

REPORT OF THE COMMITTEE ON TUBERCULOSIS

Chair: Dustin Oedekoven, SD
Vice Chair: Beth Thompson, MN

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The Committee met on Tuesday, October 18, 2016, from 1:00 to 5:30 p.m. There were 53 members and 20 guests present.

Dr. Oedekoven welcomed committee members and guests, and introduced Dr. James Averill as acting Vice Chair as Dr. Beth Thompson was not able to attend this year. Dr. Oedekoven determined there was quorum for the committee to meet and vote on resolutions.

Dr. Tyler Thacker 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. Michael Gilsdorf presented the report of the Bi-National Committee (BNC). A motion to accept the report on the BNC was made and seconded. The motion was passed. The full text of the report is included in this report.

Dr. Mark Camacho presented the National Tuberculosis Program Update.

Topics Discussed:

1. Indemnity
2. Collection of official identification at Slaughter
3. Granuloma submission rate
4. Caudal Fold Test response rate
5. Status of the proposed TB/Brucellosis Rule and the 2009 Federal Order

Dr. Scott Wells presented an update on Modeling Transmission of Bovine Tuberculosis in Uruguay Using Dynamic Cattle Movement Networks.

Dr. Noel Harrington presented the Canada National Tuberculosis Report. After almost 20 years of effort, bovine TB is approaching undetectable levels. Canada continues to have robust surveillance and aggressive response to newly identified cases.

Dr. Fernando Rivera Olvera presented the Achievements in Reducing On-farm TB Prevalence in Mexico.

Mr. Salvador Diaz Oliveros, State of Veracruz, Mexico, presented on Experiences in the Development of an Electronic System Support for Livestock Management in the Southern Region of Mexico and Its Contribution for International Recognition as TB Low Prevalence Area.

State updates were provided by:

Texas:

Discussed TB affected herds. In addition, discussed slaughter traces originating in Texas, 8 of 9 been closed.

California:

Discussed past 15 years dealing with TB and has now obtained Accredited Free status statewide.

Michigan:

Discussed Michigan's TB Program history, four herds identified past year, and efforts to enhance wildlife risk mitigation. Further details included in this report.

Indiana:

Discussed finding of TB infected cattle herd this year and in free-ranging white tailed deer. Further details attached to this report.

Committee Business:

At the conclusion of the formal presentation, Dr. Oedekoven determined there was a quorum and gave a report on the 2015 resolutions and responses.

Three resolutions were presented to the committee; all three were approved and forwarded to the Committee on Nominations and Resolutions. Topics included:

- 1) Amend importation requirements for cervids from Manitoba
- 2) Optimization and Standardization of purified protein derivative (PPD) tuberculin for IFN- γ
- 3) National Cervid TB Herd Accreditation Program

Dr. Oedekoven discussed a draft proposal from the USAHA Executive Committee for streamlining committees to be more efficient and effective.

REPORT OF THE 2016 USAHA BOVINE TUBERCULOSIS SCIENTIFIC ADVISORY SUBCOMMITTEE

Tyler Thacker, Chair

Eight presentations were made at the 2016 Tuberculosis (TB) Scientific Advisory Subcommittee meeting.

Potential for Rapid Antibody Detection to Identify Tuberculous Cattle with Non-Reactive Tuberculin Skin Test Results

W. Ray Waters, Bovine Tuberculosis Research Group, National Animal Disease Center, Agricultural Research Center, USDA

Background: Bovine tuberculosis (TB) control programs generally rely on the tuberculin skin test (TST) for ante-mortem detection of *Mycobacterium bovis*-infected cattle. **Results:** Present findings demonstrate that a rapid antibody test based on Dual-Path Platform (DPP®) technology, when applied 1-3 weeks after TST, detected 9 of 11 and 34 of 52 TST non-reactive yet *M. bovis*-infected cattle from the U.S. and Great Britain (GB), respectively. The specificity of the assay ranged from 98.9% (n = 92, U.S.) to 96.0% (n = 50, GB) with samples from TB-free herds. Multi-antigen print immunoassay (MAPIA) revealed the presence of antibodies to multiple antigens of *M. bovis* in sera from TST non-reactors diagnosed with TB. **Conclusions:** Thus, use of serologic assays in series with TST can identify a significant number of TST non-reactive tuberculous cattle for more efficient removal from TB-affected herds.

Early Detection of Circulating Antigen and IgM-Associated Immune Complexes During Experimental *Mycobacterium bovis* infection in cattle

Konstantin Lyashchenko, Senior Research and Development Director, Chembio Diagnostic Systems Inc.

The presence of circulating antigen in cattle experimentally infected with *Mycobacterium bovis* was demonstrated using dual-path platform (DPP) technology. The antigen-capture immunoassays employed rabbit polyclonal antibody recognizing predominantly *M. tuberculosis* complex specific epitopes and were able to detect soluble substances and whole cells of mycobacteria. The antigen found in serum appeared to be mostly bound to IgM, but not to IgG, within the immune complexes formed at early stages of *M. bovis* infection. The antigen was also detected in bile and urine, indicating possible clearance pathways. The data correlation analyses supported the role of IgM responses in antigen persistence during *M. bovis* infection. The antigen was detectable in serum months prior to the antibody seroconversion, suggesting potential for improved immunodiagnostics.

