REPORT OF THE COMMITTEE ON BLUETONGUE AND BOVINE RETROVIRUSES

Chair: James E. Pearson, Ames, IA
Vice Chair: William C. Wilson, Laramie, WY

T. Lynwood Barber, CO; Nathan Bauer, TX; Edward J. Dubovi, NY; James F. Evermann, WA; Robert W. Fulton, OK; Bob Gerlach, AK; Chester A. Gipson, MD; Joel Goldman, LA; Larry L. Hawkins, MO; Chris S. Hayhow, KS; Robert B. Hillman, NY; Thomas J. Holt, FL; Robert F. Kahrs, FL; Oscar Kennedy, VA; N James MacLachlan, CA; Daniel G. Mead, GA; James O. Mecham, WY; Bennie I. Osburn, CA; Eileen N. Ostlund, IA; Richard E. Pacer, MD; Laurie S. Prasnicki, WI; David E. Stallknecht, GA; Susan W. Tellez, TX; Mark C. Thurmond, CA; Mary Anne Williams, CA; George O. Winegar, MI.

The Committee met at the Minneapolis Hilton Hotel, Minneapolis, Minnesota on Tuesday October 17, 2006. There were 32 members and guests in attendance. James E. Pearson, Chair, and William C. Wilson, Vice Chair, conducted the meeting.

Jim MacLachlan, College of Veterinary Medicine, University of California, Davis, discussed development and preliminary characterization of a recombinant canarypox virus vectored vaccine for protective immunization of ruminants against bluetongue virus (BTV) infection were described. Six sheep, immunized with recombinant canarypox virus vector (BTV-CP) co-expressing synthetic genes encoding the two outer capsid proteins (VP2 and VP5) of BTV serotype 17 (BTV-17), developed high titers (40-160) of virus-specific neutralizing antibodies and were resistant to challenge with a field strain of BTV-17. In contrast, five sheep immunized with a commercial recombinant canarypox virus vector expressing the E and preM genes of West Nile virus were seronegative to BTV and developed pyrexia, lymphopenia, and extended, high-titered viremias following challenge exposure to the field strain of BTV-17. These data confirm that the BTV-CP vaccine may be useful for the protective immunization of ruminants against bluetongue, and avoids the problems inherent to live-attenuated BTV vaccines.

W.C. Wilson, Arthropod-Borne Animal Diseases Research Laboratory (ABADRL) Agriculture Research Service (ARS), U.S. Department of Agriculture (USDA), presented potential for bluetongue virus persistence in insect cells. Research being conducted at ABADRL continues to support the hypothesis that BTV overwinters in the insect vector as a core virus with reduced mammalian infectivity. The L3 and S10 RNA gene segments were detected and sequenced from in uninfected cells from the *Culicoides* cell-line. These segments were closely related to BTV serotype 17. Another core protein and a non-structural protein were also detected in uninfected cells from the *Culicoides* cell-line using immunohistochemistry. Neither the genes nor the outer capsid proteins that facilitate mammalian infection have been detected. Extremely low titer of mammalian infectious BTV has been detected after virus purification of large volume of uninfected cells from this cell-line. Dr. Wilson also presented an update on the bench validation of a real-time polymerase chain reaction (PCR) for all eight serotypes of epizootic hemorrhagic disease virus (EHDV). The goal is to combine the

EHDV PCR signature with developing BTV signatures for multiplex real-time PCR assay to detect and distinguish these related viruses.

James Mecham, ABADRL-ARS, then reviewed detection of bluetongue virus in *Culicoides* cell cultures. Cell lines derived from *C. sonor*ensis have been developed at the ABADAL. These cell lines have been shown to support BTV replication, and are potentially valuable tools for better understanding virus replication in the insect vector. However, since little or no cytopathology is noted following infection, detection of virus in these insect cells requires indirect methods, such as co-cultivation with susceptible mammalian cell culture. Immunoperoxidase staining, enzyme-linked immunosorbent assay and *In situ* fluorescent staining were used to directly detect and quantitate BTV in infected *Culicoides* cell cultures. These assays should facilitate the use of the insect cell lines for BTV isolation and studies of virus replication.

