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The Committee met on October 16, 2020, virtually, from 2:30 to 4:40 p.m. There were [x] members and [x] guests present.

Presentations and Reports

Addressing the Challenges of Emerging Vector-Borne Diseases in the United States
C. Ben Beard, Center for Disease Control and Prevention (CDC)

This presentation addresses four primary topics: 1) the burden and trends for vector-borne diseases (VBDs) in the U.S., 2) factors that are influencing VBD emergence, 3) the challenges for effective prevention and control, and 4) CDC's plans for addressing VBD concerns in the U.S.

Between 2004 and 2018, more than 760,000 cases of VBDs were reported in the U.S. The number of annual reported cases of disease from mosquito, tick, and flea bites has doubled. Tick-borne diseases accounted for over 75% of reported VBD cases, and mosquito-borne disease epidemics happen more

frequently. The reported data substantially underestimate actual disease occurrence (8 to 70-fold depending on the disease).

Vector-borne disease emergence in the U.S. has been influenced by a number of factors including 1) global travel [i.e. frequency and range of movement of infected humans], 2) poverty, living conditions, and crowding, 3) a large human population that is susceptible to exotic disease agents, 4) limited public health resources at all levels for detecting and responding to local disease outbreaks, and 5) a changing climate and ecosystem, which can alter the incidence and distribution of disease pathogens and vectors. These factors collectively contribute to the emergence and re-emergence of disease agents carried by mosquitoes and ticks.

Local and state health departments and vector control organizations face increasing demands to respond to these threats. More than 80% of vector control organizations report needing improvement in one or more of five core competencies, such as testing for pesticide resistance. More proven and publicly accepted mosquito and tick control methods are needed to prevent and control these diseases.

In summary and conclusion, VBDs are increasing in the U.S., both in incidence and in distribution. The factors that are driving VBD introduction and emergence vary among diseases but are not likely to cease, indicating that current trends will continue and likely worsen. There are a number of challenges to preventing VBDs, including the lack of vaccines and effective vector control tools, insecticide resistance, and eroding technical capacities in public health entomology at federal, state and local levels. CDC is working with other U.S. federal agencies to develop a national strategy to address VBD threats and to reverse the alarming trend in morbidity and mortality associated with these diseases.

USDA-APHIS-VS Dun & Bradstreet (D&B), National Veterinary Services Laboratories (NVSL) Bluetongue Virus (BTV) and Epizootic Hemorrhagic Disease Virus (EHDV) Isolations/PCR Positives-Calendar year 2019

Albert van Geelen, USDA-APHIS-NVSL

During calendar year 2019, BTV or Ribonucleic acid (RNA) was detected and typed in 29 samples or collected from four states, while EHDV or RNA was detected and typed in 21 samples from seven states. Individual results are listed in tables 1 and 2.

Table 1. Bluetongue virus (BTV) polymerase chain reaction (PCR) positives, calendar year 2019

State	Serotype	Species	Number	Virus Isolation
FL	BTV-1	cattle	1	N
FL	BTV-6	goat	1	Y
CA	BTV-13	cattle	6	N
FL	BTV-15	Cattle1	1	Y
CA	BTV-17	White tail deer	1	N
CA	BTV-17	Sheep	1	N
CA	BTV-17	cattle	1	N
CA	BTV-17	sheep	1	N
TX	BTV-17	Cattle2	1	N
IA	BTV-17	cattle	1	N
CA	BTV-17	sheep	1	N
FL	BTV-18	white tail deer3	1	Y
FL	BTV-18	cattle	3	Y
FL	BTV-19	Cattle4	2	Y
FL	BTV-24	white tail deer5	1	Y

1) Co-infected in herd with BTV-19, first time detected in U.S.
2) Co-infected with Epizootic hemorrhagic disease virus [EHDV] (not typed)
3) Isolated and submitted by Dr. Stallknecht from University of Georgia (UGA)

4) Co-infection in herd with BTV-15
5) Isolated and submitted by Dr. Stallknecht from UGA.

Table 2. EHDV: Epizootic Hemorrhagic Disease virus (EHDV) PCR positives, calendar year 2019

State	Serotype	Species	number	Isolate
CA	EHDV-2	cattle	**1**	**N**
IA	EHDV-2	White tail deer	12	N
MN	EHDV-2	White tail deer	13	N
NE	EHDV-2	sheep	1	N
OR	EHDV-2	White tail deer	1	N
IA	EHDV-6	cattle	1	N

Partial-year 2020 data for NVSL Orbivirus identifications is shown in Tables 3 and 4. As of October 1, 2020, BTV has been identified and typed in six samples from three states; EHDV has been identified in three samples from one state.

Table 3. Bluetongue virus (BTV) PCR positives during Calendar year 2020 (January 1 through September)

State	Serotype	Species	Number	Virus Isolation
IA	BTV-11	cattle	1	N
TX	BTV-11	cattle	1	N
TX	BTV-13	Cattle[1]	1	N
TX	BTV-17	Cattle[2]	1	N
TX	BTV-11	White tail deer	1	N
WI	BTV-11	cattle	1	Y

1) Co-infected with BTV-17
2) Co-infected with BTV-13 and EHDV (not typed)

Table 4. EHDV PCR positives during Calendar year 2020 (January 1 through September)

State	Serotype	Species	Number	Virus Isolation
MN	EHDV-6	reindeer (zoo)	**3**	**Y**

Vesicular Stomatitis/Equine Infectious Anemia/Equine Piroplasmosis
Angela Pelzel-McCluskey, USDA-APHIS-Veterinary Services (VS)
2019 and 2020 Vesicular Stomatitis Outbreaks

The 2019 vesicular stomatitis virus (VSV) outbreak in the United States was the largest in the past 40+ years of recorded history. The outbreak was entirely VSV-Indiana serotype, which hadn't been isolated in the U.S. since 1997-1998, it lasted from June 21 to December 27, 2019, and included 1,144 affected premises in eight states (Colorado, Kansas, Nebraska, New Mexico, Oklahoma, Texas, Utah, and Wyoming). Of the total affected premises, 1,128 premises had only equine species clinically affected, 15 premises had only clinically affected cattle, and one premises had both equids and cattle with clinical signs. Given the size and scope of the 2019 outbreak, it was expected that overwintering of the virus would occur and that new cases were likely to appear in the historically affected southwestern and Rocky Mountain region states beginning in the spring of 2020.