Quantiferon®-TB IGRA: Gold Standard in Human Diagnostics and Promising Candidate for Improved Bovine TB Diagnostics

Carsten Schroeder, Director Market Development Veterinary Applications, Qiagen

Use of IP-10 in TB Testing of African Buffaloes

Michele Miller, Professor, Division of Molecular Biology and Human Genetics, Stellenbosch University

African buffaloes are maintenance hosts for bovine tuberculosis (BTB). Currently approved tests for buffalo include intradermal tuberculin test and Bovigam PPD assay. However, there is suboptimal specificity of these tests in this species. Improved specificity may be achieved using *M. bovis*-specific peptides and ancillary biomarkers to Interferon gamma (IFN γ). Comparing IFN γ and IP-10 production in whole blood stimulated with Bovigam PC-EC and PC-HP peptides, enhanced sensitivity was observed using IP-10 in naturally infected buffaloes. In addition, the IP-10 assay had the highest overall sensitivity, and when used in combination with the Bovigam PPD assay, 100% of *M. bovis* confirmed infected buffalo were detected; this was greater than the combination of Bovigam PPD and skin test. IP-10 also has advantages of being stable when stored on Protein Saver Cards for up to two weeks, and showed thermal stability when plasma was heat treated at 65C for 20 minutes. Additional studies are being conducted on specificity of IP-10 in low and high prevalence buffalo herds.

Application of the Phage-PCR Assay in the Detection and Control of Bovine TB in the U.K.

Cath Rees, Faculty of Science, University Nottingham and PBD Biotech Ltd.

The phage amplification assay was originally developed as a method to detect human TB in sputum. Over the last ten years we have focused on application of this method to detect animal pathogens. The method and the evidence that it can be used to detect mycobacteria in clinical blood samples will be presented. The report included recent improvements to the assay and how the assay has been used to try and control bovine TB in the U.K. The data from this study has provided new insights that will inform bovine TB control measures in the U.K.

Detection of Mycobacterium DNA in experimentally infected Cattle using the Phage Assay

Tyler C. Thacker, Mycobacterial Diseases, National Animal Disease Center, ARC, USDA

The Mycobacterial Phage Assay was developed in the U.K. to detect viable mycobacteria in clinical samples. Working with Dr. Cath Rees, the developer of the assay, Animal Research Service (ARS) transferred the experimental technique to the Bovine TB Research group at the National Animal Disease Center. The two-day Phage Assay detected mycobacteria in PBMC from 3 of 6 experimentally infected calves at four months post infection. The assay detected 1 of 5 control cows, suggesting that additional optimization of the assay is needed.

Experiences with Gamma Interferon Testing for TB in Texas

Roger Parker, Texas State-Federal Laboratory, Texas Animal Health Commission

U.S. Bovigam Update 2016

Sunny Geiser-Novotny, National Center for Cattle Health, VS-APHIS-USDA

The Bovigam® was approved in 2003 as a supplemental test in the TB program and is primarily used as a substitute for the Comparative Cervical Test (CCT) in retesting caudal-fold test suspect cattle. In 2015, a sensitivity issue was discovered with the Bovigam® when testing a large, relatively high prevalence dairy. It was determined that there was low potency in certain lots of Central Science Laboratory (CSL) purified protein derivatives (PPD) packaged with Bovigam® kits. Subsequently, Veterinary Services (VS) issued Bulletin 2015.03, Bovigam® (interferon gamma) Blood Test for Bovine Tuberculosis, on July 31, 2015, providing interim approval to use the Bovigam® Rest of World (ROW) ELISA with Lelystad bovine and avian PPDs manufactured by Thermo Fisher Scientific as the stimulating antigens.

However, since changing to the Lelystad PPD, a higher than expected number of false positives have been observed, resulting in otherwise healthy animals being indemnified, removed, and necropsied with negative findings for tuberculosis. In addition, false negative and inconsistent results were noted in an inter-laboratory study while testing an affected Texas dairy before its depopulation in 2016. As a result of the issues with test performance using Lelystad PPD, several States have stopped using the Bovigam® and changed to using the comparative cervical tuberculin skin test, requiring increased use of state and federal staff resources. While the Veterinary Services' (VS) Cattle Health Center and Thermo Fisher Scientific had discussed the possibility of increasing the cut-off value used to designate a positive result as a solution to improve specificity, the impact on sensitivity and inconsistencies identified would need to be addressed before this option could be implemented.

VS Bulletin 2015.03 expired on July 31, 2016, and VS withdrew the interim approval to use Lelystad PPD in the Bovigam® test. Beginning September 1, 2016 approved laboratories were instructed to use National Veterinary Services Laboratories (NVSL)-produced avian and bovine PPDs and the standardized laboratory protocol issued by the NVSL for testing samples using the Bovigam® ROW ELISA test, in order to improve test specificity and ensure consistency in testing procedures between the laboratories. VS will be monitoring the performance of the Bovigam using the NVSL-produced PPDs and is assisting the approved laboratories with the transition to the new protocol. VS has had conference calls and face to face meetings with Thermo Fisher Scientific to discuss future use of Lelystad PPDs in the Bovigam® test and has requested that the company make available consistent Lelystad PPD lots for U.S. approved laboratories and improve test specificity. Once the company has addressed these issues, VS will reevaluate use of Lelystad PPDs in Bovigam® for the TB program.

