REPORT OF THE COMMITTEE ON CAPTIVE WILDLIFE AND ALTERNATIVE LIVESTOCK

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The meeting of the Committee was called to order by Chairman Bob Cook at 12:30 pm on October 24, 2004. There were approximately 150 people in attendance of which 81 signed in and 25 were Committee members. In his opening remarks Dr. Cook welcomed attendees.

Dr. Chester Gipson, Deputy Administrator of the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Services (APHIS), Animal Care (AC), presented an update on AC program activities. Information on several of these issues are available on their website (*www.aphis.usda.gov/ac*). During FY 2004, 4,361 inspections were performed at 2,542 exhibitor facilities. There are 9,424 facilities total and 15,134 total inspections were either performed or attempted, including unlicensed and pre-licensing inspections, by approximately 100 field inspectors.

AC issues in the spotlight include large exotic cats, elephants, transportation, bears and birds. AC continues to work with states regarding permits/licenses to allow private ownership of large exotic cats. Issues involving tuberculosis (TB) in captive elephants, the philosophy regarding management practices for zoo elephants, and responsible care and treatment of elephants in captivity continue to involve AC staff. New regulations have been enacted for foreign air carriers and how they care for animals in transit. A memorandum of understanding (MOU) has been signed between the Federal Aviation Administration (FAA)

and AC regarding incidents related to pets in transit. Captive bear issues have continued to receive attention and investigation by AC staff. There is ongoing development of standards for birds not used in research that will eventually be covered under the Animal Welfare Act (AWA).

Under the "E-GOV" initiative, AC continues to make more of their reports and information available electronically. Electronic annual reports are filed from the research community; on-line applications/renewals for licensure will become available; and traveling exhibitors will eventually be able to provide their itinerary on-line rather than by FAX.

Electronic Freedom of Information Act (E-FOIA) requires Federal agencies to make certain documents available to the public electronically, including inspections reports. This system became functional in October 2001. After Sept 11, 2001, there were concerns about confidentiality of some of the information in the inspection reports. In order to address these concerns, there is a delay in placing the reports on-line for 30 days to allow the facility to review and ensure the information is accurate. AC is working with the United States Department of Justice to determine what information can be made available on-line while still protecting people and facilities. It is important to determine how to address these security issues while still meeting the federal obligations to provide information.

Information that can be accessed on the AC website includes: current issues and notices; AWA, regulations, policies; lists of licensees and registrants; links to related sites; order forms; fact sheets; ability to submit annual report.

AC training events that were held during FY04 included the National Work Conference, Research Issues for Veterinary Medical Officers, Basic Training for new inspectors, Foreign Animal Diseases Awareness, and Horse Protection Act Training. Additional outreach efforts were Canine Care Workshops, Attending Veterinarian Workshops, and Big Cat Symposia. Preceptorships for AC staff were available in research, transportation and special field certification. This permitted AC staff to work with people in industry. Special topics training also covered exhibitor/exotic/wildlife and nutrition/emerging issues. Canine Care Workshops – 7 workshops were held this year throughout the country and averaged 100 attendees. Attending veterinarian workshops – 2 were co-organized with the University of Missouri. Big Cat Symposia – Various aspects of Big Cat care and maintenance were presented in several workshops offered around the country. Other topics covered were nutrition, veterinary care, training, and transportation. These workshops used outside experts and experienced USDA employees as instructors, with an average of 100 attendees per workshop.

Regulatory Activities of Interest – Amendments to the AWA that affect how rats, mice and birds, not used in research will be regulated.

Amendments to regulations that impact public contact programs with marine mammals are still being worked on. Other regulations are being developed that may look at using new technology in the Horse Protection Act.

Dr. Amy Glaser, Cornell University, presented "How to Get an Antemortem Diagnosis, Some Vaccine Titer Data, and an Update on West Nile Virus (WNV) Zoo Surveillance System." Preliminary studies have examined vaccination in non-equine species using the Ft. Dodge equine WNV vaccine (funded by Ft. Dodge). The vaccination protocol was 2 vaccinations using inactivated whole virus vaccine, 3 weeks apart; each dose was 1 ml IM. Serum was collected pre- and 42-60 days post-vaccination. All animals were antibody negative at the beginning of the respective vaccine trails. Three zoos participated in the initial study (Dickerson Park Zoo; Gladys Porter Zoo; Woodland Park Zoo). A wide range of species were vaccinated. Overall, there was a relatively low rate of seroconversion. Flamingos, raptors, and parrots seroconverted. Titers of birds that seroconverted were relatively low, although some did have higher titers near the end of the trial (ex. a few birds had titers >1:640). Since no challenge studies were performed, there is no data on protection.

