REPORT OF THE COMMITTEE ON JOHNE'S DISEASE

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Vice Chair: Scott J. Wells, St. Paul, MN

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The Committee met on Sunday, October 15, 2006 from 12:30 – 5:30 p.m. at the Minneapolis Hilton Hotel, Minneapolis, Minnesota. There were 79 attendees.

The Chair opened the meeting and welcomed Committee members and guests. The Committee was updated on progress on resolutions and recommendations from the 2005 Committee meeting.

Resolution 19: The United States Animal Health Association (USAHA) strongly encourages the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) to maintain funding for cooperative agreements with states under the National Johne's Control Program in the FY 2006 budget to the maximum extent possible. Consideration for funding may be based on compliance with Johne's Disease Control Program Standards, degree of state cost-share assistance (both direct and in-kind) and the number of herds participating in the program. A baseline would be established for all states to receive some monies for their programs.

Response: Veterinary Services (VS) is working hard to identify all the needs to sustain the program and will do our best to provide the maximum resources to the States in the face of the budget cuts to the program. Program funds used for cooperative agreements will be distributed based on compliance with the Voluntary Bovine Johne's Disease Control Program (VBJDCP) standards and the number of herds enrolled into the program. The degree of cost sharing will not be used in the determination of funding distribution but this information will be collected as a baseline measure for this year. A baseline funding level will be chosen so that all states participating will receive a minimal level of federal support

Resolution 20: The United States Animal Health Association (USAHA) requests that United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), National Veterinary Services Laboratories (NVSL) 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: The United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), National Veterinary Services Laboratories (NVSL), *Brucella* and *Mycobacterium* Reagents Team (BMRT) is currently working with the Agriculture Research Service (ARS), National Animal Disease Center (NADC) and APHIS field veterinarians on monitoring the Johne's Demonstration Herds to evaluate Johnin PPD production methods. There are several variables involved in the production process that may affect the diagnostic sensitivity and specificity of the product in sheep and cattle, and the NVSL is working towards defining an optimal and repeatable Johnin PPD production method. The BMRT is currently raising cultures of *Mycobacterium avium paratuberculosis* that will be used to create 3 to 4 experimental batches of Johnin PPD. The method of culture growth and the method of Johnin PPD production will be closely monitored and recorded. Each of the PPD products will be evaluated in the laboratory setting as well as within sheep and cattle – with the help of NADC, other Johne's research laboratories, and the Johne's Demonstration Herds. Once an optimal experimental Johnin PPD product is identified, the BMRT will use the same production method in multiple batches of Johnin PPD. The entire process for evaluating and optimizing the Johnin PPD production method is hindered by the slow growth rate of the *Mycobacterium* spp. of bacteria and the time needed to compare skin test results in animals to culture results from those animals as a measure of true infection status. The BMRT is estimating that this validation process may take at least 18-24 months before a final production method is identified and proven to be reproducible. At the current time, NVSL has not received funding to support this Johnin PPD production project, and as a result, we rely on the collaboration with other research groups to provide data on the performance of the PPD products in animals. The data that is generated must be reviewed by the APHIS Johne's Disease Control Program Staff to determine if a Johnin PPD product would be a valuable diagnostic tool within the Johne's Disease Control Program. If the APHIS Johne's Disease Control Program Staff decides to incorporate the use of a Johnin PPD into the program standards, the NVSL will at that time seek funding to produce the Johnin PPD product.

Recommendation: The Johne's Disease Integrated Program (JDIP) program be charged with leading a project to write a white paper on the direct and indirect economic impacts of Johne's disease on dairy and beef production including the marketing of dairy and beef farm products if a scientific linkage is established between Johne's Disease and human health.
Initial funding in the amount of $50,000 for costs associated with the preparation of the white paper be made available through the National Johne's Disease Control Program by USDA-APHIS and $10,000 from JDIP with matching funds to be sought from industry.

Response: The United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), agrees that a better understanding of the economic impact of Johne's disease is needed to encourage enrollment in the program. Further, industry needs to be aware of any potential economic impacts that might be expected if Johne's disease and human health are scientifically linked. Therefore, we plan to contribute $50,000 from the Johne's disease budget toward the development of a white paper on the direct and indirect economic impacts of Johne's disease on dairy and beef production.

Recommendation: USDA-APHIS continues funding the National Johne's Education Initiative through a cooperative agreement with National Institute for Animal Agriculture (NIAA).

Response: USDA-APHIS-VS recognize the value of the National Johne's Education Initiative and will continue funding for this initiative dependent upon adequate budgetary resources

Recommendation: The Committee recommends the following curriculum for Johne's Certified Veterinarian recertification>

1. To be required for recertification advance course:
   Review of Johne's basics
   Epidemiology update
   Testing and Interpretation, Part 2
   New and emerging tests
   Best tests for different scenarios
   National program review, highlighting any changes
   JD economics
   Marketing tips
2. In an effort to give all veterinarians equal education and knowledge about Johne's disease, we also recommend adding the list of topics in the Advanced Course to the certification training for first time Johne's Certified Veterinarians.
3. In response to identified needs and requests from Designated Johne's Coordinators and from veterinary practitioners, we also would like to strongly suggest that states include the following topics in their recertification education offerings. While recognizing the speculative nature of some of the topics, the National Johne's Working Group feels that practitioners need to be kept abreast of the most current research and opinions so they can better respond to and advise their clients.
   Special Challenges and Topics:
   Correcting common misconceptions (identified by DJC's) – case scenarios
   Update on research regarding the zoonotic issue
   Vaccine usage
   Potential use of Monensin

Response: VS agrees that a standard curriculum for the certification and recertification of private veterinarians would help to provide consistent training and ensure veterinarians receive current information. VS will add the proposed curriculum to the next revision of the program standards under the requirements of Johne's Certified Veterinarians.

Recommendation: USDA-APHIS-VS and JDIP utilize the knowledge gap report to assist in determining research funding guidelines.

Response: VS recognizes the value of the "Knowledge Gaps Subcommittee Report" and will use it to assist in making decisions regarding funding research on Johne's disease. VS will also consider the research priorities in determining research funding guidelines.

Charles Thoen, College of Veterinary Medicine, Iowa State University, presented a time-specific paper entitled Monitoring Responses by Use of 5-color flow cytometry in subsets of peripheral T-cells Obtained from Cattle Inoculated with a Killed *Mycobacterium avium* subspecies *paratuberculosis* Vaccine.

Ken Olson, NIAA Johne's Education Coordinator reported that the Johne's Education Initiative (JEI) and Coordinator position is a cooperative agreement between USDA-APHIS-VS and the NIAA. The purpose is to provide producers and those working with them easy access to information about Johne's Disease and programs. The program encourages participation, provides information on dealing with the disease and reducing the likelihood of Johne's introduction into uninfected herds or flocks. In addition to continued development, refinement and expansion of the Johne's Education Web page www.johnesdisease.org information on Johne's Disease was presented or provided at the following during the past year.

- Johne's Section at 2006 American Dairy Science Association-Animal Science Association Joint Meetings
    - One Invited paper on JDIP
    - 10 abstracts presented
    - Approximately 70 in attendance
- Industry meetings
    - National Milk Producers Federation Annual Meeting
    - National Dairy Herd Improvement Association Annual Meeting
    - Wisconsin Livestock Identification Consortium Annual Meeting
- Industry visits
    - Dairy Herd Improvement Association Service Affiliates
    - Dairy Management Inc. visit with Marshfield Clinic
- Producer Publications
    - Hoard's, Dairy Today, Feedstuffs
- World Dairy Expo
    - Interviews on farm radio networks
    - Media and Industry contacts
    - Information Distribution
        - Dairy Farmers of America
        - Wisconsin Milk Marketing Board
        - USDA APHIS

Michael Carter, National Johne's Program Coordinator; John Honstead, Western Region Johne's Coordinator, Veterinary Services (VS), Animal and Plant Health Inspection Service (APHIS) presented the FY 2006 United States Johne's Disease Program Update.

In 1997, USAHA National Johne's Working Group (NJWG) appointed a Subcommittee to design an affordable and flexible program based on sound scientific knowledge. The result was the U.S. 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 in April of 2002. The latest revision to the program standards occurred in June of 2006 with the include of pooled fecal samples for level 3 test negative testing and updating the laboratory approval section of the standards.

By the end of FY2006, 49 States had adapted to VBJDCP or had programs that were considered in compliance with these standards. Seventy-eight laboratories participated in the NVSL for Johne's serology check test with 7 of them international. Fifty-one (3 international) laboratories have been approved for *M. paratuberculosis* fecal culture and 15 (4 international) for PCR testing. In FY2006, the reported activities includes 784,978 cattle tested by ELISA and 125,336 cattle tested by fecal culture, 11,859 cattle tested by PCR, 8,441 enrolled herds (6,364 dairy and 2,077 beef) of which 1,792 are test negative herds (1,068 dairy and 724 beef). Herds enrolled as test negative herds are progressing through to level 4. There are 790 Johne's program level 1 (417 dairy and 373 beef), 600 Johne's program level 2 (375 dairy and 225 beef), 150 Johne's program level 3 (96 dairy and 54 beef), and 243 Johne's program level 4 herds (171 dairy and 72 beef).

In FY2006 USDA-APHIS-VS receive $13.1 Million. Of this $6.3 Million was distributed through cooperative agreements with the States for use with the National Johne's Demonstration Project ($1.2 – 17 States), and $5.1 million State Cooperative Agreements. This is also the second year for funding a Johne's Education Initiative Coordinator through a

cooperative agreement with NIAA. Accomplishments include maintaining JEI website and the inclusion of a Johne's Low Risk herd.

In the Western Region WY is the newest additional to the western States adopting the Johne's program although it is being developed as a quality assurance type program. All JD requirements included but they focus more too all fecal-oral organisms.

Processors in the West are becoming more involved in supporting the program. Tillamook Dairy Processor requires risk assessments and herd plans (RAMP) for all supplying dairies. This impacts approximately 150 producers. The Northwest Dairy Association supplies milk and has asked its members to complete voluntary RAMP for there herds. This has the potential of impacting and additional 600 producers. The thoughts behind this are that farms with RAMP's will have better quality milk and healthier animals.

The last item the West is actively pursuing is paratuberculosis vaccination. Iowa uses vaccination heavily to control Johne's and their producers, veterinarians, and researchers would like additional discussions of adding vaccination to the program to make the program more vaccine friendly.

Mark Camacho, Eastern Regions (ER) Johne's Coordinator reported that in the ER activities appear to be leveling off due to maturity of the program and decreasing funding with a stable number of producers. Only modest changes in program activity are expected unless key factors change. The ER continues to have the majority of participants in the VBJDC Program.

The dairy industry, in general, seems to prefer the management part of the program as they battle to clean up infected herds or control disease. The beef industry seems to favor the Test Negative Status part of the program as seed stock producers strive to sell a high quality product. States which continue to see high within herd prevalence Johne's Disease herds will fight to have some kind of program as federal dollars decrease. Visits to OH, WI, NY, MN and PA over the last few years show that the cattle industries of these states really want this program.

There is a lot of frustration in ER over the rapid decrease in funding of such a new program. It appears that there are not enough market forces in either the beef or dairy industry to self sustain this program at present levels if federal funding goes away. Competition for funding against other higher profile disease threats like tuberculosis, bovine spongiform encephalopathy and highly pathogenic avian influenza and tougher federal budget constraints are difficult hurdles for the program

John Adams, National Milk Producers Federation commented on the FY 2007 budget. Currently the House proposed FY 07 budget has approximately $7.7 million earmarked for Johne's Disease. The Senate proposed FY 07 budget earmarks $10M for Johne's. This is a significant decrease from the $13.1 million in FY 06. Committee members were encouraged to contact their state's congressional delegation to secure adequate funding in FY07 for the Voluntary Johne's Disease Control Program.

Dr. Robert Whitlock, Co-Chair of the National Johne's Working Group (NJWG) Subcommittee gave a summary report of the NJWG meetings and activities. Approximately 125 people attended the two day session. The full text of the Subcommittee Report is included in these proceedings.

Vivek Kapur, University of Minnesota, updated the Committee on the Progress of the JDIP. The JDIP (www.jdip.org) is a research consortium funded by the USDA-Cooperative State Research, Education, and Extension Service (CSREES), National Research Initiative (NRI). JDIP is focused on advancing knowledge on Johne's disease for the improvement of animal health and food quality. There are currently 140 scientists from 30 universities or agencies involved with various segments of the project.

A total of 10 new projects have been undertaken in addition to the 9 initial projects. Substantial activity and progress is being made in all areas. The JDIP renewal application is due October 31st. JDIP is requesting funds of $1.2 million for the next four years.

USDA provided $50,000 for the development of a "white paper" on the direct and indirect economic impacts of Johne's in July 2006. Currently data collection, meetings with key industry representatives and outlining of economic models is taking place. A draft report will be available in December for stakeholder review and comment. The final white paper will be presented at the annual JDIP meeting January 19-21, 2007 in College Station, TX. The intent is to have a paper that is readable and understandable at all levels from producer to research scientist.

Dr. Janet Payeur, National Veterinary Services Laboratories (NVSL) reported on laboratories approved to perform organism based tests for 2007. The list of approved laboratories is included in the proceedings.

Ms. Janet Marquardt, NVSL reported on laboratories approved to perform serologic tests for 2007. The list of approved laboratories is included in the proceedings.

Dr. Jason Lombard, Center for Epidemiology and Animal Health (CEAH) submitted several recommendations from the Scientific Advisory Subcommittee that were approved by the Committee.

The Committee passed a Resolution that asks USDA-APHIS-VS to encourage and fund a greater focus on research in development of quantitative-based tests for detecting *Mycobacterium avium paratuberculosis* (MAP) in bulk tank milk.

The Committee also approved a Resolution that that directs USDA-APHIS-VS to request necessary funding to provide limited indemnification of cattle producers under specific conditions for culling to slaughter any animal confirmed positive for Johne's Disease and determined to be a high or moderate MAP shedder. These Resolutions were forwarded to the Committee on Nominations and Resolutions.

The Committee passed the following recommendations:

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 Dr. Charles Fossler.
2: Laboratories that passed the Johne'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.

