

## REPORT OF THE COMMITTEE ON TUBERCULOSIS

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Vice Chair: Dustin Oedekoven, SD

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The Committee met on October 23, 2012 at the Greensboro Sheraton Hotel, Greensboro, North Carolina, from 1:00 to 5:25 p.m. Dr. William Hartmann, Chair, welcomed members and guests to the Committee on Tuberculosis. Dr. Hartmann introduced Vice-Chair, Dr. Dustin Oedekoven. There were 54 members and 63 guests present.

Dr. Mitch Palmer, Chair of the Tuberculosis (TB) Scientific Advisory Subcommittee presented the Report of the Scientific Advisory Subcommittee. A motion to accept the report of Scientific Advisory Subcommittee was made, and seconded. The motion was passed. The full text of the report is included in this report.

Dr. Dee Ellis, Texas Animal Health Commission, gave the Report of the Bi-national TB Committee. The full text of the Bi-national report is included in this report.

Dr. Alecia Naugle, National Tuberculosis Program Manager, USDA-APHIS-VS, gave the National Tuberculosis Program Update. The full text of the update is included in this report.

Dr. Jose Alfredo Gutierrez Reyes, Director of Animal Health Programs, SAGARPA/SENASICA presented "Bovine Tuberculosis in Mexico: Control and Eradication, Achievements and Challenges" to the Committee.

Dr. Noel Harrington, Canadian Food Inspection Agency was introduced and gave the Canadian Tuberculosis Program Report.

William Wallace, Chihuahua Animal Health Committee, gave the Chihuahua Animal Health committee report.

Dr. Doug Cory, Professional Rodeo Cowboys Association (PRCA) United States Team Roping Championships (USTRA) spoke to the Committee about the Use of Mexican Cattle for Recreational Purposes in the US.

Ernie Morales, Morales Feedlots, Inc. presented on the US Feeder Perspective on the Importation of Mexican Origin Feeder Cattle.

Dee Ellis, Texas Animal Health Commission, spoke about the Border State Veterinarian Issues and Concerns.

Dr. Suelee Robbe-Austerman, National Veterinary Services Laboratory (NVSL), presented Molecular Epidemiology of Bovine Tuberculosis. A summary of the presentation is included in this report.

Dr. Alecia Naugle, National Tuberculosis Program Manager, USDA, APHIS, VS, spoke on the Granuloma Submission Rates in Fed Cattle in the US. Additionally, Dr. Joyce Bowling-Heyward, Director of Import/Export Animals for USDA, APHIS spoke briefly on testing and effects on importation of cattle.

A discussion period was held with the panel of speakers, including Suelee Robbe, Dee Ellis, William Wallace, Jose Alfredo Gutierrez Reyes, Doug Cory, Alecia Naugle and Ernie Morales.

### **Committee Business**

At the conclusion of formal presentations, William Hartmann determined there was a quorum. Two resolutions were approved and forwarded to the Committee on Nominations and Resolutions. Topics included support for research on mycobacterial diseases in animals and tuberculosis testing of export cattle and the requirement for a negative culture of *M. bovis* from histopathologically negative tissues.

**REPORT OF THE BOVINE TUBERCULOSIS SCIENTIFIC ADVISORY SUBCOMMITTEE**  
Mitch Palmer, Chair

Four presentations were made at the 2012 Tuberculosis (TB) Scientific Advisory Subcommittee (SAS) meeting.

**Evaluation of Serodiagnostic Assays for *Mycobacterium bovis* Infection in Elk, White-Tailed Deer, and Reindeer in the United States- Final Report**