Ante-mortem diagnosis of WNV varies between birds and mammals. Birds generally have high levels of viremia but may not be antibody positive at the onset of clinical signs. Multiple samples can be used for diagnosis – whole blood (EDTA, citrate, heparin); oral pharyngeal swab/cloacal swab and blood feathers can be used to test for the presence of virus. Mammals generally have low viremia but may not be antibody positive at the onset of clinical signs. Multiple samples can be used for ante-mortem diagnosis – serial blood samples (serum); cerebral spinal fluid; +/- EDTA blood, can be used for virus identification by PCR or virus isolation, however, it is much harder to find virus in mammals.

The WNV surveillance working group started in 2001. The program objectives were: to build an affordable/reliable WNV testing schedule for zoos and offer a novel extensible data source for national surveillance; to enhance relationships between public health agencies and zoos; to design and implement analyses and reporting that enable characterization of outbreaks from disparate data; and to enable monitoring and prediction of epidemic outbreaks and detection of anomalies. Multiple zoos are involved in sending samples to Cornell University Animal Health Diagnostic Laboratory. Serum and plasma are tested for virus and or antibody, depending on history. Tissues are tested by reverse transcriptase polymerase chain reaction (RT-PCR) and virus isolation. Serum/plasma are tested for WNV/St. Louis Encephalitis virus antibody by plaque reduction neutralization test.

Initial accomplishments were that the project created/strengthened

relationships between zoos and local/state health officials for the detection/reporting of a zoonotic disease threat; provided data to public health system; and has given zoos an avenue for testing valuable rare species. Current directions of the project include creation of an infrastructure that is extensible to other biologic threats of concern (ex. avian influenza); expansion of the program to create regional diagnostic centers; and creation of a web-based data entry, analysis and reporting system.

Features of the new database are that it is simple and extensible (for example, another infectious disease can easily be added); contains institution's information; diagnostic lab testing information flexibility (multiple sample submissions from any institution; tracking of multiple samples from same animal; track multiple tests performed on same sample); animal history and status can be entered; data entry can be made through standardized web forms; and reports will be available through the web to program participants. Future development will include web access and reports; automated comparative reports, and advanced algorithms for establishing patterns of outbreaks and trends.

In summary, extensibility to other infectious diseases is the crux of the database and analysis design. The database is a rich data source on zoo species as sentinels of WNV (more than 10,000 tests have been analyzed to date). The method of data analysis allowed identification of associations between predictors (state, sex, species, time, etc.) and positive outcomes. Visual mining of strong associations will allow easy detailed analyses by conventional methods.

Dr. Bob McLean, Wildlife Disease Manager at USDA-APHIS-Wildlife Services (WS) National Wildlife Research Center (NWRC), presented "West Nile Virus in North America: Overview and Update." Exotic WNV was introduced into New York City in 1999. In the Old World, WNV cycled through mosquito vectors and avian reservoirs. In the United States (U.S.), WNV became more virulent and caused mortality in crows and humans. American crows experimentally infected with WNV demonstrated a much higher viremia than those infected with St. Louis Encephalitis virus. Guidelines for 2000 WNV surveillance were published in Centers for Disease Control and Prevention (CDC) Morbidity and Mortality Weekly Report with revisions in 2001-2004. These included enhanced passive surveillance for dead corvids and active surveillance with sentinel chickens and wild birds; mosquito surveillance and enhanced passive veterinary and human surveillance. Information was fed into the state public health database then updated by CDC's database as part of "ArboNet". Maps were updated weekly. However the missing part of surveillance system was the lack of live bird testing. Enhanced passive surveillance of WNV resulted from reporting, sampling and testing of sick or dead equines with compatible

signs, or clinical human cases or deaths with compatible signs. Mosquito surveillance was performed by monitoring populations and WNV infection rates. Bird surveillance consisted of samples from captive sentinel and zoo birds as well as free-ranging birds (live and dead bird testing). The American crow became the public health sentinel for WNV; it is highly susceptible to WNV infection and virus titers in tissues were high enough to allow delayed testing. Submission of dead birds by public and local agencies for testing allowed detection and tracking of WNV; however, testing of dead birds required a Biosafety Level-3 lab for testing. A test developed for mosquitoes (rapid swab test) was found to be useful in birds; due to high viral loads in tissue, an oral swab could detect virus in corvids. Using dead bird surveillance, this method provided earlier detection by weeks before sentinel birds, horses or human events. Within 6 years of introduction, WNV had reached the west coast of the U.S. Currently, WNV is endemic within the U.S. In 2004, there were more human cases west of the Rockies than in the east U.S. (ex. 583 cases in Calif.) The WNV events are probably weather driven. There were 951 total equine cases in 2004. Canada had its first WNV case in 2001. Mexico saw increased WNV activity in 2004; WNV first appeared in Yucatan in 2001. WNV appears to be disseminating south through Central America and north along the western coast of Canada. There is also a concern about mosquitoes going to Hawaii.