3. Laboratories that fail organism detection test and desire a retest should complete the following protocol through NVSL.
   - 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.
   - Each laboratory would be encouraged to seek additional training either from another local laboratory considered proficient in organism detection or at NVSL.
   - Letters from NVSL notifying each laboratory about test results will also be sent to the Designated Johne's Coordinator (DJC) for that state and to the National Johne's Coordinator (NJC) for their information. Labs 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 Johne's Disease Control Program.
   - Labs 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 Johne's Coordinator, if adequate check test kits are available at NVSL.
4. Laboratories that fail two sequential organism detection test and desire a retest should complete the following protocol through NVSL.
   - 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.
   - 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.
   - 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.
   - 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.
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.

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.
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.
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.
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.
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 labs 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.
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.

Monitoring responses by use of 5-color flow cytometry in subsets of peripheral T cells obtained from cattle inoculated with a killed *Mycobacterium avium* subspecies *paratuberculosis* vaccine.

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Abstract
The antigen-specific responses of peripheral T cell subsets in cattle inoculated with a killed *Mycobacterium avium* subspecies *paratuberculosis* (MAP) vaccine were monitored by use of 5-color flow cytometry. The results were compared with those from 2 established cell-mediated immunity assays, the skin test and the whole blood interferon-g (WB IFN-γ) assay. Forty-five female Holstein cattle with negative results for MAP in skin test conducted at time of inoculation with MAP were allocated to 4 groups. Cattle of group 1 (n = 12) were 0 to 3 months old and inoculated with a killed MAP vaccine. The 10 cattle of group 2 were the same age as those in group 1 but were not inoculated with MAP vaccine. The 11 cattle of group 3 were 9 to 12 months old and inoculated with killed MAP vaccine. The 12 cattle of group 4 were the same age as those in group 3 but were not inoculated with MAP vaccine. Flow cytometry identified T-cell subsets that responded specifically to the recall antigen. Results of assays for CD25 expression and WB IFN-γ had the strongest correlation with results for skin tests as well as results with each other. Intracellular expression of interferon-g was not as well correlated with results for the other tests. Flow cytometry can be useful for characterizing the immune response after administration of MAP vaccine and should be evaluated with regard to its sensitivity and specificity when used in detecting cattle naturally infected with MAP.

Johne's disease

- Caused by *Mycobacterium avium* ss *paratuberculosis* (MAP).
- Causes important economic losses to dairy and beef industries.
- Vaccination with killed cells in oil reduced the occurrence of clinical disease and fecal shedding.

Objectives

- To explore the effective use of the MAP vaccine in older calves
- To compare 2 conventional tests for CMI responses to MAP (skin test and WB IFN-γ assay) with a new technique (5-color flow cytometry)
- To detect up-regulation of CD25 and intracellular expression of IFN-γ in naïve cattle after vaccination at 2 different ages

Experimental design

- Blood samples were collected 11 months after vaccination for WB IFN-γ assay and flow cytometry.

- MAP- purified protein derivative (PPD) skin test was performed on the same day that the blood was collected.

Animals
Four groups of 10-12 skin test negative female Holstein calves, from a herd with Johne's disease

- Group 1 was vaccinated at 0 to 3 months of age.
- Group 2 was the same age as group 1, but was not vaccinated.
- Group 3 was vaccinated at 9 to 12 months of age.
- Group 4 was the same age as group 3, but was not vaccinated.
- Group 3 and 4 were pregnant at the time of testing.

Vaccine
USDA licensed MAP killed cells in oil adjuvant (Mycopar^{TM}, Fort Dodge Animal Health.

Antigen
MAP-PPD (NVSL Lot # 9801) was used as recall antigen in all tests.

Skin test

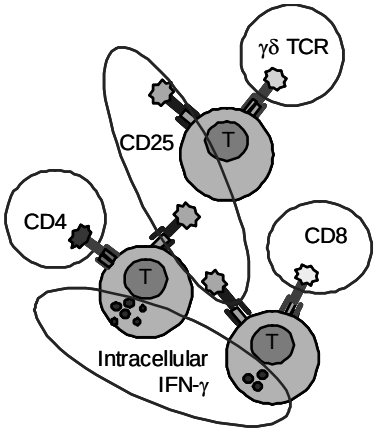
- Delayed-type hypersensitivity test was conducted on site using MAP-PPD.
- The antigen was injected intradermally in the cervical region.
- The increase in skin thickness (in mm) at 72 hours after injection of 3 mm or greater were considered positive.

Whole blood IFN-γ assay

- Performed at the National Animal Disease Center, Ames, IA.
- Whole blood was stimulated with MAP-PPD for 24 hours.
- The plasma was tested for IFN-γ by ELISA.
- Animals were considered positive when the net optical density (OD) was 0.1 and greater.

Five-color flow cytometry

- Isolate peripheral blood mononuclear cells (PBMC)
- Incubate PBMC *in vitro* 6 days with MAP-PPD in microtiter plates
- Stain cell surface markers and activation markers
- Simultaneous labeling of 3 T cell subset markers (CD4, CD8, γδ TCR), activation marker CD25 and intracellular IFN-γ.
- Detects co-expression of double positive cells, e.g. CD8 and γδ TCR.
- Identifies all T cell subset that expresses CD25 and/or intracellular IFN-γ in the same well.



Reagents for 5-color flow cytometry:
The monoclonal antibody mix:

- mouse anti-bovine CD4 isotype IgG1
- mouse anti-bovine CD8 isotype IgM
- mouse anti-bovine δγ TCR isotype IgG2b
- mouse anti-bovine CD25 isotype IgG2a

The secondary antibody conjugates mix:

- goat anti-mouse IgG1- Phycoerythrin-Texas Red (PE-TR)
- rat anti-mouse IgG2a-R-Phycoerythrin (R-PE)
- goat anti-mouse IgM-Alexa Fluor 647 (AF 647)
- goat anti-mouse IgG2b-Alexa Flour 488 (AF 488)

The antibodies for intracellular IFN-γ

- Biotinylated mouse anti-bovine IFN-γ
- Streptavidin-Alexa Fluor 700 (AF 700).

Flow cytometry analysis

CD4+, CD8+, and γδ TCR+ lymphocytes were evaluated for:

- The up-regulation of the activation marker CD25 as CD25 expression index (CD25 EI)
- The net increase of intracellular IFN-γ production in response to MAP-PPD (Δ%IFN-γ+)
- The level of significance $P<0.05$ was applied to all comparisons.

%CD25+ = Percentage of the T cell population that express CD25+
MFI: Mean fluorescence intensity of CD25 expression

Δ%IFN-γ+ = %IFN-γ+ of antigen-stimulated cells - %IFN-γ+ of unstimulated cells
%IFN-γ+: Percentage of the T cell population that is IFN-γ+.

Results:

Skin test: Skin thickness in vaccinated cattle was significantly greater than of non-vaccinated cattle.
WB IFN-γ assay: Mean net ODs of the vaccinated cattle were significantly greater than of non-vaccinated cattle. No significant differences between age group were observed in skin test and WB-IFN-γ assay results.
Flow cytometry: Vaccinated cattle showed significantly higher responses in both CD25 EI and Δ%IFN-γ+ than non-vaccinated cattle. Only flow cytometry detected significantly higher responses in CD25 EI in cattle vaccinated at 0-3 months than cattle vaccinated at 9-12 months.

All test results showed significant correlations with each other.

Conclusions:

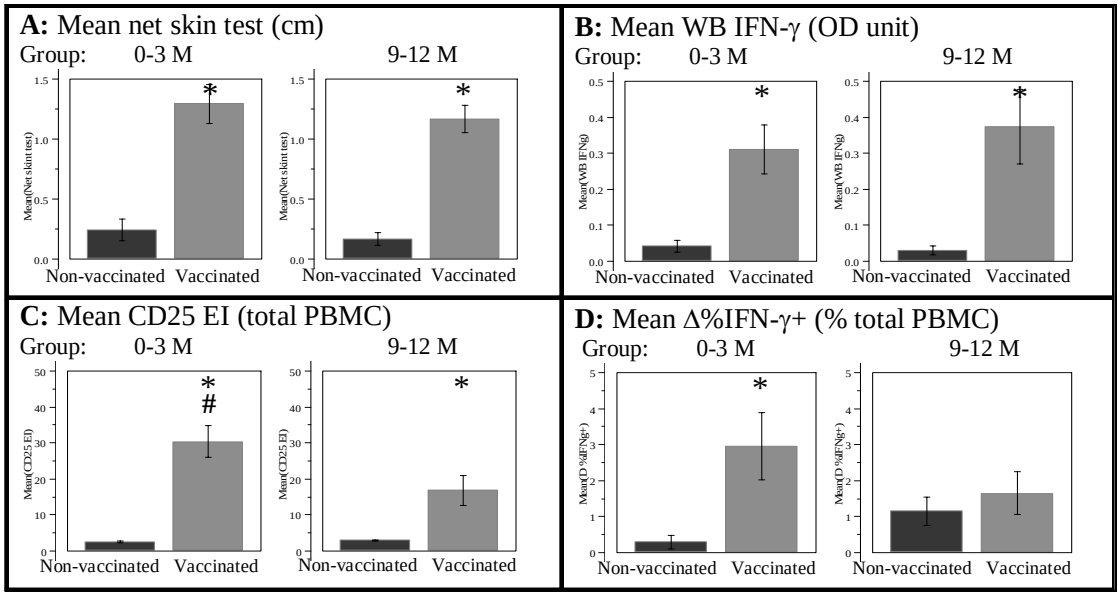
All test results showed significant correlations with each other.

- The CD25 EI had high correlation with the skin test and WB IFN-γ assays.
- The Δ%IFN-γ+ was not as sensitive in pregnant cattle vaccinated at an older age.
- Flow cytometry provided precise identification of T cell subsets responsible for specific response to MAP-PPD.
- The flow cytometry detected significant differences between vaccinated and non-vaccinated cattle both within and between age group.
- The vaccine induced *in vitro* responses in all T cell subsets and the responses were still detectable 11 months after vaccination.
- The higher responses in cattle group 1 may imply a better response to vaccination at a younger age.
- The pregnancy status of cattle group 3 may contribute to the lower *in vitro* responses.

Discussion:

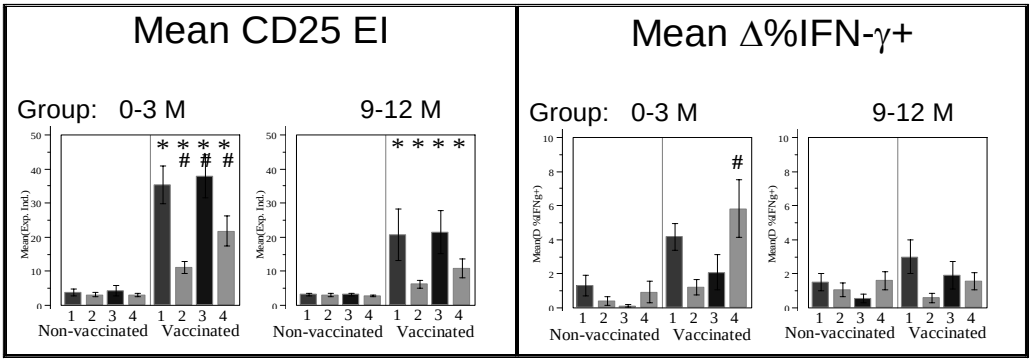
- The individual tests are able to significantly differentiate the *MAP* vaccinated group from the non-vaccinated group.
- The 5-color flow cytometry is able to identify individual T cell subsets that express the activation marker CD25 and intracellular IFN-γ, simultaneously or separately.
- The CD25 EI has higher sensitivity for measuring subtle differences in the magnitude of T cell reactivity between groups.
- The antigen specific up-regulation of CD25 expression by the CD8+ subset may be a more sensitive predictor of a memory CMI response and should be investigated further.

Results of each parameter



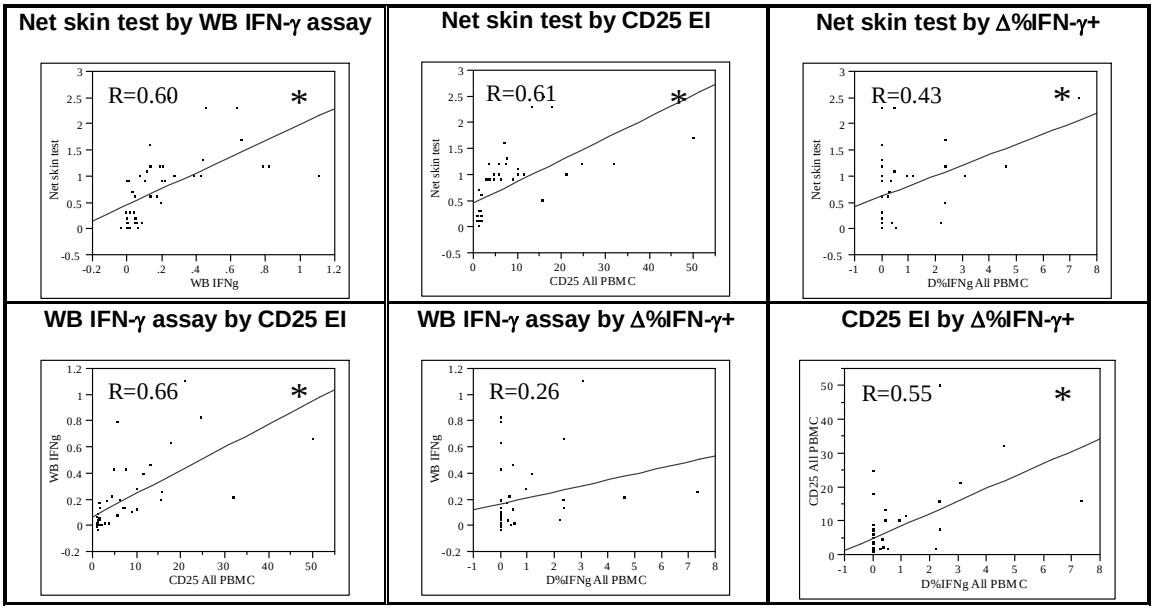
Statistically significant (P<0.05) of corresponding values: ∗ of the same age group, # of the different age group