Replacement of CSL by Lelystad Tuberculin PPD Implications on BOVIGAM™ Testing in U.S.

Björn Schröder, Thermo Fisher Scientific

On a global level, Tuberculin purified protein derivatives PPD of different sources is used as

stimulation antigens for BOVIGAM™. Until July 2015, PPD of Central Science Laboratory (CSL) origin has been used for BOVIGAM™ in U.S. The 2014 USAHA Resolution 29 recommends replacing CSL PPD with Lelystad PPD. Due to a lack of potency however CSL PPD has been discontinued in July 2015. Currently, BOVIGAM™ ROW in conjunction with Lelystad PPD has been provided to U.S. customers under Center for Veterinary Biologics (CVB) Research and Evaluation (R&E) Import Permit. The field trials indicate that the sensitivity of Lelystad PPD is better in comparison to CSL without affecting the specificity.

Due to the higher sensitivity of Lelystad PPD, more animals are now being identified as reactors. This presents a problem to the TB program in the U.S. In response, National Veterinary Services Laboratories NVSL PPD is being used with BOVIGAM™ ROW as a short-term solution. The long-term solution is to optimize Lelystad PPD for use with BOVIGAM™ to fulfill the requirements of the TB program.

Other Business:

In February 2016, the Scientific Advisory Subcommittee (SAS) of the USAHA Committee on Tuberculosis (TB) was asked to consider a proposal by the APHIS Veterinary Services (VS) Cervid Health Program to raise the dual path platform (DPP) VetTB Assay optical density (OD) cut-off value for reindeer from 200 to 500. Since the DPP VetTB Assay was approved for use in the diagnosis of *Mycobacterium bovis* infection in reindeer in 2013, 179 animals have been tested. Two animals have been considered positive with a cut-off of 200. Infection with *M. bovis* could not be demonstrated in either animal.

In setting the initial DPP cut-off values, a conservative cut-off of 200 was set for reindeer due to the lack of information on *M. bovis* infection in reindeer in the U.S. The decision was based on concerns that lowering the sensitivity of the test by increasing the cut-off, could potentially misdiagnose infected animals. At the same time, a cut-off of 500 was set for elk, white-tailed deer, and red deer based on statistical evaluation of previous DPP testing data. The fallow deer cut-off of 200 was set based on DPP testing data from a single *M. bovis* infected fallow deer herd identified in 2010. There is no new data to support a change in the cut-off points for elk, white-tailed deer, red deer or fallow deer.

The request from the Cervid Health Program was accompanied by a report entitled, "Evaluation of Current Dual Path Platform Testing Protocol for TB in Cervids" compiled by USDA, APHIS, VS, Center for Epidemiology and Animal Health (CEAH), dated December 2015. In summary, the CEAH report determined that "increasing cut-off values (for reindeer and fallow deer) results in decreased test sensitivity without much change in the test specificity. Thus, the number of undetected animals is likely to increase, even with very low disease prevalence in the population, without having much effect on the false positives."

The specific question posed to the TB SAS was: "Is it scientifically justified to change the Cervid TB DPP serological testing protocol for reindeer at National Veterinary Services Laboratories (NVSL) by raising the OD cut-off value for test positivity from 200 to 500 on either DPP test cassette line?"

In examining the question there are several items to consider:

1. The request is to comment on whether or not there is "scientific justification" for a change in cut-off values. A scientific justification would require data on both specificity and sensitivity. As data on the sensitivity of the DPP in reindeer does not exist, there is no appropriate manner to obtain valid scientific justification.
2. Although no information is available on DPP test sensitivity in *M. bovis*-infected reindeer, it is known that reindeer are susceptible to experimental infection with *M. bovis*¹. Experimentally infected reindeer produce antibodies in response to *M. bovis* infection that are directed at the *M. bovis* specific antigens used in the DPP².
3. To our knowledge there is no evidence that reindeer are immunologically more similar to fallow deer (with a cut-off of 200) than elk, red deer, and white-tailed deer (with a cutoff of 500).

¹ Palmer MV, Waters WR, Thacker TC, Stoffregen WC, Thomsen BV. Experimentally induced infection of reindeer (*Rangifer tarandus*) with *Mycobacterium bovis*. Journal of Veterinary Diagnostic Investigation, 2006; 18(1): 52-60.

² Waters WR, Palmer MV, Bannantine JP, Greenwald R, Esfandiari J, Andersen P, Pollock JM, Lyashchenko KP. Antibody responses in reindeer (*Rangifer tarandus*) infected with *Mycobacterium bovis*. Clinical and Diagnostic Laboratory Immunology, 2005; 12(6): 727-735.

4. The prevalence of *M. bovis* infection in reindeer in the U.S. is extremely low. In fact, *M. bovis* infected reindeer have never been detected in the U.S.