Jeff Nelson

USDA-APHIS-VS, NVSL, Ames, IA

In 2011, the United States Department of Agriculture conducted a project in which elk (*Cervus elaphus* spp.), white-tailed deer (WTD) (*Odocoileus virginianus*), and reindeer (*Rangifer tarandus*) were evaluated by the single cervical tuberculin test (SCT), comparative cervical tuberculin test (CCT), and serologic tests. The rapid antibody detection tests evaluated were the CervidTB Stat-Pak (Stat-Pak), and the Dual Path Platform (DPP) VetTB. Blood was collected from presumably uninfected animals prior to tuberculin injection for the SCT. A total of 1,783 animals were enrolled in the project. Of these, 1,752 (98.3%) were classified as presumably uninfected, based on originating from a captive cervid herd with no history of exposure to TB. Stat-Pak specificity estimates were 92.4% in reindeer, 96.7% in WTD, and 98.3% in elk and were not significantly different from SCT specificity estimates. Using the DPP in series on Stat-Pak antibody-positive samples improved specificity in the three species. Thirty-one animals were classified as confirmed infected, based on necropsy and laboratory results, and 27/31 were antibody positive on Stat-Pak for an estimated sensitivity of 87.1%. The study findings indicate that rapid serologic tests used in series are comparable to the SCT and CCT and may have a greater ability to detect TB-infected cervids.

**NVSL TB Serum Bank Update - 2012**

Jeff Nelson

APHIS-VS-NVSL, Ames, Iowa

The NVSL TB Serum Bank provides well-characterized serum samples with skin test results for samples from uninfected animals and skin test, histopathology, and TB culture results from infected animals. The serum bank continues to be available to researchers and diagnostic companies as they develop and evaluate serologic tests for bovine TB using the criteria recommended by the US Animal Health Association. In FY 2012 the serum bank was able to add 104 serum samples from white-tailed deer and 2,390 serum samples from cattle. The serum bank currently contains about 3,688 serum samples from cervid species of which 92 are from TB infected animals, as well as, 5,229 serum samples from cattle of which 476 are from TB infected animals. The serum bank will continue to accept blood and tissue samples from potentially infected cattle and white-tailed deer.

**Comparison of CSL and Lelystad Tuberculin in the Bovigam® Under Field Trial Conditions**

Bjoern Schroeder

Prionics USA

A total of 984 animals were investigated with BOVIGAM® using CSL and Lelystad tuberculin in parallel. Animals that were confirmed Tb positives (N=193), animals derived from confirmed positive herds but were only assessed as exposed (N= 709) and animals derived from a Tb negative region in USA and confirmed Tb negative animals (N=82) were investigated.

The results demonstrate that significantly more animals were detected in the infected group using Lelystad PPD in comparison to CSL purified protein derivatives (PPD). Forty four animals were detected exclusively with Lelystad PPD whereas only three were detected exclusively with CSL PPD. Similar results were obtained in the exposed group in which 43 animals were only detected with Lelystad PPD whereas only seven animals were exclusively detected with CSL PPD. In Tb negative herds equivalency between the two PPDs could be demonstrated. In total, 82 animals have been tested in USA and UK with the two PPD sources used in parallel. One animal was exclusively detected with either Lelystad PPD or CSL PPD. Three animals were found positive with both PPDs. The proportion of agreement between tests is given with 98%.

The field trial results show that the sensitivity of Lelystad PPD is higher in comparison to CSL PPD without negatively affecting specificity. Lelystad tuberculin is a useful tool to improve Tb diagnostic. Consequently, we request approval of Lelystad PPD to be used with BOVIGAM® for the detection of tuberculosis in cattle.

**Whole Genome Sequencing- Improving Epidemiology**

Tyler Thacker

USDA-ARS, NADC, Ames, Iowa

Next Generation Sequencing technologies enable the rapid sequencing of the genome of *Mycobacterium bovis*. In collaboration between National Animal Disease Center (NADC) and National Veterinary Services Laboratory (NVSL), over 80 strains of *M. bovis* have been sequenced. Small nucleotide polymorphisms (SNPs) were identified in each strain and

aligned. Phylogenetic relationships we determined using MrBayes. The utility of whole genome sequencing to aid epidemiological investigations was discussed.

### **IDEXX *Mycobacterium bovis* Antibody ELISA Update**

John C. Lawrence

IDEXX Livestock and Poultry Diagnostics, Westbrook, Maine

OIE General Session Approval May 2012

- Fitness for Purpose - Supplemental test, local surveys at herd level
- Data reviewed by TB expert committee and Biological Standards commission

2012 CVB Data Submissions/Approvals

- USA Field Trials (three kit lots at three sites)
- Pre-licensing serials (three kit lots)

USDA CVB Product License September 28, 2012

- Supplemental test to be used in conjunction with other methods for diagnosing bovine TB
- Sale and use is restricted to laboratories approved by state and federal animal health officials