On April 13, 2020, the National Veterinary Services Laboratories in Ames, Iowa, confirmed a finding of VSV infection (Indiana serotype) on an equine premises in Dona Ana County, New Mexico. This was the index case of VSV for the 2020 outbreak and for the state of New Mexico. As the outbreak progressed, seven additional states became confirmed as VSV-affected: Arizona on April 22, Texas on

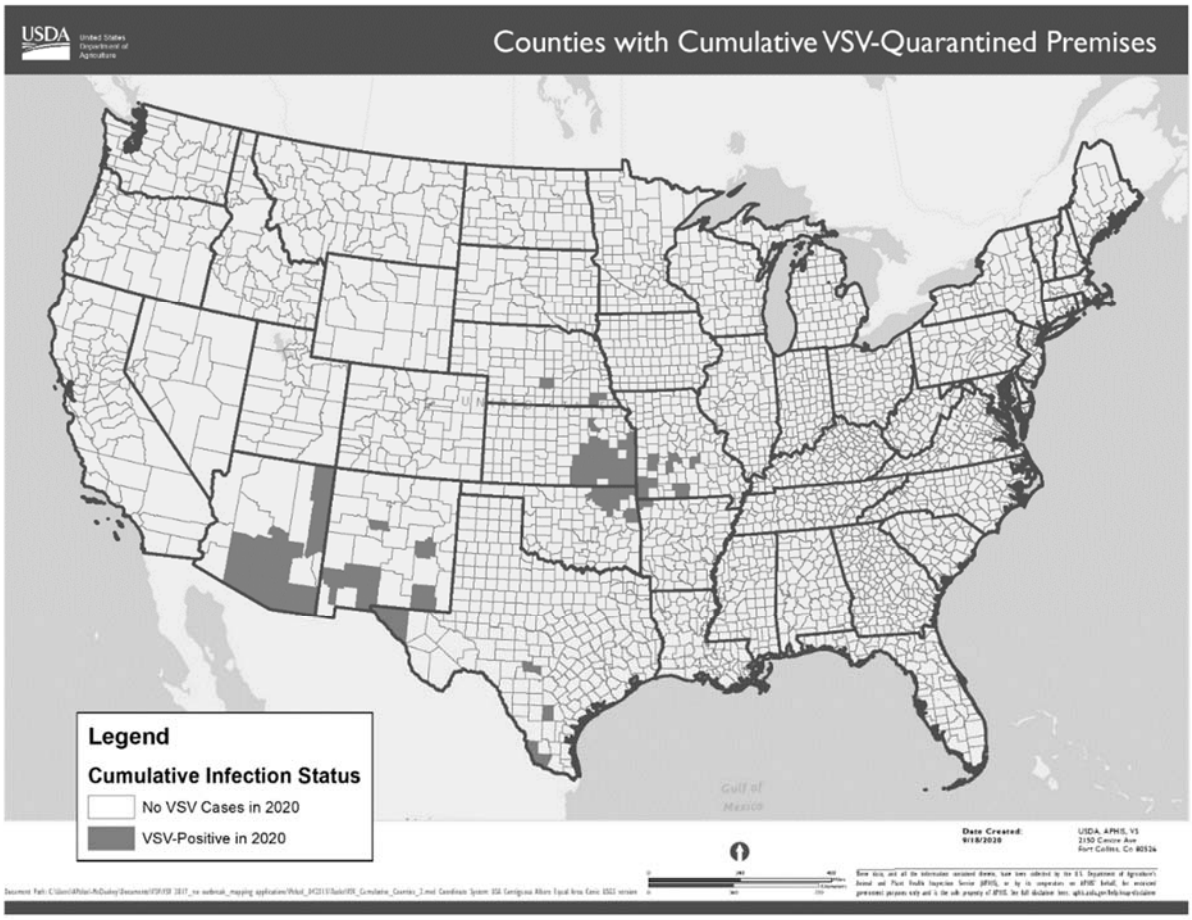
April 23, Kansas on June 16, Nebraska on June 24, Oklahoma on July 7, Missouri on July 13, and Arkansas on July 27, 2020. A total of 325 premises in these eight states have been either suspected or confirmed as VSV-infected during the outbreak to date and placed under state quarantine. Quarantines remain for a period of 14 days from the onset of lesions in the last affected animal on the premises and vector mitigation strategies and enhanced biosecurity procedures are recommended on quarantined premises to reduce within-herd spread of the disease.

The breakdown of the number of quarantined premises and affected counties by state for the VSV 2020 outbreak are shown in Table 1 below and the distribution of affected premises is shown in Figure 1.

Table 1. Total number of VSV-affected premises by state as of September 24, 2020

State	# Counties Positive	# Confirmed Positive Premises	# Suspect Premises	Total # Premises Quarantined
Arizona	7	18	1	19
Arkansas	1	4	0	4
Kansas	26	101	95	196
Missouri	11	36	17	53
Nebraska	3	5	0	5
New Mexico	6	13	3	16
Oklahoma	9	18	4	22
Texas	6	10	0	10
TOTAL:	69	205	120	325

Figure 1. Cumulative map of VSV-affected counties: April 13 – Sept 24, 2020



Of the 325 VSV-affected premises identified, 312 premises have had only equine species clinically affected, 12 premises have had only cattle clinically affected, and one premises has had both equine and cattle clinically affected. At the time of this writing, all 325 VSV-affected premises have completed the quarantine period and been released. Surveillance for additional cases potentially associated with this outbreak are ongoing.

