

REPORT OF THE COMMITTEE ON TUBERCULOSIS

Chair: Dustin Oedekoven, SD

Vice Chair: Beth Thompson, MN

John Adams, VA; Sara Ahola, CO; Bruce Akey, NY; Wilbur Amand, PA; Joan Arnoldi, WI; James Averill, MI; Kay Backues, OK; Lowell Barnes, IN; Bill Barton, ID; Peter Belinsky, RI; Warren Bluntzer, TX; Steven Bolin, MI; Joyce Bowling-Heyward, MD; Richard Breitmeyer, CA; Becky Brewer-Walker, AR; Gary Brickler, CA; Charlie Broaddus, VA; Charles Brown II, WI; William Brown, KS; Mike Chaddock, DC; John Clifford, DC; Michael Coe, UT; Jim Collins, GA; Kathleen Connell, WA; Thomas Conner, OH; Walter Cook, WY; Donald Davis, TX; Thomas DeLiberto, CO; Jere Dick, MD; Leah Dorman, OH; Brandon Doss, AR; Anita Edmondson, CA; Dee Ellis, TX; Steven England, NM; Donald Evans, KS; John Fischer, GA; James Foppoli, HI; W. Kent Fowler, CA; Nancy Frank, MI; Mallory Gaines, DC; Tam Garland, TX; Robert Gerlach, AK; Michael Gilsdorf, MD; Chelsea Good, MO; Velmar Green, MI; Stephane Guillosoy, MO; Thomas Hagerty, MN; Rod Hall, OK; Steven Halstead, MI; Noel Harrington, ONT; William Hartmann, MN; Greg Hawkins, TX; Carl Heckendorf, CO; Terry Hensley, TX; Linda Hickam, MO; Bob Hillman, ID; Christine Hoang, IL; Donald Hoenig, ME; Thomas Holt, FL; Dennis Hughes, NE; John Huntley, WA; Billy Johnson, AR; Jon Johnson, TX; Shylo Johnson, CO; Jamie Jonker, VA; Karen Jordan, NC; Susan Keller, ND; Bruce King, UT; Diane Kitchen, FL; Paul Kohrs, WA; Maria Koller-Jones, ONT; John Lawrence, ME; Maxwell Lea, Jr., LA; Rick Linscott, ME; Jason Lombard, CO; Konstantin Lyashchenko, NY; Daniel Manzanares, NM; Bret Marsh, IN; Chuck Massengill, MO; Susan McClanahan, MN; Paul McGraw, WI; Robert Meyer, WY; Susan Mikota, TN; Michele Miller, FL; Eric Mohlman, NE; Ernie Morales, TX; Henry Moreau, LA; Julie Napier, NE; Sherrie Nash, MT; Alecia Naugle, MD; Cheryl Nelson, KY; Jeffrey Nelson, IA; Kenneth Olson, IL; Mitchell Palmer, IA; Elizabeth Parker, ITA; Boyd Parr, SC; Elisabeth Patton, WI; Janet Payeur, IA; Kris Petrini, MN; Alex Raeber, CHE; John Ragsdale, NM; Jeanne Rankin, MT; M. Gatz Riddell, Jr., AL; Suelee Robbe-Austerman, IA; Keith Roehr, CO; Mo Salman, CO; Larry Samples, PA; Bill Sauble, NM; Shawn Schafer, ND; Joni Scheffel, MN; Irene Schiller, CHE; David Schmitt, IA; Dennis Schmitt, MO; Stephen Schmitt, MI; Andy Schwartz, TX; Charly Seale, TX; Laurie Seale, WI; Craig Shultz, PA; Kathryn Simmons, DC; Daryl Simon, MN; Nick Striegel, CO; Rodney Taylor, NM; Tyler Thacker, IA; Charles Thoen, IA; Kenneth Throlson, ND; Darren Turley, TX; Paul Ugstad, NC; Arnaldo Vaquer, VA; Kurt VerCauteren, CO; Jesse Vollmer, ND; Mark Walter, PA; Ray Waters, IA; Scott Wells, MN; Diana Whipple, IA; Ellen Wiedner, FL; Richard Willer, HI; Brad Williams, TX; Kyle Wilson, TN; Ross Wilson, TX; Josh Winegarner, TX; Nora Wineland, MO; David Winters, TX; Jill Bryar Wood, TX; Ching Ching Wu, IN; Stephanie Yendell, MN; Marty Zaluski, MT.

