REPORT OF THE COMMITTEE ON BLUETONGUE AND BOVINE RETROVIRUSES

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A. Welcome:

The Committee met Tuesday, November 8, 2005 at the Hershey Lodge and Convention Center, Hershey, Pennsylvania. There were 33 members and guests in attendance. James E. Pearson, Chair, and William C. Wilson, Vice Chair, conducted the meeting.

D. J. Johnson, United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), National Veterinary Services Laboratories (NVSL) reported on the first isolation of the bluetongue virus type 1 in the United States. In November 2004, a white-tailed fawn found near Belle Isle, Louisiana (marshy, remote area near the Gulf Coast) was observed stumbling and acting strangely. The animal was 'euthanized' by a hunter and tissues were submitted to the Southeast Cooperative Wildlife Disease Study (SCWDS) in Athens, GA. Bluetongue virus (BTV) was isolated from the tissue samples at the SCWDS. The isolate was forwarded to the National Veterinary Services Laboratories (NVSL) for typing where it was identified as BTV type 1 (BTV-1) by virus neutralization and (Polymerase Chain Reaction (PCR). The PCR procedure used for identification targeted the outer capsid genome (L2) which is the viral region conferring type identity. Comparison of the isolate sequence (1779 base pair region) with other published BTV types revealed 88% homology to a South African BTV-1 vaccine strain. The isolate also showed homology to Australian BTV-1, but to a lesser degree. No significant homology was observed with any other published BTV genomes, including types present in the United States. This is the first report of BTV-1 in the United States.

Dr. James Mecham USDA, Agriculture Research Service (ARS), Anthropod Borne Animal Disease Research Laboratory (ABADRL) presented information on sequence analysis of a BTV-1 isolate made in 2004 from a deer in Louisiana. Sequence data was generated from both the S5 and S7 genome segments of this isolate and compared to sequence generated from the South African BTV-1 prototype strain and to other BTV-1 sequences available from GenBank. Phylogenetic analysis based on this sequence data indicates a fairly close relationship between the U.S. BTV-1 isolate and African isolates of BTV-1.

E. N. Ostlund, USDA-APHIS-VS-NVSL reported on the Serelogical Survey of domestic ruminants in Louisana for antibodies to bluetongue virus type 1. Bluetongue virus type 1 (BTV-1) was isolated from a deer in St. Mary Parish, LA in the fall of 2004 (see report by D. J. Johnson et. al). Although bluetongue virus serotypes 2, 10, 11, 13, and 17 are considered endemic; BTV-1 had not previously been identified in the United States. In the spring of 2005, domestic ruminants in St. Mary Parish were sampled and tested for evidence of exposure to BTV-1. A total of 549 animals were tested: 460 cattle, 47 sheep and 42 goats. None of the animals were reported to have a history of bluetongue-like illness and none were vaccinated against bluetongue. Serum was screened by CELISA for antibodies to any BT serotype. Sixty-one samples (11%) tested positive in the screening CELISA. The CELISA positive samples were further examined in virus neutralization (VN) assays to detect neutralizing antibodies to BTV-1 and BTV-2. Of the 61 animals tested by VN, 20 demonstrated detectable neutralizing antibodies to BTV-1. Of these, 6 animals (5 cattle, one sheep) had significantly higher titers to BTV-1 compared to BTV-2. Presence of BTV-1 specific antibody titers in the serum from the 6 animals supports a prior exposure to

BTV-1. Additional studies of domestic and wild ruminants as well as *Culicoides* spp. are in progress; these will continue to investigate whether BTV-1 has become established in the study area.

C.Y. Kato, USDA-ARS-ABADRL presented information regarding an improved, high through put method for detection of bluetongue virus in *Culicoides* (biting midges). Current protocols for RT-PCR assay of bluetongue virus (BTV) RNA in biting midges (*Culicoides* sp.) involve time consuming steps for sample (single insect) homogenization, RNA extraction, and RT-PCR assay. Further, current RT-PCR methods for assay of BTV RNA are either not very sensitive, require multiple steps for chemical denaturation, reverse transcriptase reaction, 1° and 2° PCR amplification, and/or Real Time PCR instrumentation. We report a new, rapid (less than 5 h start-to-finish), and sensitive assay method for detecting BTV in biting midges.

