REPORT OF THE COMMITTEE ON BLUETONGUE AND RELATED ORBIVIRUSES

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The Committee met on October 21, 2013 at the Town and Country Hotel, San Diego, California, from 1:00 to 5:30 p.m. There were at least 19 members and at least 25 guests present. James Maclachlan and William Wilson, Chair and Vice-chair, respectively, introduced the meeting.

Bluetongue in Europe: Lessons Learned

Francisco Javier Reviriego Gordejo, Disease Control and Identification; European Union, Brussels, Belgium

Dr. Reviriego Gordejo presented an overview of the sequence of events that occurred in Europe during their recent bluetongue outbreaks that began in the Mediterranean Basin in 1998. He then reviewed the EU response to this event, notably mass vaccination of livestock and animal movement restrictions. He also provided an update for the current situation and stressed the importance of ongoing surveillance informed by risk assessment. Bluetongue virus (BTV) serotype 1 has recently reappeared in Sicily and portions of Italy, with severe disease and high mortality among susceptible sheep.

Gordejo then discussed the remarkable occurrence of so-called “vaccine incidents” in which apparent live-attenuated strains of BTV serotypes 6, 11 and 14 have been naturally spread amongst farm livestock in northern Europe. Whereas the strains of BTV 6 and 11 were present only transiently, BTV-14 is still present in Europe. This virus was first identified in 2011 in livestock imported into Spain from Poland, and was again detected in animals imported to Spain from Lithuania. The virus was subsequently detected among livestock in Lithuania, Estonia, Latvia and Poland, and had been actively circulating in western Russia including in the region adjacent to the border with Belarus.

The EU has concluded that these “vaccine incidents” will “die out over time”, which they consider has subsequently come to pass. There are concerns in the EU that intentional introduction of BTV strains complicate the understanding of infection and occurrence of disease.

Development and Application of an *in vivo* Model for Studies of Vector Capacity of *Culicoides* spp. for Bluetongue and other Arboviruses.

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The international profile of arboviruses that are transmitted by biting midges from the *Culicoides* genus has become much more prominent in the last decade with widespread transmission of bluetongue virus (BTV) and more recently the emergence of Schmallenberg virus in Europe. While there is little doubt that most of these viruses are transmitted by *Culicoides* spp., it can be difficult to generate the data required to satisfy criteria for the acceptance of a midge species as a competent vector of a virus. Major elements include proof that the insect of interest takes a blood meal from a particular mammalian host, infection of the insect species with the virus, replication of the virus in the insect and subsequently transmission of the virus back to the animal host. Research to support these criteria can be extremely difficult because there are few midge species that have been colonized and raised under laboratory conditions. Further, transmission experiments typically require ruminants, with associated management, husbandry and ethics considerations. The small size of *Culicoides* spp. also makes research with many

species in the genus very difficult. Consequently in most circumstances only limited indirect or partial data have been produced to support the role of an individual insect species as a vector. In recent times, this has involved detection of the virus in wild caught insects, use of semi-quantitative 'real time' PCR to demonstrate high levels of nucleic acid consistent with virus replication in the insect, and specifically high virus levels in the head or salivary glands suggesting a capacity to infect a mammal when taking another blood meal. To address these issues, we have developed an *in vivo* model using embryonated chicken eggs (ECE) and applied it to studies of vector competence of the major Australian vector of BTV, *Culicoides brevitarsis*, the smallest midge in the genus.

The kinetics of BTV replication and viraemia in inoculated embryos was monitored by qRT-PCR in individual ECE by collecting small volumes of blood several times per day. At peak viraemia, wild caught insects (raised as unfed adults, recently emerged from cattle dung) were placed in a small cage attached to the egg shell and allowed to feed on exposed blood vessels and membranes. Virus levels in the embryo blood were monitored pre and post feeding in each experiment. Engorged midges were held and fed on sucrose for the incubation period. To monitor potential virus replication, a proportion of insects were sampled at different time points and virus loads monitored by qRT-PCR. After an 8-10 day incubation period, pools of surviving midges were allowed to feed on uninfected chicken embryos. After this second feeding period, BTV ribonucleic acid (RNA) levels were quantified in individual midges. Virus replication was also monitored in the 'uninoculated' ECE to establish whether the midges had transmitted virus.