Comparison by T cell subset



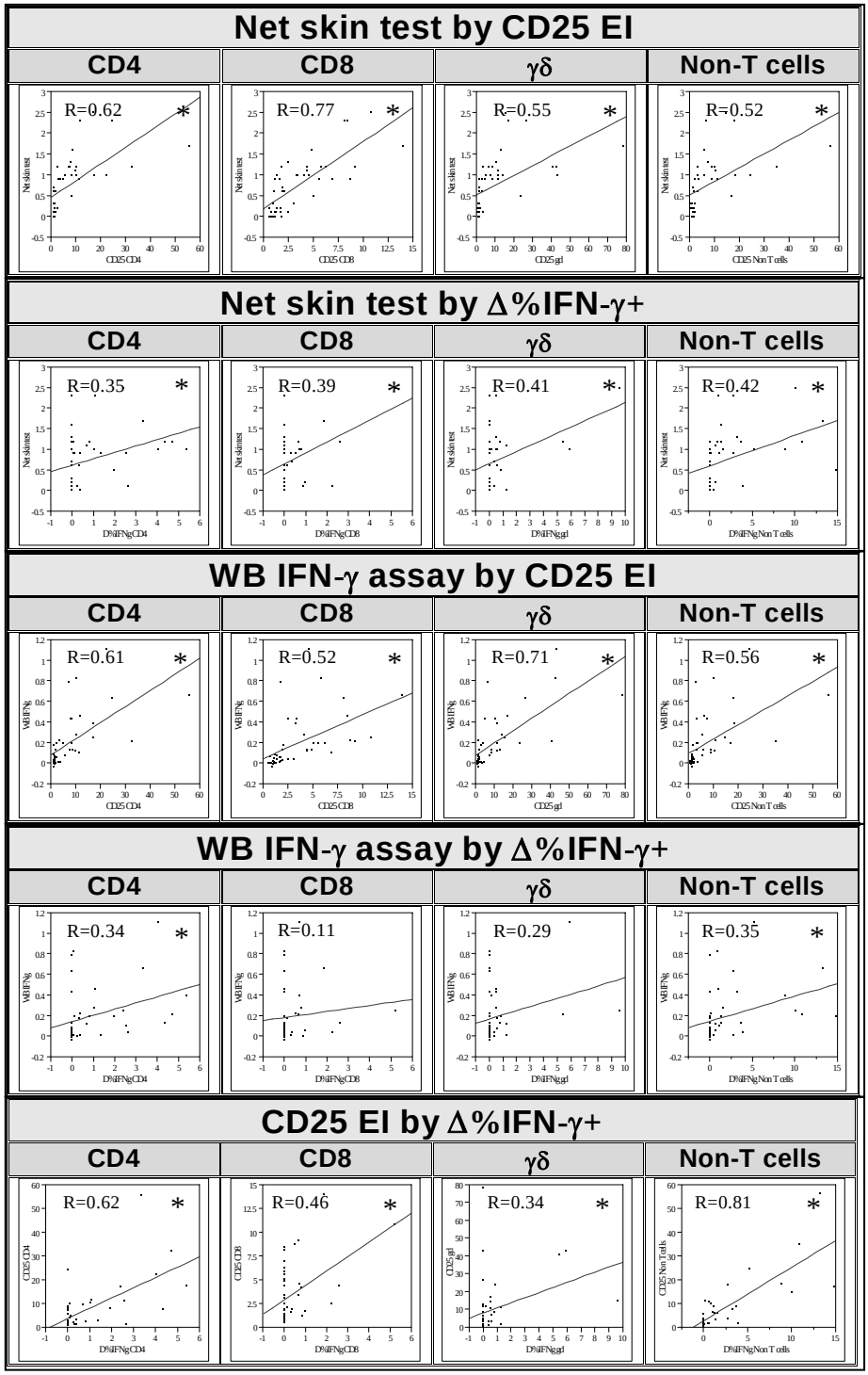
1: CD4+, 2: CD8+, 3: γδ TCR+, 4: Non T cells
Statistically significant (P<0.05) of corresponding values: ∗ of the same age group, # of the different age group

Correlation between each test



∗ Statistically significant (P<0.05)

Correlation by T cell subset



∗ Statistically significant (P<0.05)

Acknowledgements:

- Suelee Robbe-Austerman and Megan Parlett, National Animal Disease Center (NADC), Agriculture Research Services (ARS), United States Department of Agriculture (USDA).
- Thomas Skadow and Wasin Charerntantanakul, Iowa State University.

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Report of the National Johne's Working Group (NJWG)

Co-Chairs: R. H. Whitlock
John Adams

The Subcommittee met October 12-13, 2006 during the 110th Annual Meeting, Minneapolis Hilton Hotel, Minneapolis, Minnesota. Approximately 125 persons signed the attendance roster. Another 50 persons attended parts of the meeting. The two day meeting was split into 4 half day sessions.

The first half day session Thursday morning was devoted to Johne's Disease (JD) reports and a discussion of Canada's JD program. The second session on Thursday afternoon addressed the status and findings of the Johne's demonstration herd project. The Friday morning session reviewed testing methods for JD. The fourth session Friday afternoon addressed milk enzyme linked immunosorbent assay (ELISA) testing and implementation by Dairy Herd Improvement Association (DHIA)

Thursday AM Session
Johne's Disease Reports and Discussion of
Canada's Johne's Disease Programs

Bob Ehlenfeldt, Chair, Committee on Johne's Disease reported on Resolutions 19 and 20 and the four recommendations passed at last years USAHA meeting, Hershey, PA. The first recommendation: United States Department of Agriculture (USDA), Animal and Plant Health Inspections Service (APHIS), Veterinary Services (VS) provided $50,000 to help fund the Johne's White paper being coordinated through Johne's Disease Integrated Program (JDIP), Vivek Kapur-PI. The second recommendation: USDA-APHIS-VS provided funding to National Institute for Animal Agriculture (NIAA) for the Johne's Educational Initiative, Ken Olson will be responsible for this effort at NIAA. The third recommendation: proposed topics for inclusion in the recertification curriculum were approved; will be added to Program standards. The fourth recommendation: the Knowledge Gaps Report was approved and included in the 2005 Committee on Johne's Disease Report.

Ken Olson presented the treasurer's report for the NJWG. The current balance is $25,941. To date approximately 800 Johne's and Beyond CD's have been sold. The United State Animal Health Association (USAHA) office has a few additional CD's for sale.

Mike Carter, National Johne's Coordinator, summary report indicated as of October 3, 2006, 48 states have Johne's Advisory Committees with more than 8441 herds enrolled in programincluding 1792 status herds. For the past year approximately 784,978 serum ELISA tests and 125,336 fecal cultures, 4,077 pooled fecal samples and 717 composite environmental fecal samples were completed under the National Johne's program. More than 4,113 Risk Assessments (RA) and Herd Management Plans (HMP) were completed in the last year. Herds enrolled in the status program included 700 herds at level 1, 600 herds at level 2, 125 herds at level 3, and 242 herds level 4, with 1068 dairy herds and 724 beef herds enrolled. The National Johne's Demonstration Herd Project was funded at $1,280, 000.

Frank Garry and Jeannette McDonald, Co-chairs of the Education Group reported that the online Johne's Disease Veterinary Certificate Program was initiated Feb 2004, currently 45 states accept the online training for veterinarians in their states that wish to become certified for the Johne's control program. The recertification module was approved at last year's USAHA meeting and was made available in April 2006. This Johne's disease update has also been added to the Certificate Program for a total of 7 modules. At the time of the USAHA Annual Meeting 53 individuals had registered to be recertified. New veterinary modules for other species, including goats, sheep, and cervidae, have been added. Producer modules are also available for dairy, beef, sheep, goats, cervidae and dairy in Spanish. Two virtual dairy farm visits are available for veterinarians to practice risk assessments and management plans. In addition, two virtual beef production visits are currently being developed. Modules currently in progress include "producer stories" funded by JDIP, followed by a 360^{o} evaluation of JDVCP and a 360^{0} evaluation of the Dairy Producer Module. Other projects planned include a Special Topics Course to include vaccine usage, monensin usage, zoonotic issues and a stakeholders needs assessment tool.

John Adams reported that Congress has not passed a 2007 budget for Agriculture. The House has proposed $7.7 million while the Senate has requested $10 million for Johne's disease. Companies such as IDEXX, Prionics, BD, Trek, Tetracore as well as producers and producer organizations are encouraged to contact their Congressional Representatives and Senators in Washington about the proposed cut-backs in the Johne's program. Legislative contacts in Texas and California would be most helpful. The Congressional Conference Committee will include Wisconsin's, Senator Kohl and Pennsylvania's Senator Specter. The Office of Management and Budget's (OMB) budget proposal only included $3 million for APHIS for the Johne's program. National Mild Producers Federation (NMPF) continues to have Johne's disease a major interest and has hired a specialist to lobby for the Johne's program.

Jason Lombard reported the 2007 Dairy National Animal Health Monitoring System (NAHMS) study will include 1,000 cattle operations to evaluate calf health and nutrition, bovine virus diarrhea (BVD), milking procedures and Johne's disease among other objectives. The Johne's disease component will include an estimate of the national herd level prevalence (using environmental manure sampling with 6 composite manure samples per farm), bulk milk samples for polymerase chain reaction (PCR) and ELISA, and will assess producer's familiarity with Johne's disease.

Beth Patton, Chair, Program Standards Committee reported that the Scientific Advisory Committee will review the statistical confidence of being free of Johne's at each level of the status program. Major discussion points on the program standards included: (1) the A, B, C, D categories for infected herds (still used in 13 states); not all tests are the same in the same areas, i.e. Florida vs. Vermont for ELISA for example, beef cattle may not respond to Johne's infection in the same way as dairy cattle thus influencing test results; (2) bi-annual or annual risk assessment (RA) and herd management plans (HMP); (3) abbreviated forms for RA and HMP (currently used in Minnesota and Wisconsin; Pennsylvania has a modified follow-up form); (4) what is the science behind renewals for annual testing or RA and HMP? The Committee needs to develop a form (RA-HMP) for professional heifer raisers and will seek input from the Professional Dairy Heifer Growers organization.

Report of the Laboratory Committee to Program Standards Committee to the NJWG October 12, 2006. Members (18) of the Laboratory Committee include: Byrum, Bev; Capsel, Randy;

Carter, Mike; Cui, Jing; Glazer, Amy; Harris, Beth; Henderson, Louise; Marquardt, Janet; Lombard, Jason; Payeur, Janet; Stehman, Sue; Tucker-Schroeder, Linda; Rajev, Sree; Stabel, Judy; Tewari, Deep: Whitlock, Bob; Wu, Ching Ching. The Committee held 6 separate conference calls between August 11 and September 28, 2006. Each conference call was graciously hosted by Mike Carter, National Johne's Coordinator.

The Laboratory Committee recommends the following eight items.

1. Preliminary approvals for laboratories that have passed the NVSL culture or PCR check tests.
   Any laboratory that passed the Johne'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 National Veterinary Services Laboratories (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.

2. Response for laboratories that fails organism detection check test.
   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 *Mycobacterium avium paratuberculosis* (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 Johne's Coordinator (DJC) for that state and to the National Johne's Coordinator (NJC) for their information. Labs 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 Johne's Disease Control Program (VBJDCP).
   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 NJC, if adequate check test kits are available at NVSL.

3. Response for any laboratories that fails organism detection check tests in two sequential years.

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.

4. Reporting results for fecal culture by shedding category: This issue was discussed at some length but no consensus was reached except that laboratories should report an interpretation based on their experience. Since heavy shedders represent a spectrum of cfu/gm ranging from 10,000 to 10,000,000 cfu MAP per gram, perhaps laboratories should be required to detect heavy shedders as heavy shedders with less focus of sensitivity of fecal culture. The heavy shedders and perhaps super-shedders represent the greatest source of environmental contamination and thus risk spreading JD on farms to susceptible cattle (calves). SS then pointed out that laboratories should continue to have a sensitive test, especially at the herd level since detection of MAP in status herds remains an important issue.

5. American Association of Veterinary Laboratory Diagnosticians (AAVLD) standards and protocols for laboratory testing. AAVLD is in the process of implementing more rigorous standards for approved AAVLD laboratories and that some of their procedures may impact Johne's testing. 2007 is the target date to implement these validated testing protocols in laboratories. Publication of the organism based MAP detection tests would help in this effort.

6. Z-scores for each lab submitting results for organism based tests will be implemented when NVSL reports this year's check test results. The Z-scores for all labs reporting for the organism based tests would be included on a Z-score plot. Each lab would know their Z-score and be able to compare to all other labs how they compared. Z-score calculations would include: a) the mean cfu of all tubes for that sample. Some labs use 3 and others use 4 tubes, b) mean cfu for heavy shedders (above 50) would be truncated at 50 cfu to provide a more uniform method to record heavy shedders as one number, rather a wide range. Previously the Laboratory Committee reported that counting cfu above 50 was not necessary, thus for the purposes of the Z-score calculation, the truncation of heavy shedder cfu at 50; c) Both critical and non-critical samples required to pass the organism based test would be included in the Z-score calculations.

7. Quality control (QC) for organism detection tests. Low positive fecal samples could be utilized as QC samples in laboratories doing organism based testing. Several questions arose including:
    a. Frequency of inclusion of these QC sample?
    b. Ability of NVSL to provide these samples?
    c. Added cost this would represent to the laboratory?
    d. Are we able to implement this on a pilot basis?

8. Milk ELISA testing and check tests:
    a. NVSL will be obtaining milk ELISA test kits for evaluation from Antel Bio
    b. NVSL is requesting milk samples from known positive cows for their use to evaluate the milk ELISA test.
    c. Both Mike Carter and Janet Marquardt indicated the Laboratory Committee's input on his subject will not be needed at this time.
    d. At the present time, the milk ELISA test has not been licensed by VS-Center for Veterinary Biologics (CVB).

Items numbered 1, 2 and 3 were forwarded to the Program Standards Committee for incorporation into the Johne's Program Standards and were approved by the Committee on Johne's. Item 6 will be implemented this year by NVSL and Center for Epidemiology and Animal Health (CEAH) staff. Items 4, 5, 7 and 8 are informational. Near unanimous approval of the report by the NJWG on October 12, 2006 with minor edits and clarification.

