

REPORT OF THE COMMITTEE ON BLUETONGUE AND BOVINE RETROVIRUSES

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The Committee met at John Ascuaga's Nugget Hotel in Reno, Nevada on October 22, 2007. There were 41 members and guests in attendance. James E. Pearson, Chair, and William C. Wilson, Vice Chair, conducted the meeting.

The Committee agenda included three sections of presentations. These presentations included a summary of bluetongue and epizootic hemorrhagic disease situation, Canadian bluetongue import policy and a research update.

Summary of Bluetongue and Epizootic Hemorrhagic Disease Situation

Donna J. Johnson, U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), National Veterinary Services Laboratories (NVSL), presented, Exotic Bluetongue Viruses Identified from Ruminants in the Southeastern U.S. from 1999-2006. Supporting authors include Peter Mertens and Sushila Maan, Institute for Animal Health- Pirbright Laboratory and Eileen Ostlund, USDA-APHIS-VS-NVSL. This paper is included at the end of the Committee report in these proceedings.

David E. Stallknecht, Southeastern Cooperative Wildlife Disease Study (SCWDS), University of Georgia, gave an update on Bluetongue and Hemorrhagic Disease Surveillance.

This year we have received numerous reports of hemorrhagic disease (HD) in deer and have received an unprecedented number of samples for virus isolation originating mostly from both penned and wild white-tailed deer. As of October 16th, we have made 237 virus isolations we continue to receive large numbers of samples as well as reports every day. Nearly all virus isolations have been epizootic hemorrhagic disease virus-serotype 2 (EHDV-2) from white-tailed deer. We also have isolated EHDV-1 and bluetongue virus-10, -11, and -17. EHDV-1, BTV-10 and BTV-11 and BTV-17 (one each) have been isolated from Missouri. We have one isolate of BTV17 from a mule deer in New Mexico and three isolates of BTV-17 from pronghorn in Wyoming.

For the second year, we have also isolated EHDV-6. In 2006, EHDV-6 (as identified by NVSL and Pirbright) was isolated from white-tailed deer (free-ranging and captive) in Illinois and Indiana. The 2007 isolated came from a captive white-tailed deer in Missouri.

Freeda Issac, National Center for Import Export (NCIE), USDA-APHIS-VS, provided a report on the Florida Bluetongue Surveillance Study.

A bluetongue survey will be conducted by the SCWDS at the request of the USDA-APHIS-VS. The goal of this study is to determine the presence of *Culicoides spp.* and bluetongue virus (BTV) in the Southeastern United States. Surveys for *Culicoides spp.* will be conducted through the Cooperative Agreement for Exotic Arthropod Surveillance in the Southeastern United States and Puerto Rico. Surveys for BTV will be conducted through the above Cooperative Agreement and through the Cooperative Agreement for Disease Relationships that Involve Wildlife, Domestic Livestock and Poultry.

The objectives of the study is to determine the species of *Culicoides* present in the state of Florida and to further develop a surveillance system for BTV present in the Southeastern United States in white-tailed deer. The principal investigators are Dr. Joseph L. Corn and Dr. John R. Fischer.

Surveys will be conducted at selected sites in Florida at locations of previous BTV activity. Additional trapping will be conducted statewide between Spring and Fall of 2008 to account for seasonal activity of *Culicoides*. Insect trapping will be coordinated with wildlife surveys for exotic ticks and other arthropods and will employ several light traps per site at 4-8 geographic locations per month. Insect specimens will be processed and submitted for identification to a laboratory. SCWDS will maintain a database to include species collected, date and trapping locations.

SCWDS will evaluate the feasibility of collection of *Culicoides* spp. from sentinel wildlife at selected sites in Florida. Where feasible, SCWDS will collect *Culicoides* spp. and these specimens will be processed and submitted to a laboratory for identification. SCWDS will maintain a database to include species collected, sentinel host, date and sentinel locations.

