REPORT OF THE COMMITTEE ON BLUETONGUE AND RELATED ORBIVIRUSES

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The Committee met on November 15, 2010 at the Hilton Hotel, Minneapolis, Minnesota, from 1:00 to 5:20 p.m. There were 13 members and 26 guests present. James Maclachlan and William Wilson, Chair and Vice-chair, respectively, introduced the meeting. There was no discussion of previous Committee business. One resolution was developed and approved.

Presentations

Experiences in Switzerland with bluetongue virus serotype 8

Dr. Gabriella Worwa
University of California – Davis and Institute for Virology and Immunoprophylaxis, Switzerland

Dr. Worwa provided a historical perspective of BTV serotype 8 infection in Northern Europe, emphasizing reproductive aspects of the infection in ruminants as well as the expression of disease in cattle. The European strain of BTV serotype 8 can be highly virulent in livestock and infection is also characterized by high frequency occurrence of transplacental transmission that is unusual amongst field strains of BTV; the economic consequences of these virus - induced reproductive effects have been substantial. The numbers of reported cases has plummeted since the advent of widespread immunization of susceptible ruminants with inactivated BTV-8 vaccine, such that few cases have been reported to date in 2010.

The Netherlands Strain of BTV Serotype 8 in White-Tailed Deer

Barbara S. Drolet[1], Lindsey M. Reister[1], James O. Mecham[1], William C. Wilson[1], Pauline Nol[2], Kurt C. VerCauteren[2], Tara C. Ruby[2], Piet A. vanRijn[3], Richard A. Bowen[4]
[1]USDA, ARS, Arthropod Borne Animal Diseases Unit
[2]USDA, APHIS, National Wildlife Research Center
[3]Central Veterinary Institute of Wageningen UR, The Netherlands
[4]Colorado State University

To determine the susceptibility of U.S. white-tailed deer to the European strain of BTV-8 (EU-BTV-8) isolated in The Netherlands, eight seronegative deer were injected subcutaneously in the neck and intradermally in the inner left leg. Two deer were sham inoculated to serve as uninfected controls and housed with infected animals to verify the inability of this virus to spread by direct contact transmission. Body temperatures and clinical signs were recorded daily. Periodic blood samples were analyzed for BTV RNA with qRT-PCR, for BTV serum antibodies by cELISA, and for infectious virus by plaque assay. At necropsy, tissue samples were taken for histopathological examination and tested by qRT-PCR for viral RNA. Deer developed moderate to severe clinical disease from 8 to 15 days post inoculation (dpi). Peak viremia by qRT-PCR was from 7-10 dpi with detectable titers seen as far out as 28 dpi in some deer. Antibody titers were detected by cELISA starting at day 6, peaked by day 10, and continued through day 28. These results suggest that if EU-BTV-8 is accidentally or intentionally introduced into the U.S., considerable disease would be expected in our white-tailed deer and they would serve as significant virus reservoirs.

Whole genome sequence analysis of field strains of bluetongue virus

Bill Wilson[1], Dane Jasperson[1], Mark Harpster[2], Patrick Johnson[2], Donna Johnson[3], Eileen Ostlund[3], Raymond Lenhoff[4], Pejman Naraghi-Arani[4], Mark Ruder[5], Andrew Allison[5], David Stallknecht[5], and Timonthy Smith[6]

[1]USDA, ARS, Arthropod Borne Animal Diseases Unit
[2]Department of Chemical and Petroleum Engineering
[4]Lawrence Livermore National Laboratory
[5]Southeastern Cooperative Wildlife Disease Study (SCWDS), University of Georgia
[6]Meat Animal Research Center

The Arthropod Borne Animal Diseases Research Unit (ABADRU) has been developing rapid, high sensitive biosenor technology based on gold nanoparticles and Surface Enhanced Raman Scattering (SERS). The "proof-of-concept" for the nucleic acid and immunological based assays was reviewed. A more recent enhancement in this technology was also discusses. The ABADRU has also adapted the published single primer ligation - whole genome amplification protocol that allows the whole bluetongue virus genome to be amplified without prior sequence knowledge and submitted to high-throughput DNA sequencing. Preliminary data was discussed as well as the potential impact on the ability to rapidly perform molecular evolution analyses.

Epidemiology of bluetongue virus infection in California

Dr. Christie Mayo
School of Veterinary Medicine, University of California, Davis, CA

An overview of recent surveillance for bluetongue virus infection of cattle in California was provided. This was a collaborative undertaking between the University, the California Department of Food and Agriculture, and the California Animal Health and Food Safety Laboratory, and utilized some 120 sentinel calves in different regions of the state. Calves were monitored monthly for the presence of viral nucleic acid by real time RT-PCR and/or antibodies by cELISA. The study demonstrated limited perinatal transmission of BTV nucleic acid to calves via colostrum, as well as seasonal infection with BTV serotypes 11 and 17 from August until November. A risk analysis is now being undertaken to identify factors that predict the likelihood of BTV infection of calves in the region.

