

REPORT OF THE COMMITTEE ON CAPTIVE WILDLIFE AND ALTERNATIVE LIVESTOCK

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The Committee met at 8:00 a.m. on October 20, 2007 at John Ascuaga's Nugget Hotel, Reno, Nevada. The meeting was called to order by Vice Chair Michele Miller. There were approximately 120 people in attendance of which 103 signed in and 37 were Committee members. In her opening remarks, Vice Chair Miller welcomed attendees and requested a show of hands to ensure a quorum of members were present.

Dr. Chester Gibson, Deputy Administrator, Animal Care (AC), Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), presented an Update on Animal Care, USDA-APHIS. Information on several of these issues is available at www.aphis.usda.gov/animal_welfare/index.shtml. During Fiscal Year (FY) 2007, 18,343 total inspections were performed on a total of 10,063 facilities. Some of the issues in the spotlight included large exotic cat handling, elephant tuberculosis and handling, birds, pet evacuation/rescue, and avian influenza. The Pets Evacuation and Transportation Standards (PETS) Act of 2006 ensures that state and local emergency preparedness operational plans address the needs of individuals with household pets and service animals following a major disaster or emergency. Pending legislation (Haley's Act) would allow USDA to draft public safety regulations for facilities falling under the Animal Welfare Act (AWA), specifically defining big cat and direct contact. The Captive Primate Safety Act is still pending and would make it illegal to import, export, sell, acquire, or purchase nonhuman primates. The revised marine mammal sections of the AWA are still in the rulemaking process. The plan is to have the proposed rule in clearance by the end of 2007. Proposed rulemaking is being developed for birds to be covered under the AWA. Rulemaking for rats and mice will be done at a later time. The Animal Care Policy Manual is now available on the website. Public comment period closes November 8, 2007. USDA-APHIS-AC is developing a Center for Animal Welfare Education, Outreach, and Technology. The role of the Center will be to develop new technologies through cooperative agreements and collaboration with land grant colleges, veterinary schools, and other professional organizations.

Drs. Tom Gidlewski and Dean Goeldner, VS-APHIS-USDA presented, Update on the USDA-APHIS-VS Chronic Wasting Disease Program. In FY 2007, 17,189 farmed or captive cervids were tested for chronic wasting disease (CWD), up from the previous three-year average of about 15,000. No new CWD positive farmed cervid herds were detected in FY 2007 nor were any herds depopulated. Four infected elk herds in Colorado and one infected white-tailed deer herd in Wisconsin currently remain. All are under state quarantine.

New York found no additional CWD positive free ranging cervids in 2007, but West Virginia found additional cases in Hampshire County. Wisconsin continues to aggressively battle CWD with over 130,000 animals submitted for testing since 2000 and over 850 positive deer identified. The infected area appears to be slowly spreading. During 2007, Canada discovered two positive captive elk herds and one positive captive white-tailed deer herd in Saskatchewan.

After considering a United States Animal Health Association (USAHA) Resolution to approve enzyme linked immunosorbent assay (ELISA) testing for captive cervids, a decision was made to continue the use of immunohistochemistry (IHC) as the official test in order to maximize the chance of identifying positive herds and also maintain a method for oversight by the National Veterinary Services Laboratories (NVSL).

Submission of an ear with the official eartag attached or submission of fresh tissue accompanied by an appropriately executed chain of evidence document will allow DNA comparison in the event of a positive diagnosis. Archiving herd blood samples on special collection cards is also a way to compare DNA in the event of a positive diagnosis in the future. Memos describing acceptable DNA comparison procedures are in final review.

Rectal biopsy continues to be examined as a tool for CWD ante-mortem diagnosis. Many additional animals were tested in 2007 with the identification and removal of positive elk from infected herds. Eighty percent of the positive animals in a highly infected white-tailed deer herd were identified with rectal biopsy. The lower incidence of CWD in most infected elk herds complicates the evaluation of this test in elk. It appears that in deer, rectal lymphoid tissue becomes positive later than lymphoid tissue of the head suggesting that early cases may be missed with rectal biopsy. Positive rectal biopsy is indicative of disease but a negative rectal biopsy test does not rule out CWD in an individual or herd.