While the overwintering event and identification of new VSV-Indiana positive cases were expected in 2020, there were several unusual occurrences associated with this outbreak that were not predicted. Firstly, in addition to the VSV-Indiana cases that occurred in New Mexico, Arizona, and far west Texas in April/May 2020, a new incursion of VSV-New Jersey virus from Mexico simultaneously appeared in south Texas and continued northward as far as south-central Texas affecting seven premises in four counties. An outbreak involving both VSV-Indiana and VSV-New Jersey serotypes concurrently had not been seen in the U.S. since 1997-1998. Secondly, the expected continuation of the VSV-Indiana outbreak from 2019 in the Rocky Mountain region (Colorado, Utah, and Wyoming) never materialized in 2020. There were some severe drought indicators that presented in this region in late spring and early summer which may have had a significantly negative impact on the VSV-competent vector populations, but further study is needed to evaluate the climate variables that may have played a role. Finally, the appearance of an outbreak cluster in the Kansas, Missouri, Oklahoma, and Arkansas region was not expected and VSV cases this far east had not been seen since the 1930s.

Analysis of these abnormalities along with other variables involved in the 2020 outbreak are planned by the VSV Grand Challenge Team, a multidisciplinary group sponsored by USDA-Agricultural Research Service (ARS) and involving four different ARS research hubs and APHIS-VS. This team, established in 2015, explores climatic, ecological, hydrological, virus, vector, host, and epidemiological variables that drive VSV incursion and expansion in the U.S. with the goal of establishing reliable predictive information on disease transmission and outbreak scope to support the state/federal field response. The team is

currently producing several peer-reviewed publications per year that capture and share the research results.

Complete situation reports for the 2019 and 2020 VSV outbreaks can be accessed on the USDA-APHIS website at the following link: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/cattle-disease-information/vesicular-stomatitis-info

Update on Equine Piroplasmosis (EP) and Equine Infectious Anemia (EIA)

In calendar year 2019, there were 31,391 domestic U.S. horses tested for equine piroplasmosis (EP) as part of active ongoing surveillance with much of the testing focused on the previously identified high-risk groups of sanctioned and unsanctioned Quarter Horse racehorses where iatrogenic transmission of the disease is well recognized. A total of 72 horses were found to be infected with *Theileria equi* during this time period in seven states. All 72 horses were Quarter Horse racehorses with iatrogenic transmission either confirmed or suspected to have been the cause of spread and 14 of these horses were co-infected with equine infectious anemia (EIA). Eleven (11) of the 14 co-infected EP/EIA horses were epidemiologically linked to a single racehorse trainer whose unhygienic practices of needle, syringe, and IV set re-use were determined to have caused spread within the group.

More than 17,000 U.S. horses have been tested for EP so far during the 2020 calendar year (testing numbers current through June 2020) with 19 *T. equi*-positive horses found in six states as of September 30, 2020. Eighteen (18) of the EP-positives are current or former Quarter Horse racehorses with iatrogenic transmission of the disease either suspected or confirmed. One (1) positive horse was an Arabian stallion with a life-long history of ownership by several unsanctioned racing participants in two states and it is suspected the stallion may have been used previously in this population as a blood donor horse for blood doping the racehorses. The common practice in this population of reusing a single IV blood set for blood doping often leads to blood-borne disease spread not only to the blood recipient horses but also back to the donor horse. Two (2) of these 19 EP-positive horses were found to be co-infected with EIA. The horses that were co-infected with both EP and EIA have been euthanized and many of the remaining EP-positive horses have been enrolled in the USDA-APHIS EP Treatment Program. All EP-positive horses will remain quarantined until permanent clearance of *T. equi* through high-dose imidocarb dipropionate treatment is achieved and the horse maintains *T. equi*-negative status on all diagnostic testing. To date, there have been 365 horses treated in the U.S. for EP with 320 horses having met the clearance and test negative criteria for quarantine release.

In calendar year 2019, a total of 1,151,584 EIA tests were conducted in the U.S. with 89 horses confirmed as EIA-positive in 17 states. At least 75 of the 89 EIA cases occurred in Quarter Horse racehorses with iatrogenic transmission either suspected or confirmed to have been the source of spread in those cases. So far in 2020, there have been at least 853,000 EIA tests performed in the U.S. (January-July 2020 reported test data) with 22 EIA cases confirmed in five states as of September 30, 2020. Nineteen (19) of the 22 EIA positives occurred in Quarter Horse racehorses with iatrogenic transmission of the disease either suspected or confirmed. Many of the EIA-positive horses were found to be participating in unsanctioned racing. The EIA cases identified in 2019 and 2020 further highlight our recognition of a recent shift in the epidemiology of EIA in the U.S. While prior to 2017, many of the EIA cases were found to be in untested or under-tested equine populations where natural vector-borne transmission of the disease had occurred over time, since 2017 the majority of the EIA cases are now being found in Quarter Horse racehorses with iatrogenic transmission involved. Iatrogenic transmission of EIA is a preventable occurrence and targeted educational outreach is needed in these high-risk populations to reduce the incidence of EIA.

West Nile Virus (WNV) and Eastern Equine Encephalitis (EEE)

Equine case counts for WNV and EEE are sourced from the CDC's ArboNET database and summarized by APHIS-VS in consultation with state animal health officials. Annual reports for each disease are compiled by calendar year and more current case counts during the active vector season are posted bi-weekly to the APHIS website. This information can be accessed at the following links:
For WNV information: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/equine/wnv
For EEE information: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/equine/eee-wee-vee

In calendar year 2019, there were 90 equine WNV cases identified in 25 states. So far in 2020, there have been only 20 equine WNV cases identified in four states as of September 9, 2020. For EEE, there were 184 equine cases reported in 24 states in calendar year 2019 and in 2020 a total of 77 cases in ten

states have been reported as of September 9, 2020. Delays in reporting equine arboviral cases in ArboNET are routinely recognized and may be magnified this year due to the public health community's necessary prioritization of response to COVID-19.

The 2019 EEE case count in equids, while elevated, did not set any historic high records, however there were several observations surrounding EEE infections in 2019 that raised concerns both in the veterinary and human medical communities. Firstly, there were a record-setting number of human EEE infections reported in 2019; a total of 38 human cases in ten states with 15 fatalities. The number of human EEE cases across the years 2009-2018 had an average of seven cases per year recorded with the highest case count in a single year being 15 cases in 2012. Another unexplained observation was that for the first time in history, the ratio of equine WNV cases to equine EEE cases was inverted. In previous years, equine WNV cases usually outnumber equine EEE cases 2:1. In 2019, the number of EEE cases was double that of WNV in equids. Finally, the number of EEE cases confirmed in alternate and wildlife species had not been recognized at such a high level and with so many species of animals represented as were reported in 2019. These anomalies for EEE in 2019 have yet to be explained and there is concern that 2020 could also be an unusually active year for EEE infection in all species.

