

## REPORT OF THE COMMITTEE ON PARASITIC DISEASES

Chair: Dee Ellis, TX

Vice Chair: David Winters, TX

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The Committee met on [Date] at the Sheraton Hotel in Kansas City, Missouri, from 8 am to 12 noon on October 22, 2014]. There were 16 members and 37 guests present.

### **Time-Specific Paper Title.**

None

### **Presentations & Reports**

The following Presentations were given

#### **1. Developing transgenic (genetically modified), male-only, screwworm strains by Steven R. Skoda USDA ARS**

Eradicating screwworms from mainland North America using the sterile insect technique is an unprecedented achievement saving livestock producers at least \$1.6 billion annually; reinfestation is prevented by maintaining a permanent barrier at the Panama – Colombia border. It has long been recognized that developing a genetic sexing, male-only strain has great potential for containing costs and improving the quality of released sterile males. The development of transgenic techniques for several insect species other than *Drosophila*, including screwworms, has excited interest toward genetically engineering repressible, lethal, female-specific genetic systems for screwworms. Five genetic sexing screwworm strains have been developed (via collaboration between ARS and North Carolina State University) - expressing single-component, late-acting, tetracycline repressible female lethal genetic system - and are being examined to determine their utility in the eradication program. We have measured several fitness parameters that influence production - biological yield, egg hatch, and longevity - and several lines are comparable with the parental strain. Along with other tests necessary to gain regulatory approval we will perform a proof-of-principle population reduction experiment in large field cage. We have also begun development of a two-component, early-acting, tetracycline repressible, female lethal genetic system. Early expression of tTA in the embryo leads to activation of expression of a cell death gene, which leads to death of the embryo. Only females die as the cell death gene contains the sex-specifically spliced NWS tra intron. This would lead to significant savings in diet costs (up to 50%, ≥\$500,000/year at current rearing levels) and/or increase the production capacity of the plant.

#### **2. Puerto Rico Fever Tick Research by Beto Perez DeLeon USDA ARS**

#### **3. Amblyomma Ticks in the US Virgin Islands by Francisco Collazo-Mattei USDA ARS**

On Nov. 6, 2013 an adult male *Amblyomma variegatum* tick was found on a routine visit to a premise located on the western end of the island of St. Croix. This premises had a previous history of TBT during prior infestations on the island. A total of number 105 animals have been quarantined in this single farm. Wildlife surveillance conducted by Southeastern Cooperative Wildlife Disease Study (SCWDS) found no TBT on mongoose trapped on the index premises and adjacent properties to the index premises. As of

October 2014 no new affected farms have been reported. The index farm is under treatment and continual surveillance as of Oct. 2014.

#### **4. US Equine Piroplasmosis Report by Angela Pelzel USDA VS**

Since November 2009, more than 268,604 domestic U.S. horses have been tested for equine piroplasmosis (EP) through active surveillance and movement testing with 143,372 horses tested at approved NAHLN laboratories and 125,232 horses tested at NVSL. To date, 247 EP-positive horses (237 *Theileria equi*-positive, 10 *Babesia caballi*-positive) have been identified through this surveillance. These positive horses are unrelated to the 2009-2010 *T. equi* outbreak on a Texas ranch where 413 positive horses were identified in connection with the outbreak and natural tick-borne transmission on the ranch was documented to have occurred over at least 20 years. Of the 247 positive horses identified through active surveillance, 198 were Quarter Horse racehorses, 13 were Thoroughbred racehorses, 1 was a Quarter Horse roping horse, 3 were identified during an illegal importation investigation, and 32 were horses previously imported to the United States before August 2005 under the complement fixation test. The epidemiology investigations conducted in all of these cases have indicated no evidence of tick-borne transmission and the cases in racehorses specifically have involved iatrogenic transmission as the method of spread.

