

REPORT OF THE COMMITTEE ON BLUETONGUE AND BOVINE RETROVIRUSES

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Vice Chair: Dr. William C. Wilson, Laramie, WY

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The Committee met on October 26, 2004. There were 38 members and guests in attendance. James E. Pearson, Chair, and William C. Wilson, Vice Chair, conducted the meeting.

Dr. Jim MacLachlan University of California, Davis, discussed the 3rd International Bluetongue Symposium that was held in Taormina, Sicily October 26-29, 2003. The meeting was sponsored by Italian Ministry of Health, European Union, and the World Organisation for Animal Health (OIE) and organized by the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G Caporale" and Istituto Zooprofilattico Sperimentale della Sicilia "A Mirri", Teramo Italy. The program was developed by Executive and Steering Committees representing all regions of the world. The format of the meeting was invited oral presentations and poster presentations of other scientific information. The areas addressed included the current global situation; epidemiology and vectors; bluetongue virus and bluetongue disease; diagnostics; control using vaccines; and control and trade issues.

Working Groups were designated, which developed the conclusions and recommendations for the meeting. The proceedings are being published in a special color edition of *Veterinaria Italiana*, Istituto Zooprofilattico Sperimentale dell'Abruzzo del Molise G. Caporale, Teramo, Italy. There were over 300 registered participants representing all regions of the world with 45 oral presentations and over 90 posters. The points of emphasis from the meeting included that the current diagnostic technology is adequate; that viremia is of limited duration in animals; that there is a need for better surveillance worldwide; that the concept of global ecosystems needs to be developed; that there is a need to better define the precise role of insects in the

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global ecology of bluetongue virus (BTV) infection; and that there is a need for new generation of vaccines. It is anticipated that the information from this meeting will serve as a basis for the revision of the OIE *Terrestrial Animal Health Code* (Animal Health Code).

A time-specific Committee paper entitled "Bluetongue control and vaccination: What bluetongue Standard should be adopted by Office International des Epizooties" was presented by Dr. Enzo Caporale, Director, Istituto Zooprofilattico Sperimentale, dell'Abruzzo e del Molise 'G. Caporale', Via Campo Boario, 64100 Teramo, Italy and President of the OIE Scientific Commission. There was an extensive discussion after the presentation by Dr. Caporale. It was recommended by the Chair that interested parties that had comments on the changes should provide them to Dr. Michael David, United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS).

Dr. Eileen Ostlund, USDA-APHIS-VS, National Veterinary Services Laboratories (NVSL), Ames, Iowa, gave a presentation entitled "Update on Diagnostic Observations for Bluetongue, Epizootic Hemorrhagic Disease (EHD) and Bovine Leucosis Virus (BLV) in the United States."

In 2003, virus isolation attempts for BTV and/or EHDV were completed on 216 samples and 205 samples were tested by PCR. There were 195 submissions of imported fetal bovine serum for BTV safety testing by sheep inoculation requiring 345 sheep. None of the sheep inoculated with imported fetal bovine serum in 2003 developed BTV antibodies. The positive results from submissions to the NVSL are listed in the following tables:

Table 1					
BTV isolation (VI) / PCR positives, Calendar year 2003					
State	No.	Species	Type	VI	PCR
CA	1	Bighorn			x
CO	1	Sheep			x
FL	7	Cattle	2*	x	
FL	1	Cattle			x
FL	2	Sheep	**	x	
IL	3	Alpaca			x
OK	1	Cattle			x
OK	3	Sheep			x
TX	1	Deer			x

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Table 2					
EHDV isolation (VI) / PCR positives, Calendar year 2003					
BTV isolation (VI) / PCR positives, Calendar year 2003					
State	No.	Species	Type	VI	PCR
CA	1	Mule deer	2	x	x
ID	6	Cattle	2	x	
SD	1	Deer			x
TX	1	Deer			x
WA	2	Elk		x	

Calendar year 2004 BT/EHD positive submissions (January 1 – October 25, 2004):

BTV has been detected by PCR from nine specimens originating from Alabama (1), Colorado (1), Montana (1), Nebraska (3), and Texas (3). All BTV-positive samples were from cattle. The three positive samples from Nebraska were pooled dried hemoglobin being tested for export certification. The PCR-positive hemoglobin samples were negative by BTV isolation. No BTV isolates have been made through October 25. To date in 2004, one EHDV isolate from an Iowa deer has been obtained. This isolate was identified as EHDV-type 2.

