

## REPORT OF THE COMMITTEE ON TUBERCULOSIS

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Vice Chair: Beth Thompson, MN

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The Committee met on October 21, 2014 at the Sheraton, Kansas City, MO from 1:00 p.m. to 6:00 p.m. There were 70 members and 30 guests present. Dr. Oedekoven introduced himself, welcomed members and guests, and introduced the vice chair, Dr. Thompson.

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

Dr. Chuck Massengill then presented the report of the Bi-National TB Committee (BNC.) Dr. Massengill, U.S. Coordinator for the U.S.A./Mexico Bi-National Committee for the Eradication of Bovine Tuberculosis and Brucellosis (BNC) began his report with a history of the BNC, and concluded with an update on the committee's work.

Dr. Burke Healy, Director of Cattle Health Services, presented the National Tuberculosis Program Update. Dr. Healy reported on individual state updates, for Michigan and California, and also updates on total affected herds. The full text of the update is included in this report.

Dr. T.J. Myers, Associate District Administrator, APHIS Veterinary Services, reported on the cooperation between the United State and Mexico on regionalization, including the goals of the five year regionalization plan.

Dr. José Alfredo Gutiérrez Reyes, Director, Animal Health Programs, SAGARPA/SENASICA presented the committee with the Mexico National Tuberculosis Report.

Individual presentations were given on the role of *M. bovis* in zoonotic transmission, including:

Zoonotic potential of *M. bovis*, presented by Dr. Brian McCluskey;  
Phylogenetic analysis of *M. bovis*, presented by Dr. Sue Lee Robbe-Austerman;  
North Dakota TB update, presented by Dr. Susan Keller; and  
California TB update, presented by Dr. Anita Edmondson.

The panel then discussed and answered questions on *M. bovis* in humans and related issues.

**Committee Business:**

At the conclusion of formal presentations, Dr. Oedekoven determined there was a quorum.

Dr. Oedekoven reported on the status of the 2013 resolutions.

One resolution was approved and forwarded to the Committee on Nominations and Resolutions. The topic of that resolution was to urge USDA APHIS to license the Bovigam® assay so that Lelystad tuberculin may be used in the stimulation phase of the assay, as part of the official TB program procedure.

**Other business:**

A motion to adjourn was made, and seconded. The meeting adjourned at 5:30 p.m.

**Report of the 2014 USAHA Bovine Tuberculosis**

## Scientific Advisory Subcommittee

Chairman: Mitchell Palmer, DVM, PhD

Five presentations were made at the 2014 TB SAS meeting.

### **Integration of Models and Dense Phylogenetic Sampling to Understand BTB epidemiology in Cattle and Wildlife**

Professor Rowland Kao, Institute of Biodiversity Animal Health and Comparative Medicine, University of Glasgow, Glasgow, UK.

The epidemic of bovine Tuberculosis in British cattle is the most important livestock disease problem in Great Britain today, where the control of the epidemic is complicated by the presence of an important wildlife reservoir, the Eurasian badger. This system is an important exemplar of a disease with a wildlife reservoir, a problem that can be more generally framed in the context of multi-host pathogen system. Now, whole genome sequencing applied on the mass scale is being used to study the transmission dynamics of the pathogen at a hitherto unimaginable level of detail. In this presentation, I shall discuss the relative merits of evolutionary and epidemiological modeling approaches to interpreting high density phylogenetic data, and how both these approaches present new challenges and new opportunities in our efforts to control bTB.

### **Effect of Skin Test on Serum Antibody Responses to *Mycobacterium bovis* Infection in Cattle**

Ray Waters<sup>1</sup>, Jeff Nelson<sup>2</sup>, Tyler Thacker<sup>1</sup>, Mayara Maggioli<sup>1</sup>, Molly Stafne<sup>3</sup>, Kristin Bass<sup>4</sup>, Rick Linscott<sup>5</sup>, John Lawrence<sup>5</sup>, and Mitch Palmer<sup>1</sup>

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<sup>5</sup>IDEXX Laboratories, Westbrook, Maine