So far in 2014, 22,395 domestic U.S. horses were tested for EP with the identification of 31 horses positive for *T. equi*. Twenty-five (25) of those positive horses were Quarter Horse racehorses with ties to unsanctioned racing and/or Mexico, 3 positives were identified during an investigation of horses illegally imported from Mexico, and 3 horses were previously imported from EP-endemic regions before the implementation of the cELISA import test in 2005. Additionally, of the 25 *T. equi*-positive Quarter Horse racehorses found, 8 of those horses were dually infected with both *T. equi* and equine infectious anemia (EIA). Epidemiology investigations conducted have implicated iatrogenic transmission (needle/syringe reuse, blood transfusions, contamination of multi-use drug vials, etc.) as the primary method of transmission in these cases. All EP-positive horses are placed under State quarantine and the horse owners are offered four options for long-term management under state/federal regulatory oversight: 1) life-time quarantine, 2) euthanasia, 3) export from the country, or 4) long-term quarantine with enrollment in the APHIS-VS and ARS treatment research program. In February 2013, APHIS-VS established a policy to release horses previously infected with *T. equi* which had completed the official treatment program, been proven cleared of the organism by a series of methods over time, and were test negative on all available diagnostics. Of the 247 positive horses identified, 154 have either died or been euthanized, 18 have been exported, and 41 have been enrolled in the treatment research program. Twenty-two (22) of the horses enrolled in the treatment program have met all of the test-negative requirements and have been released from quarantine. From the Texas ranch outbreak, 163 horses were enrolled in the treatment research program and have completed treatment with 132 horses having met all test-negative requirements and are eligible for release. Successful results from the treatment research program were previously reported by Ueti et al. in "Re-emergence of the Apicomplexan *Theileria equi* in the U.S.: Elimination of Persistent Infection and Transmission Risk" published in *PLoS One*, September 2012.

#### **5. Texas Equine Piro report by Andy Schwartz, Texas Animal Health Commission**

Following the diagnosis of Equine Piroplasmosis (EP) in 2009 on the Texas index premises, ranch management set a goal establishing a Remuda free of the disease. Positive horses, totaling 175, were treated with imidocarb dipropionate. Post-treatment Polymerase Chain Reaction (PCR) results were negative for all but six horses. These animals were treated again, and found negative on subsequent PCR tests. Treated horses and unaffected horses were commingled, and all horses were retested annually on cELISA, CF, and PCR. None of the apparently unaffected horses have seroconverted. Twenty three (23) of the treated horses remain positive on cELISA. Only 1 of the 175 treated horses reverted to PCR positive. The plan to speed

progress toward a test negative herd is selectively re-treating 8 horses, those with the highest titers on cELISA and those with a titer no longer diminishing over time.

Since 2009, a total of 121 EP positive horses have been disclosed in Texas, not related to the index ranch. Of this total, 36 positives were found through testing horses in Kenedy and Kleburg counties. Horses in these counties were deemed to be at high risk for EP due to the presence of *Amblyomma cajennense*, a tick proven to be a competent vector for the disease. Horses in additional counties in the southern tip of the state will be tested as resources allow.

The remaining 85 positive horses were disclosed through movement testing and epidemiological investigations. The majority of these positives, 73 in all, were racing Quarter Horses. These horses are thought to have been infected through the use of contaminated needles and blood products administered by trainers. Three were dually infected with the Equine Infectious Anemia virus.

To address the issue of EP risk in the racehorse population, the Texas Animal Health Commission established a rule in 2011 requiring a 12 month negative test for *Theileria equi* on horses entering race facilities. Proposed changes to this rule would require negative EP test at all horse racing venues, not limited to facilities licensed by the Texas Racing Commission. The Texas racing industry has requested Thoroughbreds be exempted from this requirement as only two positives have been found, both in 2010.

Horses being smuggled into Texas from Mexico remain a disease threat. In 2012, officials seized 10 adult horses and 4 yearlings. All the adults tested positive for EP. In 2014, four more adult horses were seized. Again, all were test positive for EP. Information gathered in the investigation led to the discovery of additional positive horses in California. The horses involved in this investigation were Spanish Purebred, indicating a possible increased disease risk in this breed.

## **6. Theileria equi Genotyping by Nita Grause USDA VS**

Drawing on previous experience in next-gen sequencing of other organisms, NVSL opted to use this approach to genotype *Theileria equi*. NVSL has successfully sequenced a cell culture derived isolate, and on the basis of that developed some “benchmarks” for sample preparation to optimize cost and efficiency of whole genome sequencing. Several challenges must be overcome, namely, concentrating *T. equi* organisms and/or depleting horse DNA in order to get sufficient target genetic material without overwhelming the system with host DNA. NVSL has archived approximately 600 blood samples previously positive by nested PCR to be sequenced in order to build a database. Diversity in sequences will be compared to epidemiological data and other published studies.