Cattle Fever and Asian Longhorned Ticks in the U.S. 2020

Denise L. Bonilla, USDA-APHIS-VS

Cattle Fever Ticks (*Rhipicephalus* (*Boophilus*) *microplus* and *R.b. annulatus*)(CFT) in 2020

Cattle Fever Tick Eradication Program (CFTEP) Workforce: increase of 31% = two Veterinary Medical Officers, one Epidemiologist, one Field Operations Supervisor, three Supervisory Mounted Patrol Inspectors, six Mounted Patrol Inspectors, one Administrative Officer, ten Program Assistants Infestations: Total premises needing to be checked is up in 2020 (3,360) compared to 2019 (2,835). New infested premises are about the same as last year. Notably this year, the program saw a 200% increase in native exposures in the free area. In the free area, Cameron County went from 40 to 57 premises and Zapata (47) and Webb (27) are slightly down from last year. Willacy County is high (25) holding steady from last year.

Research: Through omnibus and other funding over the past few years, we have been able to help fund 17 projects with Agricultural Research Service (ARS) (NP 103 and 104) as primary cooperators and then ten other projects with non-ARS primary cooperators. These span general categories of treatment, wildlife, prevention, population genetics, surveillance, and detection with many projects spanning multiple categories. In 2020, CFTEP was able to help start projects that 1) examine animal/tick feces chemistry for CFT detection; 2) using weather stations for CFT outbreak predictions; 3) efficacy of BM86 vaccine in cattle in Texas and 4) lavender oil as a repellant/treatment for horses.

Asian Longhorned Ticks (*Haemaphysalis longcornis*)(ALHT)

New states positive in 2020: Ohio, Rhode Island and South Carolina. New hosts are black bear, gray squirrel, brown booby, great horned owl, and Peromyscus mice.

Theileria orientalis Ikeda: Has been shown to be vectored by ALHT in other parts of the world. Researchers at Virginia Veterinary Medicine School report at least 25 Virginia and four West Virginia counties with T. orientalis Ikeda. In Virginia, there is a site that has positive ALHT in the environment and sick cows but there was no direct link. ARS Pullman was recently able to show that our U.S. ALHT vectors a U.S. strain of T. orientalis Ikeda to calves in the laboratory. This concretes the role of ALHT as vectors of T. orientalis Ikeda here in the U.S.

Red Sheep Tick (*Haemaphysalis punctata*)

Through our ALHT network, Columbia University and USDA, NVSL were able to detect another exotic tick, *Haemaphysalis punctata*, the red sheep tick from Block Island, Washington County, Rhode Island. After going through their archives, Columbia found several environmental samples back to 2010. This tick can vector Babesia, Rickettsia, and Theileria in its native range. Unlike ALHT, it is non pathenogenetic. It is a three-host tick that as an adult likes feeding on cattle, horses, goats, sheep and medium size mammals. As an immature it likes to feed on birds, rodents, and lizards. It's not been detected off of the island of this time and VS will continue work with Colombia University and others to monitor the situation.

Cattle Fever Ticks: Rio Bravo Buffer Zone

Andy Schwartz, Texas Animal Health Commission

Since 1893, the U.S. has fought to eradicate and then prevent re-incursion of cattle fever ticks (CFT), vectors of Bovine babesiosis. By 1943, CFT were pushed back to the Texas-Mexico border, and a 500-

mile-long buffer zone was established in Texas. Considerable industry, state, and federal resources are expended annually in detecting and subsequently eradicating CFT that are introduced from Mexico largely by stray cattle and horses, and wildlife species that include white-tailed deer and nilgai antelope. The concept of the Rio Bravo Buffer Zone (RBBZ) was developed to address these CFT incursions by establishing a buffer zone in Mexico that mirrors the longstanding buffer zone in Texas. Establishing the RBBZ is a cooperative effort supported by industry, state, and federal representatives in both the United States and Mexico. A steering committee was established in March 2020, with representatives from Texas Animal Health Commission (TAHC), Animal and Plant Health Inspection Service (APHIS), Development, Fisheries and Food (SENASICA), National Confederation of Livestock Unions, Mexico (CNOG), Coahuila, Nuevo Leon, and Tamaulipas. Upon request, the states of Tamaulipas, Nuevo Leon, and Coahuila prepared budget proposals for establishing buffer zones in their respective states. APHIS did not support these proposals but did indicate support for pilot projects in these states. Efforts are now focused on establishing two cooperatively funded CFT eradication pilot projects, one in Tamaulipas and one in Coahuila. The pilot projects will be supported through Texas state funds and are scheduled to be conducted March 1-July 31, 2021.

ABADRU – Vector-Borne Disease Research

Research Updates from the Arthropod-Borne Animal Diseases Research Unit

Barbara S. Drolet, Lee Cohnstaedt, Bethany McGregor, Dana Mitzel, Dana Nayduch, Leela Noronha, William Wilson, USDA, Agricultural Research Service (ARS)

The Arthropod-Borne Animal Diseases Research Unit (ABADRU) has an interdisciplinary group of researchers working on a variety of viruses and vectors. Our research mission is to explore ways to detect and prevent emerging, transboundary arthropod-transmitted diseases of livestock. With expertise in microbiology, entomology, and veterinary science, we attack these arthropod-borne diseases on all three fronts. Specific diseases include Japanese encephalitis virus (JEV), Rift Valley fever virus (RVFV); epizootic hemorrhagic disease virus (EHDV), bluetongue virus (BTV), vesicular stomatitis virus (VSV), bacterial pathogens carried by house flies, and many aspects of vectors including vector biology, field ecology, surveillance, and pest management for mosquitoes and midges.