Michelle Emery, National Veterinary Services Laboratories (NVSL), Veterinary Services (VS), Animal Plant Health Inspection Service (APHIS), discussed pathogenicity of exotic BTV-1 in sheep and deer. Bluetongue virus type 1 (BTV-1) was previously identified in an isolate originally obtained from a moribund white-tailed deer in LA. (DJ Johnson, et al., JVDI, July, 2006). To assess the pathogenicity of the virus, an experimental challenge was conducted in 4 adult white-tailed deer and 6 adult sheep. One additional animal of each species served as a sham-inoculated control. BTV-1 infection and viremia were demonstrated by PCR and virus isolation in 4/4 challenged deer and in 6/6 challenged sheep. No virus or viral RNA was detected in the control animals. BTV-1 neutralizing antibodies were detected post-infection in all challenged animals but were absent in the controls. Mild, transient pyrexia was the only clinical sign observed in sheep. Infected deer showed a range of signs including hypersalivation, tongue necrosis, lameness, coronary band lesions, and recumbency.

William Wilson, ABADRL, ARS, presented Preliminary development of a real-time PCR for all serotypes of EHDV. EHDV has been associated with bluetongue-like disease in cattle. Although US EHDV strains have not been experimentally proven to cause disease in cattle there is serologic evidence of infection in cattle. BTV causes an estimated $125,000,000 annual loss to the U.S. livestock industry and about $3,000,000,000 annual losses worldwide. Therefore rapid diagnosis and differentiation of BTV and EHDV is required. Our laboratory has been developing the molecular basis for early detection of indigenous and exotic BTV and EHDV disease outbreaks. The foundation for these tests was accomplished by phylogenetic analysis of two conserved target genes; one that is highly expressed in infected mammalian cells, the other is highly expressed in infected insect cells. The analysis of all BTV and EHDV prototype strains indicated that a complex primer design will be necessary for a comprehensive gene amplification diagnostic test. This information has been used as the basis for the development of rapid EHDV real-time PCR that detects all EHDV serotypes. The EHDV detection assay does not cross-react with BTV serotypes; however, this assay is less sensitive than nested-PCR protocols. The sensitivity of 1 pg double-stranded RNA for all eight serotypes is sufficient for diagnostic applications without the contamination problems associated with standard PCR and especially nested-PCR tests.

Ray Lenhoff, Lawrence Livermore National Laboratory, Chemistry Biology and Nuclear Programs discussed agricultural vesicular/ulcerative disease assays and presented an updated on bluetongue virus assay.

Seventeen PCR or rtPCR assays are being developed for three foreign animal diseases and four endemic look-alike diseases (bovine virus diarrhea, infectious bovine rhinotrcheaitis bovne papular stomatitis and bluetongue). The tests will have four built in controls and use a 21-plex bead mix. Viral DNA and RNA targets will be screened simultaneously. The tests are being optimized for a clinical sample matrix (oral swab) from bovine, porcine, and small ruminants. They will have a high confidence level and use multiple loci per disease. They will also include internal controls that measure assay integrity and comprehensive protocols have been developed. Assay multiplexing will reduce labor, consumable and reagent costs.

The current bluetongue virus (BTV) bead based assay (2005) uses multiplex signatures against segment 1 and 9; however this method is probably inadequate to cover the genetic diversity of this virus. Another assay is being developed against segments 5 (NS1) and 10 (NS3 and NS3a), which are conserved. Twenty seven BTV segment 5 and 10 signatures (PCR reactions) were generated using a combination of ARS and genbank sequence information. These have been used to screen against background nucleic acids (from soils, aerosols, microbes and vertebrate DNAs) and nine of the signatures were removed. After screening against domestic BTV target RNA, four more signatures were removed. In collaboration with ARS (Bill Wilson), screening against all 24 BTV serotypes is underway. The best signatures will be incorporated along with two from the previous assay (2005). The resulting assay will be used in the bovine vesicular multiplex assay for 2006. These signatures should also prove useful as single taqman assays or in additional multiplex combinations for BTV detection.

Following the scientific presentations, other reports and updates were presented to the Committee. Danny Mead, Southeastern Cooperative Wildlife Disease Study (SCWDS), University of Georgia, Athens, GA provided an update on hemorrhagic disease surveillance conducted by SCWDS. During 2005, bluetongue and epizootic hemorrhagic disease viruses were isolation from white-tailed deer in six states. EHDV-2 was isolated from free-living and captive white-tailed deer in Arkansas, Illinois (two isolates) Kansas, (five isolates), Missouri (five isolates) and Texas (three isolates). BTV-17 was isolated from white-tailed deer in Louisiana and BTV-13 was isolated from two white-tailed deer in Texas.