The Committee met on October 22, 2013 at the Town and Country Hotel, San Diego, California, from 1:00 p.m. to 6:00 p.m. There were 76 members and 37 guests present. Dr. Oedekoven introduced himself, welcomed members and guests, and introduced the vice chair, Dr. Thompson.

The first presenter was Dr. Robert Meyer 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 presented the Elephant Tuberculosis (TB) Subcommittee Update. The Subcommittee approved a resolution, for presentation to the TB committee regarding the replacement of the TB Stat-Pak Assay with the DPP VetTB Assay as a presumptive or screening test for TB in elephants.

Dr. Massengill, U.S. Coordinator for the U.S.A./Mexico Bi-National Committee for the Eradication of Bovine Tuberculosis and Brucellosis (BNC) reported on the meeting held by that group during the annual meeting of Mexico's National Confederation of Livestock Organizations in May, 2013. Discussions focused on programs designed to reduce the prevalence of bovine tuberculosis in the U.S. and in Mexico. USDA proposed changing the design of "zones" or regions in Mexico from single state areas to areas which may contain more than one state or portions of more than one state. The proposal would require that two or more states would have to work in unison and share the disease status based on their joint efforts. The BNC also received reports on Mexico's national animal identification/animal disease

traceability program (SINIIGA) as well as a report on a web based system (SICAMORA) for documentation of compliance with export requirements for export of cattle from Mexico to the U.S.

The BNC made specific requests of USDA-APHIS-VS regarding the simplification of the documents which must be presented at the border for the export of cattle, consistency of requirements based the exporting on state status, and allowing the use of an 'M' brand on both steers and spayed heifer.

Dr. Lee Ann Thomas presented the National Tuberculosis Program Update. The full text of the update is included in this report.

Dr. Jose Alfredo Gutierrez Reyes presented the Mexico National Tuberculosis Report.

Individual state updates were provided as follows:

Dr. James Averill, Michigan: Five newly-detected TB-affected herds, including three beef and two dairy herds were identified in FY 2013. One dairy and one beef herd are in the modified accredited zone (MA). The dairy herd is currently under a test-and-remove herd management plan. The beef herd in the MA zone was depopulated with federal indemnity.

A dairy located in the accredited free zone (AF) was detected through slaughter surveillance and the subsequent investigation led to the detection of TB in two beef herds and in a feedlot, also located in the AF zone. The three herds were depopulated with federal indemnity. A decision is pending regarding the management plan for the feedlot. Feedlots with infected animals are not classified as affected herds. Wildlife surveillance is being conducted in the AF zone in proximity to the affected herds.

In addition, two dairies and one beef herd are continuing under 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.

Dr. Anita Edmondson, California: One newly-detected TB-affected dairy herd was identified in California during FY 2013. The affected herd was detected through slaughter surveillance, and is under a test-and-remove management plan. In addition, two TB cases were detected in adult cattle (> 2 years of age). The most likely source for one case in a culled dairy cow was a dispersed Jersey herd. The second case occurred in a beef cow and is currently under investigation. One California dairy quarantined in 2011 was released from quarantine in February 2013.

Dr. Susan Keller, North Dakota: A single infected cow has been identified in a TB-affected cow-calf operation. This animal was purchased from a TB affected beef herd located in Texas that was depopulated in FY 2012. The herd is being managed under a test-and-remove herd plan and no additional infected animals have been detected. A decision is pending regarding wildlife surveillance.

Dr. Paul Kohrs, also provided an update for Washington.