ABADRU's JEV research is led by Drs. Leela Noronha and Dana Mitzel. Endemic to Asia and Oceana, the Flavivirus, JEV, is closely related to West Nile virus and Saint Louis encephalitis virus and is transmitted by *Culex* mosquitoes. Mosquitoes can transmit JEV to humans causing almost 70,000 cases of encephalitis every year with fifty percent of those cases having significant lifelong neurological problems. In swine it results in abortion, still births, and birth defects. Infection in horses results in neurological disease. It is a foreign animal disease threat to the U.S., as we have susceptible animals and competent mosquito vectors. The virus is maintained in a cycle between mosquitoes and vertebrate hosts, primarily wading shore birds, and in pigs which greatly amplify the virus. Humans, cattle, and horses are incidental or dead-end hosts, because they usually do not develop high enough concentrations of JEV in their bloodstreams to infect feeding mosquitoes. Very little is known about how JEV is maintained in mosquitoes. This is key to understanding competence of U.S. mosquito species and the potential JEV could become established as West Nile virus did, once introduced. In most of Asia, the primary vector is *Culex tritaeniorhynchus.* Two U.S. mosquito species that have been shown to be competent for JEV are *Culex quinquefasciatus* and *Culex tarsalis.* We need to better understand differential infectivity of various mosquito species and determine strain selectivity and dominance in regions of new virus introductions with stable transmission. Toward those goals, a *Culex tarsalis* continuous cell line has been developed. These cells were shown to be susceptible to JEV infection by immunofluorescence and growth kinetics studies have been conducted. This new cell line will be instrumental helping to identify factors that affect JEV infection in mosquitoes.

The JEV team are also doing research to identify factors associated with JEV maintenance in swine. The overarching goals are to characterize susceptibility, pathogenesis, and disease dynamics in domestic and feral swine. Additionally, vector-host interactions associated with JEV transmission will be characterized. In the near-term, research is being conducted to identify and characterize a surrogate system to study JEV at a BSL-2 level, and to test target tissue cell lines and macrophage-like cell lines for susceptibility. The relative susceptibility and viral growth of various porcine target tissue cell lines has been compared to baby hamster kidney (BHK) cells. BHK cells are typically used to grow JEV, but hamsters are not a relevant host species. Establishing infection kinetics in cultured pig cells will help refine the questions to pursue *in vivo* studies in pigs. Peripheral macrophages and dendritic cells of pigs

also support JEV replication. This ability plays an important role in viremia and the ability of this virus to breach the blood brain barrier by transcellular transport into the central nervous system. A porcine macrophage-like cell line has been tested for susceptibility and viral growth compared to BHK cells. These cells are species and tissue relevant and will be extremely helpful in understanding the infection characteristics of JEV and the innate immune responses of pigs to infection.

ABADRU's RVFV research is led by Dr. William Wilson. Endemic to Africa, Rift Valley fever is mosquito-borne disease of domestic and wild ruminants, causing high mortality in newborn calves, lambs, and goats, and high abortion rates in sheep. Zoonotic transmission to humans is typically through blood, tissues, or raw milk of infected animals. There are limited vaccines available in Africa and there are no fully licensed vaccines or commercial diagnostics in the U.S. It is a foreign animal disease threat to U.S., as we have susceptible animals and competent mosquito vectors. Rift Valley fever's tripartite segmented genome can reassort to generate novel reassortant viruses. This has the potential to produce viruses that are more pathogenic, more transmissible, or that have wider vector or host range. This is especially concerning because widespread use of live attenuated vaccine strains in endemic countries allows the potential replication of vaccine and wildtype strains simultaneously within the same mosquito or animal. Identifying these reassortants is important for optimum specificity and sensitivity of diagnostic tests, and for epidemiology and predictive risk modeling. Collaborative studies with Kansas State University (KSU) were conducted using a novel genotyping assay to detect and characterize reassortants. Co-infections with three different RVFV strains are in progress in both sheep and mosquitoes. For RVFV diagnostics, in collaboration with KSU, an enzyme-linked immunoassay (ELISA) has been developed utilizing a baculovirus-expressed nucleoprotein antigen. This assay showed high specificity and sensitivity in both sheep and calves for two different viral strains. Using baculovirus-expressed antigens instead of whole virus antigens decreases the biosafety and biosecurity risks of detecting RVFV-exposed animals.

Recently, in collaboration with KSU, a subunit EHDV vaccine was developed using a baculovirus-expressed VP-2 protein for EHDV serotypes 2 and 6. Mice and cattle showed neutralizing antibody in response to vaccination. Vaccinated white-tailed deer were protected from clinical disease after challenge with wild type virus and no viral RNA was detected in blood or tissues. This subunit vaccine technology has been licensed to a commercial partner.

ABADRU's VSV research program is led by Dr. Barbara Drolet. Vesicular stomatitis (VS) is an insect-transmitted disease of cattle, horses and swine. It is endemic from northern South America to northern Mexico. Sporadically, an incursion will occur where virus from an endemic region will move north into the U.S. causing an outbreak that spreads across a large geographic area encompassing many states from south to north throughout the insect vector season. These incursive viruses can cause single year outbreaks, but more recently, multi-year outbreaks from an overwintering virus genotype are common. Vesicular lesions and saliva contain large amounts of virus, enabling animal to animal contact transmission within the herd. These shedding animals can also infect people resulting in a flu-like illness. Additionally, three insect species are known to transmit VSV: *Culicoides* midges, *Simuliidae* black flies, and *Phlebotomus* sand flies. These insects require blood in order to go through a gonotrophic cycle to lay eggs. If feeding on an infected animal, insects ingest the virus and after an extrinsic incubation period they become infected and are able to transmit VSV to naive animals during subsequent blood feedings. This blood feeding/egg laying cycle can be expected to occur three to four times over the life of an insect. Virus is cleared in animals by seven days, but premises are quarantined, restricting all animal movement, for fourteen days after the onset of lesions.