SCWDS will initiate contact with the owners of captive deer facilities in Florida to determine the feasibility of collection of *Culicoides* spp. in the vicinity of captive deer facilities and of collection of blood specimens from captive deer <1 year of age for BTV isolation. *Culicoides* specimens will be processed and submitted to a laboratory for identification. SCWDS will maintain a database to include species collected, sentinel host, date, and location of collection.

SCWDS will enhance its collection of diagnostic specimens for BTV isolation from white-tailed deer from the Southeastern United States, specifically Georgia, Florida, Alabama, Mississippi, Texas and Louisiana. Diagnostic samples are submitted by SCWDS member state wildlife management agencies and other sources throughout the United States. Specimens are collected from clinically ill or dead white-tailed deer for diagnostic testing for BTV and EHDV, a related orbivirus. SCWDS has existing diagnostic capabilities (virus isolation, polymerase chain reaction (PCR) and supporting diagnostic tests to identify known North American BTV and EHDV serotypes) to conduct this work and has partnered with National Veterinary Services Laboratory (NVSL) and Agriculture Research Service (ARS), Laramie, in previous orbivirus surveillance and research. EHD viruses utilize the same *Culicoides* vectors and risk factors for their potential range expansion or introduction into the United States are similar to those of BTV. Because EHD viruses represent a significant pathogen of white-tailed deer, their inclusion should enhance participation from wildlife agencies.

Still under discussion and development by APHIS is the sampling of sentinel cattle herds in Florida and other states to determine the presence of BTV and fluctuations in viral activity. Periodic sampling of sentinel cattle may represent the most efficient means of obtaining BTV isolates.

These surveys will help to determine which species of *Culicoides* are present in Florida, including exotic species not previously reported. In addition, surveys for BTV will help to determine which BTV serotypes are present in the southeastern United States and BTV isolates will provide much needed biological material to determine their origin. *Culicoides* and BTV identified will be reported to APHIS on a quarterly and annual basis. Any unknown BTV recovered from white-tailed deer will be immediately sent to NVSL for confirmation and identification.

An update on diagnostic observations for bluetongue, epizootic hemorrhagic disease, and bovine leukosis virus in the United States was given by Eileen Ostlund, NVSL-VS-APHIS-USDA. Details of this update are included at the end of this report.

Rudy Meiswinkel, Central Institute for Animal Disease Control, Lelystad, The Netherlands presented an update and overview of the BTV serotype 8 epidemic in Northern Europe. This paper is included in these proceedings at the end of this Committee report.

Canadian Bluetongue Import Policy

Samira Belaïssaoui, Animal Health and Production Division, Canadian Food Inspection Agency (CFIA), Ottawa, Canada, provided a report on the bluetongue import policy from Canada.

A background of the situation was first presented. After broad consultation, Canada announced in 2006 the removal of BT-related import conditions for ruminants from the United States. It has been concluded that there may be only very limited opportunities for bluetongue to spread and become established beyond a single season. The new import conditions came into effect in early 2007 after the necessary regulatory changes.

The current situation is:

- Under the Health of Animals Act, the traditional United States serotypes 2, 10, 11, 13, 17 are immediately notifiable while the other 24 serotypes are reportable;
- When the US announced the discovery of exotic serotypes in Florida, CFIA changed its BT related import conditions for that state
- This is consistent with CFIA policy that exotic BT serotypes (those not normally found in North America) will continue to be subject to risk mitigation measure

New import conditions: A negative test for BT virus infection by cELISA must be performed and documented within 30 days prior to import. In the case of a positive result, a negative PCR test performed and documented within 30 days prior to import will qualify the animal for import.

Research Presentations

Jim MacLachan, University of California-Davis, presented The Pathogenesis and Pathology of Severe Bluetongue of Sheep.

The results of experimental infection of sheep with virulent bluetongue virus serotype 4, studies were presented; the work was done collaboration with colleagues at the Faculty of Veterinary Science, University of Pretoria. The investigators induced severe bluetongue in the inoculated sheep. The disease initially was characterized by hemorrhagic manifestations where later in the course, at approximately two weeks after infection, the animals developed severe respiratory signs as a consequence of pulmonary edema. The signs and lesions in these experimentally infected sheep were very similar to those that occurred in sheep infected at the Institute of Virology and Immunoprophylaxis (IVI) facility in Switzerland with the strain of bluetongue virus serotype 8 that is currently spreading throughout northern Europe. Potential mechanisms of pathogenesis were discussed, along with future avenues for research.