## **7. SCWDS Wildlife Sampling Report by Joe Corn, SCWDS**

Dr. Joseph Corn and Ms. Stacey Vigil, Southeastern Cooperative Wildlife Disease Study (SCWDS), University of Georgia, Athens, Georgia; and Dr. James Mertins, USDA-APHIS-National Veterinary Services Laboratories, Ames, Iowa, gave a report on SCWDS Exotic Arthropod Surveys. The SCWDS, in collaboration with the USDA-APHIS- VS, conducts surveys for exotic arthropods on free-ranging wildlife in the Southeastern United States and Caribbean region. Current and upcoming programs include surveys for the tropical bont tick on wildlife in St. Croix, U.S. Virgin Islands and Vieques, Puerto Rico; surveys for cattle fever ticks on small mammals and mesomammals in the Cattle Fever Tick Quarantine Area in Texas; surveys for *Culicoides* vectors of bluetongue virus and epizootic hemorrhagic disease virus in the Southeast United States; and surveys for cattle fever ticks on feral swine in the Cattle Fever Tick Quarantine Area. Recent surveys have not detected the tropical bont tick on wildlife in St. Croix, and have not detected cattle fever ticks on small mammals and mesomammals in Texas. Surveys for *Culicoides* have detected new state records for 11 *Culicoides* species in 15 states.

## 8. Cattle Tick Genome Sequencing Project by Felix Guerrero USDA ARS

The cattle tick, *Rhipicephalus (Boophilus) microplus*, is regarded as the most economically important ectoparasite of livestock worldwide. In addition to direct effects associated with its obligate blood feeding, *R. microplus* is an invasive species that also serves as vector of the pathogens that cause bovine babesiosis and anaplasmosis. *R. microplus* ranks 6<sup>th</sup> among the most pesticide resistant arthropods globally. With the belief that the tick's genome held the key to discovery of sustainable tick control technologies, we initiated the cattle tick genome sequencing project in 2003. Initial focus was upon determining the size and characterization of the genome and profiling the transcriptome. In 2005, the genome was discovered to be almost 3 times the size of the human genome, consisting of ~70% repetitive DNA. This essentially stalled the genome sequencing component of the project until long-read sequencing technologies then at the research stage commercially matured. From 2005-2009, we focused upon obtaining the transcriptomes of larvae, adult tick gut, ovary, synganglia, and Haller's Organ. In 2010, a reassociation kinetics-based approach was developed to remove the highly repetitive fraction of the genomic DNA, enriching for the unique and low copy fractions. Sequencing was performed on this fraction, adding to information on the transcriptome and the associated exon-intron structures. In 2012-2013, an approach was designed and implemented, using Pac Bio supplemented with Illumina reads of the Cot-selected genomic DNA, to complete the sequencing of the cattle tick genome to approximately a 10X coverage. A customized bioinformatic approach is presently underway to quality filter, error correct, and assemble the Sanger-, Illumina-, 454-, and Pac Bio-based sequence datasets. Our project enabled the application of reverse vaccinology to identify molecules for testing as candidate antigens to elicit a highly effective and protective immune response against *R. microplus* infestation in immunized animals. Vaccine discovery research efforts produced patented technology that is available for transfer and development by a commercial partner. USDA is an equal opportunity employer and provider.

## 9. Amblyomma Cajennense US Distribution by Angela Pelzel USDA VS

The Cayenne tick, *Amblyomma cajennense* Fabricius, is a widely distributed tick species found in many different ecosystems such as semi-arid grasslands and tropical humid deciduous forest. Infestations of cattle by these ticks can lead to weight loss, decreased milk production, starvation, and secondary infections. The Cayenne tick was proven to be a vector of *Theileria equi*, a causative agent of equine piroplasmiasis (EP), among horses based on experimental transmission studies. This tick is a common pest of humans in South America and in Texas. The Cayenne tick transmits Rocky Mountain spotted fever, human ehrlichiosis, Venezuelan equine encephalomyelitis virus, Q fever, and *Rickettsia amblyomii*. *Ehrlichia* species and *Borrelia lonestari* have been detected in ticks collected in southern Texas.