Recently, several serologic tests designed to detect immunodominant antibodies to *M. bovis* antigens (e.g., MPB83, MPB70, ESAT-6, and CFP10) have emerged for potential use with samples from cattle. Of these, a commercial ELISA to MPB83/MPB70 (*M. bovis* antibody ELISA) has gained approval for use in cattle for bovine tuberculosis control programs by the *Office International des Epizooties* and United States Department of Agriculture. In the present study, the effect of injection of purified protein derivatives (PPD) for caudal fold (CFT) and comparative cervical (CCT) skin tests on serum antibody responses were evaluated with samples from cattle experimentally-infected with *M. bovis* (n = 8, aerosol challenge). Injection of *M. bovis* PPD for CFT (89 days after aerosol challenge) elicited serum antibody responses detectable within 1 week by the IDEXX *M. bovis* antibody ELISA. Positive responses were detectable in all animals up to 74 days after PPD administration. Injection of *M. avium* and *M. bovis* PPDs for CCT (105 days after CFT) resulted in a dramatic increase in antibody responses in all animals. Antibody avidity, as measured by an ammonium thiocyanate assay, also increased upon injection of PPDs for CCT. These findings demonstrate that the anamnestic response elicited by injection of PPD(s) for skin test results in

both qualitative and quantitative increases in serum antibody responses in *M. bovis*-infected cattle, of diagnostic relevance.

**Comparison of CSL and Lelystad tuberculin PPD in the Bovigam under field trial conditions in the US-** Dr. Bjoern Schroeder, Thermo Fisher Scientific, Prionics AG, Schlieren-Zurich, Switzerland.

**Distribution of *Mycobacterium bovis* genotypes in infected deer and the implication for whole genome sequencing epidemiology**

Tyler C Thacker<sup>1</sup>, Mitchell V. Palmer<sup>1</sup>, Suelee Robbe-Austerman<sup>2</sup>, Tod P. Stuber<sup>2</sup>, W. Ray Waters<sup>1</sup>

<sup>1</sup>National Animal Disease Center, ARS, USDA, Ames, Iowa

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*Mycobacterium bovis* (*M. bovis*) was cultured from 30 tissues originating from 14 infected deer. Whole-genome sequencing (WGS) was performed on the original inoculum, single colonies subcultured from the original inoculum as well as isolates from each culture positive tissue. Results indicate that population bottlenecks appear to be the primary driver of WGS genotype changes observed in both deer tissues and subclutured inoculum, as the majority of homogeneous SNPs identified in peripheral deer tissues and subcultures were identified in the original inoculum as heterogeneous SNPs. Furthermore, individual tissues had different WGS genotypes. These data suggest that dissemination of *M. bovis* beyond the initial site of infection may demonstrate that transmission events within the animal require few mycobacteria, creating additional bottlenecks. These results imply that specimen sampling, tissue pooling, and culture practices can impact WGS results. Consequently interpreting results with these potential biases in mind is critical.

**Update on the NVSL bTB serum bank and use of Chembio DPP in captive cervids-** Dr. Jeff Nelson, USDA, APHIS, National Veterinary Services Laboratories, Ames, IA, USA.

The National Veterinary Services Laboratories (NVSL) continues to accept serum from animals for the Tuberculosis Serum Bank. Currently the bank contains 3732 samples from cattle and 3505 from cervid species. Of the cattle samples, 524 are from *Mycobacterium bovis* infected animals. For cervid samples, 71 come from *M. bovis* infected animals. Since the inception of the TB serum bank in 2007, 3667 serum samples have been provided to 31 different requestors.

Technology advances have allowed the development of serological tests to detect antibodies specific to *Mycobacterium bovis*. In 2011, a project was completed by the United States Department of Agriculture, Veterinary Services (VS) to determine if serological tests could be an alternative to tuberculosis (TB) skin testing in cervids. The single cervical test and the comparative cervical test were compared with serological tests elk, white-tailed deer, and reindeer. Two serological tests were evaluated: the CervidTB Stat-Pak™ and the Dual Path Platform VetTB Assay™ (DPP). The specificity of both serologic tests used in series was 97.0%. Stat-Pak and DPP were designated as official tests in the U.S. bovine TB eradication program for select species in February 2013 because of the VS project. Analysis of data from samples submitted during the spring of 2013 determined that the specificity of Stat-Pak and DPP was 97.7%. However, false positive results remained a concern. Of the 43 cervids sent to necropsy, none were infected with *M. bovis*. Using this additional data, on September 1, 2013 VS established cutoff values for the DPP test using an optical reader. Values  $\geq 200$  for reindeer and fallow deer and  $\geq 500$  for white-tailed deer, red deer, and elk are positive. The cutoff values have improved specificity to 99.8% percent. Since March 2014, DPP is used exclusively for serological testing in cervids. Samples submitted to NVSL rose from 6,532 to 16,310 in fiscal years 2013 and 2014, respectively. Using

serological testing in the U.S. bovine TB eradication program may allow for increased TB testing participation rates and reduce injuries or deaths during testing events.