Active use in various regions as well as continued evaluations by country reference laboratories

### **Subcommittee Business:**

At the 2011 USAHA tuberculosis (TB) committee meeting an ad hoc subcommittee was formed to revise the document known as the "Criteria for evaluating experimental tuberculosis test performance for official test status to be approved." Minor revisions were made, primarily to add flexibility to the recommendations in situations where the number of TB infected animals of a given species is low and conducting trials with the statistical power previously required is not possible. The revised document was reviewed by the TB Scientific Advisory Subcommittee (SAS). The final draft was distributed to members of the TB committee for review. It is the recommendation of the TB SAS that the revised "Criteria for evaluating experimental tuberculosis test performance for official test status" be approved.

**REPORT OF BI-NATIONAL COMMITTEE**  
Dee Ellis, Texas Animal Health Commission

Dr. Dee Ellis, Border state veterinarian representative on the Bi-National Tick, Tuberculosis (TB), and Brucellosis committee gave a short overview of the recent meeting held in Los Cabos, Baja Sur, Mexico in June of 2012. An explanation of the structure, membership and intent of the group was given first. Ellis then shared the primary talking points that the industry group of Mexican and US industry representatives agreed on, which include the USAHA member and the border state animal health representatives. The consensus points of agreement which were presented to the SAGARPA and USDA representatives present related to TB were:

1. Mexico has made advances in decreasing TB prevalence
2. Need to manage risk of transmitting TB from Mexico feeders to US cattle
3. Continue to utilize pasture grazing as part of feeder management systems
4. Request Chihuahua discuss the management/testing process for exhibition/event cattle prior to export at USAHA TB Committee
5. Request USDA only downgrade a Mexican state after a review has been made
6. Request USDA utilize zones more often so entire state statuses are not as likely to be affected
7. Urge USDA to consider two Modified Accredited Advanced (MAA) zones for Sonora – related to brucellosis status differences to simplify internal movements.

**National Tuberculosis Program Update, Report**  
Alecia Naugle  
US Department of Agriculture  
Animal and Plant Health Inspection Service (APHIS)  
Veterinary Services (VS)

**Fiscal Year (FY) 2012 – Preliminary Report**

**Development of Proposed Brucellosis/TB Regulations**

APHIS continued to develop new regulations and supporting standards for the brucellosis and tuberculosis (TB) programs in FY2012. Under the proposed approach, The *Code of Federal Regulations* will provide the legal authority for the programs while the details of the programs will be described in a program standards document.

APHIS conducted several webinars that provided additional information about the proposed regulation in FY2012. APHIS proposed to use a national calculator to determine the fair market value for animals that are destroyed because of TB or brucellosis in the Draft Regulatory Framework published in May 2011. In response to requests from commenters, APHIS hosted two webinars in November 2011 that provided more information about the calculator and options for indemnity payments. In August 2012, APHIS presented an overview of the Proposed Rule and Program Standards for Brucellosis and Bovine Tuberculosis. The webinar presentation described the fundamental concepts underlying the proposed regulations, the content of both the Proposed Rule and the Program Standards, and significant differences from the draft regulatory framework and the rationale for these differences. Recordings of both webinars are available at: [http://www.aphis.usda.gov/animal\\_health/tb\\_bruc/webinars.shtml](http://www.aphis.usda.gov/animal_health/tb_bruc/webinars.shtml).

APHIS is hopeful that Proposed Rule and Program Standards will be published in the Federal Register in early FY2013. Both documents are currently under Agency review. Upon publication, APHIS plans to provide an extended comment period of 90 days through the [www.regulations.gov](http://www.regulations.gov) website in light of the scope of these regulations.

**Bovine State Status**

As of September 30, 2012, 48 States, two Territories, and one zone were TB accredited-free (AF), including Puerto Rico and the US Virgin Islands. California was modified accredited advanced (MAA). Michigan continued to have accredited free (AF), MAA, and modified accredited (MA) status.

**Captive Cervid State Status**

All States and territories have MA status.

**TB Program Reviews**

APHIS conducted an on-site TB program review in Michigan in July 2012. This review was conducted to evaluate the status of the TB eradication program and compliance with the memorandum of understanding that is required for split-State status.