Dr. Dee Ellis provided a presentation on Calf Ranch High Risk Evaluation and Inspection Process that is being developed and implemented in Texas.

Dr. Ken Olson spoke on the New Multistate Initiative on Mycobacterial Disease in Animals.

Committee Business:

At the conclusion of formal presentations, Dr. Oedekoven determined there was a quorum. Four resolutions were approved and forwarded to the Committee on Nominations and Resolutions. Resolution topics included:

- Modify the reporting of 'Top 40' cow kill plants to include official ID collected and recorded on VS form 6-35
- Allow the designated TB epidemiologist to consider herd and animal history along with a Dual Path Platform result when classifying animal status in farmed cervidae herds

- Replace the Elephant TB Stat-Pak with the DPP VetTB Assay as a presumptive or screening test
- Allow the evaluation of the CervidTB Stat-Pak for use in sika and mule deer

Other business:

The Elephant TB subcommittee's charge was determined to be completed. The United States Animal Health Association (USAHA) Committee on Tuberculosis recommends that the United States Department of Agriculture (USDA), Animal Plant Health Inspection Service (APHIS) collaborate with the American Association of Zoo Veterinarians (AAZV), the Management and Research Priorities of Tuberculosis for Elephants in Human Care Stakeholders Task Force (ECT), the National Assembly of State Animal Health Officials NASAHO, the National Association of State Public Health Veterinarians (NASPHV), and others to revise the Guidelines for the Control of Tuberculosis in Elephants in an effort to ensure that the most current Guidelines referenced and used by regulatory officials reflect the best science, data and research available as well as meaningful and valuable stakeholder input.

The United States Animal Health Association (USAHA) Committee on Tuberculosis recommends formation of a working group by USAHA, to determine TB risk from importation of Durango cattle, and to formulate solutions for identified issues. The group would include APHIS, industry and states, and others as needed. The group will develop recommendations within 180 days for consideration by the USAHA TB committee. Presentation of information to the USAHA Board of Directors and/or Executive Committee will be determined by the chairperson of the TB committee.

A motion to adjourn was made, and seconded.

The meeting concluded at 6:00 p.m.

**REPORT OF THE 2013 USAHA BOVINE TUBERCULOSIS (TB) SCIENTIFIC ADVISORY
SUBCOMMITTEE (SAS)**
Chair: Mitchell Palmer

Six presentations were made at the 2013 TB SAS meeting.

1. Clinical and Diagnostic Developments of a Gamma Interferon Release Assay (Bovigam™) for Use in Bovine Tuberculosis Control Programs

K. E. Bass^{1,2}, B. J. Nonnecke², M. V. Palmer², T. C. Thacker², R. Hardegger³, B. Schroeder³, A. J. Raeber³, and W. R. Waters²

¹Iowa State University, Ames, IA

²United States Department of Agriculture, Agricultural Research Service, National Animal Disease Center, Ames, IA

³Prionics AG, Schlieren, Switzerland

Currently the Bovigam assay is used as an official supplemental test within bovine tuberculosis control programs. The objectives of the present study were to evaluate two *Mycobacterium bovis* specific peptide cocktails, purified protein derivatives (PPDs) from two sources, liquid and lyophilized antigen preparations, and a second generation IFN- γ release assay (Bovigam, Prionics AG). Three strains of *M. bovis* were used for experimental challenge: *M. bovis* 95-1315, *M. bovis* Ravenel, and *M. bovis* 10-7428. Additionally, samples from a tuberculosis-affected herd (i.e. natural infection) were evaluated. Robust responses to both peptide cocktails HP (PC-HP) and ESAT-6/CFP10 (PC-EC), as well as PPDs were elicited as early as three weeks after challenge. Only minor differences in responses to Commonwealth Serum Laboratories (CSL) and Lelystad PPDs were detected with samples from experimentally infected animals. For instance, responses to Lelystad *M. avium* derived PPD (PPDa) exceeded respective response to CSL PPDa in *M. bovis* Ravenel infected and control animals. However, 1:4 dilution of stimulated plasma demonstrated greater separation of PPDb from PPDa responses (i.e., PPDb – PPDa) with use of Lelystad PPDs, suggesting that Lelystad PPDs provide greater diagnostic sensitivity than CSL PPDs. Responses to lyophilized and liquid antigen preparations did not differ. Responses detected with first and second generation IFN- γ release assay kits (Bovigam) did not differ throughout the study. In conclusion, antigens may be stored in a lyophilized state without loss in potency; PC-HP and PC-EC are dependable biomarkers for aiding detection of bovine tuberculosis, and second generation Bovigam kits are comparable to current kits.