In response to detection of *T. equi* in horses on a ranch in southern Texas and identification of a potentially new tick vector of naturally-transmissible EP, we conducted this study of the Cayenne tick (Appendix Figure 01). The goals of our study were to: (1) define the current geographical distribution of the Cayenne tick, both globally and in the United States, (2) define suitable habitat for the Cayenne tick in the United States beyond its current distribution in southern Texas, and (3) evaluate the spatial relationship between habitat in the United States that supports the Cayenne tick and the locations of various animal populations that are suitable hosts of the tick. Our working hypothesis was stated as follows: "Suitable habitat for the Cayenne tick does not extend above 30 degrees north latitude in the continental United States (Appendix Figure 02). We acquired tick identification datasets from veterinary diagnostic laboratories, and queried a tick geodatabase created by USDA-APHIS-VS for Cayenne tick identification records. We also acquired Cayenne tick data sets from the Texas Veterinary Medical Diagnostic Laboratory (TVMDL) and from the Southwestern Cooperative Wildlife Disease Survey (SCWDS). A spatial overlay analysis was

used to combine habitat data layers and create a map that classified U.S. habitat into three mutually exclusive classes: (1) high suitability, (2) moderate suitability, and (3) low suitability.

There were a total of 9,994 records in the tick geodatabase for which the submitted specimen had been identified as the Cayenne tick. The TVMDL shared 234 Cayenne tick records for inclusion in the tick geodatabase, and we received seven Cayenne tick records from the SCWDS Research Group. The regions classified as "high suitability" covered large portions of the coastal-southern United States and included the States of South Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana and Texas. Some regions in California and Arizona also were classified as "high suitability." There was substantial overlap between high suitability habitat in southern States and most counties in those States that are populated by high numbers of horses and beef cattle.

We concluded that suitable habitat for the Cayenne tick does extend above 30 degrees north latitude in the continental United States. The Cayenne tick species already appears to be established in southern Texas, and establishment of the Cayenne tick beyond its current range in southern Texas appears plausible. The overlap of economically important livestock populations and high suitability habitat should raise concerns of Cayenne tick's potential to transmit disease to livestock populations.

#### **10. Vesicular Stomatitis National Report by Angela Pelzel USDA VS**

A summary of the ongoing 2014 vesicular stomatitis (VS) outbreak in Texas and Colorado was presented including background on the disease, statistics on the current situation, and next steps for determining future management of the disease in the U.S. in light of OIE's 2014 decision to remove the disease from the list of immediately notifiable animal diseases. To date, a total of three hundred eighty-eight (388) VSV-positive premises have been identified in two U.S. states, Colorado (326 premises) and Texas (62 premises). There have been 14 counties affected in Colorado (Adams, Arapahoe, Boulder, Broomfield, Douglas, El Paso, Fremont, Jefferson, Larimer, Logan, Morgan, Otero, Pueblo, and Weld Counties) and 13 counties affected in Texas (Bastrop, Falls, Guadalupe, Hidalgo, Jim Wells, Kinney, Lee, McLennan, Nueces, San Patricio, Travis, Val Verde, and Williamson Counties). Of the 388 total VSV-positive premises, 370 have been positive equine premises, 16 have been positive bovine premises, and 2 premises have had both cattle and horses positive. Positive premises are eligible for quarantine release 21 days after lesions have healed in all affected animals. At the time of this meeting, two hundred sixty-one (261) premises in Colorado have been released from quarantine and there are an additional forty-one (41) premises in Colorado on 21-day countdown to quarantine release. As of October 13, 2014, all confirmed VSV-positive premises in Texas have been released from quarantine. Weekly situation reports and maps from the incident are publically available on the USDA-APHIS website.

#### **11. Texas Vesicular Stomatitis Report by TR Lansford, Texas Animal Health Commission**

The 2104 national index case of vesicular stomatitis virus infection (VSV) was diagnosed in equine in Kinney County, Texas, on May 23, 2014. Historical VSV outbreaks have begun in southwest Texas and progress northward and westward across Texas and into other western states. The 2014 VSV outbreak did not follow that pattern. The next cases of VSV were disclosed more than 300 miles away in the Lower Rio Grande River Valley of Texas, followed by cases located west northwest of Corpus Christi. The outbreak progressed into central Texas with the majority of the cases occurring just east of Austin in Bastrop and Travis counties. The final infected case was diagnosed on August 28. The final quarantine was released on October 13, 2014. In total, 13 Texas counties had positive premises during the 2014 outbreak.