2004 BT Proficiency Exam:

Fifty-nine laboratories participated in the 2004 BT proficiency test. The panel consisted of 20 serum samples. The passing score was one or fewer samples missed. Fifty-four laboratories passed on the first attempt. Five laboratories failed the first attempt and all five passed a retest. Fifty-nine laboratories are approved to conduct official (export) BT serology tests as of October 25, 2004.

2004 BLV Proficiency Exam:

Sixty-one laboratories participated in the 2004 BLV proficiency test. The panel consisted of 20 serum samples and the passing score was one or fewer samples missed. Fifty-five laboratories passed on the first attempt. Six laboratories failed the first attempt but passed a retest. As of October 25, 2004, there are sixty-one laboratories approved to conduct official (export) BLV serology tests.

Dr. Brian Jamieson, Senior Veterinary Officer, Imports/Exports, Animal Health and Production Division, Canadian Food Inspection Agency, Ottawa, Canada, gave a presentation entitled "Bluetongue Regulatory and Research Efforts in Canada."

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National BT Surveillance:

The triennial national serosurvey was delayed from 2002-2003 to 2004 to allow the implementation of the animal identification program. The number of samples tested was 15,105 and there were two C-ELISA positives; the samples had originated from the Okanogan Valley. The sentinel herd program in the Okanogan Valley has continued with no positives detected since 1998.

BT Research:

A collaborative project has been conducted between Agriculture Canada in Lethbridge, Canada and the USDA Agriculture Research Service (ARS) Arthropod Borne Animal Diseases Research Laboratory (ABADRL) in Laramie, WY. The objectives are to determine the prevalence, biting rate, and species abundance of *Culicoides*. The research included: monitor populations of *Culicoides* to determine seasonal abundance; determine the ovipositional status of females; monitor biting intensity every two weeks at three feedlots; develop a potential transmission model for BT based on the effects of temperature on longevity and feeding of western Canadian *Culicoides* and viral development in the vector; establish a colony of *C. sonorensis* wild populations; conduct laboratory studies to determine the relationship between air temperature, vector longevity and egg development; and conduct experiments on the duration of the extrinsic incubation of the virus at various constant and cycling temperatures.

The third year of a three year project is being completed; however, modelling work is ongoing with the final report is due by June 2005. The main findings are: *Culicoides* spp. were trapped at all 8 locations but $\pm 90\%$ were from one location (87-97%); over 90% of *C. sonorensis* trapped were from 1 site; timing of *C. sonorensis* activity/population peaks varied annually; majority of parous insects were uniparous, from 7-11% were biparous; there was a low infection rate of *C. sonorensis* from Alberta and northern Montana for BTV following virus challenge; and U.S. colony flies are more likely to take a second blood meal than wild Alberta flies. The conclusions from this research are: the proportion of *C. sonorensis* old enough to transmit the BTV is extremely low in southern Alberta; Alberta *C. sonorensis* are largely refractory to infection with BTV; flies from Alberta collection sites were not infected with BTV; and populations evaluated are largely incompetent as a vector of the BTV.

Regulatory Changes:

A new program has been established to allow year round importation. The following are some key components:

Breeding ruminants: seasonal testing required for importation into western Canada, importation into six eastern province is permitted with-

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out testing, additional changes to import requirements will be dependant upon findings of Lethbridge research

Restricted feeder cattle, importation for feeding and subsequent slaughter; Canadian cattle co-resident in importing feedlots – return to breeding herd, anaplasmosis concerns are one of the main challenges

Restricted Feeder Program: Thirty-nine source states recognized as 'minimal risk' for bluetongue based on historical data, year-round imports except from Okanogan Valley of British Columbia, importation is into previously approved feedlots, feedlot management system has been established to verify disposition of all animals, export certification by USDA accredited veterinarian and unique animal ID is required. Also required are a vector control program, water management, lagoon shoreline management, provisions for Canadian cattle departing feedlot to breeding herd, restrictions on movement of imported animals between feedlots, and a sentinel animal program within importing feedlots.