National Veterinary Services Laboratory update

Eileen Ostlund
USDA-APHIS-VS National Veterinary Services Laboratories

Bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) isolations/PCR positives

Calendar year 2009

Bluetongue virus or RNA was detected in 5 samples submitted during calendar year 2009. The positive bluetongue virus isolation and polymerase chain reaction (PCR) test results from submissions to the National Veterinary Services Laboratories (NVSL) in 2009 are listed in Table 1

Table 1. BT virus isolation (VI) / PCR positives, Calendar year 2009

State	*No.*	*Species*	*PCR*	*VI*
MS	3	Deer isolates (SCWDS)		BTV-3
OK	1	Deer	Positive	BTV-14
TX	1	Deer isolate		BTV-11

*Southeastern Cooperative Wildlife Disease Study, Athens, GA

During calendar year 2009, 4 samples tested positive for EHDV by virus isolation and/or PCR. The positive EHDV isolation and PCR test results from submissions to the National Veterinary Services Laboratories (NVSL) in 2009 are listed in Table 2.

Table 2. EHDV isolation (VI)/ PCR positives, Calendar year 2009

State	*No.*	*Species*	*PCR*	*VI*
MO	1	Deer		EHDV-2 & EHDV-6
OK	1	Elk		EHDV-2
OK	1	Deer		EHDV-1 & EHDV-6
TX	1	Deer isolate (year unknown)	Pos	EHDV-6

Calendar year 2010 (January 1– October 31)

As of 31 October 2010 bluetongue virus has been identified in 11 samples. BTV was isolated from six blood samples from cattle in CA; BTV-10 was identified in four samples, and BTV-11 was identified in two. BTV-1 was isolated from 4 samples from sheep in FL. This represents the first detection of BTV-1 since a 2004 isolate was obtained from LA. BTV-12 was identified in a FL deer sample submitted to NVSL by SCWDS. In the same time period, EHDV-2 was identified in 4 samples including a deer isolate from LA and deer samples from FL, IL, and MO.

Summary of non-endemic bluetongue virus isolates identified at NVSL 1999-2010

In the United States, bluetongue virus types 2, 10, 11, 13 and 17 are considered endemic. Some states are free or seasonally free of bluetongue activity while others experience less seasonality. Of the endemic types, BTV-2 is restricted primarily to Florida, and the other types are more widespread. Since 1999, NVSL had identified 36 isolates of non-endemic bluetongue virus from U.S. ruminant species. Of these, 9 isolates were submitted to NVSL by SCWDS. At least one isolate has occurred in each of 6 southeastern states (AR, FL, LA, MS, OK, TX); the largest number have been identified in samples originating from Florida. A total of 10 previously unrecognized bluetongue serotypes have been identified to date (BTV types 1, 3, 5, 6, 9, 12, 14, 19, 22, 24). Of these, BTV-3 has been the most frequent non-endemic isolate and has been found in 4 states; BTV-3 isolates have occurred in 7 of the past 12 years. BTV-1, BTV-12, and BTV-14 have also been found outside of FL. None of the non-endemic bluetongue types has caused widespread disease outbreaks. The *Culicoides spp.* vectors responsible for transmission of the non-endemic types are unknown.

2010 Bluetongue Serology Proficiency Test

Fifty-six laboratories participated in the 2010 bluetongue (BT) proficiency test. The panel consisted of 20 ruminant serum samples. The passing score was two or fewer samples missed. Of the 56 laboratories participating in the 2010 BT proficiency test, 39 agreed with each other and with NVSL on the positive/negative bluetongue antibody status of all 20 samples. Eight laboratories missed one sample, and seven laboratories missed two samples. Two laboratories failed the first attempt of the 2010 BT proficiency test but passed the retest. Laboratories approved to conduct official (export) bluetongue serology are listed on the website: http://www.aphis.usda.gov/animal_health/lab_info_services/approved_labs.shtml

Hemorrhagic Disease Surveillance and Research

Mark Ruder, Andrew Allison, and David Stallknecht
Southeastern Cooperative Wildlife Disease Study (SCWDS), University of Georgia

During 2009, there were 34 viruses isolated from the 103 virus isolation attempts made, representing 22 states and 5 species (92 white-tailed deer, 1 key deer, 5 mule deer, 4 cattle, 1 elk). Isolations were made from free-ranging and captive white-tailed deer in Alabama (EHDV-2), Florida (EHDV-2), Kansas (EHDV-2), Louisiana (EHDV-2), Michigan (EHDV-6), Mississippi (BTV-3), Missouri (EHDV-2), Montana (EHDV-2), Ohio (EHDV-2), Tennessee (EHDV-2), Texas (BTV-17), and West Virginia (EHDV-2). In addition, BTV-11 was isolated from a cow in Georgia. As of October 22, 2010, there have been 13 viruses isolated after 42 virus isolation attempts, representing 7 states and multiple species (34 white-tailed deer, 2 mule deer, 3 elk, 1 unspecified cervid, 1 domestic cow, and 1 domestic sheep). Isolations were made from free-ranging and

captive white-tailed deer in Alabama (EHDV-1 and EHDV-2), Arkansas (EHDV-6), Florida (BTV-12 and EHDV-2), Maryland (EHDV-2), New Jersey (EHDV-2), and North Carolina (EHDV-2). In addition, EHDV-2 was isolated from two elk in New Mexico.