Will K Reeves Arthropod-Borne Animal Diseases Research Laboratory (ABADRL), USDA-ARS, presented, Characterizing the Epidemiology of Bluetongue Virus Serotype 1 in Southern Louisiana.

In November 2004 BTV-1 was isolated from the tissues of a hunter-killed white tailed deer from southern Louisiana. There was significant concern that BTV-1 might be established in Louisiana. Unfortunately, the hurricanes season of 2005 caused so much devastation that monitoring the status of BTV-1 in southern Louisiana was impossible. *Culicoides* spp. were sampled from southern Louisiana in 2006 and 2007. Four pools of *Culicoides* tested positive for BTV but the virus serotype appears to be BTV-17. The reason for the disappearance of BTV-1 from Louisiana remains unknown.

Analysis and Characterization of the Receptor for Bluetongue Virus on Vertebrate Cells was presented by James Mecham, ABADRL, USDA-ARS.

The presentation featured research at ABADRL on characterization of mammalian cell receptor(s) for bluetongue virus. Experiments with glycan deficient cells and competitive inhibitors suggest the involvement of specific glycans in the initial interaction of virus with susceptible cells. The data also indicate that this initial interaction facilitates or enhances virus binding to a secondary receptor, which is required for virus internalization. Understanding the nature of viral receptors on susceptible cells will enhance our understanding of tissue tropism and pathology and may lead to more effective disease control strategies.

William C. Wilson, ABADRL, USDA-ARS, presented Molecular Diagnostic Tools for Early Detection of Arthropod-Borne Animal Viruses.

The presentation highlighted ABADRL efforts on the development of rapid nucleic acid detection tests for BTV and the related EHDV for all serotypes. This work has been done in collaboration with the Lawrence Livermore National Laboratories and the Southeastern Cooperative Wildlife Disease Study. Rapid real-time reverse transcriptase-polymerase chain reaction (qRT-PCR) tests that detect prototype strains of indigenous and exotic BTV and EHDV RNA have been developed. The EHDV qRT-PCR detected all 40 field strains available. The EHDV qRT-PCR was evaluated against clinical samples, and could directly viral RNA from tissues were also virus isolation. The assay is slightly less sensitive than the nested RT-PCR previously developed by ABADRL but is not as prone to cross-contamination.

An Update on the ABADRL was given by Barbara S. Drolet, ABADRL, USDA-ARS.

The mission of the ABADRL is to solve major emerging and/or exotic arthropod-borne disease problems that affect or threaten the U.S. livestock industry and wildlife. Many 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) ARS National Programs with the goal of transferring information and technology to livestock industries, and to action and regulatory control agencies. The ABADRL operates Biosafety Level 1 (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 high containment animal space. At 95 percent renovation completion of the BSL-3 laboratories, a roof structure failure was identified and is currently being addressed. Target completion date is spring or summer of 2008 and will provide ca. 1,500 ft² of BSL-3 space. In addition, the former ABSL-3Ag large animal facility (2,680 ft²) is being renovated and re-classified as ABSL-2 enhanced space.

Currently the ABADRL is addressing research gaps of several arboviruses including domestic and exotic strains of bluetongue virus, epizootic hemorrhagic disease virus, vesicular stomatitis virus, and Rift Valley fever virus. Research areas include virus-vector-host interactions; development, refinement, evaluation and validation of diagnostic tests and vaccines; characterization of viral receptors on vertebrate and invertebrate cells; characterization of viral persistence in *Culicoides*; vector competence; horizontal and vertical arbovirus transmission; vector genomics and proteomics of insect salivary glands and midguts; vector biology, ecology, and behavior; disease risk assessment; and development of effective disease and vector control management strategies.

The name of the Committee was discussed and it was pointed out that bovine retroviruses are no longer discussed in the meetings. A motion was made and passed that the name of the Committee be changed to: "Committee on Bluetongue and Related Orbiviruses."