WNV equine vaccine was introduced in 2001. Since 2001, there has been a decrease in the number of equine WNV cases and deaths. The number of avian species that have died or been infected with WNV is now 278. It is estimated that several million birds have died in the U.S. from WNV. In 2003, 73,861 dead birds were reported; 22,455 were tested (30%). Of the tested birds, 52% were WNV positive (11,597 positive birds); corvids accounted for 84% of the affected birds. American crows and blue jays are the most commonly affected avian species. Trend data are too insensitive to detect regional population effects or effects are compensated for by immigration of unaffected birds from surrounding localities because of patchy distribution of WNV. The extent of mortality in regional and national crow populations and other species and the overall significance and impact to bird species are unknown but recent evidence suggest some significant local impact and possible long-term effects.

NWRC studies are underway to look at methods to improve surveillance. One study is sampling of cliff swallow nestlings for WNV infection as an early warning surveillance for predicting human risk. An oral swab is taken from nestlings in June. This provided early warning of risk in 2003 and helped target mosquito control. Another study examined the role of small mammals in WNV transmission. Samples were collected from 20 species (600 mammals). WNV antibody was detected in 8 species of wild mammals from several regions (highest

prevalence occurred in eastern grey squirrels, fox squirrels, and Virginia opossums). Experimental infection of sage grouse with WNV resulted in 100% mortality within 3-4 days of infection. This has led to research on a vaccination to protect the captive breeding program.

Dr. W. Ray Waters, Veterinary Medical Officer and scientist in the Bovine Tuberculosis (TB) Research Group, USDA Agriculture Research Service (ARS), National Animal Disease Center (NADC), presented an update on elephant tuberculosis serology. *Mycobacterium tuberculosis* (*M. tb*) has been isolated from 30 captive elephant within 14 herds in the U.S. (1994-2004); all Asian elephants. *Mycobacterium bovis* (*M. bovis*) has been isolated from 1 African elephant. Multiple drug resistance has been reported. The only approved diagnostic test is culture of trunk wash samples. There are several challenges with elephant TB diagnosis. Culture of trunk wash has relatively poor sensitivity. Skin test is not validated in elephants and there is no confidence in these results. A gamma interferon (gamma IFN) test is currently in development. Serologic tests are appealing because: samples can be stored for future analysis; archived samples can be analyzed; various assay platforms can be directly compared; and these assays are amenable to serial analysis (i.e. to monitor therapy). There is a multiple antigen Enzyme-Linked Immunosorbant Assay (ELISA) currently in use for experimental testing in elephants.

Dr. Waters reported on a study that used archived samples from elephants with known clinical status and trunk wash culture data to compare three assays: immunoblot, multiple antigen print immunoassay (MAPIA), and rapid test.

Immunoblot assay used whole cell *M. tb* sonicate as the antigen. This preparation lacks secreted antigens (i.e. ESAT6). Using serum from one elephant that was infected with *M. tb*, positive bands were detected from serum collected in 1996. This elephant did not have a positive trunk wash culture until 2000. Antibiotic treatment was started in 2000, and a decrease in the number and intensity of bands was observed in the immunoblot. Several other *M. tb* infected elephants showed similar patterns using immunoblot.

MAPIA – In this assay, a machine prints specific antigens horizontally on a nitrocellulose membrane which can be cut into strips and used in Western blot. Strips are incubated with serum samples, then incubated with anti-Ig conjugate and color developer. Using MAPIA, similar to the immunoblots, an antibody response to multiple bands was observed in serum from the *M tb* infected elephant. After treatment, antibody response waned to certain antigens. No antibody response was detected to any antigens in non-infected elephant sera. Using a densitometry, the antibody response to ESAT6 remained relatively high, but other antibody responses (to 16kD and Mtb48) decreased with therapy. Therefore, an increase in antibody response to

any of these antigens post-therapy may indicate reactivation of infection.

Rapid test – This test uses lateral flow technology and can be used in the field with whole blood or serum. If a band is present in the test strip, it indicates a positive reaction. Rapid test detected antibody 4 years prior to positive culture in the first elephant tested. Results are similar to those seen with MAPIA. A decreased antibody response to nMPB83 and Mtb48 antigens was observed with antibiotic therapy but antibody to ESAT6 remained high. MAPIA using serum from an *M. bovis* infected elephant showed lighter bands.

MAPIA can be used on other species. Serum was obtained from a gazelle that became infected with the same *M. tb* strain as the elephant. Using MAPIA, the serum showed a similar banding pattern to the infected elephant serum. MAPIA can also be used to indicate which antigens will show the strongest reaction in the rapid test.