In FY 2007, APHIS-VS received approximately \$16.6 million in appropriated CWD funding. All earmarks were removed from the FY 2007 appropriation that was passed as a yearlong continuing resolution. In addition to the \$5.75 million made available to the states and tribes for CWD surveillance and management in wildlife, APHIS also provided \$1.7 million in end-of-year funding to twenty states to supplement CWD activities in farmed and captive cervid program. The FY 2008 appropriations have not been passed by Congress; The president's budget requests \$12.3 million for CWD.

In September 2006, APHIS-VS delayed implementation of the final CWD rule that had been published in July 2006. This delay was precipitated by three petitions to the rule received from organizations representing state agencies and officials including USAHA. On November 3, 2006, APHIS-VS published these petitions for public comment. After reviewing these comments, APHIS-VS requested additional information from the states in late June 2007 regarding their restrictions for the movement of cervids into their states. Based on all the information received, APHIS-VS has begun drafting new proposed rule language and is circulating it internally for review.

Kurt VerCautern, National Wildlife Research Center (NWRC), Wildlife Services (WS), APHIS-USDA, presented Current Research at the Fence: An Update from the USDA-APHIS-WS, NWRC. The spread of CWD, bovine tuberculosis, and other diseases in wild and captive cervids is of great nation-wide concern. Research is needed to fill information gaps associated with questions pertaining to disease transmission at the interface between wild and privately owned cervids. We are working to address some of these questions and the goal of this presentation is to provide an update on two of our current efforts.

1. We are determining the minimum fence height that is essentially 100 percent effective in keeping wild white-tailed deer out and captive white-tailed deer in. The study is nearly complete and preliminary results show that while 91 percent of deer can jump a six-foot fence it is very rare for a deer to clear an eight-foot fence. More trials are currently being done at eight feet.
2. We are determining the effectiveness of electric fencing used with woven-wire fencing to limit fence-line contact, and the probability of disease transmission, by elk. Our electric fence is one meter from the woven-wire fence and runs parallel to it. We are evaluating the fence's effectiveness with varying scenarios and motivation levels between elk in test pens. Preliminary results suggest that coupling a single woven-wire fence with electric fence virtually eliminates contact through the fence.

Our role is to find answers to questions that are of great interest to federal and state agencies responsible for managing and regulating wild and captive cervids as well as the privately owned cervid industry.

John Pilon, NWRC-WS-APHIS-USDA, presented Development of a Chronic Wasting Disease Vaccine: Progress and Promise. CWD is a transmissible spongiform encephalopathy (TSE) of domestic and wild cervids in North America. To address possible prevention regimens for CWD, we have taken an active vaccination approach using prion derived-peptide sequences, a carrier protein, and an adjuvant to overcome self-tolerance. Twenty CL57/BL6 mice per group were vaccinated and boosted with 50 µg of the carrier protein-peptide conjugate; all vaccines produced a humoral immune response as measured by ELISA. After vaccination mice were challenged with the Rocky Mountain Laboratory (RML) mouse-adapted scrapie strain. The mouse-model results demonstrate that our method could generate titers toward the prion protein peptides and most importantly, improve the life span of RML mouse adapted scrapie challenged mice. Using the insights gained from this initial mouse-model study we have recently begun evaluating a vaccine candidate in the target species, mule deer (*Odocoileus hemionus*).

Justin Greelee, National Animal Disease Center (NADC), ARS-USDA, presented prolonged CWD incubation time and unique PrP^d profile in Prnp 132LL elk. The transmissible spongiform encephalopathies including CWD in deer and elk invariably result in fatal neurodegeneration and accumulation of PrP^d, an abnormal form of the host prion protein PrP^C. In some species, polymorphisms in the open reading frame of the Prnp gene are associated with differences in the manifestation of prion disease including relative susceptibility, clinical signs,