Sue Stehman provided a brief discussion on the quantification of shedding level based on liquid culture using the Trek ESP system as follows: If time to detection (TTD) was less than 21 days, the sample was categorized as a high shedders, if TTD was between 22-28 days, a moderate shedder and if > 29 days, a low shedder. The Johne's laboratory at Cornell is collaborating with NVSL (Payeur) on a "quantification study" to compare TTD in liquid culture for both TREK & MGIT liquid culture systems. The samples initially run on TREK-ESP liquid culture system, were frozen up to six months at -70^0C. The samples were then shipped to NVSL for culture on MGIT and on HEYM using a standard protocol. The samples represent a range of TTD, 25 super-shedders (TTD 7-14 days) , 75 heavy shedders (TTD 15 – 21 days), 75 moderate shedders (TTD 22-28 days), 75 low shedders (TTD 29-33 days and 25 negative samples.

Jason Lombard reported on the ELISA Quality Control sera being used by about 31 laboratories to monitor ELISA variability over time. USDA-APHIS-VS provides the QC at no cost, if the laboratory reports results to USDA-APHIS-VS-CEAH on a regular basis. The present QC sera being supplied by NVSL for the Prionics ELISA needs to be replaced, as the optical density (OD) is too high. Well to well variation is minimal for the Prionics (BioCor) ELISA test. Nearly 40% of variation is due to kit lot for the Prionics ELISA. Idexx well to well variation was 83% compared to 98% for Prionics. IDEXX began distributing an improved kit in July of 2006. CEAH needs to provide at least a quarterly report back to laboratory participants and the overall QC test performance among participating laboratories. This report should be sent to Laboratory directors and the lab quality assurance person. As of October 12, 2006, the data should be available on the website of USDA-APHIS-VS-CEAH. CVB would also like to be aware of the QC reports to monitor variation among labs.

Mike Collins reported a meeting of the National Advisory Committee of Microbiological Criteria for Foods, Subcommittee on Paratuberculosis, David Acheson, Chair. The first meeting was in March, 2006 and the second Committee meeting was held in October 2006 in Washington, DC. Using published reports; the Committee will access the possible sources of MAP, the levels of MAP, evaluate the methods to detect MAP in foods and will determine what research needs to be done to further define the issue. The committee will not assess the zoonotic potential of MAP. A final report will be completed in 2007.

Steve Hendrick**,** Saskatoon, Saskatchewan provided an overview of the Canadian Johne's program entitled the Canadian National Voluntary Johne's Disease Prevention and Control Program (CJDPCP). A ready reference is available: Canadian Vet Journal 47:539-541, 2006. The program is being supported by the Canadian Animal Health Coalition (CAHC) which includes: Dairy Farmers of Canada (DFC), the dairy industry, the Canadian Federal Government, Provincial Government's of Ontario and Alberta, academicians and veterinary practitioners. Alberta had previously established (2001) a JD status program with four levels. One of the early objectives of the program was to facilitate easy participation by producers and to focus of infected herds with the goal of reducing herd prevalence over time. Penalties for test positive herds are being kept to a minimum. Tested results are being kept confidential. The impact of the CJDPCP will be periodically assessed by periodically determining the herd prevalence of JD.

The program includes two pathways: Status Pathway (SP) and Prevention Pathway (PP). Emphasis with both education and financial support for testing will focus on test-positive herds with the goal to minimize spread of disease both within and among herds thus lowering the national herd prevalence. The program includes risk-assessments and herd-specific herd management plans designed to reduce the spread on Johne's disease.

PP does not have any specific testing requirements. However a pre-assessment questionnaire is required to be completed by the producer to reduce the time and cost required to do the RA and HMP. No self assessment options are available. These herds will be required to complete a follow-up check list and questionnaire 10 months after the initial RA and HMP. Provincial JD coordinators are encouraged to send participation certificates to herds enrolled in the program.

SP must complete the RA and HMP in addition to the preassessment survey. The testing requirements for level 1 include ELISA testing or culture of strategically planned environmental manure samples from high cow traffic areas. If testing results do not identify JD in the herd, the herd advances to level 1. Owners may elect to remain at this level by re-testing at least once every two years with all organism based tests be negative. In order to remain in the SP, fecal culture positive cows must be culled for level one herds. For level 2, 10 fecal samples from all eligible cows individual cows are pooled and cultured, if negative and with completion of a second RA and HMP, the herd advances to level 2. No other levels are recognized at this time. Producers are recognized for the number of years enrolled in the program in either path in the program. This is a low risk program, not certified free of JD if enrolled in the SP. While implementing the program, knowledge gaps will be identified and investigated if funds become available. At some point a national Johne's coordinator will be appointed. Implementation of program is being lead at the provincial level with participation by industry/government partnership. Canadian producers also have concerns about being identified as a positive herd.

Mark Kinsel, DJC, Washington State reported that Northwest Dairyman's Association (NDA-Anton Mickleson and John Bosma) have adopted a resolution strongly recommending their producers (more than 500 herds) each have a RA and HMP in place. NDA believes this is important for sustainability of their membership to maintain markets. This is similar to the resolution adopted by Tillamook County Creamery Association (Mark Wurtenberg) in 2003 (about 150 dairies) that requires their producers to have a RA and HMP in place in order to sell milk to the association. Mark Kinsel will provide free software that allows the RA & HMP to be computerized and facilitates data retrieval to state Veterinarians and DJC's. Contact Mark by E-mail at mkinsel@agr.wa.gov if you would like a copy of the software.

Janet Marquardt at NVSL provided an update on the purified protein derivative (PPD) Johnin project that was the result of a 2005 USAHA resolution. Four batches of Johnin PPD have been prepared to date; each one has been different from the other despite very similar growing conditions for the mycobacteria. National Animal Disease Center (NADC) has and will continue to collaborate with NVSL to characterize the proteins in each lot of Johnin produced to date.

Thursday PM Session
Johne's Demonstration Herd Project

Scott Wells provided the background for the National Johne's Demonstration Herd Project that was part of Goal II of the Johne's disease strategic plan approved by USAHA in 2002. Johne's demonstration herds are critical and have the highest priority for funding for the National Johne's Program. The demonstration herd Committee of the NJWG developed four objectives for this project. That included (1) effectiveness and feasibility, (2) a mechanism to provide information and materials for educational purposes, (3) to develop and evaluate the JD program and (4) to create a mechanism for add-on projects.

Jason Lombard reported on the National Johne's Demonstration Herd Project. In the third year of the study, 17 states are participating in the demonstration project involving 70 dairy herds with 74,000 milk cows from 16 states and 27 beef herds with 6,400 beef cows from 11 states. The forms used for RA and HMP have been modified from the standard form for National program to allow better data collection and analysis. Two meetings of investigators were held; Denver, CO on October 11-12, 2005 and in St Paul, MN on September 20, 2006 in conjunction with the American Association of Bovine Practitioners (AABP) meeting. Many investigators also presented research abstracts during public sessions and poster sessions. The first manuscript outlining the National Demonstration Herd Project is being planned. A CD was made of the research presentations in St. Paul. As with the national program there is a greater focus on dairy herds with an overall fecal culture prevalence up to 10% positive cultures in some herds. The seroprevalence of JD has decreased from 9 to 6% over three years in 26 of the dairy herds. One specific question critically evaluated was associations with testing culture positive using a logistic regression model. Heifer calves born to culture positive dams were twice the risk to be culture positive compared to calves born to culture negative dams. Other lessons learned include the necessity to cull cows with clinical signs from herd as quickly as possible as well as removing calves from dams prior to nursing. Data from the project will be shared with all demonstration herd owners and their veterinarians. Proposed studies include prevalence and risk areas for transmission on the farm, cost benefits for participation in the project and gamma interferon study.

Ching Ching Wu reported data on 7 Indiana herds with two of the herds practicing Johne's vaccination. First herd receiving vaccine was an Amish herd which started vaccinating in 2001. Whole herd fecal cultures are done annually and ELISA testing quarterly. They cull clinical suspects quickly. In 2003, 13/42 (30.9%) were vaccinated, 19/42 (45.2%) fecal culture positive which 6/19 were vaccinated, 6/13 (46.2%) vaccinates were fecal positive; in 2005, 26/40 (65.0%) were vaccinated, 3/40 (7.5%) fecal culture positive which 1/3 (33.3%) was vaccinated, 14/40 (35.0%) ELISA positive. The second vaccinated herd has 900 cows and has been vaccinating since 2003. Fecal positive culture rate changed from 58% in 2003 to 17% in 2006.

Roxanne Pillars, Michigan (team members included J.B. Kaneene and D.L. Grooms) reported that 7 dairy herds have been enrolled for 4 years. Environmental monitoring of manure samples is being compared to reduction in herd fecal culture prevalence. Of 547 environmental samples 59 were culture positive. MAP cfu's decreased as prevalence decreased. MAP has been consistently detected in the manure storage areas and or cow housing area 79% of the time when the herd prevalence is greater than 2%. When herd prevalence exceeds 5%, MAP cultured from many other areas, most commonly the maternity pen floor. The number of MAP positive environmental samples and number of cfu's in those samples increased as number of cows shedding MAP in the herd increased. Thus, cleanliness and sanitation of maternity pen must be emphasized in JD control program.

In an ongoing calf study-27/1,500 (2%) of manure samples were culture positive from calves, of the 27 positive calves, 8 were repeat culture positive. Fecal culture test positive calves are 8.6 times more likely to be born to a test positive dam as from test negative dams. Their group is also estimating the costs of the Johne's control programs on these herds. An annual questionnaire (2003-2005) has been administered to herds participating in the Johne's control program assessing four categories supplies/testing, management, capital investment and labor. Preliminary estimates indicate the average cost is $61 per cow with a range of $30 to $102/cow/year.

Frank Garry with colleagues J.E. Lombard, H.L. Hirst, M.M. Dennis, M.C. Antognoli and M.D. Salman reported on antemortem identification of cattle with disseminated MAP infection. If a food safety risk for MAP is presumed to exist, which cows should be excluded from human consumption? How should cattle be screened prior to slaughter? Current antemortem tests available to screen for MAP infection include physical exam/ clinical signs, ELISA, fecal culture, tissue biopsy histopathology or culture, PCR and CMI testing. Their objective was to determine the association of antemortem test results with presence of disseminated MAP infection in cattle at slaughter.

To date 40 cows with complete BACTEC results from 4 gastro-intestinal samples (feces, mesenteric lymph nodes, ileum, and ileo-cecal lymph node) and 36 cows with conventional culture results on multiple organs. Histopathology has been completed on 36 cows. Animals were classified as infected if at least one tissue or fecal culture positive for MAP and animals had disseminated infection (DI) if intestinal and extra intestinal involvement of MAP as identified by culture. Results found 12 of 36 not infected, 8 of 36 were localized intestinal infections only and 16 of 36 cows had disseminated infection. The liver was positive in 6 of 16 cases while the hepatic lymph node was positive in 13 of 36 cows. Preliminary conclusions indicated almost 50% of the MAP infected cows had DI. Combined ELISA results detected 100% DI cows (needs further verification). Fecal culture detected high proportion of DI cows.

Preferred tissues to collect to detect DI are intestinal related but kidney and suprammamary lymph nodes are important tissues to collect to detect DI. MAP was never found in skeletal muscle. Heart muscle was found to be colonized. Peripheral lymph nodes could be included in ground meat. DI was common in serum-ELISA-positive cows without clinical signs of Johne's disease. If food safety policy moves toward exclusion of cows with MAP from the food chain, then a clearer understanding of the occurrence and identification of DI would be necessary.

Scott Wells, C. Ferrouillet and S.M. Godden, Minnesota reported one of their goals was to determine if farm management factors were effective to reduce JD transmission. Three management factors included limiting transmission during the perinatal period, limiting contamination of the environment and limit introduction of animal from infected herds. The tools used to make these assessments included: annual risk assessment, annual herd control plan and testing of adult cows (serum ELISA and fecal culture). Their study includes 6 herds enrolled between February 2000 and January 2001. Two herds range from 45 to 50 cows while four herds range between 220 and 330 cows. Herds are sampled once annually and at time of confirmed pregnancy in two herds. The mean herd ELISA prevalence was 13.5% (5.6% to 28.7%) and culture prevalence was 12.9% (3.85 to 34%) with a clinical case prevalence of 18.3% (0 – 24.8%) of all culled cows. Survival analysis by Kaplan-Meier curves and Cox proportional hazards analysis will be used. Cohorts are defined by birth date of cows and it is assumed that animals are negative until first positive test. The maximum follow-up for all cows is 45 months. Results from the Cox model suggested a decrease in seroconversion and fecal shedding over time. Birth cohorts are different in regards to when in the lives of animals the management improvements are implemented.

Sue Stehman reported for the New York (NY) program. Team members included P. Leids, R. Scrafford, R. Ellis, C. Johnson, J. Huntley. NY program includes 4 herds with 750 to 1200 cows per herd.

These four herds used different control strategies including strategic testing and integrated management, rolling herd testing (120-150 days in calf) with culling of fecal culture positive prior to dry off; rolling herd fresh cow testing prior to breeding management with minimal testing and vaccine and management. Management plans are assessed on a quarterly basis in each herd.

The diagnostic laboratory switched to TREK-ESP system in June 2004 and more recently the Trek media may have changed with an increased number of culture positive samples (16%). One herd began vaccinating for JD in 1997, with no fecal culture positive samples since birth cohort in 2001. This herd is now vaccinating only 50% of newborn calves. This herd has lowest % positive environmental samples. Identification and removal of highest shedding cattle at or before critical management decision points, especially maternity pen management. Determination of the best method to monitor herd prevalence once low prevalence is reached remains an unanswered question.

Ernest Hoving with colleagues D. Wolfgang, R. Whitlock and D. Tewari reported on the Pennsylvania demonstration herd project. Depending on the producer's goals, most laboratory tests used can identify infection at the herd level. Economic considerations will be important in future for determining a testing plan. Herds relying on testing 30 random ELISA tests on second lactation or older cattle as screening method may not be truly capturing the herd status. Individual animal culture is still gold standard in diagnosis.

Mike Collins reported on one of the two Wisconsin demonstration herd projects. The primary hypothesis: Will management changes together with ELISA testing control Johne's disease? The outcome evaluation will be comparison of rate of infection in animals born before and after start of the Johne's disease control program. Only ELISA test results will be given to producer to manage cows. Fecal culture results not given to producer; but will be used to judge infection rate independent of ELISA testing. Results will be assessed by Herd: Pre- Post- Control Program, first lactation cows ~ incidence before and after implementation of the control Program. Conclusions at this time indicate the program has been successful with a decreased apparent prevalence ($p<0.001$), cessation of clinical cases of JD and satisfied dairy producers. One side light has been the use of zip-ties in cow's ears to facilitate identification as ELISA test positive.