This model has many advantages, including the capacity to test field caught insects, study 'wild type' virus, has no need for ruminants, can be undertaken at short notice and has the capacity to test large numbers of replicates. Orthobunya and other viruses may also be studied.

National Surveillance: Bluetongue Virus (BTV) and Epizootic Hemorrhagic Disease Virus (EHDV) isolations/PCR positives; Calendar year 2012

Eileen Ostlund, USDA-APHIS-VS National Veterinary Services Laboratories

Bluetongue virus or ribonucleic acid (RNA) was detected in 51 samples submitted or collected during calendar year 2012. The positive bluetongue virus isolation (VI) and polymerase chain reaction (PCR) test results from submissions to the National Veterinary Services Laboratories (NVSL) in 2012 are listed in Table 1.

Table 1. BT virus isolation (VI) / PCR positives, Calendar year 2012

State	*No.*	*Species*	*PCR*	*VI*	*Other Info*
AZ	1	Bighorn	BTV-10	BTV-10	
AZ	1	Bighorn	BTV-11	Negative	
CA	1	Sheep	BTV-10	BTV-10	
CA	1	Mule Deer	BTV-10	BTV-10	
CA	2	Sheep	BTV-11	Negative	
FL	7	Boer goat, cattle	Positive	Negative	Unable to type
FL	1	Sheep	BTV-9	Negative	
FL	1	Key deer	BTV-12	Negative	
IL	2	Deer	BTV-13	BTV-13 (1)	Both also EHDV-6
IL	2	Cattle	BTV-17	Negative (1), Not done (1)	One also EHDV-6
IA	1	Deer	BTV-11	BTV-11	
IA	1	Cattle	BTV-17	BTV-17	
KS	1	Dog isolate	BTV-11		
LA	8	Cattle	BTV-12	Negative or not done	Frozen blood

State	No.	Species	PCR	VI	Other Info
					submitted for typing
LA	6	Deer	BTV-12	BTV-12	Isolated from 1 sample
NE	1	Cattle	BTV-13	Negative	
NE	1	Cattle	Positive	Negative	Unable to type
NM	1	Elk	Positive	Negative	Unable to type
ND	1	Cattle	Positive	Negative	Unable to type
OK	1	Cattle	BTV-13	Negative	
OK	1	Cattle	Positive	Negative	Unable to type
SD	1	Deer	BTV-3	BTV-3	
SD	2	Mule deer, elk	BTV-13	Negative	Elk also EHDV-2
TX	1	Deer	Positive	Negative	Unable to type
TX	3	Deer	BTV-12	Negative	
TX	1	Deer	BTV-17	BTV-17	

During calendar year 2012, 142 samples tested positive for EHDV by virus isolation and/or PCR. The positive EHDV isolation and PCR test results from submissions to NVSL in 2012 are listed in Table 2.

Table 2. EHDV isolation (VI)/ PCR positives, Calendar year 2012

State	No.	Species	PCR	VI	Other Info
FL	1	Deer	EHDV-1	EHDV-1	
FL	1	Deer	EHDV-6	Negative	
IL	1	Cattle	EHDV-2	Negative	
IL	2	Cattle	EHDV-6	Negative	1 also BTV-17
IL	1	Cattle	EHDV-6	EHDV-6	
IL	3	Deer	EHDV-6	Negative or not done	1 also BTV-13
IL	3	Deer	EHDV-6	EHDV-6	1 also BTV-13
IA	5	Cattle	EHDV-2	Negative	
IA	2	Cattle	EHDV-2	EHDV-2	
IA	7	Deer	EHDV-2	Negative or not done	
IA	2	Deer	EHDV-2	EHDV-2	
IA	1	Bison	EHDV-2	Negative	
IA	1	Cattle	EHDV-6	Negative	
IA	1	Deer	EHDV-6	Negative	
IA	3	Deer	EHDV-6	EHDV-6	

