

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

Chair: Howard D. Lehmkuhl, Ames, IA  
Vice Chair: James F. Evermann, Pullman, WA

Beth W. Carlson, ND; Karen Conyngham, TX; Daniel T. Crowell, NV; Edward J. Dubovi, NY; Anita J. Edmondson, CA; Darla R. Ewalt, IA; Bob Frost, CA; Robert W. Fulton, OK; Jennifer L. Greiner, IN; Burke L. Healey, NC; Del E. Hensel, CO; David L. Hunter, MT; Robert F. Kahrs, FL; John C. Lawrence, ME; James W. Leafstedt, SD; Janet Maass, CO; Chuck E. Massengill, MO; Steven C. Olsen, IA; Jeanne M. Rankin, MT; Julia F. Ridpath, IA; R. Flint Taylor, NM; George A. Teagarden, KS; Susan W. Tellez, Tx; Robert M. S. Temple, OH; Marsharee Wilcox, MD; William C. Wilson, WY.

The Committee met on Sunday October 21, 2007 at the Nugget Hotel in Reno, Nevada, from 12:30 to 5:30 pm. There were 12 members and 32 guests present. The Chair welcomed the Committee members and guests and reviewed the agenda, procedures and expectations for the meeting.

The Bovine Viral Diarrhea Virus (BVDV) Control Subcommittee on Infectious Diseases of Cattle, Bison and Camelids met and focused its discussions on testing methodology and surveillance for BVDV in cattle and genetic variation observed in BVDV isolates from alpaca. BVDV causes both persistent and acute infections in ruminants. As persistently infected (PI) animals are the main means of introduction of BVDV to naïve populations, elimination of PI animals is a basic tenet of BVDV control/reduction efforts. The standard for positive determination of persistent infection (PI) status is two positive tests from samples collected at least three weeks apart.

Dr. Robert Fulton, Oklahoma State University, reported on a BVDV surveillance study of beef cattle conducted in 31 herds in the southwest. BVDV vaccines were used in all herds in the study. In this study more than 4,344 calves were tested, of which seven were determined to be PI. These seven animals resided in six different herds. Based on this study and previous research, Dr. Fulton reported that herds using vaccines PI animals tended to occur as single individuals rather than in groups. A commercial antigen capture ELISA (ACE) test, immunohistochemistry (IHC) and virus isolation were used in this study to confirm PI status. The ACE test calls for soaking skin biopsy samples, most frequently ear notch samples, in phosphate buffered saline (PBS) solution for a minimum of two hours. The ACE test is then conducted on the PBS solution rather than on the skin biopsy directly. Dr. Fulton reported that freezing of the skin biopsy in the PBS prior to testing eliminated false positives. Dr. Fulton recommended, when using the ACE test, samples giving a raw optical density (OD) reading below that of the positive control provided by the manufacturer be retested. Dr. Fulton also noted in their study that increasing the primary antigen concentration used in IHC reduced false negatives. Dr. Daniel Givens, Auburn University, reported on the isolation and characterization of a BVDV isolate that could not be detected by the two monoclonal antibodies used in the commercially available ACE test and in most diagnostic laboratories doing IHC. A unique mutation in the region coding for the BVDV structural protein Erns was detected in this isolate. This isolate was detectable using a commercial ACE test that is licensed for use in Europe. This test is based on detection using a cocktail of monoclonal antibodies. The ACE test available in the United States, that failed to detect this isolate, uses just one monoclonal antibody for detection. Dr. Givens is currently screening samples submitted to the Alabama BVDV Control Program to determine the incidence of BVDV isolates that are not detected using the commercial ACE test available in the United States. Dr. Bruce Brodersen, University of Nebraska – Lincoln, reported that for the last five years 10,000 to 12,000 IHC tests per month have been conducted in his laboratory. The percentage of positive samples has been trending downward over the last three years. The percentage of samples testing positive has gone from 0.4 percent to 0.2 percent. Dr. Brodersen also reported that they have found that in rare cases nonpersistently infected cohort animals housed in herds harboring more than three percent PI animals accumulate BVDV antigen in skin that may persist over extended periods of time. These animals are not PI but may be incorrectly identified as PI. Such animals may be differentiated from true PI animals based on IHC staining patterns.

Dr. Dave Dargatz, Centers for Epidemiology and Animal Health (CEAH) reported that the National Animal Health Monitoring System (NAHM) will be conducting a BVDV study in cow/calf operations in 24 states. There are three components to the NAHMS study. The first is an educational component that will introduce the goals and protocols of the NAHMS study to potential participants. This component will begin in November 2007. The second component is a questionnaire surveying vaccination practices. This component will be conducted January through March of 2008. The third component is a sampling of all spring calves born in participating herds in 2008. The testing

of these samples will begin in June of 2008 and will be based on ACE tests conducted on individual ear notch samples.