Some herds controlled JD faster than others. Factors affecting rate of JD control included 1) initial prevalence as a high prevalence reduces rate of progress, 2) ability to aggressively cull ELISA-positive cows, 3) quicker culling results in faster control, 4) affected by the number of heifers successfully raised and ready to enter the milking herd, 5) diligence in following herd management plan and 6) herd owners most able (labor and money) to strictly follow recommendations, without exception, had the fastest success. Next steps include the creation of educational programs and products based on our study herds; online delivery (audio & video), lay publications, and analysis of herd data for statistical evidence in support of our clinical observations about factors affecting success of our program.

Beth Patton reported for the second Wisconsin demonstration herd study. More than 500 herds have used Johne's vaccine in Wisconsin. Three herds of these herds, each with over 300 head have enrolled in the demonstration herd project. The initial herd prevalence must exceed 7% and both the herd owner and herd veterinarian must agree to use the vaccine. A farm RA and HMP must be completed along with a whole herd tuberculosis test. Every other calf was vaccinated until a cohort of 50 head or 10% of the herd is vaccinated. After initial cohorts were established, every heifer calf is vaccinated. First lactation heifers are tested at calving time, thereafter at 90 days pregnant. Annual environmental samples are obtained from maternity pens, cow alleys, manure storage areas, hospital pens and flush systems. Preliminary results indicate there is a significant difference in infection prevalence, lower in vaccinates. Several more years will be needed to critically assess the effectiveness of the program.

Friday AM Session
Testing Strategies

Mike Collins provided a detailed report on the USDA-APHIS-VS supported project: Best Tests-Recommendations followed by comments by DJC's: Andy Schwartz-Texas, Tom Schomer-Nebraska, Beth Patton-Wisconsin and Boyd Parr-South Carolina. Comments to improve the document included: all ELISAs are not the same (regional differences), producers are always in charge of operation and will make the final decision despite the best test recommendations, and seedstock producers must be able to sell animals, among others. All agreed the final document needed to be widely distributed and made available on the web.

Sue Stehman reported that ELISA testing does not work in low JD prevalence herds. As a result demand for fecal cultures exceeds ELISA demand in NY State. NY has tested 7,100 pools (1:5) to date.

Scott Wells with Saraya Tavornpanich and Ian Gardner, reported on identification of Best Herd Testing Strategies for Detection of MAP. MAP detection using individual cow testing is costly and imperfect. Cows shed variable concentrations of MAP in feces, partly based on stage of infection, and those shedding high concentrations are easiest to detect. Culture of pools of fecal samples from individual cows detects most pools with culture-positive cows. Culture of environmental fecal pools detects most (90%) of infected dairy herds. The stochastic simulation model was developed to compare the herd sensitivity (HSe) of testing strategies for detection of MAP in Midwestern US dairies with no previous testing and culling related to paratuberculosis. ELISA serologic testing by 2 different assays (EA and EB), ELISA testing with follow-up fecal culture (EAIFC and EBIFC) Individual fecal culture (IFC) and pooled fecal culture (PFC) and culture of environmental samples (ENV).

Disease structure modeled on the basis of within-herd prevalence, proportion of infected cows in the herd that shed no, low, moderate, or high numbers of MAP in feces, and number of MAP as colony forming units per gram of feces (CPG) corresponding to MAP-shedding level of infected cows. Misclassification probability of ELISAs in herds was included. Comparison of herd-level sensitivity (HSe) with culture of 5 environmental samples per herd from model to NAHMS 2002 estimates.

Summary: The magnitude of HSe was strongly associated with within-herd prevalence, amount of Map organisms shed in feces by infected cows, and number of samples tested. ELISA alone was a sensitive and low cost testing method; however, without confirmatory fecal culture testing 30 cows per herd in non-infected herds yielded HSp of 21% and 91% for EA and EB, respectively. Testing all cows using ELISA testing with follow-up fecal culture (EAIFC and EBIFC) as commonly done in paratuberculosis-screening programs is unlikely to achieve 95% HSe in low prevalence herds.

Among evaluated testing methods with 100% herd specificity, ENV was the most cost-effective method for low (5%), moderate (16%) and high (35%) prevalence herds followed by PFC, IFC, and EAIFC and EBIFC, respectively. Culture of 6 environmental samples per herd yielded > 99% HSe in herds with > 16% within-herd prevalence, but not sufficient to achieve 95% HSe in low prevalence herds (5%). This model can be used to evaluate the impact of factors influencing the HSe of different testing strategies and provide decision makers with information about the cost-effectiveness of testing strategies for particular situations. Culture of pooled fecal samples (environment or cow samples) is efficient method of detecting infected dairy herds. Further work needed to evaluate efficient methods for detecting infected beef cattle herds and to evaluate efficient methods for herds after years of testing (Level 3).

Paul Anderson**,** Minnesota Board of Animal Health reported on fecal PCR compared to fecal culture. The PCR test detects most high shedders in a timely manner, less than a week. Thus producers are able to cull these animals more quickly. When only fecal cultures are used, the time required for producers to cull high shedding cattle ranges from 7-12 months based on Minnesota experience (Scott Wells). Minnesota is recognizing more pass-through or passive shedding cattle. In a study of 91 fecal tests that were culture positive and PCR negative, 76 samples were low positive with 1-10 cfu, most may be pass-through cows. In Minnesota, 1900

herds are enrolled in the Johne's program. The Minnesota program focuses more resources on infected herds than the 600 herds in the status program. 200 herds are at status level 1, 200 herds at status level 2, 100 herds at status level 3, and 100 herds at status level 4.

Jeff Nelson, NVSL reported on the "Validation Project" to validate NVSL decontamination protocol for Johne's disease in other laboratories, to compare BBL-HEYM media with BBL-HEYM flasks and to compare 8 weeks to 16 weeks incubation time. To date, 11 laboratories have participated in this project. Each sample (20 total replicated samples to each lab) was to be processed in the same manner and plated on one flask of BD-HEY and two tubes of BD-HEYM with mycobactin. The fecal sample size was 2.0 gms, placed in 35 ml of sterile distilled water in a 50 ml conical centrifuge tube. The tube was to be shaken vigorously to break up the large clumps then placed on a horizontal rocker for 30 minutes. 5 mls of liquid are removed from the upper 1/3 of the original centrifuge tube and placed into a new 50 ml centrifuge tube containing 25 ml of 0.9% HPC in ½ x BHI broth. Centrifuge tube incubated at 37º ± 2ºC for 18-24 hours (overnight). Incubated sample is centrifuged at 900 x *g* for 30 ± 2 minutes. Supernatant is discarded and the pellet is re-suspended in 1ml of BHI broth containing 100µg/ml naladixic acid, 100µg/ml vancomycin and 50µg/ml amphotericin B (Antibiotic Brew). Sample is shaken or vortexed for 15 seconds. Incubate at 37º ± 2ºC overnight. On day 3, Shake or vortex inoculum for 15 seconds prior to inoculating the media. Inoculate each HEY tube and HEY flask with 200µl of inoculum. Inoculum is rolled to ensure that the surfaces are covered. Media is incubated at 37º ± 2ºC for 8 wks.

All low, medium, and high samples were from three single sources, HEY media without Mycobactin J was not used since there were no "trick samples". More than 50 colonies observed are called TNTC. Cattle feces were used only. Findings included: Flasks have greater colony counts than tubes for the same inoculum; most labs have similar results but there are lab to lab variations for the same protocol. All labs had no growth on negative samples, 2 labs had no growth on samples that should have been positive, 1 lab had no growth on all HEY tubes (except one tube and 1 colony), flask colony counts were similar to what other labs counted, all labs except two had growth on at least one of the two HEY tubes for this inoculation volume when growth was expected. 8 Week Results included; Most labs had between 2-3X the amount of growth on flasks vs. tubes for the same inoculum; most labs cultured about the same amount of MAP for the low, medium, and high count feces; High count feces was not as high as was originally, but shows how effective the flasks were at allowing more MAP to grow. The 16 week results indicated most samples did not have more growth after allowing 8 more weeks and more contamination was noted after 16 weeks. Other results included; High count feces form heavy shedders was lower than previously tested; for quantifying these results, 60 colonies was used for TNTC. Results include colony counts from all of the tubes and flasks used in this study.

Some variability is noted in the amount of colonies counted. Flasks were easier to read overall than the tubes; glare and condensation made the flasks a bit difficult to read; the flask was superior to the tube(s) in the reduced time to spot visible MAP colonies, greater number of colonies, and ease of handling and observation; some found it hard to read through the glass flasks. Overall results indicated flasks are too thick to view under a microscope; Color change is indicative of egg yolk consumption by MAP growth; flasks are a great space saver in the incubator; the flask was superior to the tube(s) in the reduced time to spot visible MAP colonies, greater number of colonies, and ease of handling and observation, more difficult to

cover surface of flask with 200µl of inoculum than the tubes, overall flasks were preferred over the tubes.

Lisa Espey**,** IDEXX reported the company had improved their ELISA test kit to give a much better and consistent specificity and with a sensitivity of 27% to detect fecal culture positive cattle.

Tom Kellner**,** Prionics indicated the company would be submitting data for the milk ELISA to VS-CVB in the near future to seek licensure of the milk ELISA test.

Shiga Eda, University of Tennessee reported on their new and more sensitive ELISA test. For additional information see: New method of serological testing for MAP by flow cytometry. Foodborne Pathog Dis 2:250-62, 2005; A novel enzyme-linked immunosorbent assay for diagnosis of MAP infections in cattle. Clin Vaccine Immunol. 13:535-540, 2006; A highly sensitive and subspecies-specific surface antigen enzyme- linked immunosorbent assay for diagnosis of Johne's disease. Clin Vaccine Immunol 13:837-844, 2006.

Bev Byrum, Ohio reported on their experience with the TREK-ESP liquid culture system. Their's is a high volume laboratory processing over 20,000 samples per year with 9 ESP units. In order to detect all MAP positive samples, an AFB stain is done on every signal positive liquid culture bottle at the end of protocol (EOP). An automated shaker is used to dislodge MAP from the sponge in the tube before staining. Their laboratory prefer the Auromine O/Rhodamine stain with brilliant green or methylene blue counter stain as that gives faster visual assessment. They use an automated slide stainer. If the tube is AFB pos, then they do PCR (IS 900), if negative then the tube is re-returned to the incubator. If PCR positive on IS-900, then reconfirmed on PCR using 251. If positive for both IS 900 & 251 they are reported out as positive. If the sample is IS900 positive but 251 negative it is sub cultured. A MAP positive control is set up each week to detect any variation in the detection system. All liquid culture bottles left in the incubator for 42 days. Approximately 17-60% of over all positives are signaling negative. As many as 53-63% of the negative tubes will be AFB positive at the end of protocol (ave 36%). Not all of the signal negative tubes are low shedders. The machine does detect high/heavy shedders and doing PCR on 20,000 samples would be too expensive.

Sue Stehman reported that Cornell now has 16 TREK-ESP liquid culture units. They first began using TREK systems in January 2001 with about 1,000 cultures per month. Tubes are removed after 35 days of incubation. Each liquid culture bottle has an AFB stain done. For the first few years the average positivity rate was 10% to 12%. In the fall of 2004 they saw an increase in prevalence 14 to 18% when they added more AFB staining of non-signal tubes. The majority of these new positives are detected before 35 days. Individual cow results are reported back to the herd owner as soon as it is confirmed. Negative samples report after AF staining. False positive rate may increase due to line voltage change but using the associated graphs can help identify most of those samples. The report to practitioners included time to detection (TTD) and the relative cfu reported as many, moderate and few. Each positive sample is confirmed by PCR.

Deep Tewari, Pennsylvania reported on a robotics system he has been using for both ELISA and for Real Time (RT)-PCR (Tetracore). Their annual Johne's testing has been at 60,000 ELISAs and about 25,000 cultures per year. They use a Tecan™ Bio-Robotics for IDEXX ELISA. Four plates are processed in three hours; the results to date suggest a very consistent

performance of the assay. The Tecan system reduces operator error, increases throughput and improves turnaround time. Following their excellent experience with the automated ELISA assay they then considered automation of the Tetracore™ RT-PCR assay for Johne's disease. Their initial experience with the Tetracore assay was excellent. They chose the Bio-Robot by Qiagen BioRobot model M 48 which uses paramagnetic beads for nucleic acid extractions. In the 2005 fecal check test the Bio-Robot gave 23/25 correct while the Tetracore processed manually gave 21/25 samples correctly. Concern exists about potential PCR inhibitors, but the automated system looks promising.

Friday PM Session
Milk ELISA Testing for Johne's Disease

Bruce Dokkebakken, Minnesota Dairy Herd Improvement Association (DHIA) gave an overview of the organizational aspects of DHIA. Basic service of DHIA is to measure and test milk samples from individual cows at the farm level and report individual animal management data. Data is summarized to provide individual animal and herd management reports for producers. 80 DHIA technicians in Minnesota evaluate milk samples from 270,000 cows per month. Sample identification and integrity is a critical factor for all DHIA organizations. A national Quality Certification Program is in place to verify laboratory, field service and Dairy Records Management Systems (DRPC) performance. Minnesota DHIA, working in cooperation with the University of Minnesota and the MN Board of Animal Health, has recently made Johne's Milk ELISA testing available for producers in the state. This is added to the range of services provided including milk analysis, somatic cell counts, mastitis culturing, milk urea nitrogen, water testing, manure testing and forage analysis.

Ken Olson also gave an overview of DHIA activities from a consultant's perspective. DHIA operates through multiple organizations and is available in all states and has international links with International Committee for Animal Recording (ICAR). Data on individual animal records from birth to culling from the herd are available. The data is also used to facilitate genetic improvements; body type, body condition scoring, calving ease. Participation in DHIA has facilitated movement of cattle to Mexico. DHIA is one of the leaders in moving the National Animal Identification System forward.

Mike Collins reported on a large study he conducted to evaluate the milk ELISA compared to several serum ELISA tests and compared those results to fecal culture tests and PCR tests. Of more than 2,145 cows tested, 443 fecal samples were positive on at least one organism based test. The study also included 412 non infected cows from status level IV herds in Minnesota. Overall the milk ELISA test performed better than some blood ELISA tests and equal to the best serum ELISA test. For further information, see Evaluation of five antibody detection tests for diagnosis of bovine paratuberculosis. Clinical Diagnostic Laboratory and Medicine 12:685-692, 2005.