**TB-Affected Herds Identified in FY 2012**

Six TB-affected cattle herds, three beef and three dairy, were detected during FY 2012. These herds were located in California (two dairy), Michigan (one beef and one dairy), South Dakota (one beef), and Texas (one beef). Four (67 percent) of these TB-affected herds (two dairy and two beef herds) were detected as a result of slaughter surveillance and subsequent epidemiologic investigations.

Two cattle herds (California dairy, Texas beef) were depopulated with Federal indemnity. The remaining herds are under test-and-remove management plans, except that State indemnity funds were used to partially depopulate one beef herd in Michigan. Two cattle herds detected prior to FY 2012, one dairy herd each in California and Michigan, are continuing under test-and-remove management plans. Two captive cervid herds in Michigan remain under quarantine.

**National TB Surveillance**

**Granuloma Submissions:** From October 1, 2011, through June 30, 2012, 8,093 granulomas were identified during postmortem slaughter inspection and submitted for diagnostic testing. These lesions originated from 149 US establishments that slaughtered 21.7 million cattle, including 5.2 million adult cattle. The minimum standard for slaughter surveillance is one granuloma submitted per 2,000 adult cattle slaughtered annually. This standard is applied to each slaughter establishment. Of the 40 highest volume adult cattle slaughter establishments, 37 (92.5 percent) met or exceeded the submission standard, and three (7.5 percent) establishments did not. These 40 highest volume establishments slaughter approximately 95 percent of all adult cattle slaughtered in the United States.

Of the 8,093 granulomas submitted by slaughter establishments, 14 (0.17 percent) had histology consistent with mycobacteriosis. Of these 14 cases, TB was confirmed in 12 (85.7 percent) cattle. TB is confirmed by a combination of polymerase chain reaction testing of formalin-fixed tissue and culture of fresh tissue.

**Slaughter Cases:** Of the 12 TB cases detected in cattle at slaughter, three cases occurred in adult cattle over two years of age, and nine cases occurred in feeder cattle. The three adult cattle cases included two adult beef cows that led to detection of affected herds in South Dakota and Texas. The third adult TB case is currently under investigation in Oklahoma and Texas. One adult Holstein cow detected in late FY 2011 led to detection of a California dairy in FY 2012. The fed cattle cases were all beef-type cattle and were from slaughter establishments in Texas (eight cases) and Nebraska (one case). Six cases were in Mexican-origin cattle and an additional case was believed to have occurred in a Mexican-origin animal but the definitive State-of-origin could not be determined. The remaining two cases are under investigation.

**Mexican-Origin Slaughter Cases:** A total of six TB-infected animals identified through slaughter surveillance were determined to be of Mexican-origin. The official Mexican ear tags collected at slaughter indicated origin from the States of Chihuahua (one case), Nuevo Leon (three cases), and Zacatecas (two cases). An additional case is believed to have originated from Mexico based on the epidemiological investigations, but the definitive Mexican State-of-origin could not be determined.

**Live Animal Testing:** Tuberculin skin testing in live animals is another component of national TB surveillance in cattle and bison. As of August 31, 2012, 903,289 caudal fold tuberculin tests of cattle and bison were reported, with 9,919 responders (1.1 percent, 50 States and two Territories reporting). Tuberculin testing is the primary means of surveillance for TB in captive cervids as there are no standards for granuloma submissions for establishments that slaughter cervids. As of August 31, 2012, 19,721 single cervical tuberculin skin tests were conducted in captive cervid species with 368 suspects (1.7 percent) reported to APHIS.

The gamma interferon test has been available as an official supplemental test in the TB program since 2005. Laboratories in five States (California, Colorado, Michigan, Nevada, and Texas) and the NVSL are approved to conduct gamma interferon testing. A total of 8,827 tests were conducted in cattle in during FY 2012.

#### **Collaborations with Mexico**

APHIS continues to work with Mexico to ensure equivalency between the two countries' requirements for controlling TB. In FY2012, APHIS completed a review of the Mexican National TB Program. Although Mexico has made improvements and maintained program advancements in some areas,

APHIS identified several areas of critical concern during the review including the low efficiency of epidemiologic investigations and low caudal fold response rates for TB skin testing. APHIS and Secretaría Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA) continue to collaborate to address these concerns and are jointly developing a strategic plan designed to minimize the risk of TB while providing a framework to facilitate trade in the future. In addition to the national review, APHIS also conducted a TB program review in the Mexican State of Guerrero in September 2012. The purpose of this review was to evaluate an additional 15 municipalities for AP status. If the review results are favorable, all 25 municipalities in the coast of Guerrero will have AP status. The review report is pending.