With no wildlife reservoir found, it is believed that the only source of virus for insects is infected quarantined livestock. To account for the expansive geographic spread of the virus, far from quarantined animals by insects that only feed three to four times over their lifetime and only fly up to 2 km a day, we hypothesized that the blood feeding transmission cycle between infected animals and *Culicoides* midges, was not the only mechanism by which virus was being maintained and spread among midge populations. Experimental studies showed that female *Culicoides* midges are able to transmit VSV to male midges venereally during mating at a rate of 15.2% after two gonotrophic cycles and 76.3% after three cycles. Those infected males were able to venereally transmit VSV to naïve females at a 9.5% transmission rate. This is the first evidence for venereal transmission of any of the arboviruses transmitted by *Culicoides* biting midges and the first evidence for venereal transmission of VSV in any of the three primary competent VSV vector species (midges, black flies, sand flies). Venereal transmission potentially increases the number of VSV-positive midges within a breeding population beyond an initial blood feeding. This may account for further geographic virus spread by midges away from quarantined

premises with available VSV infected animal reservoirs. Maintenance of virus in the insect populations may also play a role in overwintering viral genotypes, the cause of multi-year outbreaks. This research shows the importance of males in VSV transmission dynamics, never considered previously, and in the maintenance of VSV in nature. Drolet's team will be doing further studies to determine the effects of venereal transmission on oviposition, fertility, and mating behavior, but these results highlight the need to incorporate alternative routes of transmission in understanding arbovirus outbreaks.

Arthropods transmit numerous viral, parasitic, and bacterial diseases, but the potential role of arthropods in SARS Coronavirus 2 (SARS-CoV-2) transmission is not fully understood. Previous work showed that SARS-CoV-2 replication is not supported in certain cultured mosquito cells and that some mosquito species did not support virus replication following intrathoracic inoculation, a very artificial route of exposure. ABADRU researchers expanded on those studies with a natural route of exposure, that being ingestion of an infectious blood meal, using *Culex tarsalis* and *Culex quinquefasciatus* mosquitoes and *Culicoides sonorensis* biting midges, all known biological vectors for numerous RNA viruses in the U.S. Fed insects were held, sorted into pools, and tested for viral RNA and infectious virus. Ten days after ingesting the infectious blood meal, qRT-PCR showed all three insect species were still positive for viral RNA, especially *Culicoides* midges at 85%. But no infectious virus was detected in any of the insects. Thus, although SARS-CoV-2 RNA persists, the virus does not replicate within these vector species and therefore they will not transmit it.

ABADRU's house fly research is led by Dr. Dana Nayduch. Her team has shown that the environmental niche impacts microbial communities carried by female house flies. Flies were collected from agricultural, urban, and mixed environments over a 3-month period and genetic analyses of bacteria in female fly gastrointestinal tracts were conducted. Numerous microbial species were identified with species diversity and richness being greatest in agricultural flies. The gut microbial communities of all flies were complex and contained pathogens, irrespective of collection site. The bacterial community composition was strongly influenced by the environment, which implies that flies access bacteria, including potential pathogens, from local sources. Thus, limiting fly access to bacterial sources, and/or controlling house fly populations, can result in reduced risk of flies harboring and transmitting bacteria that impact human and animal health. Gut bacterial communities also were analyzed from house flies that were collected at cattle operations (dairy or beef) in Nebraska, Kansas, and Oklahoma. Bacterial communities carried by flies were diverse and abundant. Community composition and species richness varied across both farm type (whether beef or dairy) and across geographic location. Flies carried numerous taxa of bacteria of significant medical and veterinary interest. Flies from feedlots in all three states carried *Moraxella*, associated with pinkeye. Flies from all states and farm types carried *Staphylococcus* and *Streptococcus* species associated with mastitis and other cutaneous infections. Flies also carried the foodborne pathogens *Campylobacter and E. coli*. Taken together, these results show that not all flies from all cattle facilities carry the same bacterial populations, but instead likely represent the microbial communities of the animals present on the site. Therefore, flies serve as significant reservoirs for bacteria at cattle operations and also pose the risk of disseminating and transmitting bacteria, including pathogens, among animals and their environment.

The Nayduch group also uses culture-based approaches to characterize bacteria carried by house flies. In 2019 they collected male and female house flies from beef cattle operations in three Kansas counties and cultured both total aerobic bacteria and coliforms, then tested a subset of coliforms for tetracycline (Tet) resistance. Overall, females carried both more total bacteria and more coliforms than males. Antimicrobial susceptibility testing showed 61% of the coliforms carried by flies were resistant to Tet. Up to 90% of males and 88% of females carried at least one Tet-resistant species. Up to 80% of males and 76% of females carried two or more Tet-resistant species. Although both male and females harbor antimicrobial resistant coliforms, females tend to harbor more coliforms overall and therefore may pose a greater risk in dissemination. Determining the role that management practices, climate factors, operation size, and other variables play in risk of bacterial transmission by flies is ongoing. This will identify key intervention points of fly control in order to reduce overall bacterial transmission, and specifically, anti-microbial resistant dissemination and persistence in cattle operations.

ABADRU's *Culicoides* biting midge field ecology research is led by Dr. Bethany McGregor. *Culicoides* are competent vectors for many arboviruses and they are important agricultural pests. With over 100 *Culicoides* species in the U.S., McGregor's team is studying the phenology of these important vectors. Specifically, the periodic lifecycle events and how they are influenced by seasonal and interannual variations in climate and habitat. *Culicoides* adults and larval mud samples are being collected on diverse

agricultural wildland sites in northeast KS. Understanding the seasonality of midge species allows researchers to determine which species are present at certain times of the year, how these communities overlap, and how population sizes change throughout the year. Potentially, this can implicate lesser known *Culicoides* vector species that are challenging to study in a laboratory environment. During this past summer a VSV outbreak occurred in eastern Kansas including sites where collections were being made for the phenology research. Very few *Culicoides sonorensis*, the confirmed VSV vector species, were found in the collections. However, *Culicoides haematopotus*, *C. crepuscularis*, and *C. stellifer* were abundant. Approximately 1300 midges were collected, identified to species and pooled by date, site, species and physiological status. These pools will be tested for VSV by realtime qRT-PCR. If any positive pools are found for new *Culicoides* vector species, it will fulfill two of the four World Health Organization vector incrimination criteria.