Texas Animal Health Commission (TAHC) and United States Department of Agriculture-Veterinary Services (USDA-VS) Foreign Animal Disease Diagnosticians (FADDs) investigated 126 cases of vesicular lesions since May 23, 2014. Investigations were performed not only on equine, but cattle, sheep, goats, and hogs as well. Sixty-two (62) premises were found to have animals infected with

VSV. Of the 62 premises, 58 were found to have infected equine and 4 were found to have infected cattle. A total of 89 horses and 8 head of cattle were disclosed as a result of these investigations.

## 12. Colorado Vesicular Stomatitis Report by Keith Roehr Colorado Dept. of Agriculture

### Colorado Vesicular Stomatitis Report

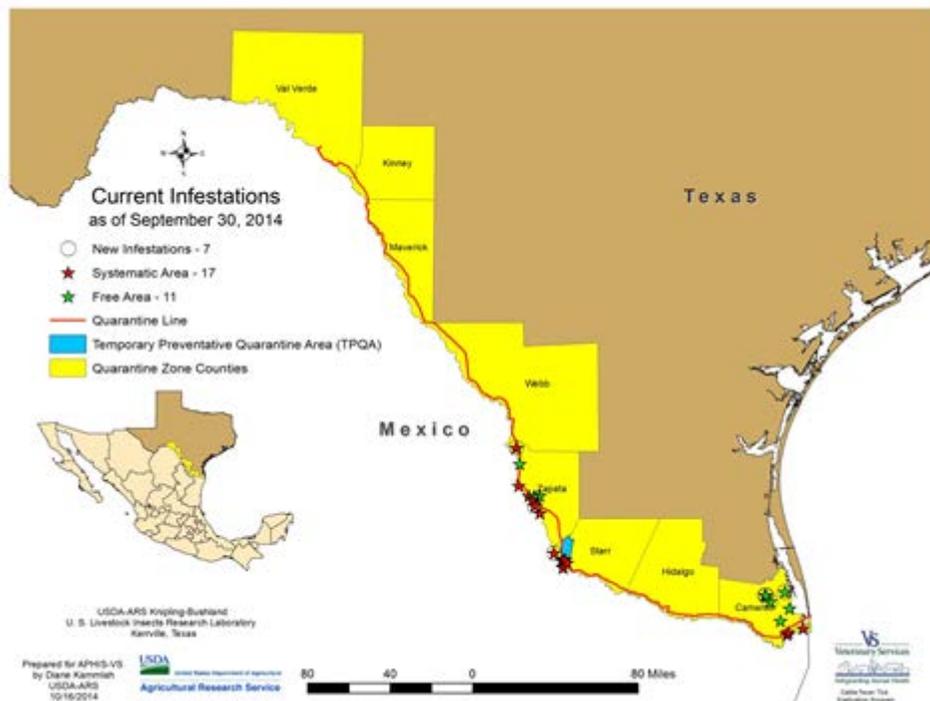
Vesicular Stomatitis in Colorado is a cyclically, endemic, foreign animal disease. This report provides epidemiologic information on the Colorado 2014 Vesicular Stomatitis Outbreak. Included are notable variations in clinical signs of disease in confirmed cases, geographic distribution, reports on case numbers and species affected. In light of the potential delisting of Vesicular Stomatitis in 2015, I have shared some generalized thoughts in potential changes in the focus of state's equine response:

- Focus on accredited veterinarians completing in equine field case work
- Positive cases would still be quarantined
- Cost of testing could be borne by the horse owner
- CSU could be approved to do horse case testing, FADDL still test bovine cases

## 13. USDA/ARS KBUSLIRL Tick/Biting Fly Research by Beto Perez de Leon, USDA ARS

Dr. Perez DeLeon provided an overview of all research activities at the USDA/ARS KBSUSLIRL Research Center headquartered in Kerrville, Texas.