incubation time, and neuropathology. The polymorphism (M/L) at Prnp 132 in Rocky Mountain elk (*Cervus elaphus nelsoni*) corresponds to the human (M/V) polymorphism at Prnp 129, where M has been associated with susceptibility to variant Creutzfeldt-Jakob Disease (vCJD). Elk with 132 M alleles are predisposed to CWD and heterozygosity is associated with a prolonged incubation time following experimental challenge. Previous studies suggest that elk homozygous for 132 L occur rarely and make up the extreme minority of elk affected with CWD. The effect of the 132 LL genotype on the development of CWD post-exposure was previously unknown. The purpose of this study was to define the course of disease in elk with various Prnp 132 allele combinations. Elk (n=8; 2MM, 2LM, 4LL) were orally inoculated at eight months of age with 15 ml of pooled brain homogenate from one 132 MM and one 132 LM elk. Elk were observed daily after inoculation and necropsies were done when clinical signs became unequivocal. IHC, western blot, and microscopic examination were used to confirm infection. Incubation time was dependent on genotype. Clinical signs were apparent in 132 MM elk after 23 months and 132 LM elk after 40 months. Rectal biopsies were done on the remaining 132 LL elk with three of four testing positive for PrP^d by IHC indicating peripheral distribution of PrP^d is apparent prior to the onset of clinical disease. Clinical signs were apparent in 132 LL elk after 59 to 63 months. One elk was euthanized 63 months post-inoculation without exhibiting clinical signs, but had PrP^d accumulation in the central nervous system (CNS) and peripheral lymphoid tissues. Differences between genotype were apparent after western blot analysis. The molecular weight of the proteinase K resistant bands of PrP^d is lower in the 132LL elk compared to 132MM or 132LM elk.

In summary, LL elk are susceptible to CWD, but have a prolonged incubation time and western blot profile unique from other genotypes of elk with CWD. Additional studies are planned to determine the mechanisms responsible for the distinct presentation of CWD in 132 LL elk.

John Fischer, Southeastern Cooperative Wildlife Disease Study (SCWDS), University of Georgia, presented Update on Epizootic Hemorrhagic Disease in Deer. Dr. Fischer reported that SCWDS has received an unprecedented number of samples for virus isolation originating from both penned and wild white-tailed deer this year. As of October 8, 2007, 214 virus isolations have been made at SCWDS. This number also is unprecedented and SCWDS continues to receive large numbers of samples as well as telephone reports and inquires every day. Nearly all virus isolations have been epizootic hemorrhagic disease virus-serotype 2 (EHDV-2) from white-tailed deer, although very low numbers of EHDV-1 and bluetongue virus (BTV)-10, -11, and -17 viruses have been isolated. The BTV-10 and 11 isolates are from Missouri and the BTV-17 isolate is from a mule deer in New Mexico. The current distribution of isolates is presented below. A distribution map that is updated weekly can be seen at the SCWDS web site, www.scwds.org.

Hemorrhagic disease (HD) occurs annually in the United States and as is occurring in 2007, most outbreaks in white-tailed deer are caused by EHDV-2. There is no reason to assume that this outbreak is associated with a particularly virulent strain; EHDV-2 can cause high mortality rates, especially when deer are infected in the northern United States. There are two aspects of this outbreak that have sparked speculation and discussion. The first involves a potential expansion of the traditional HD range due to climate change and the second involves clinical disease in cattle.

Is HD expanding its range?

The current distribution of reported HD (Figure 1) includes much of the United States. It is important to note that this distribution map is compiled from reports of clinical disease from 1980 to 2003. The map does not represent the entire distribution of HD viruses because infections in white-tailed deer in some areas, such as portions of Texas and Florida, often are subclinical. Based on the historic distribution, it appears that the current outbreak falls primarily within the historic range of HD, although some expansion may be occurring.

Although it is premature to suggest that the 2007 activity is a product of global climate change, we cannot ignore the fact that the Southeastern United States is in an unprecedented drought and that our initial cases in the eastern United States were spatially associated with areas of especially severe drought in Kentucky and Tennessee. But whether the current drought is a result of climate change is an issue yet to be determined. The drought/HD relationship is not new and has been suggested since the 1980s. SCWDS currently is analyzing its historic data to better understand this potential relationship.

Is EHDV causing disease in cattle?

