON JOHNE'S DISEASE

NVSL Approved Laboratories for Johne's Disease Centrifugation Methods October 21, 2007

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REPORT OF THE COMMITTEE ON JOHNE'S DISEASE

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REPORT OF THE COMMITTEE ON JOHNE'S DISEASE

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October 21, 2007

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REPORT OF THE COMMITTEE ON JOHNE'S DISEASE

**NVSL Approved Laboratories for
Johne's Disease
MGIT 960**
October 21, 2007

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REPORT OF THE COMMITTEE ON JOHNE'S DISEASE

**NVSL Approved Laboratories for
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Sedimentation Methods**
October 21, 2007

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REPORT OF THE COMMITTEE ON JOHNE'S DISEASE

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REPORT OF THE COMMITTEE ON JOHNE'S DISEASE

NVSL Approved Laboratories For Johne's Disease Centrifugation Methods HEY Media October 21, 2007

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REPORT OF THE COMMITTEE ON JOHNE'S DISEASE

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