

## **REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF CATTLE, BISON AND CAMELIDS**

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The Committee met on Sunday, October 15, 2006 in the Carver Room, Minneapolis Hilton Hotel, Minneapolis, Minnesota from 12:30-5:30 p.m. Eighty members and guests were in attendance. Committee members present varied from six to fourteen with ten present for the business meeting.

Talks presented in the bovine viral diarrhea virus (BVDV) Subcommittee highlighted the challenges of BVDV control. These challenges include the absence of standard criteria for validation and proficiency in BVDV testing programs, the existence of persistently infected (PI) animals in non-bovine species, the lack of diagnostic and control tools available for those non-bovine species and the need to establish cooperative efforts with producer and professional groups.

Dr. Dale Groteluchin, Chair of BVDV Control Subcommittee of the National Cattlemen's Beef Association (NCBA), presented an update on the work of the BVDV control committees of the American Association Bovine Practitioners (AABP), NCBA and the Academy of Veterinary Consultants (AVC). All three groups have adopted resolutions calling on the dairy and beef industries to focus on the control and eventual eradication of BVDV from North America. These three organizations are also currently working on resolutions calling for the full disclosure of PI status prior to commerce or movement. He also noted that currently there is no official means of communication between these control committees and the United States Animal Health Association (USAHA). Establishing such lines of communication would have significant benefits for BVDV control efforts.

Dr. Julia Ridpath, National Animal Disease Center, presented a talk on the current state of BVDV testing in the U.S. It was reported that while BVDV testing in the U.S. is expanding at an exponential rate, some laboratories - including a significant number of independent start up laboratories - are using tests that focus more on cost than sensitivity. In particular the practice of pooling ear notch samples may miss 10 to 50 percent of field samples depending on the pool size.

Dr. Donal O'Toole, President, American Association of Veterinary Laboratory Diagnosticians (AAVLD), addressed the AAVLD stance on test standards. Typically field validation requires testing of 300 positive samples and 1000 negative samples. It was noted that the low incidence of BVDV PI animals, which is thought to range between .2 and .5 percent in the U.S. herd, makes it difficult for laboratories to accrue the necessary number of samples. Dr. O'Toole emphasized that it was important for diagnostics laboratories to get it right before they made it cheap.

Dr. Ed Dubovi, Cornell University and Dr. Jim Evermann, Washington State University, presented talks on BVDV infection in alpacas. Topics covered included identification of PI alpacas, incidence rate (based on serology), clinical presentation and routes of infection and samples that are unique to this species. At this point it appears that exposure is less than 20 percent. However, these studies indicate circulation of BVDV in alpaca herds is apparently independent of exposure to cattle. The presence of BVDV in the saliva of PI alpacas suggest that saliva may be a good test sample for diagnosing persistent infection and that spitting may be a route of exposure for this species. The low incidence rate suggests that the best approach to BVDV control in alpacas is to survey for and eliminate PI animals and monitor for exposure by serology. Use of vaccines at this time is counter indicated as vaccination would interfere with monitoring by serology.

Dr. Hana Van Campen, Colorado State University, presented a talk summarizing the use of bovine vaccines and diagnostics in non-bovine species. Bovine vaccines and diagnostics are used because there is a lack of reagents for other farmed ruminants such as llamas, alpacas, bison and elk. This is also a problem with captive animals. While it has been shown to be a problem in these species little or no validation or tests of efficacy have been completed.

Pam Hullinger, Lawrence Livermore National Laboratory (LLNL) presented an update on the foot-and-mouth disease (FMD) rule-out assay and high throughput sample processing system. In addition to testing for FMD, the assay simultaneously tests for bovine viral diarrhea, bovine herpes-1, bovine parapox virus complex, bluetongue, swine vesicular disease and vesicular exanthema of swine. The new rapid diagnostic test for these important and economically devastating animal diseases was developed by LLNL in partnership with the U. S. Department of Homeland Security (DHS), the U.S. Department of Agriculture (USDA) and the University of California, Davis. The new diagnostic tool reduces the period required to detect FMD, and six indigenous diseases with similar symptoms from days to hours. In addition the test can simultaneously detect all seven diseases in one sample. Early detection of these diseases provides an opportunity to more quickly trace and minimize the spread of these diseases and enhance the nation's ability to respond to natural or terrorist introduction of these diseases into the national animal population.