To date in 2006, EHDV-1 was isolated from Mississippi and Missouri and EHDV-2 was isolated from deer from Colorado, Georgia, Illinois, Kansas, Louisiana, Missouri, and Texas. Isolations of BTV were restricted to Kentucky (BTV-17), and Missouri (BTV-10, BTV-11, BTV-17). All isolations came from free-living and captive white-tailed deer. The most noteworthy isolation during 2005 was the isolation of EHDV-2 from a white-tailed deer from Texas in March.

As a result of the detection of BTV-1 during 2004, the Southeastern Cooperative Wildlife Disease Study, in cooperation with the Louisiana Department of Wildlife and Fisheries, and NVSL is conducting follow-up surveillance in southern Louisiana. During 2004, 146 BTV ELISA positive samples from white-tailed deer (collected between 2000 and 2003) were tested for antibodies to BTV-1 by NVSL. No evidence of previous exposure to BTV-1 was detected in any of these retrospective samples. During 2005, 399 serum samples were collected from hunter-killed deer and 123 (31%) of these tested positive on the BTV-ELISA. Virus neutralization test were conducted on all of these positive animals at SCWDS (BTV-10, 11, 13, 17) and NVSL (BTV 1, 2).

Antibodies to BTV-1 were detected in 38 of these animals, but serologic data did not clearly indicate exposure to BTV-1; all of the BTV-1 positive samples reacted to one on more native BTV serotypes. However, based on antibody titers to individual serotypes, there were several (6) samples that provided possible (but not confirmatory) evidence of BTV-1 exposure. These were detected from samples collected in Vernon (1), Allen (1), Cameron (1) and St. Mary (3) Parishes. The cluster in St. Mary Parish (only 32 deer were sampled in this parish) is suggestive of BTV-1 exposure. All evidence, to date, suggests that if currently present, BTV-1 in very localize to the immediate area where the virus was initially detected. During 2006, additional samples will be collected in the immediate vicinity of St. Mary Parish.

Eileen Ostlund, NVSL, VS, gave an update on diagnostic observations for bluetongue, epizootic hemorrhagic disease, and bovine leukosis virus in the United States. Bluetongue virus or RNA was detected in 24 samples submitted during calendar year 2005. The positive bluetongue virus isolation and PCR test results from submissions to the NVSL in 2005 are listed below (Table 1):

Table 1
BT virus isolation (VI) / PCR positives, Calendar year 2005

Location	No.	Species	Type	VI	PCR
California	6	Bighorn Sheep		Negative	Positive
Illinois	1	Cattle		Negative	Positive
Maryland	2	Cattle		Not done	Positive
South Dakota	2	Deer*	11	Positive	Positive
Nebraska	12	Bovine hemoglobin		Negative	Positive
Ohio	1	Bovine serum albumin		Negative	Positive

**also positive for EHDV type 2*

During calendar year 2005, two samples tested positive for EHDV by virus isolation and/or PCR. One sample was submitted from a bovine in IA and was PCR positive for EHDV RNA. The second sample, listed in the table above, was from a deer in SD. Both BTV serotype 11 and EHDV serotype 2 were isolated from the SD deer sample. During the calendar year 2006 (January 1– September 30, 2006), bluetongue virus or viral RNA been detected by PCR from 10 specimens submitted to date in 2006. The positive bluetongue virus isolation and polymerase chain reaction (PCR) test results are listed below (Table 2):

Table 2
BT virus isolation (VI) / PCR positives, January-September, 2006

Location	No.	Species	Type	VI	PCR
Illinois	3	Cattle		Negative	Positive
Florida	1	Cattle		Not done	Positive

South Dakota	1	Cattle		Not done	Positive
Missouri	1	Deer	17	Positive	Not done
Texas	1	Cattle		Negative	Positive
New Mexico	1	Sheep		Not done	Positive
Nebraska	1	Bovine hemoglobin		Negative	Positive
Oregon	1	Mule deer	17	Positive	Positive

To date, EHDV RNA was confirmed in a virus isolate from a deer in MO. In addition, EHDV type 2 was identified in a deer isolate submitted to NVSL from MN.

2006 Bluetongue Serology Proficiency Exam

Fifty eight laboratories participated in the 2006 bluetongue (BT) proficiency test. The panel consisted of 20 bovine serum samples. The passing score was two or fewer samples missed. Two laboratories failed the 2006 bluetongue proficiency panel on the first attempt. Both laboratories passed on a retest. As of October 5, 2005, there are 58 laboratories approved to conduct official (export) BT serology tests.