State	*No.*	*Species*	*PCR*	*VI*	*Other Info*
IA	1	Elk	EHDV-6	Negative	
MN	1	Cattle	EHDV-2	EHDV-2	
MO	1	Deer isolate	EHDV-2		
NE	5	Cattle	EHDV-2	Negative or not done	
NE	4	Cattle	EHDV-2	EHDV-2	
NE	5	Deer	EHDV-2	Negative	
NE	1	Deer	EHDV-2	EHDV-2	
NE	2	Deer	EHDV-6	Negative	
NC	2	Deer	EHDV-2	Negative	
OH	1	Cattle	EHDV-2	EHDV-2	
SD	1	Cattle	EHDV-1	Negative	
SD	35	Cattle	EHDV-2	Negative or not done	
SD	21	Cattle	EHDV-2	EHDV-2	
SD	13	Deer	EHDV-2	Negative or not done	
SD	7	Deer	EHDV-2	EHDV-2	
SD	2	Elk	EHDV-2	Negative	1 also BTV-13
SD	3	Bison	EHDV-2	Negative	
SD	1	Bison	EHDV-2	EHDV-2	
SD	1	Deer	EHDV-6	EHDV-6	
VA	1	Cattle	EHDV-2	Negative	

SCWDS Update: Hemorrhagic Disease and *Culicoides* sp. Surveillance

Drs. Joseph Corn, David Stallknecht, and Ms. Stacey Vigil, Southeastern Cooperative Wildlife Disease Study (SCWDS), University of Georgia

An update hemorrhagic disease and *Culicoides* surveillance was given. The SCWDS, in collaboration with the USDA-APHIS- VS, conducts surveillance for epizootic hemorrhagic disease and bluetongue viruses, and for *Culicoides* vectors of these viruses. Collection of data for the 2012 National Report on Hemorrhagic Disease (HD) Activity in the United States has been completed. This annual summary of HD activity, which is conducted through a questionnaire sent to multiple facilities and personnel in every state, has been assembled and distributed annually since 1980. Diagnostic work related to HD conducted during 2013 has included virus isolation attempts from animals (primarily white-tailed deer) submitted from Florida, Georgia, Indiana, Iowa, Kansas, Louisiana, Maryland, Michigan, Mississippi, Missouri, Montana, New Jersey, New Mexico, North Carolina, Tennessee, Virginia, and West Virginia. To date, EHDV-1 (Indiana, Mississippi), EHDV-2 (Iowa, Missouri, Mississippi, Montana), and BTV-17 (Montana) have been isolated from white-tailed deer. In view of the isolation of several exotic EHD and BT viruses from deer, as well as detection of exotic orbiviruses among livestock in the USA in recent years, SCWDS, following consultation with USDA-APHIS-VS, has implemented a surveillance program for endemic and exotic species of *Culicoides* midges in the Southeast United States. New state records for nine species of *Culicoides* in 13 states have been identified and surveillance is ongoing.

Molecular Evolution of Field Strains of Bluetongue of Epizootic Hemorrhagic Disease Viruses

Bill Wilson[1], Dane Jasperson[1], Donna Johnson[2], Eileen Ostlund[2], Raymond Lenhoff[3], Pejman Naraghi-Arani[3], Mark Ruder[1], Andrew Allison[4], David Stallknecht[4], and Timonthy Smith[5]
[1]USDA, ARS, Arthropod-Borne Animal Diseases Unit
[2]USDA, APHIS, VS, National Veterinary Services Laboratory
[3]Lawrence Livermore National Laboratory
[4]Southeastern Cooperative Wildlife Disease Study (SCWDS), University of Georgia
[5]Meat Animal Research Center