Dr. Sabrina Swenson, National Veterinary Services Laboratory (NVSL), presented information on a voluntary nation-wide BVDV check test. NVSL, in collaboration with the National Animal Disease Center (NADC), developed a proficiency panel for detection of BVDV. Animals positive for BVDV were identified for inclusion in the panel by two positive tests by either ACE, IHC or Polymerase Chain Reaction (PCR). At least two weeks after the initial identification, virus isolation (VI) from buffy coat was performed at NADC and all isolated viruses were genotyped based on comparison of 5' UTR data. Nine animals were selected from across the United States included genotypes 1a (1 animal), 1b (2 animals), 1a and 1b (2 animals), 2a (2 animals), and negative (2 animals). Panel samples consisted of serum or buffy coat. Thirty-two laboratory participants used the panel for BVDV detection by ACE, virus isolates (VI), and/or PCR. Overall 10 of 27 laboratories identified all samples correctly by ACE with eight of the 16 samples identified correctly by all laboratories. Three of 19 labs identified all samples correctly by VI with eight of the 16 samples identified correctly by all laboratories. Fourteen of 26 labs identified all samples correctly by PCR with eight of the 16 samples identified correctly by all labs. Laboratories consistently identified negative samples, with one false positive by ACE, two false positives (from 1 lab) by virus isolation, and no false positives by PCR.

Dr. Edward Dubovi, Cornell University, reported the results of testing 12,000 alpaca samples for the presence of BVDV. This testing occurred between January 2006 and the present. His laboratory confirmed PI in 18 alpacas (0.15 percent). In addition they detected serum neutralizing antibodies in 14 percent of 268 alpaca serology samples. Phylogenetic analysis of 43 BVDV strains isolated from alpaca in a wide geographic region reveal that 42 of these strains could be grouped into two different genetic groups. Strains within these two groups were highly similar suggesting that the majority of BVDV outbreaks examined in this study could be traced to one of two point source. The one exception was a strain isolated from an alpaca residing in Canada. The results suggest that eradication of BVDV from alpacas is achievable via testing and elimination of infected animals and that vaccination is counter indicated as it would preclude surveillance for reintroduction of BVDV by serology.

Dr. James Evermann, Washington Animal Disease Diagnostic Laboratory, has been tracking the causes of coronavirus associated diarrhea in alpaca crias since 2005. There has been increased recognition of neonatal criad diarrhea in Northwestern farms. The predominant age range is four days to four weeks of age. The seasonal prevalence appears to be higher in March to April and August to September. In addition to neonatal diarrhea, coronavirus has been associated with alpacas after shows (coronavirus-associated show diarrhea). The predominant mode of diagnosis is electron microscopy (EM) on fecal samples. There is a lack of coronavirus diagnostic assays, which severely limits the detection of coronavirus in many laboratories. The industry needs more rapid and accurate diagnostic assays for coronavirus induced disease, such as antigen ELISA. Coronavirus infections of zoo animals and captive wildlife have also been highly dependant upon EM. Some research laboratories, according to Dr. Linda Safe, Ohio State University, have isolation capabilities which allows for detection of unique coronaviruses. More recent detection of unique coronaviruses has included ferret, mink, pygmy rabbit, bats and giraffe.

Dr. Konstantin Lyashchenko, Chembio Diagnostic Systems, Inc., presented information on serologic detection of tuberculosis (TB) in bison and camelids. The intradermal tuberculin test has serious limitations in non-bovid species. Chembio developed a novel serological assay, ElephantTB STAT-PAK kit, using lateral-flow technology to detect specific antibody in elephants and other captive wildlife within 20 minutes. This animal-side test was approved by USDA, Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), Centers for Veterinary Biologics (CVB) in 2007. In addition, the Multi-Antigen Print ImmunoAssay (MAPIA) was proposed for elephants, particularly, as confirmatory test and treatment monitoring tool. Extended studies with ElephantTB STAT-PAK (100 percent sensitivity and 97 percent specificity in elephants) confirmed its potential to be a valuable animal-side diagnostic test in multiple zoo animals as well as in a number of free-ranging wildlife species involved in maintaining bovine TB reservoirs worldwide. Several serological studies on animals naturally infected with *Mycobacterium bovis* (bison, camel, and llama) or *M. microti* (llama, alpaca) were shown to be detectable by the Chembio rapid test and MAPIA for early detection of TB in these species in which the skin test has failed.

Three resolutions were passed unanimously by the Committee and submitted to the Committee on Nominations and Resolutions. They addressed 1) Funding and Planning of Integrated and Comprehensive Animal Health Surveillance, 2) Bovine Viral Diarrhea Virus (BVDV) Control Cost Benefit Analysis in Beef and Dairy Production, and 3) Establishment of Check Test Panel for Testing Cattle for Bovine Viral Diarrhea Virus (BVDV) Persistent Infection (PI).