**Exotic Bluetongue Viruses Identified from Ruminants in the Southeastern U.S.
from 1999-2006.**

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U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services,

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World-wide, 24 serotypes of bluetongue virus (BTV) have been identified, and five (BTV-2, BTV-10, BTV-11, BTV-13, and BTV-17) are considered endemic in the United States. Isolation and identification of BTV isolates is routinely performed at the National Veterinary Services Laboratories (NVSL), Ames, Iowa. From 1999 to 2005 several isolates of BTV originating from Florida sheep, cattle or deer could not be identified at the NVSL as one of the U.S. BTV serotypes. Virus neutralization tests conducted on the isolates using type-specific reagents to BTV serotypes that had been identified in the Caribbean and Central American regions were inconclusive.

For BTV, the serotype identification is conferred by the major outer capsid protein, VP2. Until recently, genetic sequences for the VP2 region of all 24 serotypes of BTV were not available. Using newly published sequences of all 24 VP2 genes, PCR primers were developed for the exotic BTV VP2 regions. Subsequent PCR testing and sequencing of the PCR products were performed with the previously untypeable isolates. The archived Florida isolates as well as recent isolates from Florida and Mississippi were successfully identified. Several of the isolates were also submitted to the Institute for Animal Health, Pirbright, United Kingdom for identification and/or confirmation of the NVSL results.

BTV serotypes previously believed to be exotic to the United States that have been identified are:

BTV-3: Highlands County Florida, 1999 (sheep); Martin County Florida, 2001 (deer); Volusia County Florida, 2002 (deer); Okeechobee County Florida, 2002 (cattle); and Manatee County Florida, 2003 (cattle); Yalobusha County Mississippi, 2006 (deer).

BTV-5: Manatee County Florida, 2003 (cattle).

BTV-6: Okeechobee County Florida, 2006 (cattle).

BTV-14: Marion County Florida, 2003 (sheep)

BTV-19: Manatee County Florida, 2003 (cattle)

BTV-22: Okeechobee County Florida, 2002 (cattle); Marion County Florida, 2005 (sheep)

Culicoides insignis, the common vector of BTV in Caribbean and Central American regions and extreme southeastern United States, has traditionally been restricted to those areas. *Culicoides sonorensis* is considered the BTV vector for midwestern, western and other southern regions of North America. The limited range of *C. insignis* in the United States may account for the initial isolation of these exotic BTV serotypes only from southeastern animals, however, it is not known if the range of *C. insignis* is expanding as a result of global warming. Additionally, the potential for infection of *C. sonorensis* with any of these viruses is not known.

Update on Diagnostic Observations for Bluetongue, Epizootic Hemorrhagic Disease, and Bovine Leukosis Virus in the United States.

Eileen Ostlund

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Bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) isolations/PCR positives, Calendar year 2006

Bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) isolations/PCR positives, calendar year 2006 are as follows:

Bluetongue virus or RNA was detected in 37 samples submitted during calendar year 2006. The positive bluetongue virus isolation and polymerase chain reaction (PCR) test results from submissions to the National Veterinary Services Laboratories (NVSL) in 2006 are listed below:

Table 1 BT virus isolation (VI) / PCR positives, Calendar year 2006

<i>State</i>	<i>No.</i>	<i>Species</i>	<i>PCR</i>	<i>VI</i>
FL	1	Cattle	Pos	Not done
FL	2	Cattle	Pos	BTV 6
FL	3	Cattle	Pos	Neg
FL	1	Sheep	Not done	BTV 2
IA	1	Sheep	Not done	BTV 17
IL	3	Cattle	Pos	Neg
IL	1	Cattle	Pos	Not done
KS	1	Sheep	Not done	BTV 11
KY	1	Deer isolate	Not done	BTV 17
MO	1	Deer isolate	Not done	BTV 17
MS	1	Deer isolate	Pos	BTV 3
NE	17	Bovine hemoglobin	Pos	Neg
NM	1	Sheep	Pos	Not done
OR	1	Mule deer	Pos	BTV 17
SD	1	Cattle	Pos	Not done
TX	1	Cattle	Pos	Neg
WA	2	Sheep	Not done	BTV 17

During calendar year 2006, nine samples tested positive for EHDV by virus isolation and/or PCR. Six of these were virus isolates identified as EHDV 6, a virus type not previously reported in the United States. The positive EHDV isolation and PCR test results from submissions to the National Veterinary Services Laboratories (NVSL) in 2006 are listed in Table 2.