Sugar feeding is done by both male and female *Culicoides*, even blood feeding females, but very little is known about their sugar feeding ecology. It is not known what plants they are using, whether they feed opportunistically on whatever is available, or preferentially seek specific colors and odors, whether they use flowers only or if they also use extrafloral nectaries, which are specialized nectar secreting plant glands. McGregor's team are conducting a series of laboratory, field, and behavioral studies to learn more about this understudied phenomenon. This research will generate valuable ecological data that can be leveraged to develop targeted control strategies specific to midges, decreasing pesticide impacts on non-target species.

ABADRU's *Culicoides* management strategy efforts are led by Dr. Lee Cohnstaedt. Extensive testing of mosquito larval products for their effectiveness on midge larvae is ongoing. Insect growth regulators and monomolecular films were highly effective in killing *Culicoides* larvae. Follow up studies are in progress for the chitinase inhibitors Diflubenzuron and Novaluron.

Most recently, in collaboration with KSU, Colorado State University, and Texas A&M, Cohnstaedt's team looked at the return on investment from the boom/bust funding that occurs when a new arbovirus emerges in the U.S. Emergence of mosquito-borne viruses will continue and the reactionary responses increase attention, funding, publications, innovations, and preventive measures for public health. Long-term impacts, or returns on investment, are seen in scientific advancements, such as publications, and in innovations, such as traps. It was determined that a more sustainable, economical, and effective approach is needed to minimize the boom and bust in funding and capacity. The U.S. should strive to optimize the cost-effectiveness of budgetary spending by securing resources for biosecurity threats. This would maximize benefits while minimizing the total costs of anticipated expenditures incurred during mosquito-borne viral outbreaks.

SCWDS Update on 2020 Hemorrhagic Disease Activity and Tick Surveillance

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In collaboration with the USDA-APHIS-VS and SCWDS member state wildlife agencies, SCWDS conducts surveys for exotic arthropods across the United States. Here we provide an update on ongoing surveillance and related to the Asian longhorned tick (*Haemaphysalis longicornis*). Since the fall/winter of 2017, SCWDS has worked with numerous state, federal and private groups to conduct surveys of wildlife for *H. longicornis*. Methods have included 1) live animal trapping and environmental sampling in localized areas where *H. longicornis* has been documented, 2) passive regional surveillance of white-tailed deer and other wildlife, and 3) tick collections from wildlife presented to wildlife rehabilitation facilities in areas where *H. longicornis* has been documented. As of October 2020, we have examined ticks from ~2000 individuals representing 53 species from 22 states resulting in numerous new state, county, and host records. Although the situation is dynamic, to date, we have detected *H. longicornis* in seven states (New Jersey, Maryland, West Virginia, Virginia, North Carolina, Kentucky, and Pennsylvania) on black bear, brown booby, coyote, domestic dog, eastern cottontail, elk, gray fox, great-horned owl, raccoon, red fox, red-tailed hawk, Virginia opossum, white-tailed deer, and woodchuck.

Since 2019, SCWDS has screened host-seeking *H. longicornis* and other native tick species collected from a cattle farm in Albemarle County, Virginia where an outbreak of theileriosis (caused by *Theileria orientalis* Ikeda genotype) in cattle previously occurred. *Theileria orientalis* Ikeda genotype was detected in 13% (15/113) of *H. longicornis* nymphs, providing evidence this tick may serve as a vector for this parasite in the U.S. Native tick species collected from this site were all negative for *T. orientalis*, but

related native protozoan parasites were detected. SCWDS also screened white-tailed deer from the region to investigate their potential role in the epidemiology of exotic *T. orientalis* Ikeda. No deer sampled (n=350) were positive for *T. orientalis.*

Since spring 2019, SCWDS has conducted surveys on this same cattle farm (Albemarle County, Virginia) to investigate *H. longicornis* phenology, host associations, and habitat associations. Results indicate seasonal variation of *H. longicornis* is consistent with previous studies where nymph life stages are present across seasons but most active in the spring, followed by a peak in adult activity in the summer and larval activity in the fall. Among three habitat types (forest, edge, pasture) included in the study, we observed *H. longicornis* in all habitats but observed a lower probability of detecting *H. longicornis* in pasture habitat. In addition, we detect *H. longicornis* on various wildlife hosts including coyote, eastern cottontail, raccoon, Virginia opossum, white-tailed deer, and woodchuck. Further, we recovered a single *H. longicornis* larva from *Peromyscus* sp. (n=1). However, the tick was not attached and the importance of this detection, if any, remains unclear.

Annually, SCWDS processes tissue samples from throughout the United States from wild ruminants with suspected orbiviral hemorrhagic disease. Submissions are initially tested for epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) by molecular methods (e.g., conventional and quantitative reverse transcription PCR). For samples that test positive by RT-PCR, virus isolation is attempted, and isolates are identified to serotype. Samples with no virus isolate are not further typed. Findings from the 2019 and 2020 transmission seasons are reported here.

2019 SCWDS EHDV & BTV Diagnostics
Virus Serotypes Detected

STATE	SPECIES	VIRUS
Alabama	white-tailed deer	EHDV-2
Arkansas	white-tailed deer	EHDV-2
Florida	white-tailed deer	BTV-3
		BTV-13
Georgia	white-tailed deer	EHDV-1
		BTV-2
Idaho	white-tailed deer	EHDV-2
	pronghorn	EHDV-2
Indiana	white-tailed deer	EHDV-2
Kansas	white-tailed deer	EHDV-2
Kentucky	white-tailed deer	EHDV-2
Louisiana	white-tailed deer	EHDV-2
Maryland	white-tailed deer	EHDV-2
Missouri	white-tailed deer	EHDV-2
North Carolina	white-tailed deer	EHDV-2
North Dakota	white-tailed deer	EHDV-2
Virginia	white-tailed deer	EHDV-2
Wisconsin	white-tailed deer	EHDV-2
West Virginia	white-tailed deer	EHDV-2
	cattle	EHDV-2

2020 SCWDS EHDV & BTV Diagnostics
Virus Serotypes Detected as of 10/13/20

STATE	SPECIES	VIRUS
Delaware	white-tailed deer	EHDV-6
Indiana	white-tailed deer	EHDV-6
Kentucky	white-tailed deer	EHDV-2
Maryland	white-tailed deer	EHDV-6
Missouri	white-tailed deer	EHDV-2