It is the opinion of the TB SAS that the question of scientific justification cannot be addressed in the absence of data on test sensitivity. The more relevant question is whether or not there is justification for the risk of raising the DPP cut-off value for reindeer. According to the CEAH report, "the consequence of raising the DPP cut-off value is an increased risk of missing infected animals." The question of risk justification represents policy and is separate from scientific justification. Evaluating risk involves decisions outside the scope of the TB SAS. However, given the available knowledge, it would appear that the risk of missing *M. bovis* infected reindeer by increasing the DPP cut-off from 200 to 500 is very low. In a setting of very low risk, the TB SAS has no specific objection to raising the DPP cut-off for reindeer from 200 to 500, making it consistent with elk, red deer, and white-tailed deer.

It is recommended that if *M. bovis* were to be detected in reindeer in the U.S. or another country that would cooperate with the U.S., it should be a high priority to conduct an evaluation of DPP test performance in naturally infected reindeer.

BNC Issues and Updates as of October 2016

Michael Gilsdorf, International Animal Health Solutions LLC

During 2016, the Mexican/U.S. Binational Committee (BNC) on Tuberculosis (TB), Brucellosis and Ticks met in San Diego on Tuesday January 26, 2016 at the NCBA convention and also in Tijuana, Mexico, on Wednesday, May 11, 2016 at the National Confederation of Livestock Organizations (CNOG) convention.

The following comments represent the consensus agreement reached at these meetings of the BNC. October updates are provided as well.

Agreements from the BNC: January 2016

1. The BNC is concerned about USDA Animal and Plant Health Inspection Service's (APHIS) aggressive timeline regarding completing regionalization of the Mexican States. The BNC would like to know how USDA APHIS intends to complete Mexican regionalization within this time line.
 - a. **Response:** *APHIS is continuing to pursue a policy of pre-certification reviews conducted by National Service of Health Food Safety and Quality (SENASICA) followed by an APHIS regionalization review. The intent is to classify as many regions as possible by the time of publication of the pending final TB/BR rule, and all Mexican regions within the following year. APHIS is on track to meet these goals. SENASICA has conducted pre-certification reviews with APHIS participation in at least 10 of the 16 proposed regions. APHIS has conducted 9 regionalization reviews, focusing on the high-exporting status. Pre-certification reviews are planned in Coahuila and Colima in CY 2016; APHIS reviews are currently planned in the Baja Peninsula region in December 2016 and Tamaulipas in January 2017.*
2. The BNC is concerned that USDA APHIS is moving forward with Mexican State regionalization without a finalized TB/BR rule. Furthermore, BNC is concerned that comments from U.S. and Mexican stakeholders will not have time to be considered while regionalization is being conducted. Although, the BNC recognizes that these are two separate issues, they are inter-related.
 - a. **Response:** *Consideration of public comment: APHIS is in the process of considering all of the comments received from stakeholders on regionalization under the proposed TB/BR rule. We do not consider that any of the comments will require substantive changes to the proposed approach, although some adjustments may be warranted. APHIS continues to promote transparency in the process of regionalizing Mexico for bovine TB by including a U.S. State animal health representative on each review and providing the review reports to the National Assembly of State Animal Health Officials (NASAHO).*
3. When will USDA be ready to go back to Piedras Negras and Acuna to inspect the export cattle? If not, Coahuila would request that a long-term lease be negotiated for the current pens in Del Rio and Eagle Pass.
 - a. **Response:** *APHIS has finalized a long-term lease for the current inspection facility in Del Rio, Texas, and expects to continue to work with the lessor on mutually agreed improvements into the future. APHIS is also looking to lease space on a longer-term basis in Eagle Pass, Texas, with an expected end point in 2017. The federal lease process continues steadily, with a lease award expected in late 2016.*
4. When will USDA be doing their onsite review of Coahuila to change the TB status?
 - a. **Response:** *Coahuila review: SENASICA scheduled a pre-certification review of the Coahuila AP zone in July 2016 that was subsequently postponed at Coahuila's request. SENASICA has confirmed the dates for a pre-certification review the week of November 14-19, 2016.*

Agreements from The BNC: May 2016

1. Establish a working group to analyze how to implement the use of the National System of Individual Identification of Cattle (SINIIGA) ear tags in Mexican cattle at the U.S. ports of entry.
 - a. **Response:** *An electronic identification meeting was held in Tijuana Mexico on September 8, 2016. A separate meeting summary is available. Action Items:*
 1. *The Mexican BNC members intend to use the electronic ear tags but want assurance from APHIS and SAGARPA that they will be used.*
 2. *APHIS plans to initiate a pilot project to read rodeo cattle identification because these cattle already have the radio-frequency identification (RFID) tags.*
 3. *Mexico industry asked APHIS to conduct a pilot project immediately using imported cattle that would follow all the criteria mentioned here at this meeting.*
 4. *An action plan timeline for implementing an electronic identification and certification system at the ports was requested and that it be provided to the BNC members after the Fed-Fed meeting on September 9, 2016. APHIS stated they could not provide a timeline at this time.*
2. Establish a working group to plan a tick summit meeting in November (with 2 to 4 participants each from Mexico and the U.S.)
 - a. **Response:** *The Tick Summit Meeting is scheduled on November 29-30, 2016 in Weslaco, Texas.*
3. Sonora requests that USDA-APHIS recognize their brucellosis status under current regulations before the next USAHA meeting
 - a. **Response:** *Sonora brucellosis: APHIS conducted a regionalization review of Sonora for bovine brucellosis status in May 2016. The review team concluded that Sonora is free of Brucella abortus in cattle. APHIS is taking a 2-pronged approach to ensure lifting of the testing requirements for cattle exported to the United States from Sonora as quickly as possible. We plan to list Sonora as Level I for brucellosis under the pending final TB/BR rule (test exempt); in the interim, we are pursuing rulemaking to add Sonora to the list of test-exempt regions in 9 CFR 93.406(d).*
4. Tamaulipas requests a response regarding the TB review conducted by SAGARPA in February 2016. They want to know if that review qualifies them to maintain their TB status without further review by USDA.
5. The BNC members request that USDA report the origin of the exported cattle lots that are detected with ticks at the border to the SENASICA and the Mexican States. The BNC members would also like to know the phase development of those ticks.