Virtually all diagnostic methods for transmissible spongiform encephalopathies (TSE) rely on immunodetection of the disease associated form of the prion protein (PrP<sup>Sc</sup>). Eric Nicholson, National Animal Disease Center (NADC), Agricultural Research Service (ARS) reported on a method to detect PrP<sup>Sc</sup> in formalin-fixed tissues by western blot. Both immunohistochemistry (IHC), considered by some to be the gold standard for diagnosis of TSEs, and Western blot analysis are employed in a comprehensive diagnosis of a TSE. IHC relies on formalin fixed tissue for preservation of cellular architecture, an important aspect for the accurate and reliable diagnosis of TSEs. Alternatively, freezing of tissue samples is ideal for Western blot analysis but results in disruption of cellular architecture. Formalin fixation is the most prevalent form of tissue preservative, thus represents an important source of archival material for study.

Karen Conyngham, International Lama Registry (ILR), presented a brief review of the industry-developed minimum standards of care and recommended practices in caring for llamas and alpacas. These documents were designed for use by animal welfare and health agency professionals as well as llama and alpaca owners. The Minimum Standards are mandatory to llama and alpaca survival and humane treatment and are the most basic requirements the animals must have for physical well-being. The Recommended Practices offer more details and are intended as an educational foundation for camelid care. The full

text of both documents and a copy of the PowerPoint presentation can be found on the Internet at: [www.camelidcare.info](http://www.camelidcare.info)

A short overview of biosecurity practices among camelid owners was also presented. The confirmation of a small number of BVDV persistently infected alpaca crias over the past 2 years has raised the level of owner awareness regarding biosecurity. The Alpaca Owners & Breeders Association, now requires negative BVDV tests for animals entered in their sanctioned shows. Some shows have banned exhibition of animals less than 6 months of age. Alpaca transporters likewise require negative testing before hauling. Owners are strongly encouraged to euthanize any BVDV persistent infected crias.

Biosecurity recommendations include having a separate quarantine facility on any farm that will be receiving either new additions to their herds or performing outside breeding. New animals should be quarantined for at least 30 days. Those arriving for breeding should be dewormed before and after breeding and close monitoring of the weight and IgG status of any crias who accompany their dams. Farms that host events for the public and other camelid owners should keep a visitors log, provide foot coverings for any people who enter animal areas and restrict access to the main herd. Animals that attend shows should be isolated from the rest of the herd for 14-21 days upon return. While at the show, use of communal dung piles should be avoided and camelids should not be walked in areas used by other species.

Hong Li and Naomi Taus presented an update on their malignant catarrhal fever (MCF) research in American bison. MCF is a devastating disease for American bison. With a steady increase in bison population, MCF is becoming one of the most important infectious diseases for bison producers in North America due to their extreme disease susceptibility. Virtually all bison MCF cases in the U.S. are caused by the virus known as ovine herpesvirus 2 (OvHV-2) with domestic sheep as the reservoir. The inability to grow OvHV-2 in cell culture has severely limited research progress in understanding the epidemiology, pathogenesis and control of the disease.

Research scientists at the Animal Disease Research Unit, Pullman, Washington and their collaborators at Washington State University and the University of Wyoming have been conducting collaborative studies on MCF in bison. The following are the highlights of the MCF research progress recently made in our laboratory: 1) validated non-nested polymerase chain reaction (PCR) and real-time PCR test for diagnosis of clinical MCF in bison and other ruminant species; 2) established infectious OvHV-2 inoculum pools and developed a bison experimental model for research; 3) completed OvHV-2 genome sequencing; 4) characterized bison major histocompatibility (MHC) class I and class II haplotypes and determined the association between specific MHC class II *DRB3* alleles and MCF resistance/susceptibility.