Montana	white-tailed deer, pronghorn	EHDV-2 EHDV-2
North Carolina	white-tailed deer	EHDV-2 EHDV-6
North Dakota	white-tailed deer	EHDV-2
Nebraska	white-tailed deer	EHDV-2
New York	white-tailed deer	EHDV-6
Pennsylvania	white-tailed deer	EHDV-6
Tennessee	white-tailed deer	EHDV-2
Virginia	white-tailed deer	EHDV-2
Wisconsin	white-tailed deer	EHDV-2
West Virginia	white-tailed deer	EHDV-6

During 2019, 219 viruses were detected from 402 tissue samples, representing five species (369 white-tailed deer, 16 elk, ten mule deer, four pronghorn, and 3 cattle) from 26 states. Isolations of EHDV-1 (1), EHDV-2 (137), BTV-2 (1), BTV-3 (1), and BTV-13 (1) were made from white-tailed deer or pronghorn (see Table). An additional 21 untyped BTVs were detected in white-tailed deer (AR, FL, GA, IN, KY, NC, NE, PA, and WV), and 58 untyped EHDVs were detected in white-tailed deer, mule deer, elk, and cattle (AR, FL, GA, ID, KS, KY, MI, MO, NC, WI, and WV). During the 2020 transmission season (as of October 13, 2020) 100 viruses have been detected from 164 tissue samples, representing 23 states and five species (143 white-tailed deer, 12 pronghorn, 7 mule deer, 1 elk, and 1 moose). Isolations of EHDV-2 (31) and EHDV-6 (14) were made from white-tailed deer or pronghorn (see Table). An additional 13 untyped BTVs have been detected in white-tailed deer, mule deer, or pronghorn (FL, GA, IN, KS, LA, and NE) and 42 untyped EHDVs have been detected in white-tailed deer, mule deer, pronghorn, or elk (FL, GA, ID, IN, KY, MI, MO, MT, NC, ND, NE, NY, PA, SC, TN, and WV).

LAPRU Research Update

Kimberly Lohmeyer, USDA-APHIS, Agricultural Research Service (ARS)

The Livestock Arthropod Pest Research Unit (LAPRU) is composed of three separate research facilities: The Knipling Bushland U.S. Livestock Insects Research Laboratory (KBUSLIRL), Kerrville, Texas, conducts research on biting fly and tick pests of cattle and wildlife, the Cattle Fever Tick Research Laboratory (CFTRL), Edinburg, Texas, focuses on research to develop novel control methods for cattle fever ticks, and the Sterile Screwworm Production Facility, Pecora, Panama, conducts research to improve screwworm mass rearing techniques.

Significant changes are planned for both the KBUSLIRL and CFTRL facilities for FY21. At the KBUSLIRL, the vacant geneticist and laboratory director positions are slated to be filled. Additionally, the long-planned facility modernization project is slated to break ground in early 2021. This project includes the construction of a large new administrative and laboratory structure that will house the scientific staff in one building as well as a new fly and tick rearing facility and a large research stanchion barn. At the CFTRL, the vacant entomologist and toxicologist positions are slated to be filled in FY21. Additionally, thanks to new funding that has been appropriated for the cattle fever tick research program, six new scientist positions will be created and filled over the next three years. These positions include an epidemiologist and an immunologist position that will be filled in FY21, a wildlife biologist and a computational biologist position that will be filled in FY22, and a physical geographer and an agricultural engineer position that will be filled in FY23. The significant increase in funds will also be used to build new laboratory and administrative spaces, two new research stanchion barns, and a wildlife handling facility. The additional barn and laboratory research space, the wildlife handling facility, and the new scientific staff positions will greatly enhance the cattle fever tick research program and will allow for more studies to be conducted on site with both cattle and wildlife hosts of cattle fever ticks.

Ongoing research efforts of the LAPRU continue to include applied and genomic research on ticks, biting flies, and screwworms. Scientists at all three locations are involved in research to find novel control techniques for tick and fly pests as well as techniques to improve lures and mass rearing techniques for screwworms. Alternative treatments for cattle and wildlife to traditional acaricide treatments such as CoRal and Dectomax are being investigated as well as the efficacy of novel antigen vaccines. Additionally, research is being conducted to help combat insecticide and acaricide resistance and to find longer acting cattle fever tick treatments that allow a reduction in the frequency, and thus the cost, of rounding up cattle for treatment. Basic tick and fly biology studies are also underway, in particular studies to evaluate what larval ticks are doing while off host and to determine if this vulnerable life stage can be

manipulated to enhance control. Novel control strategies for ticks and flies such as desiccant dusts and essential oils are being evaluated. Modeling studies as well as field studies that incorporate “big data” collection from cattle and the environment are being conducted that will help further refine cattle fever tick life cycle models as well as models for treatment scenarios. If larval tick refugia or consistent patterns in how hosts like cattle and wildlife utilize the south Texas landscape can be identified, then control techniques can be targeted at these areas to increase the efficiency and efficacy of tick treatments. Genomic studies continue to provide information about the source of cattle fever ticks collected from new infestations along the border. This information can used to compare the genetic signature of ticks within and between counties and help trace their origin. Continued efforts to improve the genomes of cattle fever ticks, biting flies, and screwworm flies will lead to increased information about potential targets or vulnerabilities that can be exploited to develop new control tactics.

Committee Business:

The Committee called the business meeting to order at 2:35PM EDT. The mission statement was reviewed, and a quorum was established.

New Business:

The response to 2019 Resolution #38: Equine Infectious Anemia and Equine Piroplasmosis Control Strategies was discussed. It was decided that Response is sufficient for the current time; however additional follow-up will be needed. Recommend timeframe for follow-up: request timeline of Spring/Summer 2021 for completion of Uniform Standards was agreed upon by majority vote.

A new resolution titled Re-evaluation of endemic bluetongue virus serotypes in the United States was brought forward and the background was discussed. The resolution was also brought up in two other committees. Motion to adopt was moved and seconded. Vote carried unanimously.

With no further business, there was a motion to adjourn and seconded. Meeting concluded at 4:40 p.m. EDT.