USDA-APHIS-VS ANNUAL UPDATE FOR THE STATE AND FEDERAL COOPERATIVE BOVINE TUBERCULOSIS (TB) ERADICATION PROGRAM

Fiscal Year (FY) 2016

Mark Camacho

USDA-APHIS-VS Epidemiologist

Development of Proposed Brucellosis/TB Regulations

APHIS completed new regulations and supporting standards for the brucellosis and tuberculosis (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 2014-15. APHIS is hopeful that Proposed Rule and Program Standards will be published in 2015. Upon publication, APHIS plans to provide an extended comment period of 90 days.

Bovine State Status

As of September 30, 2016, 49 States, two Territories (Puerto Rico and the U.S. Virgin Islands), and one zone (Michigan) were TB accredited-free. California advanced from modified accredited advanced (MAA) status as of July 2016. The MAA zone of Michigan (MI) was advanced to accredited-free status on September 10, 2014. With this advancement, Michigan has an accredited-free and a modified accredited (MA) zone. MI TB memorandum of understanding (MOU) was re-negotiated in 2016.

Captive Cervid State Status

All States and territories have modified accredited (MA) status.

TB Program Reviews

The Michigan TB program was reviewed in FY 2015.

TB-Affected Herds Identified in FY 2016

Five TB-affected cattle herds were identified during FY 2016 including four Michigan beef herds in the MA zone and one small Indiana beef herd. No Michigan herds were depopulated with Federal indemnity but one was depopulated with state funds. Two Michigan beef herds were placed under test-and-remove management plans and one herd is still pending a decision on how to manage. Two captive cervid herds in the Michigan MA zone remain under quarantine.

National TB Surveillance

Granuloma Submissions: For FY 2016, 4,682 granulomas from 163 federally inspected establishments were identified through three quarters of the Fiscal year. Overall, 2.24 granulomas were submitted per 2,000 adult cattle (culled dairy and beef cows and bulls) slaughtered, a decrease for the third consecutive year. The granuloma submission rate was 2.6 in FY 2014. TB slaughter surveillance during FY 2014 and 2015 have experienced the lowest submission rates 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 one granuloma submitted per 2,000 adult cattle slaughtered annually. Thirty-three (33) of the 40 highest volume adult cattle slaughter establishments met or exceeded the submission standard in FY 2016, compared to 31 in FY 2015. These 40 highest volume establishments slaughter approximately 95 percent of adult cattle processed with federal inspection in the United States.

Slaughter Cases: During FY 2016, a total of 14 granuloma submissions had histology compatible with mycobacteriosis, out of 4,682 granuloma submissions (0.3 percent). Of these, TB was confirmed in ten (71.4 percent) cases. TB is confirmed by polymerase chain reaction (PCR) testing of formalin-fixed and direct PCR and culture of fresh tissue. Of the remaining two cases, other *Mycobacterium* species were identified for one case and one case could not be cultured because only formalin fixed tissue was submitted.

One of the ten confirmed cases occurred in an adult cow over two years of age from Canada, and nine cases occurred in feeder cattle. Of the nine fed cattle cases, five occurred in Mexican-origin cattle and four were in domestic origin steers. Six infected steers came through a Pennsylvania slaughter plant in one lot and led to the identification of a new Indiana affected beef herd. Two Texas and one Arizona steers were found but USDA was not able to find an affected herd of origin for any of those traces.

Mexican-Origin Slaughter Cases: A total of five 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 State of Nuevo Leon (one case), Coahuila (one case), and Nayarit (one case). Two cases were from Mexico, though the state of origin could not be determined.

Animal Identification Collection for Slaughter Cases: This data was not available at the time of this report.

Live Animal Testing, Cattle: Tuberculin skin testing in live animals is another component of national TB surveillance in cattle and bison. During October 1, 2015 through August 31, 2016, a total of 644,399 caudal fold tuberculin skin tests (CFT) of cattle and bison were reported, with 10,242 responders (1.6 percent, 46 states and one Territory reporting, data not available for four states). During FY2015, 557,395 CFT tests of cattle and bison were reported, with 7,868 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 2003. Laboratories in seven States (California, Colorado, Michigan, Nevada, Pennsylvania, Texas, and Washington) and the National Veterinary Services Laboratories (NVSL) in Iowa are approved to conduct gamma interferon testing. These laboratories completed approximately 5,331 tests for cattle residing in 20 states during FY 2016 (data incomplete for some laboratories).