A panel of sera from healthy and TB infected elephants showed good correlation between the MAPIA and the rapid test. However, one Asian elephant with chronic nail infection, joint disease, and osteomyelitis was positive on the rapid test but negative on MAPIA.

In summary, elephants produce a robust antibody response to TB infection. Of the antigens tested, ESAT6 and CFP10 are the most immunodominant. The rapid test format appears promising as a screening test in elephants. MAPIA will be useful as a primary or confirmatory test. MAPIA may be used to measure waning response upon therapy and relapses post-therapy.

Recommendations:

Recent advances need to be presented to the National TB Working Group for Zoo and Wildlife Species. Discussion should center on whether further studies are needed for validation. Additional evaluation of archived samples could be used but funds will be needed for testing. Serologic testing could be used in combination with trunk washes for a period of time but may have the potential to replace trunk wash culture as the screening test with antibody positive animals being tested more rigorously by culture. These tests can also be used to monitor therapy and as an indicator of relapses. Additional investigation of its use with other zoo species (i.e. rhinos, hoofstock) should also be done.

Dr. Candace McCombs, Sequella Inc., presented “Developing a New TB Test for Non-human Primates.” The development of the new test uses lateral flow technology ELISA and has been a collaborative effort between Sequella Inc., Chembio Inc, Univ. of South Alabama and Tulane National Primate Center. TB is the most important bacterial disease of captive primates, although it is rare in wild primates. It can be caused by either *M. tb* or *M. bovis*. A single bacterium has been shown to cause infection in rhesus macaques. TB is usually spread

by airborne contagion and can spread rapidly through a colony. Non-human primates (NHP) with TB will cough and produce infective aerosol. Current TB testing in NHP uses the tuberculin skin test. This test requires anesthesia and intradermal injection into the eyelid. It is prone to false positives and false negatives. Sensitivity is considered very poor; therefore, serial testing is usually performed (ex. 3 serial negative tests required in quarantine). Primagam is a blood-based assay that has received provisional USDA approval. On day 1, whole blood is incubated with antigens in vitro; on day 2, gamma IFN is quantitated by ELISA. The assay is more technically and logistically difficult; it requires that fresh blood must be mixed with antigens within 12 hrs; and the assay requires special laboratory equipment. Therefore, it is apparent that there is an urgent need for the development of TB diagnostics in NHP.

Rapid test for NHP for TB diagnosis – This test is stable for 1 year at room temperature. It is technically easy to perform and interpret. A sample is added to the well – if one line appears, it is negative; if two lines appear, the test is positive. The assay uses one-step, lateral flow technology. A unique cocktail of TB-specific antigens are mixed with one drop of blood, serum, or plasma. Results can be obtained within 20 minutes. This test can be used in the field while an animal is still in a cage.

Diagnostic sensitivity of the rapid test was evaluated in 6 studies and the rapid test detected a total of 46/51 infected monkeys (overall 90.2% sensitivity). Most of these monkeys had been experimentally infected while the remainder were naturally infected. The majority of animals became positive by 6-8 weeks post-infection. Animals had small granulomas and probably were not yet infectious at this point in time. In an evaluation of 7 studies, 154/157 negative monkeys were correctly classified by the rapid test in 4 different primate species (overall specificity of 98.1%).

Current studies are being performed with potentially cross-reactive mycobacteria. Rhesus monkeys are being infected with strains of *M. avium, M. kansasii*; and squirrel monkeys are being infected *with M. kansasii, M. gordonae, and M. scrofulaceum*. The studies will use the rapid test to follow serologic responses to determine if cross-reactivity occurs in skin test, Primagam, and rapid test.

A request for collaboration was made; samples are needed from TB infected primates and control samples from healthy animals. Test kits can be provided for on-site testing. Contact Konstantin Lyashchenko (*kl@chembio.com*) for more information.

In summary, the rapid test for TB in NHP is a novel quick point of care diagnostic with high specificity and sensitivity. More positive and negative samples are needed for USDA approval.

Dr. Mitch Palmer, Veterinary Medical Officer and lead scientist in

the Bovine TB Research Group, USDA-ARS-NADC, presented "Experimental Infection of Reindeer (*Tangifer tarandus*) with *Mycobacterium bovis*: Pathologic and Immunologic Findings." TB in reindeer is extremely rare. There is one report of a case in the London Zoo in 1930. TB has never been diagnosed in reindeer in the U.S. or Canada. However, mycobacterial testing in reindeer falls under USDA regulations for cervids. False positive results are common and may result in quarantine and slaughter of reindeer. To date, all necropsied reactor animals have been negative for TB. Studies performed in Alaska using comparative cervical testing (CCT) on sensitized reindeer showed 62-100% sensitivity and 80% specificity. Changes in CCT classification during the study was common. The objectives in this study were to evaluate tuberculin skin testing and in vitro blood based assay (Cervigam) using experimentally infected reindeer; evaluate use of recombinant proteins (ESAT6, CFP10, and ESAT6:CFP10), with the Cervigam assay; and describe tuberculous lesions in experimentally infected reindeer. Dr. Palmer's complete paper is included in the Scientific Papers section of these proceedings.