The method enables the extraction of nucleic acids from individual *Culicoides* specimens in a 96 - well plate format, followed by the novel use of infrared (IR) dye-labeled primers for virus detection. Specialized beads are used for mechanical disruption of insect tissues in a homogenization buffer that is compatible with cell culture and RNA extraction. The process begins with a 1 min homogenization of the 96 insects (individuals) in a 96 well plate. One tenth of the homogenate from each specimen is saved for cell culture virus isolation, while the remainder of each sample is used for rapid RNA extraction (Qiagen, Valencia, CA). An aliquot of the extracted RNA is heat denatured, then used in a single step reverse transcriptase PCR (RT-PCR) reaction with IRdye-labeled primers. The RT-PCR products are separated by agarose gel electrophoresis and visualized by an infrared scanner). The adaptation of IR-dye-labeled primers (Licor, Lincoln, NE) and a one step RT-PCR increases the sensitivity of a highly selective primer set for BTV serotypes 2, 4, 6, 10, 11, 13, 16, and 17 by 500-fold (i.e., from 100 fg (femptograms, 5000 viral particles); Akita *et al*., 1992) to a sensitivity of 0.2 fg of purified BTV RNA (5 viral particles). This new method is especially useful for epidemiologic studies involving arboviruses in that it allows for sensitive and specific detection of virus in 96 insect individual specimens by RT-PCR in less than 5 h, and the ability to perform virus isolations on the original samples.

W.C. Wilson, USDA-ARS-ABADRL gave an update on BTV persistence in *Culicoides Sonovensis*. Research being conducted at ABADRL supports 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 in uninfected cells from the Culicoides cell-line by RT/PCR amplification. These segments were sequenced and are 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. No mammalian infectious BTV has been detected in uninfected cells from this cell-line. Dr. Wilson also presented an update on the development of real-time PCR for all 8 serotypes of epizootic hemorrhagic disease virus.

Eileen Ostlund, USDA-APHIS-VS-NVSL presented on update on diagnostic observations for bluetongue, epizootic hemorrhagic disease and bovine leucosis virus in the United States.

Bluetongue (BT) virus and epizootic hemorrhagic disease (EHD) virus isolations/PCR positives, calendar year 2004

In 2004, virus isolation attempts for BT and/or EHD were completed on 226 samples and 165 samples were tested by PCR. There were 233 submissions of imported fetal bovine serum for BT virus safety testing by sheep inoculation requiring 430 sheep. None of the sheep inoculated with imported fetal bovine serum in 2004 developed BT virus antibodies. In 2004, epizootic hemorrhagic disease virus (EHDV), serotype 2 was isolated from a white-tailed deer sample. The deer was in Iowa. The positive bluetongue virus isolation and polymerase chain reaction (PCR) test results from submissions to the National Veterinary Services Laboratories (NVSL) in 2004 are listed below:

Table 1
BT virus isolation (VI) / PCR positives, Calendar year 2004

Location	No.	Species	Type	VI	PCR
AL	1	Cattle			X
CO	2	Sheep	17	X	
CO	1	Cattle			X
FL	93	Cattle			X
LA	1	White-tailed deer	1	X*	
MT	1	Cattle			X
NE	6	Bovine hemoglobin			X
TX	1	Sheep	11	X	
TX	3	Cattle			X
Costa Rica	1	Cattle			X

*submitted to NVSL as a virus isolate

Bluetongue (BT) virus and epizootic hemorrhagic disease (EHD) virus isolations/PCR positives, calendar year 2005 (January 1 – November 2, 2005)

Bluetongue virus has been detected by PCR from 9 specimens submitted to date in 2005. The PCR positive samples were from deer, bighorn sheep, dried bovine hemoglobin and bovine albumin. Bluetongue virus type 11 was isolated from a deer in South Dakota. Bluetongue virus was also isolated from three Florida sheep in October of 2005; the isolates have not been typed. To date in 2005, BTV and EHDV mixed infections have been identified by PCR in two deer from South Dakota. Virus isolation was attempted on the first case and both BTV and EHDV were isolated. The BTV isolate was serotype 11 (as noted above); the EHDV isolate has not yet been typed.

2005 Bluetongue Proficiency Examination

Fifty eight laboratories participated in the 2005 bluetongue proficiency test. The panel consisted of 20 serum samples. The passing score was one or fewer samples missed. All 58 laboratories passed the 2005 bluetongue proficiency panel on the first attempt. As of October 31, 2005, there are 58 laboratories approved to conduct official (export) BT serology tests.

2005 Bovine Leukosis Virus Proficiency Exam

Sixty one laboratories participated in the 2005 BLV proficiency test. The panel consisted of 20 serum samples and the passing score was one or fewer samples missed. All 61 laboratories passed the 2005 proficiency panel on the first attempt. As of October 31, 2005, there are 61 laboratories approved to conduct official (export) BLV serology tests.