2. Impact of the cut-off value on the performance of the interferon-gamma detection assay for diagnosis of bovine tuberculosis

Dr. Julio Alvarez

Centro de Vigilancia Sanitaria Veterinaria (VISAVET), Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain.

Due to the limitations of the tuberculin skin test, typically performed as the first-line screening technique, the interferon-gamma assay has been increasingly applied in several countries for either maximization of diagnostic test sensitivity (parallel use) or specificity (serial use) in the diagnosis of bovine tuberculosis. However, alternative protocols have been used for test interpretation, including different cut-offs. The effect of using different thresholds is evaluated here using field data (more than 66,000 tests performed on more than 30,000 cattle from infected and TB-free herds) by the alternative application of those cut-offs in place in different countries of Europe and in the USA. The use of different thresholds leads to significant differences in the number of reactors detected. Proportion of animals in which tuberculosis-infection was confirmed by bacteriology also revealed a significant effect of the cut-off associated with the age of the animal and the number of herd-tests performed in the herd since disclosure of the outbreak. Results from officially free of bovine tuberculosis (OTF) herds indicated a major impact of the threshold also in terms of specificity: those thresholds performing better in infected herds yielded significant higher numbers of false positive reactors in OTF herds. Therefore, in order to maximize the performance of the test a feasible option may be the alternative use of different thresholds depending on the epidemiological setting and the purpose of its use (maximize sensitivity in the case of infected settings and/or maximize specificity if the test is going to be used in situations in which the disease is not expected).

3. Evaluation of new diagnostic blood tests for bovine tuberculosis in cattle.

Dr. Om Surujballi, Canadian Food Inspection Agency

There are a number of serological tests for bovine tuberculosis (TB) that are currently commercially available or are in development for use in a variety of animal species. Serological tests offer a number of advantages over the technologies that are currently being used for diagnosis of this disease. Government regulators, including the Canadian Food Inspection Agency (CFIA) are interested in examining the potential of these emerging technologies for use in bovine TB control and eradication programmes. This report describes the evaluation of two serological tests, the *Mycobacterium bovis*- *Mycobacterium tuberculosis* Antibody Test Kit, DPP® BovidTB-M Assay (Chembio Diagnostic Systems Inc., currently available for experimental use only) and the *Mycobacterium bovis* Antibody Test Kit, an OIE-certified test that is commercially available from IDEXX Laboratories. A blinded panel comprised of 400 sera from cattle from which the *Mycobacterium bovis* bacterium was isolated, and 909 sera from cattle with no previous history of bovine TB, provided by the USDA Bovine Tuberculosis Serum Bank, was examined in this study. A second panel comprised of 1549 sera from cattle with no previous history of bovine TB, provided by the CFIA Epidemiology and Surveillance Section Serum Bank was also examined in this study. The performances of the DPP® BovidTB-M Assay and the IDEXX ELISA were evaluated with these two serum panels in this study and the findings will be discussed in this presentation.