Treatment groups consisted of 12 uninfected control reindeer and 13 *M. bovis*-infected reindeer (10^5 cfu administered intratonsillarly). Blood was collected for serology and Cervigam, and a CCT was performed at 3 and 8 months.

Scattergrams for cervids and bison/cattle were used to compare experimental groups. At 3 months, all infected reindeer had CCT responses in the infected zone using either scattergram. Controls were negative except for 1 suspect, using the cattle/bison scattergram. Using the cervid scattergram, only 2 controls were considered negative, 1 positive, and all the rest were suspect. If these same control reindeer were placed on the proposed reindeer scattergram, it would only require retesting of one control animal (suspect).

At 8 months, only 4 control animals remained. All infected animals were far into the reactor zone. Using the cervid scattergram, there were 2 negative controls and 2 positive reactors. Using the bison/cattle scattergram, 2 negative and 2 suspect controls. If the proposed reindeer scattergram is used, controls would be classified as 2 negative, 1 suspect, and 1 positive. Therefore, use of either the cattle/bison or reindeer scattergram provide increased specificity over traditional cervid scattergram.

The Cervigam assay was modified to incubate whole blood with purified protein derivative (PPD) or specific antigens or fusion protein. Infected animals showed increased gamma interferon (IFN) production over controls to PPD bovis, except in a few cases (during summer months due to increased reactivity to PPD by controls). If fusion protein was used for stimulation, increased gamma IFN production in infected animals was observed with very little background response by

controls.

The most common site of infection in reindeer was the medial retropharyngeal lymph node. White-tailed deer often have more severe and extensive lesions/infection than reindeer. The type of lesion is similar to that observed in other cervids. Pulmonary lesions were not very severe.

In conclusion, all CCT scattergrams identified infected reindeer. The CCT scattergram modified for reindeer provides increased specificity as compared to the standard form 6-22D. The Cervigam assay should prove useful and requires only a single handling event. Use of recombinant ESAT6:CFP10 may decrease the number of false positives detected with the Cervigam assay. Lesions can range from caseonecrotic to abscess-like. Lesion distribution may be less widespread than that seen in white-tailed deer.

Dr. Pam Dennis, Ohio State University and co-chair of the American Association of Zoo Veterinarians (AAZV) Infectious Disease Committee, reviewed "Disease Surveillance in American Zoo Association (AZA)-Accredited Zoos." Disease surveillance in zoos occurs on a national, institutional and species/taxon level. Examples of specific disease monitoring programs that occur on a national level in zoos are WNV, TB, and Chronic Wasting disease (CWD). The WNV surveillance data from zoos has been incorporated into the national public health database through the combined effort of multiple groups including Centers for Disease Control, USDA, United States Geological Survey, state and local public health and wildlife agencies, Cornell University, AZA, and AAZV. There are a number of reasons that zoo populations are useful as potential disease sentinels: collections may contain a variety of susceptible species; it is usually a stationary population; often animals can be serially sampled; zoos are located in both rural and urban locations spread over the U.S.; and often zoos are in close proximity to human populations. In the case of WNV surveillance data, in 2001, there were 1,500 zoo animals tested in 64 zoos (30 states + Washington D.C.). In 2002, there were 6,629 animals tested in 157 institutions, representing 1,163 different species. In 2003, over 10,000 animals were tested in over 185 institutions.

The National TB Working Group for Zoos is another example of how the zoo community provides disease surveillance. This advisory group is working to help establish intradermal skin testing standards for different non-program species and provide recommendations for secondary testing. It is also provides guidance for the zoo and regulatory communities for assessing and managing risk groups. Currently, the group is prospectively collecting data from zoos that are testing different species using a centralized reporting mechanism to establish prevalence, incidence, and provide information about appropriate testing and interpretation of results in different non-program species. A

subgroup has also served as an advisory group for control of TB in elephants. They have helped standardize testing, management and treatment guidelines for these species.

The zoological community has also proactively developed guidelines to mirror programs being developed for CWD. These guidelines recommend CWD testing via a certified laboratory of all cervid species that die. These data may eventually help identify which cervid species are susceptible to this disease.