David Stallknecht, Southern Cooperative Wildlife Disease Study (SCWDS) gave an update on hemorrhagic disease surveillance conducted by SCWDS. During 2004, bluetongue and epizootic hemorrhagic disease viruses were isolation from white-tailed deer in five states. EHDV-2 was isolated from white-tailed deer in Illinois (5 isolates), Kansas (1 isolate), and Texas (1 isolate). BTV-17 was isolated from white-tailed deer in Idaho (1 isolate) and Texas (1 isolate). BTV-17 was also isolated from a single mule deer from Idaho. The most noteworthy isolation during 2004 was BTV-1 from a white-tailed deer in Saint Mary Parish, Louisiana. This virus was identified by the National Veterinary Services Laboratory, USDA.

As a result of the detection of BTV-1, 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. To date, 146 BTV ELISA positive samples from white-tailed deer

(collected between 2000 and 2003) have been tested for antibodies to BTV-1 by NVSL. Twenty four of these samples tested positive for BTV-1 on serum neutralization tests but it possible that these positive results represent cross-reactions with native BTV serotypes. Titers were generally low (10-40) and when compared to SN results from SCWDS, all but one of these positive samples tested positive for multiple BTV serotypes. These results are currently being confirmed by NVSL. Collections of serum and tissue from hunter-killed white-tailed deer in Louisiana, including Saint Mary Parish are in progress. Samples will be tested by serology and virus isolation.

Samira Belaissaoui, Canadian Food Inspection Agency (CFIA), Animal Health and Protection Division, Import-Export on Canada's bluetongue regulatory programs. Bluetongue is a reportable disease in Canada. Canada is still free from bluetongue, outside of the Okanagan Valley in British Colombia, based on surveys of our national cattle herd. Surveys are conducted every 3-4 years and the last survey was conducted in 2003. In 2004, there was a change in the Canadian import regulations to allow the importation of feeder cattle from all of the US without bluetongue testing, except for 11 high incidence states. The animals must be identified with a Canadian eartag at the feedlot, have not been residence in a non high-incidence state for at least 60 days, and must go directly to a CFIA approved feedlot and from there to slaughter or re-exported to the US. The restrictions on feeder cattle from high incidence states and on breeder animals have not changed; testing for bluetongue is still required.

Arnoldo Vaquer, USDA-APHIS-VS-National Center for Import-Export (NCIE) reported on the NCIEs' import-export issues. As reported by Dr. Belaissaoui, feeder cattle can be imported from the non high-incidence states. Dr. Vaquer stated that all sheep and goats that are exported to Canada from all US states must be tested and the testing requirements for export of animals for breeding have not changed. The NCIE is working with CFIA to reduce these restrictions. Dr. Vaquer also reported that procedures were established to allow the importation of semen form Tobago and to ship 400 live animals to Cuba. Canada is holding a bluetongue summit on November 17, 2005 and the US will be represented at the meeting.

Richard Mayer, USDA-ARS-ABADRL gave an update on ABADRL. The mission of the ABADRL is to solve major emerging and/or exotic arthropod-borne disease problems that affect the U.S. livestock industry, wildlife, and human health. Currently there are 30 employees, seven of which are scientists that are responsible for the conduct of the research mission. The laboratory leases facilities and land from the University of Wyoming and owns six buildings. Currently the large animal and laboratory containment facilities are undergoing extensive renovation and repairs. When completed the BSL-3 laboratory will be approximately 1,500 ft^2 while the large animal isolation facility will be 2,680 ft^2 of BSL-3 Ag space. Additionally, a new insectary (2,000 ft^2) is being constructed that will allow for a broader research program on a larger number of insect vector species. Research areas include: virus-vector-host interactions; development of new and improvement of existing diagnostic methods; development of effective disease/vector control and management strategies; vaccine development; vector genomics; vector-virus ecology. In addition to working on bluetongue virus, epizootic hemorrhagic disease virus, vesicular stomatitis virus, and West Nile virus, Rift Valley fever virus will be added to the ABADRL's responsibilities in FY 2006. This year field surveys of mosquito vectors of West Nile virus (WNV) included species determinations, PCR determination of WNV, and PCR determination of the source of the host bloodmeal. Mosquitoes (9-10 species) with bloodmeals (484 examined) revealed all of the mosquitoes species examined fed on large animals (identified hosts were cows, pronghorn antelope, horses, moose, sheep, and humans). Three species fed on small mammals (i.e., cats, voles, rabbits and skunks) and five species fed on birds. Research on pyrethroids showed that low-pressure sprays of 0.25% Permethrin applied every five days to cattle were effective in suppressing mosquito bloodfeeding by ca. 87%. A new scientist (Kristine Bennett) has joined the staff and she will be performing research on *Culicoides* genomics, which will initially be directed towards *C. sonorensis* linkage maps and a mitochondrial sequencing project. *C. sonorensis* was validated as a competent vector for vesicular stomatitis and the report has been accepted in the Journal of Medical Entomology, 2005.