Dr. Jeff Nelson of the National Veterinary Services Laboratory, USDA-APHIS, gave two presentations via web-conference titled:

Detection of Bovine Tuberculosis Antibody Response in Sensitized Cattle Using the IDEXX *M. bovis* Ab Test, and

Impact of Blood Sample Storage Time and Temperature on Detection of Bovine Tuberculosis Antibodies Using the IDEXX *M. bovis* Ab Test

Other business:

Use of optical density (OD) reader to interpret the Chembio dual path platform (DPP) results in cervid TB testing

On August 26, 2013 the Tuberculosis (TB) Scientific Advisory Subcommittee (SAS) received a document from APHIS-VS TB Program Staff concerning use of the newly licensed Chembio dual path platform (DPP) diagnostic assay for tuberculosis in cervids. Current testing protocols involve primary testing with the Chembio CervidTB Stat-Pak® (visual interpretation) with DPP as the secondary test (visual interpretation) on all Stat-Pak® positive animals. A positive result on the first DPP is followed by a second DPP (visual interpretation), using serum collected no sooner than 30 days after the first DPP. The APHIS-VS document describes an unacceptably high number of false positive results in preliminary field-testing. Recently, over 5,200 cervids were tested, as described, with 16.2% of the samples judged positive by Stat-Pak® and 2.3% of these samples judged positive on the first DPP test. Retesting DPP responders with a second DPP yielded 1.08% positive, leaving 52 of the animals as reactors and recommended for postmortem examination. To date, 36 animals have been examined with no gross lesions of TB seen. From these examinations, 23 tissue samples have been processed for mycobacteriological isolation with no *M. bovis* identified. This large number of false positive results was unanticipated and is unacceptable. APHIS-VS has requested comment from the TB SAS on the acceptability of use of a calibrated optical density (OD) reader to measure reflectance in relative light units (RLU) of colored antigen bands, producing a numeric value as the final DPP result as described elsewhere.¹ Use of an OD reader is not currently included in the APHIS approved protocol for cervid TB testing. Numerical cut-off values for DPP results are suggested in the accompanying document.

TB Program Staff posed 4 specific questions found below with responses.

1. Will it be scientifically sound and justifiable to replace visual DPP test readings with OD reader values for designating a DPP test as positive or negative?

It is the opinion of the TB SAS that use of a calibrated, properly functional OD reader to analyze DPP results, is superior to interpretation by visual inspection. Adjustment of the protocol to use numeric OD

values to classify a DPP test as positive or negative would be a useful change to current practices. The OD cut-off values proposed will accomplish the goals to decrease the number of false positive results, decrease the number of quarantined herds, and decrease the number of deer sent for postmortem examination. Some practical questions can be raised concerning the instrument type, frequency of calibration, maintenance and proficiency testing. However, assuming APHIS, VS addresses these issues in the lab(s) that are approved for testing, use of OD numerical values should be superior to visual inspection and interpretation.

2. If OD cutoff values are used, are the proposed cut-off values appropriate for achieving the necessary specificity while maintaining reasonable sensitivity?

The ROC analysis provided (attached document), even with irregularities in the data sets, shows that attempting to optimize both Se and Sp results in an unacceptably low Sp with resultant high false positive rates. At the same time, ROC analysis illustrates that increased Sp comes at the cost of significantly decreased Se. Use of the proposed DPP cut-off values for each species increases Sp 1-4%, maintaining a Se $\geq 70\%$, similar to that seen in previous studies.¹ The proposed values clearly accomplish the goals listed above, but decrease the test's ability to identify infected animals. The reasonableness of this decrease in Se must be viewed in the context of both scientific and non-scientific factors, including advantages of serological tests such as decreased number of handling events, decreased animal stress, and decreased risk of injury to animals compared to skin testing. In addition to these practical advantages, one must also consider regulatory, policy, and logistic elements that are clearly relevant in TB testing of cervids. Given all the factors that must be considered, the TB SAS concludes that it is reasonable to accept the proposed OD cut-off values for the DPP with their associated Sp and Se.

3. Can these OD cutoff values be statistically justified from the number of animals tested to date in each species group?