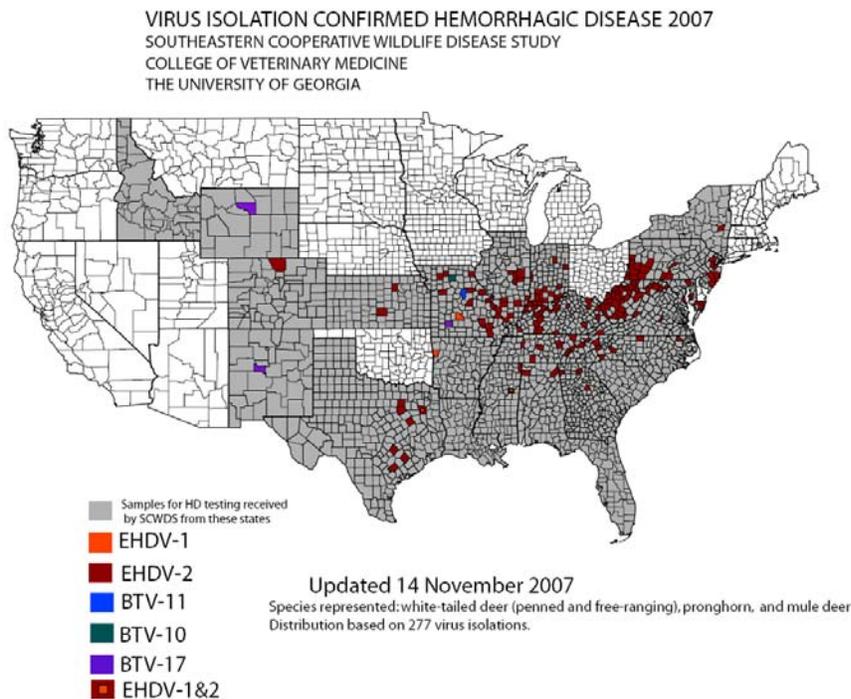
Epizootic hemorrhagic disease is not a recognized disease of cattle, but it is well established that they can be infected. There are two contrasting observations that cause confusion related to the issue of clinical disease in cattle. First, as is occurring this year, suspected cattle disease associated with EHDV-2 infection is a common occurrence during large scale EHDV epizootics. Such reports occur routinely when the virus is causing deer mortality in the northern United States. In most cases, cattle show mild disease, but occasional reports of abortion and even death (unconfirmed) do occur. Such reports are not obtained from HD-endemic areas. Unfortunately, suspected cattle cases are seldom confirmed and it needs to be clearly understood that the

presence of antibodies in such animals does not confirm EHDV as a cause of either morbidity or mortality. On several occasions, including one this year, we have isolated EHDV-2 from a cow with bluetongue-like disease, but even this may not confirm that the virus was the cause of the disease.

In contrast, clinical disease never has been associated with experimental EHDV infections of cattle, including one SCWDS study (Abdy, M.J., E.W. Howerth, and D.E. Stallknecht. 1999. Experimental infection of Calves with Epizootic Hemorrhagic Disease Virus. *American Journal of Veterinary Research* 60(5) 621-626). The truth likely lies somewhere between the field observations and the results of these experimental studies and the following hypothesis would fit with the limited data currently available: The reported disease in the field is bluetongue like and it is not unreasonable to speculate that EHDV would cause similar signs and lesions. With BTV infections, clinical disease in cattle is not common but is mild when it occurs. However, even mild disease is the exception rather than the rule. If EHDV causes a generally mild disease in a very small proportion of those cattle infected, it may well be that the disease would not be detected in the small number of animals included in experimental studies and would only be detected in the field under exceptional challenge conditions as is occurring now. If this hypothesis is correct, EHDV would be of minor concern to cattle producers, but could be responsible for sporadic disease in certain areas of the United States. All reports that received at SCWDS concerning suspected disease in cattle have been associated with the northern edge of the HD range (as defined by reported disease in white-tailed deer) and it is possible that such potential problem areas could be defined by vector distribution and herd immunity.

The reports of suspected EHD in cattle and confirmed HD in wild and penned deer can lead to one group of producers/managers blaming the other for their problem cattle, wild deer, and penned deer can all be infected with EHDV and can serve as a source of virus to vectors. It is not cattle, penned deer, or wild deer that represent the reservoir for these viruses. Rather, all ungulate species can be involved in viral amplification. In reality, the population dynamics of the biting midge vector, *Culicoides sonorensis*, may be the most important factor in these outbreak situations.

Figure 1. Virus Isolation Confirmed Hemorrhagic Disease 2007.