Seth C. Britch, Center for Medical, Agricultural, and Veterinary Entomology, Agriculture Research Service (ARS), Gainesville, FL presented information on a geographic information system (GIS) being developed for an early warning system for potential vectors of Rift Valley fever (RVF) virus. RVF is a mosquito-borne zoonotic hemorrhagic disease that causes 100 percent abortions in ungulates such as cattle, sheep, and goats, and is often fatal to young animals. Though currently confined mainly to Africa this disease could be introduced into the U.S. and spread via mosquitoes at least as rapidly as West Nile virus (WNV). Unlike WNV, RVF is also transmitted by contact with infected tissues or aerosolized material, and there is no approved vaccine for humans or animals. Work being done on RVF by collaborators in agencies within and outside of the USDA was presented. Studies include pathways analysis, development of vaccines and test kits, and GIS modeling of vectors and vector habitat. Of particular concern is the relationship between the geography of human settlement and the livestock industry, the biogeography of wild ungulates, and the biogeography of potential RVF

vectors. Developing a GIS and remote sensing platform for early warning of elevated vector populations in the U.S. combining satellite climate data and long-term mosquito surveillance data from mosquito control and public health agencies is critical. By monitoring climate in Africa and the U.S., reports of RVF activity around the world, and vector populations in the U.S., we can target and implement control and containment resources to minimize effects of Rift should it appear here. Importantly, many of the systems being develop in preparation for RVF can be laterally transferred to inform strategies against any mosquito-borne disease threat.

Dr. E. M. Nicholson, National Animal Disease Center (NADC), Agricultural Research Services (ARS) presented the Committee's Time Specific paper entitled Detection of PrP<sup>Sc</sup> in Formalin-fixed Tissues by Western Blot. The complete text of this paper is included in these Proceedings.

Three resolutions were passed unanimously by the Committee and submitted to the Committee on Nominations and Resolutions. They addressed 1) Eradication of bovine viral diarrhea virus from North America, 2) BVDV PI animal status disclosure, and 3) Vaccine development for malignant catarrhal fever in Bison.

The Committee made a recommendation, by unanimous vote, to adamantly discourage marketing or movement of animals persistently infected (PI) with BVDV in any manner that potentially exposes at-risk animals.

#### Detection of PrP<sup>Sc</sup> in Formalin-fixed Tissues by Western Blot

**E.M. Nicholson**, A.N. Hamir, and R.A. Kunkle  
Virus and Prion Diseases of Livestock Research Unit  
National Animal Disease Center  
Agricultural Research Service  
United States Department of Agriculture

Formalin fixation is the most prevalent form of tissue preservative. As such, formalin fixed tissue represents an important source of archival material for study. Formalin fixation requires little environmental control and preserves the cellular architecture of a wide range of tissues, an important aspect for the accurate and reliable diagnosis of transmissible spongiform encephalopathies (TSEs) by Immunohistochemistry (IHC), considered by some to be the gold standard for diagnosis of TSEs. Alternatively, freezing of tissue samples is ideal for Western blot analysis but results in disruption of cellular architecture. Both methods are employed in a comprehensive diagnosis of a TSE.

Virtually all diagnostic methods for TSEs rely on immunodetection of the disease associated form of the prion protein (PrP<sup>Sc</sup>). Since the prion protein is a host encoded protein, an animal affected with a prion disease will have both the normal cellular form of the prion protein (PrP<sup>C</sup>) and PrP<sup>Sc</sup>. To date no commercially available antibody can distinguish these two isoforms of the protein. IHC relies on a highly trained and experienced user to distinguish disease associated staining from background PrP<sup>C</sup> while Western blot is dependent upon limited digestion of the sample with proteinase K removing PrP<sup>C</sup> leaving only PrP<sup>Sc</sup>. These two methods have different strengths and weaknesses and as such are best used in a complimentary manner.

Under field conditions, the only available tissue samples may be formalin fixed. A method for detecting PrP<sup>Sc</sup> in formalin fixed tissues would allow analysis of numerous archived samples by Western blot, would simplify preservation of field collected samples for TSE detection, allow both Western blot and IHC analysis of the same preserved sample, and allow

adjacent regions of brain to be analyzed by both IHC and Western blot enhancing the study of TSEs

Approaches to prepare formalin fixed tissues for Western blot suitable for various proteins have been reported, however, none are applicable to the detection of PrP<sup>Sc</sup> as they employ conditions known to render PrP<sup>Sc</sup> proteinase K sensitive. Here we present an approach that recovers the signal of PrP<sup>Sc</sup> while retaining the associated proteinase K resistance such that PrP<sup>C</sup> signal may be removed *via* proteinase K digestion.