Live Animal Testing, Cervids: 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 2013. During FY2016, a total of 10,750 cervid serological TB tests were completed. These samples were submitted from 8,168 white-tailed deer (76 percent), 1,897 elk (18 percent), 456 fallow deer (4 percent), 81 red deer (1 percent), and 148 reindeer (1 percent). Five animals with positive DPP test results were necropsied in FY 2016. Of these, laboratory tests and culture for *M. bovis* have been negative for four animals and are pending for one animal.

Statistical analysis was performed on DPP test performance for tests administered during FY2013-15. The specificity of the first DPP test is 99.6 percent. The specificity after the second DPP test is 99.86 percent. Raising the DPP test cutoff would decrease sensitivity, while having very little effect on improving specificity; therefore, the DPP cutoff values will not be changed in FY 2016.

Collaborations with Mexico

In FY 2016, APHIS teams conducted reviews in Chihuahua, Nuevo Leon, Durango, and Sinaloa. In addition, APHIS and International Services (IS) staff assisted the Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA) in conducting pre-certification reviews in Baja California and Baja California Sur, Tierra Caliente Region (Guerrero, Michoacan), the Nayarit MA zone, the Huasteca Region (Veracruz, Hidalgo, San Luis Potosi, Puebla), the Centro-Occidente Region (Zacatecas, Aguascalientes, Jalisco, and San Luis Potosi), and the Guanajuato AP zone.

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 VS Guidance 6702.1 - The IDEXX Antibody (Ab) Test Serological Test for Diagnosing Bovine Tuberculosis (TB) in TB-Affected Cattle Herds. The serology test continues to be evaluated in affected herds, to determine if its use in conjunction with skin testing will reduce the risk of not detecting truly infected animals that are skin test negative. The test was used in TB affected herds in FY 2015, as part of the test and remove herd management plan.

Selected State Updates

Michigan: Four new affected herds were identified in FY 2016 described by the summary table listed below:

State	County	Herd Type	Size	Disclosed By	Herd Plan
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MI	Alpena	Beef	81	Annual test	State Depop
MI	Oscoda	Beef	180	Annual test	Test & remove
IN	Franklin	Beef	49	Slaughter	Depopulation
MI	Alcona	Beef	65	Trace/Epi	Test & remove
MI	Alcona	Beef	215	Movement	Pending

Indiana: One new infected beef herd in Franklin County was identified in April 2016 at slaughter and has since been depopulated. In addition, one deer and one raccoon were identified as TB positive triggering a 10mile surveillance effort that must be done within six months.

Gamma Interferon Testing Issue

In the course of tuberculosis testing the first Texas dairy quarantined in FY 2015, relatively lower sensitivity of the U.S. gamma interferon assay (34% and 28%) for lesions of tuberculosis was noted on the first two herd tests. As a result of extensive investigation and study over several months with collaboration of the Cattle Health Center, National Veterinary Services Laboratories (NVSL), and gamma interferon testing laboratories in Texas, Michigan, and California, a problem with lower activity of one of the lots of stimulating tuberculin in the gamma interferon assay was discovered. A notice from Veterinary Services (VS) revoked the official status of tests conducted with this particular lot after July 31, 2015. The notice described procedures to replace this testing with either the comparative cervical test or a gamma interferon assay that included a ROW (Lelystad) tuberculin for stimulation. All laboratories were verified as conducting gamma interferon assays with the ROW tuberculin by August 9, 2015.

Since changing to the Lelystad PPD high numbers of false positives have been seen at almost four times the responder rate of the previous Central Science Laboratory (CSL) purified protein derivatives (PPD). This has caused decrease in use of Bovigam[®] arising from high number of positive tests. In addition, VS has seen inconsistent results across different laboratories. VS is addressing the stimulation portion of Bovigam[®] by substituting NVSL PPD for Lelystad PPD (September 1, 2016). In addition, VS will attempt to harmonize differences in testing protocol among approved laboratories. A panel of stimulated plasma samples sent to approved labs performing the Bovigam ROW ELISA using the standardized testing protocol and VS will continued monitoring of test results.

Michigan Tuberculosis Program Update

James Averill, Michigan Department of Agriculture and Rural Development

Affected Herds

63rd Herd:

- A medium-sized beef herd in Alpena County was designated by Michigan Department of Agriculture and Rural Development (MDARD) as infected with bovine tuberculosis (TB) following routine annual surveillance testing.
- Herd was partially depopulated by MDARD funds and remainder sold through slaughter channel

64th Herd:

- A medium-sized beef herd in Oscoda County was designated by MDARD as infected with bovine TB following routine annual surveillance testing.
- The herd is currently undergoing a test and removal program.

65th Herd

- A medium-sized beef herd in Alcona County was designated by MDARD as infected with bovine TB following a trace investigation from the 64th TB infected herd.
- The herd is currently undergoing a test and removal program.

66th Herd

- A large beef herd in Alcona County was confirmed bovine TB positive in an Alcona County herd when one of the animals was tested to be transported off the farm.
- Due to the location of the infected herd, Animal Industry Division (AID) established a special surveillance area which involved a small number of herds in the northern portion of Iosco County which is outside the modified accredited zone (MAZ).
- Determination of herd is yet unknown as a whole herd test was just conducted.