Conclusions

1. BT remains an important disease for international trade considerations and Canada must be able to demonstrate the adequacy of its sanitary requirements for BT as well as ensuring the protection of susceptible domestic and wildlife species.
2. New scientific information has allowed for extensive changes in Canada's import requirements for U.S. feeder cattle.
3. Canada will continue to explore every opportunity to facilitate trade through the development of less restrictive import policies for BT.

Dr. David Stallknecht, Southeastern Cooperative Wildlife Disease Study (SCWDS), Athens, Georgia updated the committee on hemorrhagic disease (HD) surveillance conducted by SCWDS. During 2003, BTV and EHDV were isolated from four wildlife species in eight states. EHDV-2 was isolated from white-tailed deer in Idaho (17 isolates), Kansas (5 isolates), Texas (3 isolates), Washington (2 isolates), Georgia (2 isolates), Missouri (2 isolates), South Carolina (2 isolates), and Tennessee (2 isolates). EHDV-2 also was isolated from a mule deer in Idaho. BTV-10 was isolated from a pronghorn and bighorn sheep in Idaho and a white-tailed deer (penned) in Texas. BTV-13 was isolated from a bighorn in Idaho and BTV-17 was isolated from white-tailed deer in Idaho, Kansas, Texas, and Washington. To date during 2004, EHDV-2 has been isolated from white-tailed deer in Illinois and BTV-17 has been isolated from a mule deer and white-tailed deer in Idaho. With the exception of Illinois, there have been very few reports of HD this year.

Reports of HD in wildlife from 1980 to 2003 (obtained from an an-

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nual survey of state wildlife agencies) were recently mapped to update the distribution of this disease within the United States. In deer and other wild North American ungulates HD can be caused by either BTV or EHDV. A strong spatial pattern is evident with HD occurring most frequently in a diagonal band extending from the Southeast through eastern Montana. With over 23 years represented in this survey, it is interesting to note that reports are rare to absent from the Northeastern United States, and despite the fact that deer are highly susceptible and abundant in this area; a confirmed case of HD has never been reported from any of these populations.

Jim MacLachlan, University of California, Davis, California presented a report entitled "An Update on Bluetongue Research University of California, Davis". A program is underway to develop recombinant vaccines; this program is a joint program with the Instituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G Caporale" and Instituto Zooprofilattico Sperimentale della Sicilia "A Mirri, Teramo Italy. Sequencing of global isolates of BTV is being conducted. Preliminary data from this sequencing study indicates that there is regionalization of BTV with little or no movement of the isolates between regions.

Dr. Bill Wilson, USDA-ARS-ABADRL made a presentation on "Early Warning Devices for Bluetongue". Molecular biology of BT and related viruses has provided the foundation for the development early warning technologies for indigenous and exotic disease outbreaks.. The phylogenetic analysis of two-conserved target genes, one that is highly expressed in infected mammalian cells the other highly expressed in infected insect cells, from BTV prototype strains indicated that a complex primer design will be necessary for a comprehensive gene amplification diagnostic test. Status of the application of real-time RT/PCR and other existing and developing technologies for early warning of an exotic BTV outbreak was discussed.

Dr. Richard Mayer, Research Leader, USDA-ARS, Laramie, Wyoming gave an update on ABADRL in Laramie, Wyoming. ABADRL is the only laboratory within the USDA mandated to perform research on livestock diseases transmitted by insects. The mission of the laboratory is to develop effective disease diagnostic, control and management strategies than can be transferred to the livestock industry, and regulatory agencies. Currently research involves BTV, EHDV, vesicular stomatitis virus, and West Nile virus (WNV). The laboratory is participating in several cooperative projects including one involving the University of Wyoming, Montana State University, the University of Montana, the Wyoming Game and Fish Department, Wyoming Public Health Department, and the Bureau of Land Management to assess the effects of coal bed methane wells on insect vector populations and vector-borne disease transmission.

ABADRL has also been developing more simplified and sensitive RT-PCR BTV detection methods including a one-step reverse transcription-PCR reaction that employs infrared dye labeled primers (C.