The Arthropod-Borne Animal Diseases Research Unit (ABADRU) has adapted the published single primer ligation - whole genome amplification protocol that allows the whole bluetongue and epizootic hemorrhagic disease virus genome to be amplified without prior sequence knowledge and submitted to high-throughput DNA sequencing. This technology was applied to bluetongue virus serotype 3 isolates from Florida, Arkansas, Mississippi, Central American and the Caribbean basin. The analysis dataset is consistent with hypothesis that these viruses were introduced from the Central America and the Caribbean basin. The BTV-2 in California was also submitted to a similar analysis that indicated that this virus was likely introduced into FL then moved south to the Caribbean and West to CA. A historical molecular characterization of EHDV strains has been completed and used to compare recent 2012 strains causing clinical disease in cattle. Finally, this analysis was performed on BTV-11 isolated from two separate canine cases that demonstrated that the virus isolates were almost identical. These studies indicate the value of this technology in understanding virus epidemiology and ecology.

The Arthropod-Borne Animal Diseases Research Unit: Research Program Update and Current Status

William C. Wilson, Lee W. Cohnstaedt, Barbara S. Drolet, Robert Pfannenstiel, Dana Nayduch, Mark G. Ruder and D. Scott McVey
USDA-ARS, Arthropod-Borne Animal Diseases Research Unit

The Arthropod Borne Animal Diseases Research Unit's (ABADRU) research mission is to solve major endemic, emerging, and exotic arthropod-borne disease problems in livestock. The Unit completed the move to Manhattan, Kansas in 2010 and now the ABADRU is well established at the Center for Grain and Animal Health Research (CGAHR). Five new scientists that were hired to replace the scientific staff that did not relocate to Kansas are well on the way to establish new research ABADRU programs under the Agricultural Research Service (ARS) National Research Programs: NP103 and Animal Health and NP104, Veterinary, Medical, and Urban Entomology. The areas of research range from vector biology to understand virus-host interactions to better control these important diseases. The ABADRU and Kansas State University hosted an Orbivirus Gap analysis meeting last year. This unfunded workshop organized by the USDA-APHIS, USDA-ARS, and Department of Interior (DOI) was in response to the resolution made last year regarding the need for a gap analysis and research efforts to control Orbiviruses, and was successful because of participants from the U.S. and around the world. The result of this analysis will be published and available at the following link: www.ars.usda.gov/OrbivirusesGapAnalysis.pdf. The analysis addressed surveillance, diagnostics, virology, vector control and vaccines. The diagnostic and surveillance group determined that there are well standardize diagnostics for BTV in terms of export testing; however, this could be improved for EHDV and for routine diagnostic testing. There is a need for a coordinated network for surveillance of activity. The NAHLN is designed for surge capacity in an event of an outbreak but is not utilized for national surveillance. Although there is good science in the virology and vector biology around these viruses, there are still many basic science questions that need to be addressed. Tools to develop vaccines are available and the ideal characteristics of these vaccines were agreed upon. Although no formal alliance was established, it was generally agreed that international communications and coordination of efforts will be continued to facilitate progress toward addressing these important vector transmitted diseases.

Committee Business:

The Committee reviewed the response to the Resolution passed in 2012 and Bill Wilson updated results of the Gap Analysis that was convened by USDA in response to this resolution (see above). The Resolution encouraging vaccine development and increased study of bluetongue and epizootic hemorrhagic disease was also again forwarded to the Committee on Nominations and Resolutions. No additional resolutions were proposed.

The issue of Committee leadership was discussed because both the Chair and Vice-chair positions were subject to five year review. Given the highly unusual nature of this year's meeting with the Federal shutdown, and the considerable "unfinished business" of the original Committee agenda for the 2013 meeting, it was proposed, seconded, and supported unanimously that Drs. Maclachlan and Wilson continue as Chair and Vice Chair of the committee for the coming year, which will be referred to the President.