#### **TB Serum Bank**

APHIS continues to obtain well-characterized serum samples including skin test results for both uninfected and infected animals. Histopathology and TB culture results are also obtained for samples from TB-infected animals. A total of 104 samples from white tailed deer and 2,390 samples from cattle were added to the serum bank in FY 2012. The serum bank contains 5,229 serum samples from cattle, of which 476 are from TB-infected animals, and 3,688 samples from cervids, of which 92 are TB-infected. Serum bank samples continue to be available to researchers and diagnostic companies for serologic test development. States are encouraged to submit blood and tissue samples from potentially infected cattle and captive cervids, as well as blood samples from presumably uninfected cattle and white-tailed deer from AF States during FY 2013.

#### **Update on Program Approval of Serological Tests**

**ChemBio CervidTB Stat-Pak® and Dual Path Platform (DPP®):** The Center for Veterinary Biologics (CVB) previously licensed the CervidTB Stat-Pak for use in elk, red deer, and white-tailed deer. In October 2012, the CVB licensed the DPP as a secondary test for bovine TB. APHIS plans to approve both tests for use in series in elk, red deer, white tailed deer, fallow deer, and reindeer. APHIS will conduct outreach with State and Federal Animal Health Officials, accredited veterinarians, cervid producers, and cervid industry organizations to provide additional information and instructions on the use and interpretation of the tests. The National Veterinary Services Laboratories (NVSL) will conduct all Stat-Pak testing during the initial phase of program use.

**IDEXX® *M. bovis* Antibody Test Kit:** The Center for Veterinary Biologics licensed the IDEXX® *M. bovis* Antibody Test Kit for use in cattle in September 2012. The TB Program is evaluating this test to determine how it can be used in the TB Program. If the test is approved, the TB program will likely initially restrict the use of this test to specific situations (for example, in affected herds) and pilot projects that allow us to further evaluate the suitability of this test. We will also limit testing to the NVSL. As we become more familiar with its performance in our hands, VS may eventually approve additional uses for the test and additional laboratories to conduct the test. VS will continue to update State and Federal animal health officials and our stakeholders as new information becomes available concerning the approval and implementation of the IDEXX *M. bovis* Ab Test as an official TB test.

#### **Selected State Updates**

**California Update:** Two newly-detected TB-affected dairy herds were identified in California during FY 2012. The first herd was detected through slaughter surveillance, and was depopulated with Federal indemnity funds due to a moderately high within herd apparent prevalence. The second herd was detected through TB testing conducted because it was a fence line contact of the first herd. This herd is under a test-and-remove management plan. One additional dairy detected in 2011 is continuing under a test-and-remove herd plan. This herd was scheduled for quarantine release in 2012, but an infected animal was detected during the final herd test so the quarantine remains in effect.

**Michigan Update:** Two newly-detected TB-affected herds, one beef and one dairy herd were identified through ongoing surveillance testing in FY 2012. Both herds are currently under test-and-remove herd management plans. One dairy is continuing under a test-and-remove herd plan from 2004; the herd was scheduled for quarantine release in 2009 but an infected animal was detected during the final herd test so the quarantine remains in effect. One affected beef herd detected in FY 2010 completed a test-and-remove herd management plan and was released from quarantine in October 2011. Two affected captive cervid herds that were detected in FY 2009 remain under quarantine in the MA zone. All of these herds described in this summary are located in the MA zone in Michigan.

**South Dakota Update:** A TB-affected cow-calf operation that raised club calves was detected through slaughter surveillance. The herd was managed under a test-and-remove herd plan. Wildlife surveillance was conducted in the area surrounding the herd premises, and TB was not detected in the animals that were tested. The herd was released from quarantine in September 2012.