Zoos continuously provide disease surveillance at an institutional level through their preventive medicine programs, review of medical records, monitoring wildlife, and retrospective evaluation of tissue/serum banks. During quarantine, incoming animals are screened for disease and baseline data is collected. Routine exams provide follow-up diagnostic screening for disease surveillance. Necropsy of collection animals provides information on cause of death but also may provide additional information about diseases and tissue/serum for future testing. Monitoring of local wildlife aids in detection of diseases of concern occurring in the area and potential sources of pathogens.

There are also programs based on surveillance on a species or taxonomic group level. These are usually administered on a national level by veterinary advisors or species advisory groups. These advisory groups provide recommendations for health monitoring, pre-shipment, quarantine, necropsy and research protocols. Disease surveillance is usually an important part of in situ conservation programs for many of these same species.

As the zoological community continues to increase its ability to provide disease surveillance, it will also need to strength collaborative efforts with regulatory, public health, wildlife, and other groups to share information and expertise.

Peter Butchko, director of Wildlife Services in Michigan, USDA-APHIS-WS, presented “Eradication of a Bovine TB-Positive Captive Cervid Herd in Northeast Michigan.” Bovine TB was detected in a deer found on a 1,500 acre commercial hunting facility in northeast lower Michigan in December 1997. Gross lesions were present in the lung. At the time, there were an estimated 600 animals present on the ranch, mostly white-tailed deer, with a few sika deer and elk also present. Deer had been enclosed by fencing the area and then bought from the state when the ranch started. Challenges of eradication in this population were the goal of 100% depopulation; dealing with a heavily forested habitat; and verification of complete depopulation. The initial strategy used selective sharp shooting at night from vehicles and baited blinds. Occasionally, hunters would pass by the herd if they thought that they couldn’t shoot all the deer to avoid educating deer to the shooting. Additional removals occurred by client hunts and ranch personnel, which had been allowed by agreement with the state. Fencing

was constructed to restrict deer to open areas of the ranch. Shooting started February 1998 with an expected date of completion in the winter 2001. Most shooting occurred in winter months when leaves were off the trees. Results from the initial phase – 286 deer removed by January 1999.

Second phase – a helicopter was brought in to shoot the remaining deer. Additional fencing was erected to exclude deer from heavy cover. Four deer were captured and radio-collared to help locate additional deer. Radio-collared deer were removed once all other deer were removed (March 1999). These deer were effective in leading hunters to other deer but eventually learned helicopter avoidance.

The verification phase started in March 1999. Ground personnel and the helicopter conducted systematic sweeps of fenced units for deer sign; no deer sign was found. Fresh snowfall aided in verification; no tracks were seen. In May 1999, deer dogs from South Carolina were deployed to confirm depopulation; again, no deer sign was found. In February 2000, a systematic ground search for deer sign revealed no deer. The 12 month quarantine period was completed.

Accomplishments – 325 deer were removed, eliminating a potential source of infection. This provided for a significantly earlier resumption of commercial activity by the ranch. A successful partnership was formed with the helicopter company, Michigan Department of Natural Resources, Michigan Department of Agriculture, and USDA-APHIS-VS.

In conclusion, a successful depopulation plan resulted from the use of selective sharp shooting, strategic use of fencing, and aerial gunning using "Judas" deer. Depopulation can be expedited by using a helicopter in the operation.

Dr. Arnold Gertonson, Yellowstone Brucellosis Coordinator, USDA-APHIS-VS, provided a "Greater Yellowstone Brucellosis Update." In 2003-2004, the bison hazing operations in Montana (cooperative effort between state and federal agencies) consisted of 59 operations in the western boundary area (1,516 bison) and 1 operation in the northern boundary area (14 bison). Bison captures in the western boundary area during this same time period occurred in 4 operations; 8 seronegative bison were released and 12 seropositive animals were sent to slaughter. Of the 464 bison captured in the northern boundary area during 2003-04, 264 seropositive bison were sent to slaughter, 198 were released, and 111 seronegative nonpregnant yearlings were vaccinated and released. There were 2 lethal operations that involved 2 bulls.

The brucellosis vaccination program is expected to be expanded in the future. The Montana Environmental Protection Act process should be completed in early 2005. Vaccination was started in Yellowstone National Park (YNP) in 2003. The vaccine will be delivered remotely

and is currently under research and development. There is consideration of incorporation into a biobullet or possible oral delivery.

There is a three-phase quarantine feasibility study. This will occur at 3 different sites. The intent is to determine if latent infections are present in bison. Animals will be held at the first site until they are yearlings. They would move to a second site to be bred and continued to be tested. The third site has not yet been identified. Seronegative bison would eventually be released to Native American tribes and onto other public lands. This will be a soft release process and testing will continue for a period after release.