Steve Hendrick, Western College of Veterinary Medicine, Saskatoon reported on the Canadian Dairy Industry (CanWest DHI) implementation of the AntelBio Milk ELISA, "Milk ELISA Project". The Canadian Johne's program has focused on education and awareness with involvement of producers, veterinarians and government. The Canadian dairy industry has about 1 million dairy cows in 20,000 herds. Milk supply is based on supply management (quota) system. CanWest DHI was originally Ontario Dairy Herd Improvement that expanded to western Canada in 2003 to include BC, AB, SK, MB and ON servicing 4,900 herds (378,500

cows) as a traditional milk recording agency with value-added services: return-over feed and herd management clubs and Johne's milk ELISA testing.

Ontario DHI (2002) Initially compared AntelBio Milk ELISA to fecal culture results in 6 herds from 2002-2004 and then screened 126 herds to compare to serum ELISA in 82 herds and compared to fecal culture (HEYM) in 9 herds. This study randomly surveyed 50 dairy herds in Ontario; 18% and 30% of herds had 2 or more milk or serum enzyme-linked immunosorbent assay (ELISA)-positive cows, respectively. The apparent cow level prevalence was 1.7% and 2.6% on the milk and serum ELISA, respectively. The serum and milk assays agreed moderately. For further information see "The prevalence of milk and serum antibodies to Mycobacterium avium subspecies paratuberculosis in dairy herds in Ontario". Can Vet J. 46(12):1126-9, 2005. CanWest DHI began to offer the milk ELISA as a regular service to Ontario producers in June 2005 and expanded to Western Canada in December 2005. The "Milk ELISA Project" received federal funding (ACAAF) as an extension project incorporating the milk ELISA, training veterinarians and producers about Johne's disease. The milk ELISA project was initiated in Ontario (June 2005) with 80 producer-veterinarian pairs to focus on education and JD awareness, Risk assessment and implementation of BMPs. Milk ELISA testing was subsidized ($400) and compensation to veterinarians for RA and HMP training ($200), a one time payment. The project was expanded to Western Canada (Dec 2005). The provincial coordinator keep paper work in order, collects risk assessments and consent forms and arranges for veterinary training sessions. Provincial veterinary trainer facilitates completed on-farm training with veterinarians as one-on-one or in small groups.

The second phase of the project includes a revisit to a sub-set of herds in each province
And a re-test in subsequent years, assesses changes in management and re-administer risk assessments. This will serve as the basis for a PhD research project. From producers perspectives on the milk ELISA include: 1) the project is convenient, relatively cheap and provides quick turn-around time, 2) has a risk of false-positive results, 3) culling decisions may be impacted and 4) some producers just don't want to know if they have JD. For veterinarians: 1) profit made from diagnostic testing is not significant, 2) more income from consulting doing the herd plan and completing risk assessments, 3) this becomes part of the "herd health" program and 4) education is important, explaining how to interpret the test results, completing RAs and making management plans. From the Government perspective: 1) extension veterinarians see value in the test and participate in the project and 2) regulatory veterinarians accept the test as a herd screening tool not a certification test. There is no standardization for Johne's disease testing in Canada and commercial labs may participate in the NVSL check-test program. In summary, the "Milk ELISA Project" is an extension program provincially administered, provides education and awareness about JD to veterinarians and producers with a focus on management. The opinions of producers, veterinarians and regulators are quite favorable to this point. For further information on the milk ELISA project, see http://www.canwestdhi.com/johnes.htm

Jason Lombard reported on the results of the milk ELISA study as one part of the 2002 dairy NAHMS study. Milk and serum samples from 35 dairy herds in 17 states were evaluated for cow- and herd-level MAP antibody test agreement. Evaluation of 6,349 samples suggested moderate agreement between milk and serum ELISA results, with a kappa value of 0.50. Cow-level sensitivity (Se) for 18 dairy operations with 1,921 animals was evaluated relative to fecal culture results. At the cow level, the milk ELISA relative Se was not significantly different from that of the serum ELISA (21.2 and 23.5%, respectively). Logistic regression models revealed a

positive association between lactation number and milk ELISA status. Non-Holstein cows were more likely to test milk ELISA positive than Holstein cows. Cows in the first 2 weeks of lactation and after week 45 of lactation were more likely to test milk ELISA positive than cows between 3 and 12 weeks of lactation. Milk production > 80% of herd average was negatively associated with testing milk ELISA positive. Animals in the West and Midwest regions were less likely than animals in the Southeast region to test ELISA positive by either test. Estimates for herd-level sensitivity for the milk and serum ELISA, relative to fecal culture results, ranged from 56 to 83%. At the cow and herd levels, milk ELISA performed equivalent to serum ELISA using fecal culture as a reference for MAP infection and has the advantage of decreased labor costs on farms that use Dairy Herd Improvement Association testing. For further information see Journal of Veterinary Diagnostic Investigations 18(5):448-58, 2006.

Todd Bryem, Antel Bio reported on the milk ELISA that was developed at their laboratory in Lansing, Michigan. Five laboratories now run milk ELISA tests. Next year Antel Bio will do about 100,000 milk ELISA tests. The DHIA technician is able to order JD Milk ELISA. DHIA technicians are offered a monetary incentive to have producers run the milk ELISA tests. Milk ELISA testing at the time of “dry off” seems to offer one of the best times to test. In an effort to make herd veterinarians aware of the milk ELISA test data, post cards were sent to vets for a number of months with only one response to request copies of report. The state veterinarians from Wisconsin and Michigan have requested copies of the data.

From the herd veterinarian’s perspective, the milk ELISA is another tool to use in the war against JD Milk ELISA testing is a viable alternative to official program. Very few herds that use the milk ELISA have done RA and developed a HMP. Confidentiality of test results is a major issue for some farms, even though an un-official test and the majority of herd owners do not see JD as a problem (low cost benefit). Very few herds using the milk ELISA have entered status program, even if eligible. For further information see: http://www.antelbio.com

Janet Marquardt, NVSL reported on some of the issues involved in establishing a check test for a milk ELISA. Issues include: 1) long term storage of milk samples, 2) acquisition of animals to provide regular availability of milk samples (presently only have room for 4 animals) 3) milking these cows on a regular basis at a federal facility, and 4) logistical issues. Since the primary difference between milk and serum ELISA is the dilution rate used, 1:1 for milk vs. 1:20 for milk, could serum be used as a surrogate for milk? Staff at NVSL has discussed these issues and are planning to implement a milk ELISA check test in the near future.

Louise Henderson, VS-CVB reported on Licensing Veterinary Diagnostic Test Kits for Johne’s disease. To qualify for CVB licensure, diagnostic test kits should with reasonable certainty yield the results intended when used according to label (insert) instructions. The design, architecture, claims, recommendations, target disease, target animal, sample source, and intended uses determine specific requirements. CVB goal is to license tools of value to users.

Different standards are often appropriate for a specific test. Each serial of all licensed kits must pass NVSL proficiency panel (firm and CVB); kits licensed for milk samples will have to pass milk proficiency panel. USDA licenses kits for diagnosis of disease in animals, not food safety. Each sample type must be validated independently. Data must support performance characteristics of the test. Variations need to be assessed between plate, assay, run, day and laboratory. The analytical sensitivity and specificity for each sample type needs to be estimated. The test must be able to distinguish target from non-target.

The test must have the ability to correctly identify samples from positive animals and from negative animals. The dynamic range and test ruggedness need to be defined. Prior to licensure, the firms must report how they make the test (Outline of Production), how they have established the cutoff data points and how they have established performance data and how the test performs in field on each sample type data. Serials of production for consistency of performance (Outline testing) must be provided.

Pre-license validation should be done on a large number of “known” positive and “known” negative animal samples covering a range of reactivity and all sample types. They need to determine and justify cutoff values, determine performance in different populations, address matrix effects, cross reactivity and estimate performance characteristics. The test needs to be evaluated in the field in expert labs (2 serials in 3 labs); the labs must have expertise in disease testing and be cooperative in the evaluation. These evaluations need to assess suitability of test kit, adequacy of instructions and confirm performance characteristics. A rigorous evaluation at this stage is critical. USDA licensed Kits are validated for recommended purposes prior to licensure.

Claims supported with data are independently evaluated. Each test kit is documented and must have controlled manufacturing protocols of all kits for consistency. CVB monitors field performance and investigates variations. Independent testing of performance characteristics for each serial prior to release for sale is required. For further information call CVB-Inspection and Compliance, 1-800-752-6255.

National Johne's Disease Demonstration Herd Project

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Background and Justification

Goal II of the Report of the Ad Hoc Steering Subcommittee of the USAHA Committee on Johne's Disease (2002) is "to define critical knowledge gaps that influence producer participation and affect Johne's disease (JD) control." One objective under this goal is "to develop and validate model strategies for control of Johne's disease," stating that "demonstration herds … are critical and of the highest priority to provide the validated management tools to implement a science-based National Johne's Disease Program."

The importance of the Johne's Disease Demonstration Herd Project was affirmed at a meeting with the Veterinary Services Management Team in Ames, IA on May 1, 2003. This project has a 2003 budget of $1.5 million to United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) and states plus additional funding to Centers for Epidemiology and Animal Health (CEAH) for personnel to support this study.

Demonstration Herd Committee Task and Timelines

The designated task of the Demonstration Herd Committee of National Johne's Working Group (NJWG) is to provide oversight and guidance to National Johne's Disease Demonstration Herd Project. Our timeline for producing an initial model for the program is July 1, 2003. Committee members include: Scott Wells-Chair; Ian Gardner, Bill Shulaw, Sue Stehman, Mike Carter, Andy Schwartz, Dave Dargatz, Brian McCluskey and Jason Lombard.

Project considerations

The feasibility and success of Johne's control programs on farms is highly dependent on the interrelationships between attitudes, motivation, farm resources (human, facilities and financial), farm goals, management and health priorities, the perceived and real impact of Johne's on the farm, and clearly defined Johne's goals. Factors vary with each farm situation requiring a customized approach based on common principles. While recognizing that Johne's disease control is more a process than a program, our goal is to put together a core program for the National Johne's Disease Demonstration Herd Project. Recognizing differences in cattle herd production systems regionally and state needs and priorities, our goal was to keep the core aspects of this program to a minimum, while maintaining the minimum level of high quality data to achieve the highest priority (primary) objective of this project.

Demonstration Herd Project Objectives:

Primary objective:

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.

The primary hypothesis to be tested is that control of JD can be achieved through implementation of on-farm management practices to reduce transmission of infection to susceptible cattle. This project will evaluate whether a flexible, voluntary, farm-specific process using different Johne's disease control and testing strategies can be shown to consistently decrease the impact of Johne's infection in herds in different states with different testing approaches and management systems. Culling data, testing data and management data will be used to evaluate the effectiveness and feasibility of the Johne's disease control program.

We recognize that effective and feasible control of JD is dependent upon many factors including 1) degree of implementation (limited resources of facilities and labor and conflicting herd health or other farm priorities), 2) biosecurity challenges including purchased cattle, 3) testing strategy (definition of testing strategy and adequacy for meeting goals, utilization of test results for monitoring or selection of cattle for management decision-making, poor test performance in herds), and 4) limitations of state resources in amount or type of support that can be provided to farms (insufficient testing capacity or access to testing and insufficient personnel).

We estimate that a minimum of 100 herds nationally is necessary to test the hypothesis that a reduction in the incidence of clinical disease or prevalence of infection will be evident 4 years after the adoption of a JD herd control program by each herd owner. This estimate is based on the assumption that the best *a priori* guess of the point estimate (reduction) is 70% and the goal is to have an error margin of no more than +/- 10% on the final estimate. With these inputs, the required number of herds is approximately 84 but we assume that there will be a 15 to 20% loss of herds attributable to factors such as sales and, hence, we recommend upward adjustment to100. If a goal is to compare estimated proportions between subgroups of herds (cattle type, herd size, etc), many more herds will be necessary to ensure statistical significance. Our group has not specifically addressed the latter situation.

Criteria for states involved in demonstration herd program

1. Active Johne's Disease Advisory Committee within state
2. Minimum infrastructure within state to conduct program
    - Priority will be given to states with a substantial cattle population. Demonstration herds will be considered in states with fewer cattle if funds are available and the states also meet the following criteria.
    - Trained Johne's Disease Designated Coordinator/epidemiologist
    - Laboratory capacity or access to capacity to perform required testing
3. Strength of proposal

Secondary objectives:

1. Provide information and materials for education and training of public and private practice veterinarians and cattle producers.

2. Develop and evaluate management, testing, and monitoring strategies for use in control of Johne's disease in cattle herds.
3. Create the opportunity for add-on projects within states to address important research objectives. Specific questions that could be answered include:
    a. How well do the test data collected over time correlate with the information gathered from the Johne's disease history and risk assessment (including clinical cull data) about the level of Johne's infection on a farm?
    b. How well does environmental sampling correlate with shedding prevalence over time?
    c. Can we accurately measure implementation of management practices or risk mitigation?
    d. How often do we see test conversions – positive to negative with fecal culture and serology?
    e. What is the longterm outcome of a test result relative to advancing infection? Do low shedders progress and at what rate? How well does serology correlate with fecal shedding in longitudinal studies?

To achieve these objectives, core information will be shared across states participating in this project. States will submit Demonstration Farm proposals to the USDA Johne's coordinator, Dr Michael Carter, for review. CEAH will serve as the coordinating center for data compilation, validation, and analysis. Data will be captured via the Microsoft Access database program available from Dr. Michael Carter, VS-APHIS-USDA. Each state should work out confidentiality issues to ensure farm identifiers are not made available to public without consent. The goal is to share core information from demonstration herds to achieve the objectives above, while allowing for flexibility and creativity among states in achieving additional specific goals and objectives.