**Other business:**

**1. Use of the Chembio Dual Path Platform (DPP) VetTB Assay® as a primary test.**

On Dec 20, 2013, the Scientific Advisory Subcommittee (SAS) of the USAHA Tuberculosis Committee received a request from USDA APHIS VS TB Program Staff (Staff) to evaluate a proposed change in the Cervid TB Program. Specifically, Staff requested that SAS comment on the use of the Dual Path Platform (DPP) VetTB Assay® as a stand-alone primary and secondary test for *Mycobacterium bovis* (bTB) in cervids. Previously the CervidTB Stat-Pak® was used as a primary test and the DPP as a secondary test. The proposed change would use DPP test readings from a calibrated optical density (OD) reader as both the primary and secondary tests (30 days apart) in approved cervid species.

The specific question posed by Staff to the TB SAS: "Is it scientifically valid to change the Cervid TB serological testing protocol at NVSL by eliminating the CervidTB Stat-Pak® as the primary test and instituting the use of the DPP VetTB Assay® as the primary serological test and also using the DPP VetTB Assay® as the confirmatory test 30 days after the initial positive serological test?"

**TB SAS Comments:**

1. As Chembio will discontinue the manufacture of the CervidTB Stat-Pak®, a change in protocol is requisite. Replacing a subjective, visually interpreted assay with an objective assay that uses electronically determined, numeric values for status classification is considered a positive change.
2. From a regulatory perspective, terminology may be important; therefore, categorizing the second DPP as a "repeat test" is more accurate than using terminology such as "confirmatory test".
3. It appears from the testing of 150 samples each of white-tailed deer and elk, that the DPP does not categorize as positive, samples categorized as negative by the Stat-Pak. This conclusion is based on a sample size determined to be statistically valid by a CVB statistician (i.e 150 samples each of the two most commonly tested species). The validity of this evaluation is important, as it is the basis of the critical assumption that 8285 Stat-Pak negative samples would have also been DPP negative if tested, thus yielding the specificities of 99.76% for the 1<sup>st</sup> DPP and 99.89% for the 2<sup>nd</sup> DPP. Presuming this assumption is valid; it appears scientifically acceptable to make the proposed change in the Cervid TB testing protocol.

**2. USAHA TB Scientific Advisory Subcommittee Report on:**

**Correlation of *Mycobacterium bovis* gamma interferon test kit, for cattle (Bovigam®), Product Code 5A64.00 (permitted), utilizing CSL PPD, and *Mycobacterium bovis* gamma interferon test kit (Bovigam®), Product Code 5A64.01 (not permitted), utilizing Lelystad PPD for the detection of tuberculosis in cattle.**

On September 19, 2014, Prionics USA provided to the chairman of the USAHA Tuberculosis Committee and the Scientific Advisory Subcommittee (SAS), documentation on field trial comparisons of CSL tuberculin<sup>1</sup> and Lelystad tuberculin in the Bovigam® assay. Bovigam® is licensed and approved for use in the USDA bovine tuberculosis eradication program for the identification of *Mycobacterium bovis* infected cattle.

In 2012, the TB SAS reviewed similar data that demonstrated increased sensitivity of the Bovigam<sup>®</sup> when Lelystad tuberculin was used compared to CSL tuberculin. It was the opinion of the TB SAS that Lelystad tuberculin be approved for use in the stimulation phase of the Bovigam<sup>®</sup> assay.

The objective of the current submission was to compare tuberculins from 2 different sources (CSL and Lelystad) in order to demonstrate equivalence of product performance. This was done to fulfill requirements for approval of the Bovigam<sup>®</sup> kit containing Lelystad tuberculin in place of CSL tuberculin.

**TB SAS Comments:**

The 2014 submission describes side-by-side comparisons of Lelystad and CSL tuberculins using samples from confirmed *M. bovis* infected cattle (confirmation by culture or PCR) from herds in Michigan and Colorado, and presumed non-infected herds in Texas, Idaho, Minnesota and Pennsylvania. Data from a total of 84 confirmed *M. bovis* infected animals and 711 non-infected animals are presented.