**Texas Update:** A TB-affected cow-calf herd in west Texas was detected through slaughter surveillance. The herd was maintained on two separate, epidemiologically distinct premises. Infected animals only occurred at one of the premises; and the remainder of the cattle on the affected premises were depopulated with Federal funds. Wildlife surveillance was conducted in feral swine and white tailed deer in the area surrounding the herd premises, and TB was not detected in the wildlife that was tested. Slaughter surveillance in a Texas establishment identified a TB-infected adult cow; however, the individual animal identification was not collected at the time of slaughter. The investigation for this case is ongoing, and testing of herds that contributed to the slaughter lot is underway in Oklahoma and Texas.

## Canadian Tuberculosis Program Report for 2012

Noel Harrington

Canadian Food Inspection Agency

Canada's official animal health service resides as part of the Canadian Food Inspection Agency (CFIA). The Agency is mandated to safeguard the food of Canadians and the health of the animals and plants on which safe food depends. The goal of that CFIA's National Bovine Tuberculosis Eradication Program is the detection and eradication of bovine tuberculosis (TB) from Canada thereby ensuring the health of Canada's people, livestock, and wildlife.

The current National Bovine Tuberculosis Eradication Program consists of five major elements: disease reporting, disease detection (surveillance), stamping-out responses to disease outbreaks, movement controls, and wildlife reservoir management. The collective result of these elements is reflected in Canada's area disease status classification.

Detection of TB in Canada occurs through both passive and active surveillance. Passive surveillance consists of routine post-mortem inspection by private practitioners and diagnostic laboratories, as well as the tuberculin testing of individual animals for reasons such as export, entry into artificial insemination centres, or changes of ownership. The foundation of active surveillance is the routine post-mortem inspection of animals at slaughter for the presence of suspect tuberculosis lesions which are submitted to the CFIA's Mycobacterial Disease Centre of Expertise for laboratory examination. The program's performance standard requires submission of a minimum of one granulomatous lesion for every 2,000 adult cattle slaughtered.

Slaughter surveillance is augmented through periodic on-farm testing of livestock herds. This testing is targeted at: a) those livestock sectors with insufficient slaughter volumes to support slaughter surveillance as the sole mode of active surveillance; and b) those geographic areas where the risk of bovine TB being present in, or introduced into, the area's livestock warrants a higher level of surveillance. Additional opportunities for surveillance occur through testing for export, entry into artificial insemination centres, and as part of disease investigations.

In Canada, the caudal fold tuberculin (CFT) test is the screening test for cattle/bison and the mid-cervical tuberculin (MCT) test is the screening test for cervids. Comparative cervical tuberculin (CCT) test is the ancillary test for cattle/bison/cervids. The performance standard for tuberculin testing requires a minimum false positive reactor rate of 1% for caudal fold testing. The CFIA is working to develop a more efficient approach to track individual reactor rates as well as improved training for new and existing staff on tuberculin test methods.

The CFIA is contributing to an industry (Canadian Cattlemen's Association) project entitled *The Evaluation of new Diagnostic Blood Tests for Bovine Tuberculosis in Cattle*. Using serum samples provided by the USDA TB Serum Bank, the project will evaluate and compare the performance of several commercial and non-commercial serological tests. The goal is to identify one or more tests that are suitable candidates for potential adoption as an official bovine TB test in Canada.

Since 2009, a single TB-affected herd has been identified in Canada. A Canadian origin beef cow exported from British Columbia (BC) to the US for immediate slaughter was found to have TB lesions on post-mortem inspection. Laboratory testing by the USDA found the lesions to PCR positive and *M. bovis* was subsequently isolated by mycobacterial culture. The CFIA's subsequent actions included whole herd depopulation ( $n=318$ ) with a further six infected animals identified. The investigation of trace-in, trace-out, contact, and perimeter herds ( $n=143$ ) resulted in tuberculin testing an additional 4,000 animals. No additional cases of *M. bovis* infection were identified through the investigation. The source of the infection to the index premises has not been identified.

For the purpose of monitoring the performance and progress of the National Bovine TB Eradication Program, every province is considered to be a separate eradication area. Each eradication area is assigned a bovine TB status that reflects the adequacy of the disease surveillance and eradication measures implemented in the province/area (bovine TB free, bovine TB-accredited advanced, or bovine TB-accredited). Since 2006, all provinces/eradication areas have been classified as bovine TB-free.