The limited number of each species available for testing complicates statistical justification in this case. This is especially true in the case of fallow deer and reindeer. Furthermore, the apparent prevalence of disease in the cervid population is so low that it is impossible to acquire a sufficient number of naturally infected animals to truly evaluate Se. As for Sp, the VS National Surveillance analysis determined that if no infection was found in 30 animals examined postmortem, there was a 95% chance the current testing protocol had a Sp $< 97.5\%$. This Sp was shown to be unacceptable. More than the required 30 animals have been examined postmortem and tissues processed for mycobacterial isolation, with all results being negative. Therefore, the TB SAS concludes that a sufficient number of the various species have been tested and examined to reasonably, but not statistically, justify the proposed cut-off values.

4. Is there any problem using currently held serum samples to reevaluate the existing suspect and reactor animals retroactively with the newly established cutoff values and clearing those below the new cutoff levels?

It is the opinion of the TB SAS that if currently stored serum samples are re-analyzed using the same standards as all other samples being analyzed (i.e. same reader, cut-off values, etc.), retroactive re-classification of test results would not be improper. From a policy viewpoint, re-testing using OD reader values for re-classification should be done in close collaboration with individual state animal health officials, and in keeping with any state regulations that may be relevant. From a sample quality viewpoint, the usefulness of stored serum samples is a function of collection and storage. Assuming that samples were collected, processed and stored appropriately, the samples should be suitable for use in retrospective sampling.

References

1. Lyashchenko KP, Greenwald R, Esfandiari J, O'Brien DJ, Schmitt SM, Palmer MV, Waters WR. Rapid detection of serum antibody by DPP VetTB assay in white-tailed deer infected with *Mycobacterium bovis*. *Clinical and Vaccine Immunology* 2013; 20(6): 907-911.

**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) 2013**

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 legal 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 2013. APHIS is hopeful that Proposed Rule and Program Standards will be published in early 2014. Upon publication, APHIS plans to provide an extended comment period of 90 days.

Bovine State Status

As of September 30, 2013, 48 States, two Territories, and one zone were TB accredited-free (AF), including Puerto Rico and the U.S. Virgin Islands. California was modified accredited advanced (MAA). Michigan continued to have 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 California during September 2013. This review was conducted to evaluate the status of the TB eradication program in California, which has modified accredited advanced status.

TB-Affected Herds Identified in FY 2013

Seven TB-affected cattle herds, four beef and three dairy, were detected during FY 2013. These herds were located in California (one dairy), Michigan (three beef and two dairy), and North Dakota (one beef). Five of these TB-affected herds (two dairy and three beef herds) were detected as a result of slaughter surveillance and the subsequent epidemiologic investigations. In addition, TB infection was detected in one Michigan feedlot subsequent to the investigation of an affected Michigan dairy.

Four cattle herds (one dairy and three beef herds, Michigan) were depopulated with Federal indemnity. The three remaining herds are under test-and-remove management plans (one dairy, California; one beef herd, North Dakota, one dairy, Michigan). Three cattle herds detected prior to FY 2013, including two dairies and one beef herd in 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, 2012, through June 30, 2013, 8,804 granulomas were identified during postmortem slaughter inspection and submitted for diagnostic testing from 146 federally inspected establishments. In addition, 200 granulomas were submitted from 13 state inspected establishments. The minimum standard for slaughter surveillance is 1 granuloma submitted per 2,000 adult cattle slaughtered annually. This standard is applied to each slaughter establishment. The 40 highest volume adult cattle slaughter establishments met or exceeded the submission standard through the third quarter of FY 2013. These 40 highest volume establishments slaughter approximately 95 percent of all adult cattle slaughtered in the United States.

Slaughter Cases: During FY 2013, a total of 29 granuloma submissions had histology consistent with mycobacteriosis. Of these, TB was confirmed in 22 (75.9 percent) cases. TB is confirmed by polymerase chain reaction testing of formalin-fixed tissue and culture of fresh tissue. Of the remaining 7 cases, other *Mycobacterium* species were identified for 6 cases and fresh tissue was not available for culture for one case.