Using the Bovigam<sup>®</sup> assay as directed by the manufacturer, assay sensitivity in confirmed *M. bovis* infected cattle was 73.8% and 45.2% for Lelystad and CSL tuberculins, respectively. This difference was statistically significant. Assay specificity in presumed *M. bovis* negative cattle was 96.9% and 95.1%, respectively for Lelystad and CSL tuberculins. This difference was not statistically significant.

This new data, combined with that reviewed in 2012, demonstrates that Lelystad tuberculin performs with superior sensitivity and equivalent specificity to CSL tuberculin in the Bovigam<sup>®</sup> assay. Therefore, it is the opinion of the TB SAS that approval of Lelystad tuberculin for use in the Bovigam<sup>®</sup> assay would be appropriate. It is important to note that a positive recommendation from the TB SAS in no way implies approval by the USDA Center for Veterinary Biologics or USDA APHIS TB Program staff.

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<sup>1</sup> For purposes of this report the terms tuberculin and purified protein derivative (PPD) are synonymous.

**U.S. Department of Agriculture  
Animal and Plant Health Inspection Service (APHIS)  
Veterinary Services  
Annual Update for the State and Federal Cooperative  
Bovine Tuberculosis (TB) Eradication Program  
Fiscal Year (FY) 2014**

### **Development of Proposed Brucellosis/TB Regulations**

APHIS completed new regulations and supporting standards for the brucellosis and TB programs in FY 2012. Under the proposed approach, The *Code of Federal Regulations* will provide the regulatory authority for the programs while the details of the programs will be described in a program standards document. These new regulations and supporting standards were under departmental review during FY 2014. APHIS is hopeful that Proposed Rule and Program Standards will be published in early 2015. Upon publication, APHIS plans to provide an extended comment period of 90 days.

### **Bovine State Status**

As of September 30, 2014, 48 States, two Territories (Puerto Rico and the U.S. Virgin Islands), and one zone (Michigan) were TB accredited-free. California has modified accredited advanced (MAA) status. The MAA zone of Michigan was advanced to accredited-free status on September 10, 2014. With this advancement, Michigan has an accredited-free and a modified accredited (MA) zone.

### **Captive Cervid State Status**

All States and territories have MA status.

### **TB Program Reviews**

No TB program reviews were conducted in FY 2014.

### **TB-Affected Herds Identified in FY 2014**

Two TB-affected cattle herds, one dairy in North Dakota and a small bison herd in the MA zone of Michigan, were detected during FY 2014. The dairy is under a test-and-remove management plan, and the bison herd was depopulated with Federal indemnity. This is the fewest number of new herds since 1998; however, infected herds have not yet been identified for three culled adult cattle detected through slaughter surveillance. Two captive cervid herds in the Michigan MA zone remain under quarantine.

### **National TB Surveillance**

**Granuloma Submissions:** From October 1, 2013, through June 30, 2014, 6,096 granulomas from 123 federally inspected establishments were identified during postmortem slaughter inspection and submitted for diagnostic testing. In addition, 106 granulomas were submitted from three state inspected establishments for a total of 6,202 granuloma submissions. Overall, 2.6 granulomas were submitted per 2,000 adult cattle (culled dairy and beef cows and bulls) slaughtered. This is the lowest submission rate since 2006. During FY 2006-13, the submission rate ranged from 2.9-3.5 per 2,000 culled adult cattle slaughtered. The minimum standard for slaughter surveillance is 1 granuloma submitted per 2,000 adult cattle slaughtered annually. Thirty-six of the 40 highest volume adult cattle slaughter establishments met or exceeded the submission standard through the third quarter of FY 2014. These 40 highest volume establishments slaughter approximately 95 percent of adult cattle processed with federal inspection in the United States.

**Slaughter Cases:** During FY 2014, a total of 16 granuloma submissions had histology consistent with mycobacteriosis. Of these, TB was confirmed in 11 (68.8 percent) cases. TB is confirmed by polymerase chain reaction testing of formalin-fixed tissue and culture of fresh tissue. Of the remaining 5 cases, other *Mycobacterium* species were identified for 3 cases and no organism was isolated for two cases.