Johne's Disease Demonstration Herds – Dairy Cattle

Outcomes to be measured:

1. **Monitor disease**
    a. **Incidence of clinical disease** –
        i. Number of clinical JD cases in herd by year within each age cohort (lactations 1, 2, 3+) and by source (home reared or purchased) divided by mean cow inventory during year.
        ii. Exit monitoring of clinical suspects by fecal culture, or Enzyme-Linked ImmunoSorbent Assay (ELISA) with confirmation by organism detection method, is recommended until a producer has a good sense of the percent of clinical suspects that are in fact Johne's cases.
    b. **Prevalence of infection** – preferably monitored by whole herd fecal culture or other organism detection methods, as well as antibody detection method (e.g., ELISA).
        i. The target is at least 80% of adult cattle in herd tested per year. With very large program herds, a random subset of the adult cattle in these herds may be tested if resources in state are limited, using statistical subset sampling as described in Voluntary Johne's Disease Herd Status Program (VJDHSP).
        ii. At minimum, all cattle (or statistical subset) should be tested using ELISA and all ELISA-positive cattle in herd must be confirmed using fecal culture or an approved agent detection method.
2. **Monitor secondary culls –** (subclinically infected cattle)
    a. Culling due to test results needs to be defined and distinguished as a separate (secondary) reason for Johne's culling
        i. If fecal culture is used – describe/categorize quantities shed and basis of culling
        ii. If serology is used for culling – recommend culture confirmation of ELISA-positives as a goal.
3. **Monitor Risks and Management** –
    a. Define risks using risk assessment tools
    b. Document interventions and management events

Dairy Herd Selection criteria:

1) **Basic enrollment criteria:** The herd must have a history of clinical disease in the herd. More than presence of disease in a purchased herd addition. Herds must be documented as infected by organism detection methods.
2) **Owner profile**: The herd owner must be willing to keep good records and have in place a method (not necessarily computerized) to do so. This means individual animal identification (ID) and records of animal movement sales, culls, including reasons and individual animal health events. The herd owner must plan to be in business for at least the next 5 years, and must have the time, labor, facility and cash resources necessary to implement a control plan. The herd owner uses and cooperates with a herd veterinarian and is willing to cooperate with university, state, or federal veterinarians involved with the demonstration herd program.
    - The herd owner must be willing to allow the data to be shared in the national data collection effort. Anonymity of herd identity would be assured.
    - The herd owner must be willing to allow his herd to be used for educational purposes such as producer education programs, etc. Participating herd owners should allow data, photographs,

etc. to be used for educational purposes. Educational programs involving farm visits may be helpful in the educational effort but are not mandatory.

- The herd owner must be willing to initially set some herd goals for control of the disease and be willing to annually review these and assess degree of progress.

3) The herd must use a Herd Veterinarian or Accredited Program Planner that is actively supporting the farm and the Johne's disease control program.
4) Farm Demographics: We expect that states will include herds of different sizes and management systems and infection challenge, though not necessarily all of the categories below within each state. Because demonstrating change over time in a herd with small cow numbers or very low prevalence is difficult, a minimum herd size of 50 cows with an estimated prevalence of infection of 5% or greater is strongly encouraged. However, smaller herds with higher prevalence or larger herds with somewhat lower prevalence may be acceptable. If the product of cow numbers times estimated prevalence equals 300 the herd could be enrolled. Examples are: 30 cows with 10% prevalence or 100 cows with 3% estimated herd prevalence. If the herd prevalence is unknown at the beginning of the program, infected herds having a minimum of 5% of the cows culled the previous year having signs compatible with clinical Johne's disease would be an acceptable alternative.
    - Examples -
        - Adult milking cow herd size <100, 100 - 1000, >1000 milk cows
        - Tie stall vs. freestall cattle housing
        - Commercial producer vs. registered seedstock producers
        - Infection Status/ Challenge/ Impact – based on fecal shedding and clinical cull rate due to Johne's disease
            a. Higher Prevalence (10-50%+) based on fecal shedding or ELISA-positive or >5% of adult cow herd culled with clinical Johne's disease per year)
            b. Lower prevalence (<10% of cows fecal shedding or ELISA-positive or ≤1% of adult cow herd with clinical infection per year)

Procedures:

Outline

1. Define overall herd goals and specific goals relative to Johne's Disease.
2. Collect baseline farm data as outlined in the Herd Plan Manual.
3. Complete Johne's Risk Assessment and farm walk-through and document risks.
4. Define risks and recommend management changes to address those risks in a written herd plan. Risks are prioritized and the management changes recommended that are in line with farm goals, resources. Review Quarterly. Document events.
5. Define Testing Strategies to meet Johne's control objectives
    - Document how test results will be used in the farm plan
6. Annual review to revisit farm goals, resources, commitment, management and progress.

1) **Define Herd Goals -** A herd plan and farm goals for the control of Johne's disease should be developed at the initiation of the program and reviewed at least annually. This should be done with the herd veterinarian and the demonstration herd coordinator/risk assessor. The USAHA/NJWG Dairy Manual describes general management/control program strategies used by producers for control of their Johne's disease problem:

- Maintain and manage infection prevalence – The overall goal is to minimize existing risks and manage introduced infection. This strategy may be most useful in herds with low prevalence or with fewer identified risks.
- Control – The overall goal is to reduce prevalence; reduce clinical infection and losses; and reduce premise contamination and potential for transmission.
- Reduce or eliminate infection – aggressive testing, management strategies and timeline.

2) The **standardized risk assessment** form developed by the NJDWG must be used for each herd.
   a) States can use their own risk assessment form too, but must use the standardized one in order to assist in the data collection effort.
   b) Define risks using quantitative risk assessment tools (available through USDA-APHIS-VS and on USAHA website) performed at least annually.
   c) Inter-rater variation should be minimized by risk assessment in these herds by the same person, if possible, each year. These individuals should have participated in training conducted by USDA-APHIS-VS.
   d) Recommended management changes should be in line with farm goals and resources, and these should be reviewed quarterly with one of these serving as an annual review.
3) **Testing -** All adult animals (including bulls) must be tested by culture (or other organism detection method) and ELISA at least annually. Colony counts, or some standardized method, to categorize shedding intensity should be used. If states cannot comply with this because of herd size constraints or laboratory capability, an alternative strategy of whole herd ELISA testing with culture of ELISA-positives is acceptable. If test results are used for culling or herd management, a description of how test results are used should be included in the herd plan.
4) **Environmental samples** should be collected for culture at least annually and may be collected more frequently. These samples can be a composite of fecal material, not a single fecal pile collected from calving pens, feeding/loafing areas, alleys, and input to manure handling systems spreaders, pits, lagoons.
5) **Minimum record keeping requirement** –
   - Herd Demographics
     1) current production per year
     2) production per cow
     3) Facilities – tie stall/stanchion, free stall, mixed
     4) % replacements purchased
     5) % replacements reared
   - Culling Data
     1) Culling rate
     2) Culling rate due to clinical Johne's
     3) Culling rate due to Secondary Johne's culling (subclinical cows culled as a result of a Johne's test result).
     4) Age at culling, date at culling, purchased or home reared, Johne's test results
   - Testing data -
     1) Test Date
     2) Age or lactation number
     3) Days in milk on sample date
6) **Minimum intervention strategies** used: Use common minimum standards for interventions across all herds. Suggest modification of the same minimum management practices as used in the Program Standards for Beef and Dairy Herds p 13.
   - Herd plans should emphasize risks from fecal contamination - Identify and mitigate risks from exposure to and ingestion of adult manure in environment, feed and water – especially exposure to young stock.

1) Maternity area must be clean and dry and separate, or protected from manure from, other adult animals.
2) Separate newborn calves from adults immediately or as soon as possible
3) No pooled colostrum; Use colostrum form single identified, healthy, low risk or test negative cow
4) Calves must be fed milk replacer or pasteurized milk
5) Calves and heifers must be kept free from exposure to the manure of mature cattle. House separately or, if housed together, prevent contact with adult manure by physical barriers.
6) Prevent contamination of feed and water fed to young stock by manure from adults. Do not feed refusals from adult cattle
7) Adult Cattle
    a) Identify animals contributing most to farm's infection load (testing or clinical observation) and market early or separate and use targeted management to mitigate exposure risks.
    b) Acquire new animals from low risk herds or submit a fecal culture and blood for ELISA at the time of purchase and include these animals in the regular testing thereafter.
8) Use separate equipment for manure cleaning and feed handling. An acceptable alternative is to thoroughly clean and then disinfect this equipment after manure handling and before handling feed. Acceptable disinfectants include a substituted phenol (Amphyl, Osyl, Wexcide, One Stroke, TekTrol), Virkon S (Trifectant), or other product labeled as being tuberculocidal.

7) **Monitor Risks and Management**
    a) Document interventions and management events on quarterly basis.
    b) Measure of herd implementation of priority management through reduction in herd Risk Assessment score at least annually.
8) **Other perceived benefits of Johne's control** (Optional, not part of core program)
    a) Improved calf health. Good records and analysis will be needed to document this.
    b) Decreased other clinical diseases (e.g., salmonellosis) in cows and/or calves
    c) Increased longevity of cattle in herd

Johne's Disease Demonstration Herds - Beef Cattle

Outcomes to be measured:

1) **Monitor disease**
    - Incidence of clinical disease – preferably confirmed by fecal culture or other organism detection methods
    - Prevalence of infection – preferably monitored by whole herd fecal culture or other organism detection methods. However, in some cases, whole herd ELISA with fecal culture of ELISA-positive animals may be the only practical method.
2) **Monitor culls –**
    - Confirm the status of all culled cows with fecal culture if at all possible
    - ELISA can be used as an alternate if culture is just not possible but cultural confirmation of ELISA-positives should be the goal.
3) **Monitor Risks and Management** –
    - Define risks using risk assessment tools
    - Document interventions and management events

Herd selection criteria:

1) The herd must have a history of clinical disease in the herd more than presence of disease in a purchased herd addition. Herds must be documented to be infected by organism detection methods.
2) Owner profile: The herd owner must be willing to keep good records and have in place a method (not necessarily computerized) to do so. This means individual animal ID and records of animal movement (sales, culls [including reasons], etc.) and individual animal health events. The herd owner must plan to be in business for at least the next 5 years, and must have the time/labor/facility/cash resources necessary to implement some portion of a control plan. The herd owner uses and cooperates with a herd veterinarian and is willing to cooperate with university, state, or federal veterinarians involved with the demonstration herd program.
3) Because demonstrating change over time in a herd with small cow numbers or very low prevalence is difficult, a minimum herd size of 50 cows with an estimated prevalence of infection of 5% or greater is strongly encouraged. However, smaller herds with higher prevalence or larger herds with somewhat less prevalence may be acceptable. If the product of cow numbers times estimated prevalence equals 300 the herd could be enrolled (examples: 30 cows with 10% prevalence or 100 cows with 3% estimated herd prevalence. If the herd prevalence is unknown at the beginning of the program, infected herds having a minimum of 5% of the cows culled the previous year having signs compatible with clinical Johne's disease would be an acceptable alternative.
4) The herd owner must be willing to allow the data to be shared in the national data collection effort. Anonymity of herd identity would be assured.
5) The herd owner must be willing to allow his herd to be used for educational purposes producer education programs, etc. Participating herd owners should allow data, photographs, and etc. to be used for educational purposes. Educational programs involving farm visits may be helpful in the educational effort but are not mandatory.
6) The herd owner must be willing to initially set some herd goals for control of the disease and be willing to annually review these and assess degree of progress.

Procedures:

1) Define Herd Goals

A herd plan and farm goals for the control of Johne's disease should be developed at the initiation of the program and reviewed at least annually. This should be done with the herd veterinarian and the demonstration herd coordinator/risk assessor. The USAHA/NJWG Beef Manual describes general management/control program strategies used by producers for control of their Johne's disease problem:

- Maintain and manage infection prevalence – The overall goal is to minimize existing risks and manage introduced infection. This strategy may be most useful in herds with low prevalence or with fewer identified risks.
- Control – The overall goal is to reduce prevalence; reduce clinical infection and losses; and reduce premise contamination and potential for transmission.
- Reduce or eliminate infection – aggressive testing, management strategies and timeline.

2) The **standardized risk assessment** form developed by the NJDWG must be used for each herd.
   (a) States can use their own risk assessment form too, but must use the standardized one in order to assist in the data collection effort.
   (b) The risk assessment for all herds initially enrolled in a state should be done by the same person, and this person should also perform this in subsequent years.
   (c) Recommended management changes should be in line with farm goals and resources, and these should be reviewed twice yearly with one of these serving as an annual review.
   (d) At least one of the farm visits or risk assessments should be done near calving time, if possible, to assess what is actually being done at this critical time.
3) Testing - All adult animals (including bulls) must be tested by culture (or other organism detection method) and ELISA at least annually. Colony counts, or some standardized method, to categorize shedding intensity should be used. If states cannot comply with this because of herd size constraints or laboratory capability, an alternative strategy of whole herd ELISA testing with culture of ELISA-positives is acceptable.
4) Every attempt should be made to get a sample for fecal culture (or other organism detection method) from all culled animals before they leave the farm and the reason for leaving, age, date, if the animal was purchased or home-reared, and test result must be recorded. If samples from all culls cannot be obtained, the proportion sampled and the results must be recorded.
5) Environmental samples should be collected for culture at least annually. These should preferably be taken near calving time. These samples can be a composite of fecal material collected from calving pens (not a single fecal pile) or soil samples contaminated with feces which have been collected in the calving area or feeding/loafing areas. Protocols for culturing soil are not well established, but recent work from Australia shows success with treating soil as feces.
6) Minimum record keeping requirement – In states where the expertise and resources are available to conduct a full Standardized Production Analysis (SPA) are available, this is encouraged. An alternative is the SPA-EZ which can be found at http://gpvec.unl.edu/spa/spaez.htm#cows . Minimum data to be collected annually include:
   a. % of cows diagnosed pregnant that calve (if pregnancy checking is done)
   b. % of cows that calve of those exposed to bulls
   c. % of cows that abort
   d. % of calves weaned of those born
   e. average weight of calves weaned
   f. average age of calves weaned
   g. % cows culled and culling data as described above
   h. % replacements reared
   i. % replacements purchased

7) **Minimum intervention strategies used**:
   a. Cull heavy fecal shedders.
   b. For herds using individual calving pens, these should be cleaned and re-bedded between cows. Keep calving area as clean and dry as possible. Minimize the density of cow and calf pairs as much as possible.
   c. Use feeding practices that reduce manure contamination of feed, water, and feeding areas as much as possible.
   d. Use separate equipment for manure cleaning and feed handling. An acceptable alternative is to thoroughly clean and then disinfect this equipment after manure handling and before handling feed. Acceptable disinfectants include a substituted phenol (Amphyl, Osyl, Wexcide, One Stroke, TekTrol), Virkon S (Trifectant), or other product labeled as being tuberculocidal.
   e. Cull (send to feedlot) the most recent progeny of animals with clinical Johne's disease and those determined to be heavy shedders.
   f. Avoid spreading manure on pastures; at least those that will be grazed by heifers.
   g. Isolate scouring or unthrifty animals promptly. Do not place in paddocks with heifers and do not return them to the herd until a provisional diagnosis is made. These animals should not be isolated in a calving area.
   h. Raised weaned replacements separated from older animals if possible. Avoid grazing weaned calves behind, or with, cows (3 month temporal separation).
   i. Acquire new animals from test negative herds or submit a fecal culture and blood for ELISA at the time of purchase and include these animals in the regular testing thereafter.