Konstantin Lyashchenko, Chembio Diagnostic Systems, Inc. presented Update on Tuberculosis (TB) Serodiagnostics. Numerous animal species are susceptible to TB that has serious zoonotic and regulatory concerns. The current TB testing methodologies are inadequate for most non-domestic animals. To improve TB control programs, new diagnostic tools that would be simple, rapid, accurate, inexpensive, and host species-independent are urgently needed. Chembio developed a novel serological assay, ElephantTB STAT-PAK kit, using lateral-flow technology to detect specific antibody in elephants and other captive wildlife. This test was approved by USDA-APHIS-VS-Center for Veterinary Biologics (CVB) in 2007. In addition, the MultiAntigen Print

ImmunoAssay (MAPIA) was proposed for elephants, particularly, as confirmatory test and treatment monitoring tool. The results of extended evaluation of the Chembio immunoassays in a number of zoo species (rhino, tapir, gazelle, and jaguar) as well as free-ranging wildlife (cervids, possum, wild boar, and lion) confirmed our earlier findings strongly suggesting the potential for rapid serodiagnosis of TB in multiple host species. Diagnostic potential of MAPIA for serologic detection of TB and non-TB mycobacterial infections in marine mammals (sea lion, whale, and dolphin) was also demonstrated.

Mitch Palmer, National Animal Disease Center (NADC), ARS-USDA, presented Update on TB Vaccines in Deer. The presence of tuberculosis due to *Mycobacterium bovis* in captive and free-ranging wildlife remains one of the greatest challenges to eradication of tuberculosis in the United States. A possible addition to current control measures could be vaccination of deer to prevent infection, disease, or transmission. To evaluate the efficacy of *M. bovis* Bacillus Calmette-Guerin (BCG) vaccination of white-tailed deer, 61 yearling white-tailed deer were randomly assigned to one of three groups; two doses of 10^7 colony-forming unit (CFU) of BCG (Pasteur) administered six weeks apart SC (n=11); one dose of 10^7 CFU of BCG (Pasteur) SC (n=10), one dose of 10^9 CFU BCG (Danish) orally in a lipid based bait (n=8), one dose of 10^9 CFU BCG (Danish) orally in liquid suspension (n=8), one dose of 10^6 CFU BCG Danish SC (n=7), and unvaccinated deer (n=17). Additionally to examine the comparative efficacies of BCG (Danish) and BCG (Pasteur), additional deer were vaccinated with 10^7 CFU BCG (Pasteur) (n=9) or BCG (Danish) (n=8). All deer were intratonsilarly inoculated with 300 CFU of virulent *M. bovis* three months after vaccination. Decreased lesion severity scores compared to unvaccinated deer were seen in all orally vaccinated deer, deer receiving a single dose of BCG Danish and deer receiving two doses of BCG Pasteur. In protected deer, medial retropharyngeal lymph node granulomas were smaller, less necrotic with rare acid-fast bacilli compared to lesions in lymph nodes from unprotected deer. *Mycobacterium bovis* BCG administered parenterally or orally can be effective in reducing lesion severity in *M. bovis*-inoculated deer. Decreased lesion severity with less necrosis and fewer acid fast bacilli would likely decrease the ability of vaccinated deer to shed virulent *M. bovis* thus decreasing intraspecies and interspecies transmission. It is also evident from the current study that not all strains of BCG are equally protective in white-tailed deer.

Dave Hunter, Turner Enterprises, Inc., presented Veterinary Score Card: How Are Veterinarians Helping Your Industry?

Turner discussed how the Committee has evolved to include not only regulatory agencies and veterinarians concerned with wildlife, but industries that work with captive wildlife. He proposed that the Committee's function was similar to the approach that should be taken to wildlife health and management, which should be holistic. The ecosystem health management should include all the stakeholders in livestock, wildlife, human, and environmental health.

Charley Seale, Exotic Wildlife Association, presented Scimitar Horned Oryx Reintroduction Program. This was an overview of the partnership that the private exotic wildlife industry in Texas has with the Sahara Conservation Fund in reintroducing endangered and rare species back to native countries. The first reintroduction was conducted in 2005 in which 44 dama gazelle, 40 addax, 35 markhor and 10 scimitar horned oryx were transported to Dubai. These animals originated from private ranches in Texas. Another reintroduction program is under negotiation for a reserve in Senegal. The success of the private game ranch industry in breeding rare and endangered species is an example of how private conservation can affect worldwide preservation.

The Committee reviewed and discussed five resolutions. All were approved and referred to the Committee on Nominations and Resolutions.