Three of the 11 confirmed cases occurred in adult cattle over two years of age, and 8 cases occurred in feeder cattle. Of the 3 adult cases, the herd of origin was located for two cases, but infection was not confirmed in the source herds. Of these two, one case occurred in a beef cow from a recently dispersed Nebraska herd. The second case occurred in a beef cow that originated from Texas; herd testing found no additional infected animals. The third case occurred in an adult dairy cow slaughtered in California and is currently under investigation.

The 8 fed cattle cases were detected at slaughter establishments in Michigan (three cases), Texas (three cases) and Wisconsin (two cases). Three cases were in Mexican-origin cattle and five were in domestic origin Holstein steers. Whole genome sequencing of isolates from the Holstein steers were a close match to isolates from the 2013 affected dairy in Saginaw county, Michigan.

**Mexican-Origin Slaughter Cases:** A total of three 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 Coahuila (one case), Sinaloa (one case), and Veracruz (one case). This is the fewest number of new cases since FY 2010, when three cases were detected.

**Animal Identification Collection for Slaughter Cases:**

As a result of USAHA Resolution 29, the National Veterinary Services Laboratories (NVSL) developed a process to record information on the presence or absence of official animal ID on animals sampled for TB slaughter surveillance. Database modification and personnel training were completed early in 2014. Data entry began in late April for the third quarter of 2014. During this time period, 520/935 (56 percent) submissions had official animal identification collected at the time of slaughter, 224 (24 percent) had unofficial identification and 191 (20 percent) had no identification collected.

A complete analysis of the data will be available in FY 2015 and information on animal identification for slaughter cases will be included in future annual reports.

**Live Animal Testing, Cattle:**

Tuberculin skin testing in live animals is another component of national TB surveillance in cattle and bison. During October 1, 2013 through August 31, 2014, 474,574 caudal fold tuberculin skin tests (CFT) of cattle and bison were reported, with 6,174 responders (1.3 percent, 41 states and 1 Territory reporting). During FY 2013, 944,678 CFT tests of cattle and bison were reported, with 12,757 responders (1.4 percent, 50 States and 1 Territory reporting).

The gamma interferon test has been approved for use in cattle only as an official supplemental test in the TB program since 2005. Laboratories in six States (California, Colorado, Michigan, Nevada, Texas, and Washington) and the NVSL in Iowa are approved to conduct gamma interferon testing. These laboratories completed 5,208 tests for cattle residing in 22 states during the time period October 1, 2013 through July 31, 2014.

**Live Animal Testing, Cervids:** Information for tuberculin skin testing in captive cervids for FY 2014 was not available at the time of this report.

The CervidTB Stat-Pak® and Dual Path Platform® (DPP) tests were approved for program use in elk, red deer, white-tailed deer, fallow deer, and reindeer. Official program testing began on February 4, 2013. During FY 2014, through September 27, 2014, a total of 16,300 cervid serological TB tests were completed. These

samples were submitted from 13,142 white-tailed deer (80.5 percent), 2,604 elk (16.0 percent), 375 fallow deer (2.3 percent), 156 red deer (1.0 percent), and 23 reindeer (0.1 percent).

The production of the CervidTB Stat-Pak was discontinued by the manufacturer in early 2014 and NVSL exhausted its supply in March 2014. VS provided statistical evidence to the USAHA TB SAS in support of replacing the Stat-Pak as the primary serological TB test for cervids with the DPP test. The TB SAS approved of this change in testing protocol and subsequently VS revised the cervid TB serological test guidance document and amended the CFR to make the DPP the primary test and also the secondary post 30 day test. NVSL started testing of serum samples with the DPP as both the primary test and secondary test in March 2014.

Thirteen animals were positive of 7,239 tested (0.2 percent) by the DPP during FY 2014, through September 27, 2014. Of these, 8 of 8,424 (0.1 percent) white-tailed deer were positive, 4 of 1503 (0.3 percent) of elk tested, 1 of 256 (0.4 percent) fallow deer, and no positives of 87 red deer and 20 reindeer tested.

As of September 27, 2014, a total of eight animals have been submitted for necropsy in FY 2014. Representative lymph nodes and grossly lesioned tissues were evaluated by histopathology and culture. All samples were negative for TB by histopathology. Six cultures have been completed and *M. bovis* has not been identified; the remaining cultures are pending.