NVSL Approved Laboratories for Johne's Disease – Organism Based Tests - 2007
(October 15, 2006)

Janet Payeur
National Veterinary Services Laboratory
Veterinary Services
Animal and Plant Health Inspection Service
United States Department of Agriculture

NVSL Approved Laboratories using PCR

Veterinary Diagnostic Lab
AR Livestock and Poultry Commission
Little Rock, AR 72205
cunqin@arlpc.org

CA Animal Health & Food Safety Lab
University of CA
Davis, CA 95616
mrvillanueva@ucdavis.edu

Rocky Mountain Regional Animal
Health Laboratory
CO Dept. of Agriculture,
Division of Animal Industry
Denver, CO 80211
tiffany.brigner@ag.state.co.us

Galesburg Animal Disease Lab
IL Dept. of Agriculture
Galesburg, IL 61401
svinson@agri.state.il.us

Animal Disease Diagnostic Laboratory
Purdue University
West Lafayette, IN 47907-7440
wuc@purdue.edu

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Murray State University
Hopkinsville, KY 42240
shri.singh@murraystate.edu

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Louisiana State University
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bmangold@tetracore.com

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University of Minnesota
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MS Veterinary Research & Diagnostic Lab
MS State University
Jackson, MS 39216
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Dept. of Veterinary & Biomedical Sciences
University of Nebraska
Lincoln, NE 68583-0907
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Division of Animal Health Laboratory
NJ Dept of Agriculture
Trenton, NJ 08625
aghdica@ag.state.nj.us

PA Veterinary Laboratory
PA Dept of Agriculture
Harrisburg, PA 17110
ehue@state.pa.us

New Bolton Center
University of PA
Kennett Square, PA 19348
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tfyock@vet.upenn.edu

Animal Disease Research Diagnostic Lab
SD State University
Brookings, SD 57007
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BioGene
Brookings, SD
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TX Vet Med Diagnostic Lab
Texas A & M University
College Station, TX 77843
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UT Veterinary Diagnostic Laboratory
UT State University
Logan, UT 84322
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VA Dept. of Agriculture
Lynchburg, VA 24504
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Agri-food Laboratories Branch
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rashed.cassis@gov.ab.ca

SVA, PARATB Lab
Uppsala, Sweden, 76189
Goran.bolske@sva.se

NVSL Approved Laboratories for Johne's Disease - ELISA Based Test - 2007
(November 22, 2006)

Ms. Janet Marquardt
National Veterinary Services Laboratory
Veterinary Services
Animal and Plant Health Inspection Service
United States Department of Agriculture

State	Laboratory
Alabama	Thompson/Bishop/Sparks Veterinary Diagnostic Laboratory 890 Simms Road Auburn University Auburn, AL 36832
Arizona	Arizona Veterinary Diagnostic Laboratory University of Arizona 2831 N. Freeway Tucson, AZ 85705
	Arizona Dairy Herd Improvement Association Department of Microbiology 2465 W 12th Street, Suite #1 Tempe, AZ 85281
Arkansas	Arkansas Livestock & Poultry Commission One Natural Resources Drive Little Rock, AR 72205
California	California Animal Health & Food Safety Laboratory University of California 2789 S. Orange Avenue Fresno, CA 93725
	California Animal Health & Food Safety Laboratory University of California 105 West Central Avenue San Bernardino, CA 92408
	California Animal Health & Food Safety Laboratory University of California - Davis West Health Science Drive Davis, CA 95616
Colorado	Colorado State University Diagnostic Laboratory Colorado State University 300 West Drake Fort Collins, CO 80523
	Colorado State University Veterinary Diagnostic Laboratory Rocky Ford Branch 27847 Road 21 Rocky Ford, CO 81067

	Rocky Mountain Regional Animal Health Laboratory Colorado Department of Agriculture 2331 West 31st Avenue Denver, CO 80211
	The Dairy Authority 2525 West 16th Street, Suite E Greeley, CO 80634
Connecticut	Connecticut Veterinary Medical Diagnostic Laboratory University of Connecticut 61 North Eagleville Road, Unit 3203 Storrs, CT 06269
Delaware	Delaware Department of Agriculture 2320 South Dupont Highway Dover, DE 19901
Florida	University of Florida Research Laboratory 2015 South West 16th Avenue Deriso Hall, Room 139 Gainesville, FL 32610
	Live Oak Diagnostic Laboratory 912 Nobles Ferry Road Live Oak, FL 32064
Georgia	Athens Veterinary Diagnostic Laboratory College of Veterinary Medicine University of Georgia Agriculture Drive Athens, GA 30602
	University of Georgia Veterinary Diagnostic Laboratory College of Veterinary Medicine 43 Brighton Road P.O. Box 1389 Tifton, GA 31793
Hawaii	Veterinary Laboratory Branch Hawaii State Department of Agriculture 99-941 Halawa Valley Street Aiea, HI 96701
Idaho	Caine Veterinary Teaching Center University of Idaho 1020 East Homedale Road Caldwell, ID 83607
	Idaho Bureau of Animal Health Laboratories 2230 Old Penitentiary Road Boise, ID 83712
Illinois	Animal Disease Laboratory Illinois Department of Agriculture 9732 Shattuc Road Centralia, IL 62801

	Galesburg Animal Disease Laboratory Illinois Department of Agriculture 2100 South Lake Storey Road Galesburg, IL 61401
	Veterinary Diagnostic Laboratory University of Illinois 1412 VMBSB 2001 South Lincoln Avenue Urbana, IL 61802
Indiana	Animal Disease Diagnostic Laboratory Purdue University 406 South University Street Room 164 West Lafayette, IN 47907-2065
Iowa	Diagnostic Bacteriology Laboratory National Veterinary Services Laboratories 1800 Dayton Avenue P.O. Box 844 Ames, IA 50010
	Iowa State University – Veterinary Diagnostic Laboratory 1600 South 16th Street College of Veterinary Medicine, Room 2651 Ames, IA 50011
Kansas	Kansas State Diagnostic Laboratory Kansas State University 1800 Denison Avenue Mosier Hall Manhattan, KS 66506
Kentucky	Murray State University Breathitt Veterinary Center 715 North Drive P.O. Box 2000 Hopkinsville, KY 42240
	Livestock Disease Diagnostic Center University of Kentucky 1490 Bull Lea Road P.O. Box 14125 Lexington, KY 40512-4125
Louisiana	Louisiana Veterinary Medicine Diagnostic Laboratory 1909 Skip Bertman Drive Baton Rouge, LA 70803
Maine	IDEXX Laboratories, Inc. One IDEXX Drive Westbrook, ME 04092
	State-Federal Diagnostic Laboratory 28 State House Station Augusta, ME 04333
Maryland	Animal Health Diagnostic Laboratory Maryland Department of Agriculture

	8077 Greenmead Drive College Park, MD 20740
Michigan	Diagnostic Center for Population & Animal Health Michigan State University 4125 Beaumont Road Lansing, MI 48910
	Antel Biosystems, Inc. 3655 Forest Road East Lansing, MI 48823
	Laboratory Division Michigan Department of Agriculture 1615 South Harrison Road East Lansing, MI 48823
Minnesota	Minnesota Veterinary Diagnostic Laboratory University of Minnesota 1333 Gortner Avenue St. Paul, MN 55108
	DQCI Services Diversified Laboratory Testing 5205 Quincy Street Mounds View, MN 55112
Mississippi	Mississippi Veterinary Diagnostic Laboratory 2531 North West Street P.O. Box 4389 Jackson, MS 39216
Missouri	Animal Health Laboratory State-Federal Cooperative Laboratory 216 El Mercado Plaza Jefferson City, MO 65109
	Missouri Veterinary Diagnostic Laboratory Missouri Department of Agriculture 701 North Miller Avenue P.O. Box 2510 Springfield, MO 65802
	Veterinary Medicine Diagnostic Laboratory University of Missouri 1600 East Rollins Road P.O. Box 6023 Columbia, MO 65211
Montana	Diagnostic Laboratory Division Montana Department of Livestock 19th and Lincoln Street Bozeman, MT 59718-0997
Nebraska	Veterinary Diagnostic Center University of Nebraska - VDC East Campus Loop & Fair Street Lincoln, NE 68583
Nevada	Nevada Animal Disease Laboratory Nevada Division of Agriculture

	350 Capitol Hill Avenue Reno, NV 89502
New Hampshire	New Hampshire Veterinary Diagnostic Laboratory University of New Hampshire 319 Kendall Hall 129 Main Street Durham, NH 03824
New Jersey	New Jersey Division of Animal Health P.O. Box 330 John Fitch Plaza Trenton, NJ 08625
New Mexico	Veterinary Diagnostic Services New Mexico Department of Agriculture 700 Camino de Salud, North East Albuquerque, NM 87106
New York	New York State Animal Health Diagnostic Center College of Veterinary Medicine, Cornell University Upper Tower Road Ithaca, NY 14853
North Carolina	Rollins Animal Disease Diagnostic Laboratory 2101 Blue Ridge Road Raleigh, NC 27607
North Dakota	North Dakota State Veterinary Diagnostic Laboratory North Dakota State University Van Es Hall, Room 185 1523 Centennial Boulevard Fargo, ND 58105
Ohio	Animal Disease Diagnostic Laboratory Ohio Department of Agriculture 8995 East Main Street, Building 6 Reynoldsburg, OH 43068
Oklahoma	Oklahoma Animal Disease Diagnostic Laboratory Oklahoma State University Farm at Ridge Road Stillwater, OK 74078
Oregon	Oregon Department Agriculture Animal Health Laboratory 635 Capitol Street North East Salem, OR 97301
Pennsylvania	Johne's Research Laboratory University of Pennsylvania 382 West Street Road 47 Myrin Building Kennett Square, PA 19348
	Pennsylvania Veterinary Diagnostic Laboratory Pennsylvania Department of Agriculture 2305 North Cameron Street Harrisburg, PA 17110

Puerto Rico	Government of Puerto Rico, Department of Agriculture Veterinary Diagnostic Laboratory 9 Carr 693, Barrio Higuillar By Sabanera De Dorado Dorado, PR 00646
South Carolina	Clemson University Veterinary Diagnostic Center 500 Clemson Road P.O. Box 102406 Columbia, SC 29224
South Dakota	Animal Disease Research & Diagnostic Laboratory South Dakota State University 105 North Campus Drive P.O. Box 2175 Brookings, SD 57007
Tennessee	Kord Animal Disease Diagnostic Laboratory Ellington Agricultural Center, Porter/Ivy Building P.O. Box 40627, 440 Hogan Road Nashville, TN 37220
Texas	Texas Veterinary Medicine Diagnostic Laboratory Texas A & M University 6610 Amarillo Boulevard West Amarillo, TX 79106
	Texas Veterinary Medicine Diagnostic Laboratory Texas A & M University 1 Sippel Road P.O. Drawer 3040 College Station, TX 77843
Utah	Utah State Veterinary Diagnostic Laboratory Utah State University 950 East 1400 North Logan, UT 84341
Virginia	Virginia Department of Agriculture 4832 Tyreanna Road Lynchburg, VA 24504
	Virginia Department of Agriculture and Consumer Services Harrisonburg Regional Laboratory 116 Reservoir Street Harrisonburg, VA 22801
Washington	State-Federal Laboratory Washington Department of Agriculture 3939 Cleveland Avenue South East Olympia, WA 98501

	Washington Animal Disease Diagnostic Laboratory Washington State University 100 Dairy Road 155N Bustad Hall Pullman, WA 99164 Ag Health Labs, Inc. 445 Barnard Boulevard Sunnyside, WA 98944 Exact Scientific Services, Inc. 3929 Spur Ridge Lane Suite 101 Bellingham, WA 98226
West Virginia	West Virginia Department of Agriculture 4720 Brenda Lane, Building 12 Charleston, WV 25305-0172
Wisconsin	Eastern Wisconsin Dairy Herd Improvement Cooperative 718 West First Street Waldo, WI 53093
	School of Veterinary Medicine, Johne's Testing Center University of Wisconsin 2015 Linden Drive Madison, WI 53706
	University of Wisconsin Veterinary Diagnostic Laboratory 6101 Mineral Point Road Madison, WI 53705
Wyoming	Wyoming State-Federal Laboratory 1174 Snowy Range Road Laramie, WY 82072
Country	**Laboratory**
Canada - Alberta	Alberta Agriculture Food and Rural Development Immunology-Virology Unit Room 414, O.S. Longman Building 6909 116th Street Edmonton, Alberta Canada T6H 4P2
	Central Veterinary Pathology Laboratory LTD. 8131 Roper Road Edmonton, Alberta Canada T6E 6S4
Canada – British Columbia	Animal Health Monitoring Laboratory 1767 Angus Campbell Road Abbotsford, British Columbia Canada V3G 2M3

Canada – Ontario	Animal Health Laboratory University of Guelph, Ontario Door P2, McIntosh Lane Guelph, Ontario Canada N1G 2W8
	Vita-Tech Canada 1345 Denison Street Markham, Ontario Canada L3R 5V2
Canada - Saskatchewan	Prairie Diagnostic Services 4840 Wascana Parkway, Suite 1 Regina, Saskatchewan Canada S4S 7J6
Chile	Cooprinsem Manuel Rodriguez 1040 Osorno, Chile 00827
The Netherlands	Animal Health Service Arnsbergstraat 7 P.O. Box 7 Deventer The Netherlands 7400 AA

Recommended Test Regimen for the Detection of Paratuberculosis in Cattle

Pat: The two page chart that you did goes here