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Kato and R.T. Mayer). This approach has also been successful for detection of WNV, EHDV, and St. Louis encephalitis. The advantages of this method are speed and sensitivity for BTB detection, and this method is highly adaptable for an immediate response to potential and emerging threats.

ABADRL has mounted an effort on vector (*C. sonorensis*) genomics and has focused on midgut and salivary gland tissues. Over 2,000 genes have been identified for a number of different functions including cell communication, cell cycle, cell death, cytoskeleton biogenesis, development, defense, metabolism, peritrophic membrane, protein metabolism, proteolysis, etc. The expression of specific genes in these tissues has been verified by quantitative PCR and/or *in situ* hybridization. This data provides the foundation for ongoing vector biology research. These sequences are available to researchers via the GenBank national database.

In regard to facilities, ABADRL has had about \$1.7 million dollars of security upgrades and renovation improvements made over the last 18 months. These expenditures have been made to meet BSL-3 certification and select agent regulations. These efforts will result in a smaller facility but will allow the ABADRL to pursue its research mandate over the short term. Long-term research goals will require new facilities. Two major reports have been published recently in regard to the threat of biological terrorism to poultry and livestock and recognized the national need for an expanded research effort on insect/arthropod transmitted diseases to prevent and protect the U.S. against naturally or purposely introduced exotic pathogenic agents. Critical infrastructure facilities with adequate biosecurity and capability to work with large animals are needed. As the only federal laboratory mandated to study insect/arthropod transmitted diseases, ABADRL will likely be more involved with this research. The current facilities cannot accommodate such expanded programs because of space limitations and the age of the facilities. The FY 2005 Senate Agriculture, Rural Development, Food and Drug Administration, and Related Agencies Appropriations Bill states that "The Committee has been made aware of the need for a state-of-the-art animal disease laboratory at Laramie, Wyoming. The Committee directs ARS to provide a prospectus on this project." Such a facility would accommodate an expanded research program with sufficient over-capacity to accommodate state agency, university, and other federal agency cooperators. The estimated cost is \$100 million. Direct benefits of a new facility would be expansion of staff and resources, expansion the research program, greater interaction with state, university, and other federal government collaborators, greater ability to respond to emergency situations, faster development of disease detection methods, better capability to develop and validate vaccines, increased capacity for development of vector control methods.

A Committee scientific paper, "Persistence of Bluetongue Virus in

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the Insect Vector and its Implications for Disease Control” by Mecham, J.O., White, D.M., Drolet, B.S. and W.C. Wilson was presented in an American Association of Veterinary Laboratory Diagnosticians scientific session and is published in these proceedings.

The Chair reported on the response to a resolution submitted at the 2003 Annual Meeting. That resolution urged USDA-ARS to develop a strategic plan to define the facilities needed to do the research on arthropod-borne diseases of livestock performed at ABADRL; to identify the costs of such facilities; and to identify the most appropriate location in the Western United States for such facilities dedicated to animal health research. ARS responded that they shared the concern of the Committee that the work of ABADRL not be impeded by substandard facilities; that emergency repairs to both of the off-campus biological containment facilities of the ABADRL were being completed; that in response to the expert panel report, the ARS Northern Plains Area Director had approved the concept of a phased plan that would result in a new facility by 2012; that the cost of rebuilding and the source of funds have not yet been determined; and that the location for rebuilding would ultimately depend on a number of factors, including where ABADRL can best fulfill its unique mission.

The Committee discussed bovine retrovirus. The Committee has had very few reports on disease or trade restrictions due to these viruses and the question was raised if they should continue to be included. There was also a question raised if other arboviruses should be addressed by the Committee. It was decided that this will be considered over the next year and discussed at the next meeting.

Dr. Caporale reported that a revised Bluetongue Chapter to the Animal Health Code has been proposed. The primary changes are: decrease the period of infectivity for BTV to 60 days from 100 days; the northern limit of BTV distribution is increased from 45° to 50°; a new Chapter in the Animal Health Code that addresses bluetongue (BT) surveillance and monitoring; that portion will be deleted from the current BT Chapter; allow the movement of vaccinated animals with few restrictions; allow the movement of vaccinated animals 30 days after vaccination; and change the designation of *Culicoides* to *Culicoides* likely to be competent BTV vectors.