Fluorescent polarization assay (FPA) is in the final rule form. Publication in the Federal Register is expected soon. The FPA test for brucellosis has been validated for use in cattle, bison and swine. Work is being done to get this test validated for cervids.

Greater Yellowstone Interagency Bison Committee MOU draft has been sent forward by the executive committee. Signers are the Secretaries of USDA and Department of Interior, and the Governors of Wyoming, Montana, and Idaho. Idaho Governor Kempthorne requested assistance from USDA-APHIS in August 2004. There is concern about brucellosis transmission from wildlife to livestock in the Greater Yellowstone Area (GYA). APHIS will work with the States and other federal agencies in addressing the elimination of brucellosis from the GYA. Grand Teton NP and National Elk Refuge are developing elk and bison management plans; the draft for public comment is expected out next year.

Wyoming has lost class free status due to detection of Brucellosis in livestock herds. The first livestock herd was detected in December 2003. The second livestock herd was a trace-out from the first herd. Wyoming lost its class free status in February 2004. A third herd was detected in May 2004. The fourth herd was detected in June 2004. A Wyoming Brucellosis Coordination Team was appointed by the governor of Wyoming. They have been meeting since early last spring. Completion of their report is expected in December 2004.

Currently YNP estimates their bison population to be approximately 4,000 head. There are 700-800 in Grand Teton National Park. Weather will play a huge role in future developments. 1996-97 was the last severe winter.

Dr. Dean Goeldner, Coordinator of the CWD Program, USDA-APHIS-VS, gave a presentation on “Chronic Wasting Disease – APHIS-VS Program Update.” CWD was first recognized as a clinical syndrome in mule deer in a research facility in Colorado in 1967. Currently eight states have CWD in wild cervids and eight states have the disease in farmed cervids. There have been 34 positive herds; most have been depopulated. There are currently 5 known CWD postive captive cervid herds; 3 elk herds in CO and 2 white tailed deer herds in WI.

Transmission is most likely horizontal. Environmental contamination may play an important role. Vertical transmission does not appear to be important. Minimum incubation period is 15 months (mule deer) and 12 months (elk) in experimental infections. Maximum incubation period is unknown; 25 months (mule deer) to 34 months (elk) in high dose oral inoculation. Time from infection to shedding is unknown. There is evidence of transmission in both directions across fence-lines (between captive and free-ranging cervids). Movement of infected animals is the primary means for spread of disease in the captive cervid industry.

Challenges associated with a CWD program are that the disease occurs in multiple species, both free-ranging and captive; there are multiple regulatory authorities and fragmented jurisdictional frameworks; farmed cervids are a relatively new livestock industry; there are critical gaps in disease knowledge; limited diagnostic tools; and the impacts of public/media perceptions.

USDA-APHIS goals for CWD are to eradicate CWD from captive cervids and assist states and Tribes in addressing CWD in free-ranging cervids. In FY 2003, $14.8 million was added to APHIS budget for CWD; this was the first time CWD was a line item for funding. In FY 2004, $18.5 million was budgeted (including $2.25 million earmarked).

The FY 2004 Farmed Program will pay for surveillance testing for all farmed cervids. It will also cover indemnity, depopulation, disposal and testing for positive/exposed herds and trace animals. Approximately $1.2 million in cooperative agreement assistance was provided to 17 state farmed cervid programs. Each year the number of captive cervids tested has increased; in FY 04, 15,172 captive elk/deer were tested.

Immunohistochemistry (IHC) continues to be considered the gold standard diagnostic test for CWD. Four ELISA –based test kits are currently licensed for wild cervids; they are licensed for specific species and tissues. For captive cervids, IHC will continue to be used since there is sufficient capacity to meet need; test results can be highly contentious and may result in regulatory action; APHIS may consider use of alternative tests in the future. Test kits are used for free-ranging species because it allows for faster testing of large numbers of samples. Confirmatory IHC testing is still required for positives. Tonsillar biopsy is used by some states. There are 26 labs in the contract group. Online sample submission applications are available for samples from farmed cervids, which will direct samples to labs based on capacity; wildlife agencies can use any contract lab.

VS memo 574.2 was signed Aug 17, 2004; this sets procedures for defining areas where CWD is established in wildlife. It also makes purchase and depopulation an option for captive/farmed herds in these areas based on available funding.

The proposed APHIS CWD herd certification program was published in the Federal Register Dec 24, 2003. The goal is to eliminate CWD from captive cervids in the United States. This is a voluntary

program for captive/farmed elk and deer. There are fencing requirements, animal ID and herd inventory, and surveillance of deaths over 16 months. Herd status will be based on years of surveillance. Interstate movement of cervids will be allowed only if participating in a herd certification program. Surveillance requirements will be "ramped up" to 5 years. Qualifying State programs will be "grandfathered". After 5 years of compliance with no evidence of disease, herds will be designated "low risk" for CWD. Slaughter and shooter animal surveillance would no longer be required. This facilitates interstate movement and trade.

