REPORT OF THE COMMITTEE ON CAPTIVE WILDLIFE & ALTERNATIVE LIVESTOCK

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The meeting of the Committee was called to order by Chair Bob Cook at 12:35 p.m. on November 6, 2005. There were approximately 85 people in attendance, including 23 Committee members. In his opening remarks, the chair welcomed attendees.

Dr. Chester Gipson, United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Deputy Administrator for Animal Care (AC) presented an update on the AC program. Information on several of the issues he discussed were also available on their website (www.aphis.usda.gov/ac). His report included:

- In FY05, there were 9,994 licensed facilities and a total of 13,944 inspections conducted. All AC inspection reports will eventually be posted on the web, with some information removed for confidentiality. The most common Animal Welfare Act (AWA) problem noted during inspections was associated with primary enclosures.
- Public contact with captive wildlife continues to be an issue, especially as it pertains to big cats, bears and other wildlife. A policy addressing potentially dangerous animals was amended in July 2004. It requires that licensees demonstrate adequate experience and knowledge of the species they maintain. Big cats include lions, tigers, jaguars, leopards, cougars, cheetahs and any hybrids of the above. The Secretary of Agriculture has determined that there is an inherent risk in contact with big cats and therefore, there must be sufficient distance between the public and big cats.
- The Captive Wildlife Safety Act was passed in 2003 but has not been enacted. This prohibits private pet ownership of big cats and interstate movement. A Captive Primate Safety Act is under consideration in Congress.
- The 2002 Farm Bill mandates that the AWA covers rats, mice and birds not being used for research. A work plan is currently being developed to implement this new requirement.
- AC hosted a workshop in March 2005 to initiate a network at all levels of government involved in regulating and implementing laws pertaining to wildlife and exotic animals.
- AC continues to develop their role in companion animal rescues during and after disasters and assist in disaster management. They also are partnering with World Organization for Animal Health (OIE) regarding the issues of land transport of animals, humane killing of animals for disease purposes, slaughter of animals for human consumption, and transport of animals by sea.
- A recent audit of the AC program by the Office of the Inspector General is available at www.usda.gov/oig/webdocs/33002-03-sf.pdf.

Dr. Dan Baca, USDA, APHIS, Veterinary Services (VS), presented an update on the proposed revisions to the Uniform Methods and Rules (UM&R) for Tuberculosis in Captive Cervids. The United States Animal Health Association (USAHA) Committee on Tuberculosis passed recommendations in 2002 and 2003 that encouraged USDA to adopt standards for recognition of a state's progress towards eradication of tuberculosis in captive cervids by establishing state status levels, similar to existing standards for cattle and bison, and to incorporate

those standards in a revised UM&R. The USDA, in cooperation with USAHA, assigned a state/federal/industry working group to develop those standards and guidelines for consideration by the Committee on Tuberculosis.

Following a two year process, the working group developed a set of proposed standards for state status based primarily on herd prevalence of cervid tuberculosis in a state or zone, and requirements for levels of surveillance that support detection of the disease at those thresholds. The proposed plan provides for four state status levels with corresponding herd prevalence infection rates: Accredited-free (0 percent), Modified Accredited Advanced (less than 1 percent), Modified Accredited (less than 6 percent), and Non-accredited (greater than 6 percent). The associated surveillance requirements are 0.1, 1.0, and 6.0 percent for Accredited-free, Modified Accredited Advanced, and Modified Accredited status, respectively. In addition, the working group proposed revisions in the UM&R to incorporate proposed state status standards, with additional revisions regarding interstate movement and herd status standards. These proposed revisions will be discussed in greater detail at the Committee on Tuberculosis meeting during this Annual Meeting.

Dr. Tyler Thacker, USDA, Agriculture Research Service (ARS), National Animal Disease Center (NADC), made a presentation on research at NADC being conducted to study the role of two cytokines in the immune response of White Tailed Deer (WTD); interferon-γ (IFN-γ) and IL-4. IFN-γ has been reported to be critical in immune responses against mycobacterial infection and is also used as an indicator of infection. A second cytokine IL-4, the antithesis of IFN-γ, has been reported to inhibit IFN-γ production as well as increase pathogenesis. An understanding of the role these cytokines in WTD immune responses would be important in vaccine development and evaluation.

Leukocytes from infected and uninfected WTD were analyzed for IFN-γ and IL-4 expression over a 6 month period and responses compared to pathological findings. Leukocytes from animals with more severe pathology expressed more IFN-γ than animals with less severe pathology. The opposite was true for IL-4, animals with less severe pathology expressed more IL-4 than animals with more severe pathology. These data are contrary to the prevailing hypothesis that greater IFN-γ expression results in greater protection.