Table 2. EHDV isolation (VI)/ PCR positives, Calendar year 2006

<i>State</i>	<i>No.</i>	<i>Species</i>	<i>PCR</i>	<i>VI</i>
IN, IL	6	Deer isolates	Pos	EHDV 6

Unknown	1	Deer	Pos	EHDV 2
MO	2	Isolate – species not pr	Pos	Pos – typing not att

Calendar year 2007 (January 1– October 20)

Bluetongue virus or viral RNA has been detected by PCR from 26 specimens submitted thus far in 2007. Serotype 17 was isolated from several species in Montana. The positive bluetongue virus isolation and polymerase chain reaction (PCR) test results are listed in Table 3.

Table 3. BT virus isolation (VI) / PCR positives, January-October, 2007

<i>State</i>	<i>No.</i>	<i>Species</i>	<i>PCR</i>	<i>VI</i>
MO	2	Cattle	Pos	Neg
MT	4	Sheep	Pos	BTV 17
MT	3	Antelope	Pos	BTV 17
MT	2	White-tailed deer	Pos	BTV 17
MT	1	Mule deer	Pos	BTV 17
MT	2	Sheep	Pos	Neg
NE	11	Bovine hemoglobin	Pos	Neg
SC	1	Cattle	Pos	Neg

As of October 20, 2007, EHDV has been detected in 35 samples submitted to NVSL. With the exception of one virus isolate submission from Texas, for which the collection date is unknown, all 2007 EHDV isolates at the NVSL in 2007 have been type 2. The positive EHDV isolation and PCR test results are listed in Table 4.

Table 4. EHDV isolation (VI) / PCR positives, January – October, 2007

<i>State</i>	<i>No.</i>	<i>Species</i>	<i>PCR</i>	<i>VI</i>
FL	1	Deer	Pos	EHDV 2
FL	1	Deer	Pos	Pos-type pending
IA	1	Deer	Pos	EHDV 2
IN	1	Deer isolate	Pos	EHDV 2
IN	1	Cattle	Pos	EHDV 2
KY	1	Deer isolate	Pos	EHDV 2
KY	1	Deer	Pos	EHDV 2
NY	2	Deer	Pos	Pending
OH	4	Cattle	Pos	Neg
OH	2	Deer	Pos	EHDV 2
OH	2	Cattle	Pos	EHDV 2
PA	1	Deer	Pos	Neg
PA	1	Deer	Pos	EHDV 2

PA	1	Deer	Pos	Not done
SD	1	Deer	Pos	EHDV 2
SD	1	Deer	Pos	EHDV 2
SD	1	Deer	Pos	Not done
TN	2	Elk	Pos	EHDV 2
TN	1	Cattle	Pos	EHDV 2
TN	1	Cattle	Pos	Pending
TX	1	Deer isolate (date unknown)	Pos	EHDV 1
WI	3	Deer	Pos	EHDV 2
WI	1	Deer	Pos	Not typed (same owner as above)
Wash., DC	1	Deer	Pos	Pending
Unknown	2	Deer isolate	Pos	EHDV 2

2007 Bluetongue Serology Proficiency Test

Fifty eight laboratories participated in the 2006 bluetongue (BT) proficiency test. The panel consisted of 20 serum samples. The passing score was two or fewer samples missed. Three laboratories failed the 2006 bluetongue proficiency panel on the first attempt. Two laboratories passed the retest. One laboratory failed the proficiency retest and a serologist from the laboratory received refresher training at NVSL. As of October 20, 2007, there are 58 laboratories approved to conduct official (export) BT serology tests.