Fifty-nine laboratories participated in the 2006 bovine leukosis (BLV) proficiency test. The panel consisted of 20 serum samples and the passing score was one or fewer samples missed. Three laboratories failed the 2006 bovine leukosis proficiency panel on the first attempt. To date, two of these laboratories have successfully completed a retest. As of October 5, 2005, there are 58 laboratories approved to conduct official (export) BLV serology tests.

Samira Belaissaoui**,** Imports / Exports, Animal Health and Production Division, Canadian Food Inspection Agency (CFIA), Ottawa, Canada, presented on bluetongue regulatory programs in Canada.

On July 10, 2006, after stakeholder consultation, CFIA announced a revised import policy for bluetongue. The following are the components of that policy:

1. CFIA concluded that there may be only very limited opportunities for bluetongue to spread and become established beyond a single season.
2. The revised policy will permit the importation of ruminants from the United States without testing for bluetongue.
3. Bluetongue due to serotypes endemic in the United States will be changed from being a Reportable Disease to Immediately Notifiable
   - The Reportable Diseases Regulations require animal owners, veterinarians and laboratories to report the presence or suspicion of a listed disease to the CFIA immediately so that control or eradication measures can be applied. The diseases listed under these regulations are usually of significant importance to human or animal health or to the Canadian economy.
   - Immediately Notifiable Diseases are animal diseases that are exotic to Canada, but unlike the reportable diseases there are no control or eradication programs in place. Laboratories will continue to be required to report any bluetongue reactors to the CFIA.
4. The role of the CFIA would be limited to investigating mortalities in domestic ruminants such as deer and sheep in the event of an outbreak. As a precaution, the CFIA will enhance its bluetongue surveillance, moving from triennial to

annual monitoring. This activity, coupled with ongoing research is intended to monitor for the absence of the disease.

5. The CFIA would still be able to fulfill its international reporting obligations to trading partners and the World Organization for Animal Health (OIE) by making bluetongue U.S. serotypes immediately notifiable.
6. The announced policy will be implemented over the coming months after finalizing the necessary regulatory amendments.

Arnaldo Vaquer, National Center for Import Export (NCIE), VS-APHIS, gave a report on bluetongue import/export issues. In the United States, there are no outstanding BT export issues. For Canadian exports, there are no changes in export requirements to date. Currently, no BT testing is required for feeder cattle from 39 low incidence states or feeder cattle from 11 high incidence states if the cattle reside in a qualifying state for 60 days prior to export. No BT testing is required for feeder sheep and goats (neutered males only) exported between October 1 and March 31. For issues relating to imports, the European Union (EU) BT situation doesn't impact import requirements due to bovine spongiform encephalopathy (BSE) prohibition for live animals. The import of semen is not affected. Around the world, countries that are reporting BT include Germany, Belgium, France, Netherlands and Spain.

Vincenzo Caporale, European Commission, Brussels presented on behalf of Bernard Van Goethem, the response of the European Union to recent bluetongue outbreaks in Belgium, Netherlands, Germany and France.

The situation in Netherland, Belgium, Germany and France continues to garner attention. The first outbreaks were detected in Holland in mid-August, in which the virus wasidentified as BTV-8. A very warm July (36°C/97°F in Brussels), followed by a fresh and wet August and again warm in September and early October contributed to the problem. There were apparently two peaks of disease: end of August and end of September/early October. The number of outbreaks, as of October 11, 2006, is as follows:

- Netherlands 211 cases
- Belgium 297 cases
- Germany 215 cases
- France 5 cases

This is the first time that BT has been detected in the northwestern part of the EU (53°N). Most of the outbreaks located between 50°N and 52°N. Previously, BTV 1, 2, 4, 9 and 16 have been found in the EU in southern Europe/Mediterranean area. BTV-8 is known to occur in sub-Saharian Africa, South America and perhaps India, however it has never reported before in Europe, North Africa or Middle East. This begs the question of "where has it come from?" Many epidemiological features of this serotype are still unknown, although clinical symptoms have been found in both cattle and sheep. Potential vectors seem to be *C. obsoletus* (most likely), *C. pulicaris* and possibly *C. dewulfi.* No *C. imicola* was detected in the affected zones. The current outbreak is likely to elapse within one-two months, but what happens next year is yet to be seen.
The EU as taken measure to control BT, including:

- Restrictions to movement of animals to or from the infected holding
- Restrictions to movement of animals from the areas where virus is

circulating
- Confinement of animals of the susceptible species to prevent contact with the vectors
- Insecticides treatment

Regarding zoning, EU Member States must extend measures also to holdings located within a radius of 20 kilometres (km) around the infected holding. In addition, protection (100 km radius) and surveillance zones (+ 50 km) are also established. For transparency reasons, restricted zones are also laid down in Commission legal acts. For trade purposes, restricted zones are identified on the basis of the BT virus serotype(s) circulating in the zone: several zones currently in place in the affected Member States.