Deer surveillance:

In 2015 has shown apparent prevalence in the free-ranging, white-tailed deer population in the core area of the MAZ was 2.7 percent in 2015, the highest it has been since 1997.

Enhanced Wildlife Risk Mitigation:

- In cooperation with Michigan State University Extension, the USDA's Natural Resources Conservation Service and local producers, a voluntary enhanced Wildlife Risk Mitigation (WRM) program is being offered to the highest risk herds in the MAZ.
- With a team of disease control experts and local producers, herd owners work to further assess the potential vulnerabilities on their farm. The team provides education on bovine TB transmission, examines deer behavior on their farm, and suggests changes to the farmer, which will help them heighten their biosecurity.
- So far, 17 TB core area farms have had an enhanced WRM team visit them. The goal is to have two teams conduct 25 farm visits by the end of fall.

Indiana Tuberculosis Update

Bret D. Marsh, Indiana State Board of Animal Health

Background Information

On April 28, 2016, the Pennsylvania State Veterinarian, Dr. Craig Schultz, notified the Indiana State Veterinarian, Dr. Bret Marsh, that six steers slaughtered at a Pennsylvania processing facility demonstrated lesions consistent with bovine tuberculosis (TB) and were retained at slaughter. The group of six steers was part of a group of 11 from which nine were initially retained. Three of the nine steers were released. Tissues submitted to the National Veterinary Services Laboratories (NVSL) from the six retained animals were confirmed to be histologically compatible with tuberculosis and PCR positive for *Mycobacterium bovis*. The lot of 11 steers, including the six retained animals, had backtags with a 32 prefix indicating they had been sold through an Indiana market. Documents from the slaughter plant enabled the lot to be traced to the Indiana market and market records enabled the Indiana State Board of Animal Health (BOAH) staff to definitively identify the single herd of origin.

Affected Herd

The single beef herd resided on two premises in Franklin County, Indiana. These premises are approximately 3.5 miles from one another. Premises A housed breeding animals and Premises B housed feeders. BOAH placed a quarantine on both premises on April 29, 2016. The herd was comprised of 49 cattle. Caudal fold (CF) testing on 41 test eligible cattle (≥ 2 months of age) was performed on May 6, 2016 and read on May 9, 2016. Of the 41 tested, 27 responded (65.8%) and were classified as reactors. Based on the lesions observed in the cattle at slaughter and this high response rate, the entire herd was appraised and depopulated. All reactors and the eight calves (plus a ninth bottle calf recently transferred to another Franklin County farm) were removed, euthanized, and necropsied as of May 26, 2016. The remaining 14 non-reactors were removed and euthanized as of June 1, 2016. Necropsies of reactors and calves were performed by pathologists at the Indiana Animal Disease Diagnostic Laboratory (ADDL) at Purdue University, pathologists at the Ohio Department of Agriculture ADDL, and by VS and BOAH field personnel. Of the 27 reactors, 24 had gross lesions (88.9%). In addition, 2 of the 9 calves had gross lesions. Carcasses were disposed of using incineration, alkaline digestion, or rendering. All 49 animals from the affected herd and the bottle calf were indemnified by the USDA. A herd plan was signed by the farm owners, Dr. Bret Marsh, and Dr. Frank Wilson on August 29, 2016. This plan details the requirements for cleaning and disinfection, 90-day post cleaning and disinfection fallow period, and testing of restocked cattle.

Laboratory Testing

Serum, plasma, lymph node pools, and lesioned tissue (lung, liver, granulomas) samples were collected from all reactors and calves and submitted to NVSL. Lymph node pools were submitted from

five of the 14 non-reactors. Of the reactors, 100% were positive on the Bovigam assay and 88.9% were positive for *M. bovis* on either direct tissue PCR or mycobacterial culture. Of the calves, 50% were positive on the Bovigam assay and 44.4% were positive for *M. bovis* on mycobacterial culture. Of the non-reactors sampled, 60% were positive for *M. bovis* on mycobacterial culture. A detailed summary of the laboratory results is presented below.

	CFT Reactors (n=27/27 sampled)	CFT Non-Reactors (n=5/14 sampled)	Calves (n=9/9 sampled) ¹
<i>M. bovis</i> ELISA, No. (% of animals sampled)			
Positive	12 (44.4)	NT	1 (12.5)
Negative	15 (55.6)	NT	7 (87.5)
<i>M. bovis</i> λ Interferon Test, No. (% of animals sampled)			
Positive	27 (100)	NT	4 (50)
Negative	0 (0)	NT	4 (50)
<i>M. bovis</i> direct PCR², No. (% of animals sampled)			
Detected	15 (55.6)	NT	NT
Not Detected	0 (0)	NT	NT
Mycobacterial Culture², No. (% of animals sampled)			
<i>M. bovis</i> Isolated ³	10 (37.0)	3 (60)	4 (44.4)
<i>M. bovis</i> Not Isolated ⁴	3 (11.1)	2 (40)	5 (55.6)

Abbreviations: No., Number; NT, Not tested

¹ A blood sample was not collected from 1 of 9 calves necropsied. Tissue samples were collected from all 9 calves.

² Either direct tissue PCR for *M. bovis* was performed OR mycobacterial culture. Samples from one CFT reactor had both direct PCR and mycobacterial culture performed.