2007 Bovine Leukosis Proficiency Test

Fifty nine laboratories participated in the 2007 bovine leukosis (BLV) proficiency test. Fifty nine laboratories participated in the 2007 BLV proficiency test. The panel consisted of 20 serum samples and the passing score was one or fewer samples missed. Two laboratories failed the 2007 bovine leukosis proficiency panel on the first attempt. Both of these laboratories have successfully completed a retest. As of October 20, 2007, there are 58 laboratories approved to conduct official (export) BLV serology tests.

Overview of the Bluetongue Situation in Europe with Emphasis on *Culicoides* Vectors.

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“What is disturbing about the metaphor of relations between human beings and viruses as a chess game is that the virus always plays with the white pieces and we human beings with the black. The virus makes its move, and we react”. (J. M. Coetzee, *Diary of a Bad Year*, 2007).

INTRODUCTION

Bluetongue (represented by serotype 8) appeared in northern Europe in August 2006. Subsequently, it spread across five Member States (MSs) and by December had affected an area of approximately 170 000 km². Both cattle and sheep showed clinical signs and at least two species of *Culicoides* i.e. *C. obsoletus* and *C. dewulfi* were shown to be involved in its transmission. All affected MSs initiated national entomological surveillance programmes with the result that *Culicoides* are now monitored widely using mainly Onderstepoort-type blacklight traps. The most significant findings made over the past year are summarised and discussed with emphasis on The Netherlands, where 20 farms are sampled weekly.

RESULTS

***Culicoides* activity during the winter months of 2006/7**

In Holland (and in Belgium) low numbers of *Culicoides* (almost exclusively of the *Obsoletus* Complex and excluding *C. dewulfi*) were captured almost each week between January and March; 99% were freshly emerged nullipars indicating low-level breeding to have continued throughout the winter.

How did BTV-8 overwinter between 2006 and 2007?

Between January and March (\pm 90 days) the absence of older parous, potentially BTV-infected, previous-season adult midges in light trap collections led to the (false!) hope that BTV would not survive the winter. However, its ferocious recrudescence in 2007 invites many questions, which are discussed.

More Culicoides in a cooler and wetter 2007...!

The average number of vectors captured in Holland in 2007 is approximately 10x greater than the number collected in 2006 despite it being cooler and wetter, quite unlike last year (the hottest on record since measurements began in 1706). This would indicate that warmer winters and moderate ‘normal’ summers favour vector proliferation and perhaps also the endemisation of viruses exotic to Europe.

Marked changes in some vector Culicoides abundances

The *Obsoletus* Complex is the most prevalent vector in Holland and dominant on half the farms surveyed. However, in parts of southern Holland, *C. dewulfi* has this year superseded *C. obsoletus*. If a similar reversal has occurred also elsewhere in Europe, it may in part explain the intensity of the current outbreak.

Diurnal biting activity in Culicoides

C. dewulfi and *C. obsoletus* attack livestock in broad daylight while they are at pasture, especially on overcast days. Aggravating the situation is that they enter also animal houses after dark. Therefore, the attack of livestock by day and at night, and both indoors and outdoors, complicates our fight against BT. At this stage vector control seems to hold little promise for halting the spread of the disease.

CONCLUSIONS

In 2007 BT continued to spread and included a jump across the English Channel. The BT restriction zone now covers an area of almost one million km². There are no obvious geographical or topographic boundaries that might halt the advance of BTV-8, making it likely that it will continue to do so in 2008 (and beyond) until it reaches the — as yet unknown — limits of its range. This is daunting when it is considered that vector *Culicoides* (and susceptible ruminant hosts) occur across the entire Holarctic Region, which includes the Mediterranean Basin where *C. imicola* lies in waiting, and North America, where outbreaks of BTV and Epizootic Haemorrhagic Disease of Deer virus (EHDV — another *Culicoides*-borne pathogen), are occurring also. In this respect it would seem that warmer winters will only

add to the conundrum in future promoting rather than suppressing virus survival and vector longevity. Vaccination still seems to be the best defence available to us. But have we waited too long?