## 14. Texas Cattle Fever Tick Eradication Program update by Kevin Varner, USDA VS



The Texas Cattle Fever Tick Eradication Program (CFTEP) reports a total of 11 Infested premises in the Free Area of Texas and 17 infested premises in the Permanent Quarantine Zone on 09/30/2014. The tick activity level varies along the length of the Permanent Quarantine Zone. In recent years the bulk of the new infestations have been found in Starr and Zapata counties. In May of 2014 an infested pasture was discovered in the Free Area of Cameron County. This 6000 acre pasture is leased land owned by the Brownsville Ship Channel. Adjacent to this property is the Bahia Grande Fish and Wildlife Services (FWS) Wildlife Refuge.

Later in the summer of 2014, two Cameron County cattle owners reported suspected fever tick infestations on their properties. The CFTEP confirmed fever tick infestations at both locations. By late summer 2014 fever ticks had been identified in the free area of Cameron County at three locations---near the Quarantine zone, 4 miles north and 8 miles north of the Quarantine zone. By the end of FY 2014 a 220,000 acre temporary blanket quarantine had been established and manned by a joint TAHC and USDA Task Force. An additional three infested pastures were identified by 30 September 2014.

Early speculation is that free-ranging exotic Nilgai Antelope are using the FWS Wildlife refuges to move from infested areas in Mexico into the free areas of Texas.

**Additionally in FY 2014:**

---The CFTEP worked with USDA, State and Industry partners to develop and deploy a tick vaccine in the Permanent Quarantine Zone. The vaccine project was significantly delayed by residual adjuvant droplets found at the necropsy of the animals used in the pen test. The vaccine was reformulated with a water-based adjuvant and was scheduled for a field safety trial early in FY 2015.

**15. Cameron County Temporary Fever Tick Quarantine update by Dee Ellis, TAHC**

The Cameron County Temporary Quarantine Zone (blanket) was created October 8, in far S. Texas. There are currently 6 infested premises in the blanket zone which is in the eastern part of Cameron County north of the permanent quarantine zone which borders the Brownsville ship channel. The blanket zone comprises approximately 223, 000 acres and runs to the north up to Willacy County and is bordered on the east by the Laguna Madre. There are 2 newly infested premises found in October just outside the northern border of the blanket and the pastures will range into southern Willacy County. Epidemiologic studies are underway to determine the risk of fever ticks into the "Free area" in portions of Cameron and Willacy County which may ultimately require amendment or enlargement of the blanket boundaries. Free ranging nilgai are considered to be part of the problem and more studies are needed related to the biology of the species, its range and possible treatment options in the wild. An Incident Command system of organization is being used, and is being managed by USDA VS and TAHC personnel in partnership to accommodate necessary movements of horses and cattle, servicing hunter surveillance 24/7 and evaluating all premises with susceptible species within the blanket.

**Committee Business:**

There were no resolutions considered. One recommendation was proposed and passed in support of the creation of a new USAHA Bovine Trichomoniasis committee and also creation of a supporting AAVLD Scientific Advisory Committee. The text of the "Recommendation" passed in entirety is included below.

**Creation of a USAHA Committee on Trichomoniasis in Cattle**

*Trichomonas foetus* is an obligate parasite of the bovine reproductive tract that causes a highly contagious venereal disease with significant economic impact to the cattle industry. The importance of the disease is reflected by the dramatic increase in the number of states that have recently developed Trichomoniasis regulatory programs.

Effectively addressing Trichomoniasis in the cattle industry requires a national forum for sharing information and developing best management plans. The creation of a USAHA committee where cattle

producers can work together with members of the scientific community as well as state and federal animal health officials to solve the problems faced by the industry is critical.

The Committee must contain a strong Scientific Advisory Subcommittee supported by the AAVLD.

**Mission Statement**

The purpose of the Trichomoniasis committee is:

- 1) Discuss scientific, laboratory, regulatory, commerce and political issues regarding T fetus and its effect on the cattle industry.
- 2) Evaluate interstate and intrastate regulatory issues.
- 3) Recommend effective disease control and management programs.

Addendums to the committee report should be in the following order:

Subcommittee Reports - none

Time-Specific Papers- none

Other Presentations/Papers - none

Supplemental information - none

END OF REPORT