The EU is also controlling the movement of animals by restrictions put on the movement of domestic (national) movements, for Intra-Community trade within restricted zones, for Intra-Community trade from restricted zones to free zones and derogations on animal movements. Animals can be moved out of the restricted zones under certain conditions, such as vaccination, pre-movement isolation, protection from attack from *Culicoides* and laboratory testing, per the OIE. Similar conditions can be applied for the movement of semen, ova and embryos.

Disease surveillance is an important part of the EU's efforts. It is fundamental to assess the actual risk posed by the disease and develop control measures. Current surveillance is in place to monitor the dynamics of the disease in restricted zones and modify the zones accordingly; confirm the absence of the disease in free zones; and provide for early detection of the entry of virus into free zones. Surveillance is based on four fundamental tools: serological and virological surveillance; entomological surveillance; clinical surveillance; and wildlife.

The OIE guidelines on BT surveillance are not yet adopted (work is ongoing)The Commission and the EU Member States are also working on a EU harmonised and enhanced BT monitoring and surveillance scheme. More information on the EU animal health policy and on the bluetongue measures can be found on the website of the EU Commission's DG Health and Consumer Protection
http://ec.europa.eu/food/animal/index_en.htm
Slides of BTV 8 induced lesions observed in cattle in Belgium and a map of the location of all the cases were shown

Jim Mecham, ABADRL-ARS, provided an update on the Arthropod-Borne Animal Diseases Research Laboratory (ABADRL), Laramie, Wyoming. The mission of the laboratory is to solve major emerging and/or exotic arthropod-borne disease problems that affect the U.S. livestock industry and wildlife. Many of these arthropod-borne diseases also have an effect on human health. Research is conducted in the Animal Health (NP-103) and the Veterinary, Medical, and Urban Entomology (NP-104) National Programs. The ABADRL operates Biosafety Level (BSL)-1, BSL-2 and BSL-3 facilities. Contracts are also in place with cooperators for use of BSL-3Ag and BSL-4 laboratory and animal space. The ABADRL owns six buildings and leases additional buildings and space from the University of Wyoming. Major renovation efforts are currently under way on the BSL-3 laboratories in Laramie and will be completed this year. In addition the large animal facilities are being renovated to provide ABSL-2 enhanced space. The costs of the renovation are $2.1 million. Of this total, $1.5 million is being spent to renovate the Round Building, which will have ca. 1,500 ft^2 of BSL-3 space and ca. 5410

ft ^{2} of BSL-2 space when completed. The BSL-3 area will be separate from the BSL-2 space. The remaining $0.6 million is being used to renovate the Large Animal Building, and includes new wall barrier coatings, new steam lines, a new controller for the alkaline tissue digester, new animal pens, new roof, backup power, etc. When completed, the building will have 2,680 ft^2 and will be re-classified from BSL-3Ag to ABSL-2.

Current research at the ABADRL includes studies on virus-vector-host interactions, diagnostic development, development of effective disease and vector control and management strategies, vaccine development, vector genomic studies, and studies on vector-virus ecology. The goal of the research is to transfer information and technology to the livestock industries, and to action and regulatory control agencies. The majority of the Animal Health Program at the ABADRL is being redirected to emphasize research on Rift Valley fever virus and exotic bluetongue viruses. The exact program details are being determined, but will include aspects of new diagnostic development, risk assessment, pathogen characterization, and vector-host interactions. The Veterinary, Medical, and Urban Entomology component of the ABADRL research mission, which studies vector competence and protection of U.S. livestock and wildlife from arthropod-borne diseases, is currently active, but may be revised to align more closely with changes in the Animal Health Program. During 2006, a number of research accomplishments were made at the ABADRL. These included development and refinement of diagnostic techniques for BTV; DNA and subunit vaccine development for West Nile virus detection; characterization of BTV receptors on vertebrate cells; studies on BTV persistence in *Culicoides*; studies on mosquito repellants applied to cattle in the field; studies on the effect of temperature on *Culicoides* competence for BTV infection and transmission; and studies on genetic variation of *Aedes triseriatus* and vector competence for LaCrosse encephalitis virus as a model for Rift Valley fever virus.