Four of the 22 confirmed cases occurred in adult cattle over two years of age, and 18 cases occurred in feeder cattle. The four adult cattle cases included one adult dairy cow that led to detection of an affected dairy in Michigan. One adult TB case occurred in a dairy cow that traced to a Washington state dairy; infection was not confirmed in the herd. The third and fourth cases occurred in a dairy and a beef cow, both from California. The 18 fed cattle cases occurred in beef-type cattle and were detected at

slaughter establishments in Colorado (four cases), Texas (12 cases) and Nebraska (two cases). Fifteen cases were in Mexican-origin cattle and the remaining three cases are under investigation.

Mexican-Origin Slaughter Cases: A total of 15 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), Coahuila (two cases), Durango (six cases), Nuevo Leon (two cases), Tamaulipas (two cases), and Veracruz (one case). An additional case originated from Mexico based on the epidemiological investigations; however, the Mexican State of origin could not be identified.

Live Animal Testing, Cattle: Information for tuberculin skin testing in cattle for FY 2013 was not available at the time of this report.

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, Texas, and Washington) and the NVSL in Iowa are approved to conduct gamma interferon testing. A total of 11,456 tests were conducted in cattle in during FY 2013.

Live Animal Testing, Cervids: Information for tuberculin skin testing in captive cervids for FY 2013 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. The specificity of this testing protocol, which initially utilized a visual reading of the DPP test to interpret the result as positive or negative, did not meet the anticipated specificity indicated by previous studies. An evaluation of FY 2013 testing data determined that a colorimetric optical density (OD) reader could be used on the DPP test to determine a numerical value of the positive visible line on the DPP cassette. Therefore, a cutoff value using OD values was developed for each approved cervid species, improving the specificity of the testing protocol without a major loss of sensitivity. This change was reviewed and approved by the USAHA TB Scientific Advisory Subcommittee and implemented retroactively in September 2013.

During February 4 through August 31, 2013, a total of 5,214 Stat-Pak tests were completed and 841 samples (16.1 percent) were positive. These samples were submitted from 3,170 white-tailed deer (60.8 percent), 1,482 elk (28.4 percent), 391 fallow deer (7.5 percent), 146 red deer (2.8 percent), and 25 reindeer (0.5 percent).

Stat-Pak positive samples were tested by the DPP as a secondary test and 118 samples (2.3 percent) were positive based on the visual test interpretation. Of these, 8 animals were submitted to necropsy based on their first positive DPP test, 88 were tested with a second DPP after 30 days, and the status of one animal was pending at the time of this report. The remaining 21 animals continued under quarantine and their first DPP result was later reclassified from positive to negative in September, as a result of changes to the testing protocol described above.

Of the 88 animals tested with a second DPP, 51 (58.0 percent) were positive by the DPP visual criteria and classified as reactors; of these, 35 were submitted to necropsy and 16 remained under quarantine. After the DPP test protocol was changed in September, 14 animals were reclassified from DPP positive to negative and 2 animals remained classified as reactors and their status is pending.

A total of 43 animals have been submitted for necropsy. Representative lymph nodes and grossly lesioned tissues were evaluated by histopathology and culture. All samples were negative for TB by histopathology. Thirty one cultures have been completed and *M. bovis* has not been identified; the remaining cultures are pending.

Collaborations with Mexico

In FY 2013, APHIS and Secretaría Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA) developed a joint strategic plan designed to minimize the risk of TB while providing a framework to facilitate trade in the future. There were no official reviews of Mexican State TB programs; however, APHIS personnel assisted SAGARPA with completed pre-certification reviews in Durango, Nuevo Leon and Tamaulipas during FY 2013.

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 49 samples from cervid species and 111 samples from cattle were

added to the serum bank in FY 2013. 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 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 cervid species from accredited free States during FY 2014.

IDEXX® M. bovis Antibody Test Kit

The IDEXX® *M. bovis* Antibody Test Kit was approved for official TB program use in cattle during 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 test is available at NVSL and has been used in two TB affected herds in FY 2013. Based on evaluation of the performance of this test, additional uses for the test and additional laboratories to conduct the test may be approved.