Because IFN-γ expression was high, the utility of using IFN-γ RNA as a diagnostic tool was investigated. Both IFN-γ protein and RNA was measured in both infected and uninfected deer. Over the course of infection IFN-γ protein reached a peak at 60 days post-infection (p.i.) then declined, whereas, IFN-γ RNA expression peaked at 60 days p.i. then remained high throughout the remainder of the infection. At 14 days p.i. 2 out of 10 animals were positive by Cervigam, while 4 out of 10 were positive by RT-PCR. By 2 months post infection (60 days p.i.) both Cervigam and RT-PCR were able to detect 8 out of the 10 infected animals. However, 6 months p.i., (168 days) RT-PCR detected 9 out of 10 infected animals as positive but Cervigam only detected 3 out of the 10 infected animals. Because IFN-γ RNA expression does not wane during infection as does IFN-γ protein, measurement of IFN-γ RNA may provide a more sensitive assay for the detection of infected white-tailed deer.

Dr. R. Scott Larsen, University of California-Davis, presented an update on the serologic detection of tuberculosis in elephants.Tuberculosis has become an important disease in captive elephants, particularly Asian elephants (*Elephas maximus*). Diagnosing tuberculosis in elephants has been problematic as many tests have inadequate sensitivity or specificity. A multiple-antigen enzyme-linked immunosorbent assay (ELISA) was previously investigated for detecting infection in Asian elephants and African elephants (*Loxodonta africana*); this test had excellent sensitivity and specificity, but needed further evaluation.

Modifications to the multiple-antigen ELISA panel have since been made. Valuable antigens were retained, other antigens were removed, and new ones were added. This modified ELISA was re-evaluated, using serum from 68 Asian elephants. Sixteen had *M. tuberculosis*-positive trunk cultures, while 52 were either culture negative at necropsy or had a history of negative trunk cultures and no contact with infected elephants. Seven elephants were evaluated over time.

The test was 100% (95% CI; 95-100%) specific and 94% (95% CI; 79-100%) sensitive using two of the six antigens (*M. bovis* strain AN5 culture filtrate and *M. tuberculosis* early secretory antigenic target 6). “Effectively-treated” elephants had decreasing seroreactivity, but those that were culture-positive post-treatment were more consistently seroreactive. Although “effectively-treated” elephants had declining seroreactivity, they still usually had higher values than animals that had never been infected.

Serology continues to show great promise in detecting tuberculosis in elephants, often detecting infection months-to-years sooner than trunk wash culture. Advances in techniques may soon make serology even more practical.

While serology should not replace trunk-wash culture, it is a useful adjunct for early detection of infection in elephants and for monitoring treatment.

Dr. Konstantin Lyashchenko, Chembio Diagnostic Systems, Inc., made a presentation on tuberculosis diagnostics in non-domestic species. Not only are a broad range of animal species susceptible to tuberculosis, it has serious zoonotic and regulatory concerns. In addition, the current testing methodologies are inadequate. To improve tuberculosis control programs, new diagnostic tools that would be simple, rapid, accurate, inexpensive, and host species-independent are urgently needed.

Chembio is proposing a novel serological assay, Vet TB Stat-Pak, which is based on the lateral-flow technology to detect specific antibody using colored latex particles. To select immunodominant antigens, they used the multi-antigen print immunoassay (MAPIA) and sera from cattle, WTD, reindeer, elk, and badgers experimentally or naturally infected with *M. bovis* as well as from elephants with culture-confirmed tuberculosis due to *M. tuberculosis*. The results demonstrated the remarkable animal-to-animal variations of antibody responses during infection in these species. Several key antigens that were commonly recognized by all of them were selected to develop VetTB Stat-Pak. This test is easy to perform and it requires no laboratory equipment. The test can use serum, plasma, or whole blood samples to deliver result within 20 minutes. Diagnostic sensitivity of VetTB Stat-Pak evaluated in over 20 species varied from species to species, ranging from 53% in badgers to 100% in elephants. The specificity ranged between 94% in badgers or reindeer to 98% in elk or white-tailed deer. The proposed assay format is most suitable for surveillance in a variety of wildlife and zoo species, especially where an instant result is needed.

Dr. Kurt Vercauteren, USDA-APHIS, Wildlife Services (WS), made a presentation of a study entitled, "Interaction of Free-Ranging and Captive Cervids Across Fences with Respect to Chronic Wasting Disease." The objective of the study was to document the amount and type of interactions occurring across fences with captive cervids. There were 9 study sites in Colorado with captive elk and 6 sites in Michigan with WTD. Two methods were used: track plots and motion activated cameras. Contact was measured during a 16 month period as direct or indirect (<5 m). The average was 1-1.5 intrusions/plot on the inside of the fence for elk and 0.75 intrusions/plot for WTD. There were 77 direct contacts for the total study period in Colorado and 274 indirect contacts. There were no statistically significant seasonal differences, although there was a difference between the sites. No contacts were seen when a double fence was used. In Michigan, there was a decreased total number of contacts. Stocking rates, proximity of females and males, feeding practices, fence construction, and geographic location, cervid population and other demographic factors all played a role in determining the contact frequency.