Some changes are being developed based on 105 substantive, multi-faceted comments to the proposed CWD rule. Since this is a significant rule it requires additional clearances. BSE has also slowed the process. Therefore, the final rule is still in process.

The CWD Uniform Methods and Rules (UM&R) internal review has been completed. It will require adjustments to comply with changes in the final rule. The current plan is to post the draft UM&R on the website after the final rule clears the Office of General Council.

A new web-based, user-friendly database has been developed for CWD. This will soon to be piloted at the state level. They are working with U.S. Geological Survey to determine how summary data will be shared with NBII CWD Data Clearinghouse.

APHIS is working with states and federal agencies and tribes to implement CWD plan (June 2002). They helped pay for some of the testing done during the hunting seasons. (2002/2003 and 2003/2004).

Research support to USDA-APHIS-WS National Wildlife Research Center continues in areas of research on transmission, barriers, census techniques, vaccine, scavengers and predators, and decontamination. APHIS has been in discussions with the Environmental Protection Agency on a variety of CWD issues including – defining prions as pests; CWD waste from diagnostic labs; Federal Insecticide, Fungicide, Rodenticide Act sec. 18 exemptions for using bleach, sodium hydroxide and Environ LpH for prion disinfection; landfilling of CWD carcasses.

Dr. Beth Williams, University of Wyoming, presented "Update on Chronic Wasting Disease Research." Research is on-going in many areas due to increased interest and funding. These include surveillance/geographic distribution of disease; improved diagnostic methods/strain typing – similarities to scrapie, molecular techniques to examine banding patterns from CWD tissue; host range studies – intracerebral inoculation; transgenic mouse models – developed to express prion proteins to understand natural history but also bioassays; pathogenesis/genetics – to determine if there are genetically resistant strains similar to those that occur with scrapie; epidemiology (local and landscape) – to understand transmission; spatial modeling – what factors influence occurrence of CWD; behavior – males tend to have higher incidence of infection/clinical effects; control methods – fencing, de-

creasing population in hotspots, barriers; and vaccines/therapeutics. Diagnostic IHC comparing samples from different species indicate that multiple samples may be required. In elk, CWD was detected in 69% of cases when both lymph node and brain were examined; 19% LN only; and 12% brain only. In mule deer, 84% of cases were detected if both lymph node and brain were tested, and 16% were detected if only lymph node was tested. No cases were detected when only brain was tested. Therefore, there is a need to test both LN and brain to pick up positive tissues in elk. CWD susceptibility of cattle was examined by exposing them to CWD infected tissue orally; 7 years post-oral inoculation, none of the cattle showed evidence of CWD. Cattle were also exposed to infected deer on range; 7 years post-exposure contact, there was no evidence of CWD in these cattle.

Preliminary genetic analysis have shown 1 polymorphism in mule deer, 3 polymorphisms in white-tailed deer, and 1 polymorphism in elk that may play a role in CWD. No genotype has been identified that is completely CWD resistant.

Mule deer pathogenesis studies were performed using oral CWD inoculation. By 3 months Post Innoculation, homozygous animals were positive for CWD in lymph nodes. The heterozygous animals seemed to have slightly more prolonged incubation of CWD in lymph node and brain, as well as clinical signs when experimentally infected in preliminary study. Other studies examined transmission by direct or indirect contact or contact with carcasses. After one year of contact with CWD infection was detected by tonsil biopsy. When deer were in direct contact with infected animals, 2/10 contact animals showed evidence of CWD infection by tonsil biopsy. When deer were in indirect contact with infected animals, 1/9 contact animals showed evidence of CWD infection. If animals were in contact with CWD infected carcasses, 3/12 animals showed evidence of CWD infection.

A test and cull program using tonsil biopsy is being used in Estes Park; animals are darted and collared, then culled if they are biopsy positive.

Dr. Tom Meehan, Brookfield Zoo, Chicago, IL presented an update on shigatoxigenic E. coli in contact animals at AZA-accredited zoological institutions' petting zoos. Screening included samples from 36 different institutions (976 animals); 4 zoos with Salmonella (7 animals); none positive on follow-up testing; no 0157 E. coli in contact setting.

One resolution was approved by the Committee and submitted to the Committee on Nominations and Resolutions for approval by the general membership. The resolution requested federal agencies with responsibility for implementation of Homeland Security Presidential Directive-9 to include state fish and wildlife management agencies in planning activities, add representation from these state agencies to the Food and Agriculture Sector Government Coordinating Council, and to provide funding to these state agencies to assist them with homeland security activities.