Of the viruses isolated during 2009 and 2010, EHDV-6, BTV-3 and -12 were considered exotic to the United States prior to their initial detection in 2006, 1999, and 2008, respectively. Between 2006 and 2010, EHDV-6 (Indiana) has been isolated from white-tailed deer in Arkansas, Kansas, Illinois, Indiana, Michigan, Missouri, and Texas. BTV-3 has been isolated by personnel at NVSL from sentinel cattle in Florida over multiple years since 1999 (Johnson et al, *Proc USAHA*, 2007), and has subsequently been detected from white-tailed deer in Arkansas and Oklahoma (2008), and Mississippi (2006 and 2009). This year's BTV-12 isolation from a white-tailed deer in Florida is the second detection of this serotype since it was first isolated from a white-tailed deer in Texas during 2008. The isolation of these different viruses over multiple years and a broad geographic area suggests that these viruses are likely established in the United States.

During the spring of 2009, SCWDS personnel completed an experimental infection of white-tailed deer with EHDV-7 (Israel). In the fall of 2006, this virus was the cause of an intense and widespread epizootic in Israeli cattle. Although mortality was <1%, in-herd morbidity rates ranged from 5-80% and a 10-20% drop in milk production was documented in dairy herds (Yadin et al, *Vet. Rec*., 2008). The results of the study, including viral dynamics, clinical signs, and postmortem findings, were similar to previous experimental and field findings with EHDV-1, -2, and -6. Briefly, morbidity was 100% (n=7) and 4 of 7 (58%) deer died or had to be euthanized during the study. All animals had a detectable viremia beginning on PID 3, although duration was variable among animals surviving infection, ranging from PID 12 to PID 46. Peak viremia occurred on PID 6 and ranged from <2.3 to 7.6 $\log_{10}$ $TCID_{50}$/ml. Colonized *Culicoides sonorensis* were allowed to take a blood meal from infected deer during peak viremia. Preliminary results indicate that *C. sonorensis* is susceptible to oral infection with EHDV-7 (Israel), and midges were able to transmit the virus to a naïve deer following incubation. These results indicate that white-tailed deer are susceptible to infection and severe clinical disease with this exotic EHDV and that *C. sonorensis* may biologically transmit the virus. Further, the clinical similarities observed in this study with disease caused by endemic EHDV serotypes highlight the importance of laboratory confirmation of suspected HD mortality events and the use of serotype-specific diagnostics.

The Arthropod-borne Animal Diseases Laboratory: research program update and current status

Dr. Barbara Drolet
USDA, ARS, Arthropod Borne Animal Diseases Unit

To accomplish the continuing high containment research mission of the Arthropod Borne Animal Diseases Laboratory (ABADRL) in solving major endemic, emerging, and exotic arthropod-borne disease problems in livestock, the U.S. Senate made the decision to relocate the ABADRL from Laramie, WY to Manhattan, KS. The decision was the result of an extensive analysis by ARS involving four possible relocation sites for the laboratory. Relocation was initiated and completed in FY2010. The ABADRL became one of five units at the Center for Grain and Animal Health Research (CGAHR) and was renamed the Arthropod-Borne Animal Diseases Research Unit (ABADRU). The ABADRU is doing BSL-2 research at CGAHR and will soon begin BSL-3 laboratory, animal, and insect research at the new Biosecurity Research Institute at Kansas State University. The ABADRU has three 5-year project plans under two ARS National Research Programs; Animal Health NP103 and Veterinary, Medical, and Urban Entomology NP 104. These plans include research on bluetongue virus (BTV; exotic and domestic), vesicular stomatitis virus (VSV), and Rift Valley fever virus (RVFV). Research progress to date for exotic BTV include a susceptibility study of white-tailed deer with BTV serotype 8 originally isolated in The Netherlands. Research progress to date for RVFV includes vector competence studies, animal infection model studies, production of BSL-2 diagnostic assays including qRT-PCR, ELISA, and immunohistochemistry. The ABADRU is rapidly recruiting to replace the scientific staff who chose not relocate to Manhattan. The ABADRU continues to have the highest level of funding in its history, thanks to additional funding sources such as Department of Homeland Security, ARS Office of International Research Projects, and the Department of State Biosecurity

Engagement Program. Additionally, the lab has the largest number of national and international collaborations in its history, and continues to have a productive research program addressing the needs of our stakeholders.

Recent changes to the OIE Code for Bluetongue

Dr. Dorothy Geale
Canadian Food Inspection Agency

Dr. Geale introduced aspects of the recently revised OIE Code that is currently being circulated for discussion. Of particular note is that the current Code no longer uses the terminology “vectors likely to be competent for bluetongue virus,” which necessitates animal surveillance to assure freedom from infection. There was considerable discussion regarding the impact of this change on different countries.

Committee Business

The Committee discussed and approved a resolution that the USAHA support efforts to remove the serotypes of BTV that have been identified since 1998 in the Southeastern United States from the USDA select agent list.