³ Results presented here indicate that *M. bovis* was isolated from at least one sampled tissue.

⁴ Results presented here indicate that no *M. bovis* isolation was made from any of the sampled tissues or a non-tuberculous mycobacteria (NTM) was recovered. NTM species identified include *M. engbaekii*, *M. nonchromogenicum*, and *M. monacense*. In two instances, the mycobacterial culture was reported as “contaminated.” In one instance, acid fast bacteria were recovered, but not speciated.

History of TB in Southeast Indiana as Linked to Recent Detection

After being absent from Indiana since 1984, bovine TB was identified in November 2008 in a single cow at slaughter. Soon after, TB was detected in farmed deer in 2009 in a nearby Franklin County farm consisting of red deer, elk, and fallow deer. This farm was depopulated. As a result, the Indiana Department of Natural Resources (IDNR), Board of Animal Health (BOAH), and USDA-APHIS, Wildlife Services (WS) and VS began a wildlife surveillance program. In 2010, slaughter detection of TB in two black steers traced to Indiana and Ohio premises. Since there were no recorded ID's on the steers, the premises were not able to be definitively identified. In 2011, TB was detected in a Dearborn County cattle farm. Recent genetic sequencing results suggest the two black steers originated from this farm. Surveillance of white-tailed deer was expanded to Dearborn County. From 2008-2015, over 1,400 deer from this region were tested and all were negative for TB. In response to the current detection of TB in the Franklin County beef herd, efforts are ongoing to remove wildlife from the affected premises. To date, over 70 animals have been removed and tested from Premises A and B. On August 12, 2016, NVSL reported that *M. bovis* was isolated from a thoracic lymph node of a white-tailed deer removed from one of the affected premises. This animal was a two-year-old doe removed from Premises A. The animal had no gross lesions. On September 15, 2016, NVSL reported that *M. bovis* was isolated from thoracic and

abdominal lymph nodes of a raccoon. This animal was also removed from Premises A. NVSL has performed whole genome sequencing on *M. bovis* isolates collected from recent and historic cases of TB in this region. Results indicate that all of the *M. bovis* isolates collected from Indiana animals are of the same strain and that these detection events are likely epidemiologically linked.

Zone Testing

Upon detection of the affected Franklin County herd, BOAH established a 3-mile testing zone surrounding both Premises A and B. Since 2006, Indiana has required the registration of all premises associated with the sale, purchase, or exhibition of livestock. BOAH's review of their database indicated that this 3-mile zone contains 78 cattle herds of which 40 contain test-eligible cattle (cattle > 2 years of age). Upon detection of *M. bovis* in the white-tailed deer collected from premises A, this testing zone was expanded to a 10-mile radius surrounding Premises A. This zone is also extended with a 2-mile buffer on either side of the west fork of the Whitewater River to the Ohio-Indiana border. BOAH's review of their database indicated that these zones (10-mile and river corridor) contain approximately 400 cattle herds. BOAH continues to make contact with the premises owners to identify those herds that contain test-eligible cattle. In addition, there are four farmed cervid operations within this zone, one of which is TB accredited. BOAH will test all herds with test eligible cattle and captive cervids in the 10-mile and river corridor zones within a 6-month time frame.

Wildlife Surveillance and Management

The IDNR has established two zones in which surveillance and management activities will be employed to detect and eliminate bovine TB in wildlife. Details of these activities can be found in the IDNR management plan titled *Bovine Tuberculosis Surveillance and Management in Franklin, Fayette, and Dearborn counties, 2016* and at: <http://www.in.gov/dnr/fishwild/9320.htm>. The management and surveillance zones are depicted in the attached map (Map 2) prepared by IDNR. In the Bovine Tuberculosis Management Zone, the primary activity will focus on reducing the prevalence of the disease by reducing the population of wild white-tailed deer. Specific population reduction activities include providing hunters additional opportunities to harvest deer, issuing permits to landowners to reduce the deer population on their properties, and utilization of Wildlife Services (WS) sharpshooters to remove additional deer from the affected and surrounding properties. Active management in the Bovine Tuberculosis Management Zone will begin immediately on the affected properties. Management activities will initially occur along the Whitewater River corridor from approximately the Fayette/Franklin county line to south of Metamora, which appears to most likely locations where affected white-tailed deer may be present. Surveillance activities will additionally be executed within the Bovine Tuberculosis Surveillance Zone. Sampling protocols have been redesigned in an attempt to detect bovine tuberculosis at lower prevalence rates. The IDNR will need to collect samples from between 350 and 1,100 deer, depending on sex and age class of the animal. While any age and sex of white-tailed deer can become infected with bovine TB, sampling bucks older than two years of age is more likely to detect the disease. A buck older than two years old equals about ten yearling bucks from a bovine TB surveillance perspective. The objective is to sample as many hunter-harvested bucks greater than two years old as possible and obtain the remaining samples with hunter-harvested does and younger bucks. To meet this objective, the following strategies will be employed:

- Mandatory check-in of deer will be required at IDNR Biological Check Stations on September 24 & 25, 2016 and from November 4 through November 27, 2016.
- Voluntary sample submission will occur October 1 through November 3, 2016 and December 3 through December 11, 2016.
- A second buck tag will be issued to anyone submitting a buck that meets the established requirements.