### **Collaborations with Mexico**

In FY 2014, APHIS and Secretaría Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA) finalized a joint strategic plan designed to minimize the risk of TB while providing a framework to facilitate trade in the future. APHIS teams conducted reviews in Sonora and Chihuahua and assisted SAGARPA with pre-certification reviews in Baja California Sur, Chihuahua, Coahuila, Durango, Sonora, Tabasco, and Yucatan.

As a result of the APHIS VS review of the State of Chihuahua, APHIS has granted provisional MA status there for one year beginning on July 1, 2014. APHIS provided a series of essential recommendations to Chihuahua to be completed by June 30, 2015 in order to maintain the MA status. These recommendations include that Chihuahua must complete the area testing of all cattle herds, including dairies, in MA zone no later than June 30, 2015. In addition, VS recognized four non-accredited zones within Chihuahua's MA zone for a period of 12 months beginning July 1, 2014. After July 1, 2015 VS will recognize only three non-accredited zones.

While under provisional MA status VS will accept whole herd tests conducted on or after January 1, 2013 for cattle being exported to the US. These whole herd tests will expire in 24 months, at which time an additional whole herd test would be required to export to the United States. Whole herd test charts and applicable individual animal test charts are required in order for animals to be presented at the border. TB testing for exporting to the United States must be completed by veterinarians meeting the requirement for caudal fold testing as submitted on the approved veterinarian list provided by SAGARPA to APHIS.

Regular progress on these items will be monitored by quarterly reports submitted by Chihuahua animal health officials. Each quarterly report will include a list of all veterinarians conducting caudal fold testing in the quarter and a list of all herds tested during the quarter. Failure to comply with these will result in an immediate revocation of provisional MA status. APHIS will conduct a follow-up review in Chihuahua, in approximately July 2015.

APHIS participated in two courses organized by Mexico on bovine TB epidemiology, infected herd management, and TB surveillance in Zacatecas and Hidalgo.

**TB Serum Bank** APHIS continues to obtain well-characterized serum samples for both uninfected and infected animals. The serum bank contains 5,340 serum samples from cattle, of which 524 are from TB-infected animals, and 3,737 samples from cervids, of which 92 are from confirmed TB-infected animals. 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 cervid species from accredited-free States during FY 2015.

**IDEXX® *M. bovis* Antibody Test Kit:** The IDEXX® *M. bovis* Antibody Test Kit was approved for official TB program use in TB-affected cattle herds in FY 2013. Guidance for the use of the test can be found in VSG 6702.1 - The IDEXX Antibody (Ab) Test Serological Test for Diagnosing Bovine Tuberculosis (TB) in TB-Affected Cattle Herds. The serology test is being used in addition to traditional skin testing to reduce the risk of not detecting truly infected animals that are skin test negative. The test was used in one TB affected herd in FY 2014, as part of the test and remove herd management plan. Four of 172 serology positive (2.3 percent) animals were euthanized, in addition to 4/562 caudal fold responders (0.7 percent) (one animal was positive on both tests). No evidence of TB infection was found by histology and culture in the seven positive animals.

## **Selected State Updates**

### ***California and Utah:***

Three dairies were tested in Utah by regulatory veterinarians without finding evidence of tuberculosis, as a result of finding TB in a culled adult dairy cow slaughtered in California in November 2013. Herd testing is underway of potential source herds in California and Arizona. The quarantine was released in FY 2014 for a TB-affected California dairy herd that was identified in FY 2013.

***Michigan:*** One new affected herd occurred in a small bison herd located in the MA zone. The herd was detected through inspection of an animal harvested for consumption. Quarantines have been released for two dairies and one beef herd that had a test-and-remove herd plan in the MA zone. The dairies were originally detected in 2004 and 2012 and the beef herd was detected in 2012. Two affected captive cervid herds that were detected in FY 2009 remain under quarantine in the MA zone.

***Nebraska:*** TB was confirmed in a culled adult Nebraska beef cow that originated from a recently dispersed herd. The herd had been tested in 2009 as part of an affected captive cervid herd investigation in that state; however, this cow had not received a test at that time. Whole genome sequencing results indicate the recent isolate was a close match to 2009 isolates from the cervid herd.

***North Dakota:*** A dairy was confirmed infected after herd testing subsequent to a dairy worker being diagnosed with *Mycobacterium bovis* infection. The herd is being managed by test-and-remove. An affected beef herd detected in FY 2013 was released from quarantine in 2014.