Dr. John Huntley, New York State Veterinarian, discussed New York's Chronic Wasting Disease (CWD) program. The program goals are to; 1) detect existing infection; 2) prevent introduction; and 3) eradicate CWD infection in captive deer populations. There are two types of captive cervid herds in New York. CWD monitored herds are usually deer held on a shooting or nature preserve. It is usually impractical to perform an annual inventory. CWD certified herds can be inventoried on an annual basis and may be permitted to move animals. Requirements for a CWD certified herd include herd enrollment, requirements for fencing and facilities, individual animal identification, sampling of clinical cases and all natural deaths for CWD testing, and requirements that all animal additions come from CWD certified herds. Requirements for CWD monitored herds include herd enrollment, fencing, and surveillance of 10% or 30 animals (whichever is less) for CWD, with additions of new animals coming from CWD certified herds. New York requires that all captive cervid herds perform CWD surveillance at one of the two levels.

In early March 2005, the WTD from the index herd were sampled as part of the monitoring program. In mid-March, a WTD from a second farm died and was sampled as part of the surveillance program. CWD was detected in both samples and both herds were depopulated. In total, there were 100 herd contacts in 8 herds. The most likely origin of the agent was introduction through taxidermy specimens. Fawns were exposed prior to weaning in the same area. Surveillance of free-ranging deer in a 10 mile radius around the two index farms revealed 2 positive cases out of 292 samples. Genetic testing revealed that the isolates from the free-ranging WTD were different than those from the index herd.

New York based their program and information on that obtained from Wisconsin and Colorado. They continue implementation of their captive deer surveillance program that includes both CWD and TB. There is rigorous enforcement of the program and continued development of additional techniques and procedures.

Dr. Thomas Gidlewski, USDA-APHIS-VS, presented an update on the USDA-APHIS Chronic Wasting Disease program as well as the status of CWD in North America with particular emphasis on the events of last year. The proposed rule on CWD has almost cleared departmental review and is about to enter the extra-departmental clearance process. As soon as the rule is finalized, the Uniform Methods and Rules will be reconciled with the rule and the grandfathering process of existing state CWD programs will begin. Appropriate sample collection includes obex, medial retropharyngeal lymph nodes and tonsil and the relative importance of each kind of tissue varies by species. The quality assurance measures that the USDA-APHIS-VS, National Veterinary Services Laboratory (NVSL) uses to verify internal and contract laboratory results involve verification of the positive results by two pathologists followed by completely repeating the test starting with the originally submitted samples. A review of the current status of the long term CWD interspecies transmission experiments suggests that a relatively strong species barrier exists even when the direct intracerebral inoculation route is utilized. Primary passage in a non-cervid species does appear to make the agent more virulent in subsequent passage in that same species. They have demonstrated positive rectal biopsies in three of the four LL (leucine/leucine) elk homozygous at codon 132 providing further evidence that this genotype does not convey absolute resistance. In 2005, CWD was discovered in New York, West Virginia and in free ranging deer in Alberta, Canada, as well as in a free ranging Colorado moose.

Dr. Tom Meehan, Brookfield Zoo, made a presentation entitled “Fecal prevalence of shiga-toxigenic E coli (STEC) and *Salmonella enterica* of animals displayed in public contact areas of US AZA-accredited zoos, 2003-2004.” He reported that animal exhibit human enteric disease outbreaks have been increasing over the past 10 years in North America and Europe. Diseases reported include non-typhoid *Salmonella, Cryptosporidium parvum, Campylobacter* and STEC O157. STEC O157 contact animal exhibit outbreaks since 1999 have been an increasing proportion of U.S. cases. They include more than 13 fair, petting zoo or open farm outbreaks. These outbreaks have sickened over 2,000 individuals and caused over 100 cases of hemolytic uremic syndrome and 2 deaths. He reported that a study was undertaken in order to determine the prevalence of STEC O157 and *Salmonella enterica* in zoos accredited by the American Zoo and Aquarium Association (AZA). Thirty-six U.S. AZA-accredited institutions from twenty states volunteered to participate during 2003 and 2004. Individual fecal samples from a census of animals in contact settings were collected and cultured for STEC O157 and *Salmonella*. A total of 997 animals were sampled with 871 being domestic livestock species. STEC O157 was isolated from 1/997 (0.10%) and *Salmonella enterica* was isolated from 6/997 (0.60%). This fecal prevalence was low in absolute terms and low relative to livestock in production settings and livestock in state and county fair settings. The study concluded that AZA-accredited animal contact areas present a low risk of enteric zoonotic bacteria risk at current time.

No recommendations or resolutions were presented to the committee and the meeting was adjourned at 5:10 p.m.