As a result of multiple TB-affected herds in BC since 2007 and the uncertainty as to the source of the infection, the CFIA is preparing to re-designate the TB status of British Columbia from bovine TB-free to bovine TB-accredited advanced. The re-designation will result in the requirement for movement permits for all cattle and farmed bison being moved from BC to other provinces/eradication areas of Canada with higher TB status. Restoration of bovine TB-free status in B.C. will require that there are no further cases of bovine TB during a period of 3 consecutive years.

Canada has two known wildlife reservoirs of bovine TB. The first, Wood Buffalo National Park (WBNP), is located at the northern boundary of Alberta and is home to approximately 6,000 free-ranging bison with a prevalence of TB estimated at 50%. A bison management and containment plan is in place, which includes a no-bison buffer zone, the killing of stray bison, and other measures to prevent the spread of infection to healthy wild free ranging bison. Livestock herds are not located in the vicinity of the park and no livestock cases of bovine TB have been detected that are attributable to bison of WBNP.

The second reservoir, Riding Mountain National Park located in south-western Manitoba has a very low prevalence of disease the wild cervid population. In response to several TB infected cattle herds in the area surrounding the park, the Riding Mountain TB Eradication Area was established around the park in 2003. Surveillance plans have been developed each year for livestock and wildlife collaboratively amongst the CFIA, Parks Canada and provincial authorities. These

have been risk based and prescribe the frequency of testing based on proximity to identified cases of TB in wildlife or cattle herds. In addition, risk mitigation measures have been employed including barrier fencing, bans of baiting around the park, education, and wildlife population reduction. After more than three years of surveillance testing without additional cases of bovine TB identified in livestock, the CFIA designated the RMEA as TB-free in 2006. Currently, the CFIA is applying a scenario tree surveillance model that incorporates multiple sources of information to guide surveillance in the RMEA. By targeting those herds at highest risk, the sensitivity of the surveillance program has increased, leading to greater confidence of disease freedom in the domestic livestock population.

## **Molecular Tools Used in Identifying Bovine Tuberculosis**

Sue Lee Robbe

For the last 10 years, NVSL has been genotyping isolates for the TB program, and this talk summarizes these data. NVSL currently uses spoligotyping and MIRU-VNTR-24 to genotype isolates. NVSL has 1678 isolates currently in the database. Just over 800 isolates were recovered from cattle born in the USA. Another 642 are Mexican origin animals with 465 isolates from cattle killed in Mexico, and 177 were from cattle slaughtered in the USA. Cattle of unknown origin make up 112 of the isolates. Spoligotyping isolates from animals slaughtered or who died within the USA separates out the isolates into 45 different spoligotype patterns. However, 76% of the isolates fall into only five spoligotype patterns, SB0673, SB0145, SB0271, SB0265, and SB2011.

NVSL has detected seven spoligotypes that have been identified in livestock born within the USA and as yet, these spoligotype patterns have not been identified by NVSL in Mexico origin cattle. Three of the seven, SB0292, SB0815 and an unnamed genotype 640013777601600 are variants of the endemic Michigan strain and have only been identified in Michigan. Two of the seven spoligotypes, SB1069, and SB0265 have been recovered primarily in farmed cervids at irregular intervals since the early 1990's. The last two spoligotypes are closely related to isolates previously recovered from primarily Mexican origin cattle and a few sporadic cases from USA origin cattle; they are SB0271- associated with the outbreak in Minnesota cattle and wild white-tailed deer, and SB2011-associated with an outbreak in a Colorado dairy.

There are five strains currently endemic within the USA, three of them have a wildlife component; SB0145 and variants in Michigan, SB0271 in Minnesota, and SB0145 in Molokai, Hawaii. The two other strains that do not have a wildlife component were mentioned earlier, SB1069 and SB0265, and occur primarily in farmed cervids. There is no molecular evidence that bTB is circulating within the national cattle herd. It appears that cases of bTB within the US National cattle herd are the result of new infections. Based on genotyping, bTB infections within the US National cattle herd are associated with either: wildlife, cattle of Mexican origin or farmed cervids. It appears that the adult slaughter surveillance program has been effective at identifying new infections within the US National cattle herd prior to these new infections spreading beyond the index herd and the traces from that herd. The molecular epidemiological information from genotyping can not only be used to help answer local questions in the field, but also to help guide and focus our national TB state federal cooperative program.