REPORT TO THE IAFWA Committee on Wildlife Disease
Current and Topical Information for Managers Interested in Wildlife Diseases
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The following information is of a topical nature to provide wildlife management agencies and entities timely information on wildlife disease; many partners and collaborators are involved in gathering the information presented here.

Avian Influenza (AI)

Avian influenza subtype H5N1 has been responsible for the mortality or culling of more than 100 million domestic fowl in several Asian countries since 2003. The virus has infected 40 humans who had contact with infected poultry in two countries, Viet Nam and Thailand, resulting in 29 deaths to date. The disease has not spread between/among humans. The disease has also caused mortality in cats in zoological collections in Thailand that were fed virus contaminated poultry. Some sporadic wild bird mortality has been reported in the region including storks, crows, pigeons, and a peregrine falcon, all associated with domestic fowl mortality. So far there is no evidence that this virulent avian influenza is being spread by wild birds.

In North America avian influenza has been detected in domestic poultry during 2004 in six states, Delaware, New Jersey, Pennsylvania, Texas, Maryland, and Missouri, and British Columbia, Canada, but the subtypes involved were not the same as the H5N1 subtype causing bird mortality in Asian. There is concern that the virulent H5N1 subtype will be spread by migratory waterfowl from Asia to the United States. Although the chance for this seems remote at this time, the National Wildlife Health Center (NWHC) and other agencies are monitoring wildlife mortality for early detection of the H5N1 subtype. The NWHC has formed an Avian Influenza Team that has participated in CDC/USDA Emergency Response Team teleconferences during 2004. For more detailed information on this topic, go to the National Wildlife Health Center website at http://www.nwhc.usgs.gov, where you can find additional general avian influenza information, updates on reports of avian influenza in North America, a list of frequently asked questions, and recommendations for handling wild birds that may be infected with avian influenza. The Avian Influenza Team at the NWHC continues to monitor the situation.

Avian Vacuolar Myelinopathy (AVM)

Avian vacuolar myelinopathy (AVM) is an emerging neurologic disease of wild birds in the southeastern U.S.. The disease was first recognized in bald eagles (*Haliaeetus leucocephalus*) at DeGray Lake, Arkansas in 1994, and 2 yrs later, was confirmed in a number of American coots (*Fulica americana*) on this and another lake in Arkansas (Thomas, et al. 1998). Since then, AVM has been confirmed in coots on ten lakes in four states (Arkansas, North Carolina, South Carolina, and Georgia; Rocke, et al. 2002) and also in asymptomatic birds at one reservoir in Texas (Fischer, et al. 2002). Besides coots and eagles, the disease has also occurred in several species of waterfowl, including mallards (*Anas platyrhynchos*), ring-necked ducks (*Aythya collaris*), bufflehead ducks (*Bucephala albeola)* and Canada geese (*Branta canadensis*), a great-horned owl (*Bubo virginianus*) and a killdeer (*Charadrius vociferous*) (Fischer, et al. 2002, Augspurger et al. 2003).

Coots affected with AVM exhibit profound motor dysfunction and incoordination (Thomas et al. 1998, Larsen et al. 2002); they are reluctant to fly, ataxic on land and may swim in circles or on their backs. Histologically, the disease is characterized by diffuse, spongy degeneration throughout the white matter of

the central nervous system (CNS) of affected birds. Despite extensive diagnostic and field investigations, the causative agent of AVM is still unknown. Recently, the disease was experimentally reproduced in red-tailed hawks (*Buteo jamaicensis*) upon ingestion of tissues from AVM-affected coots, providing evidence that eagles contract the disease by consuming affected coots or ducks (Fischer, et al. 2003). A subsequent experiment in which chickens (*Gallus* spp.) were fed different tissues from affected coots demonstrated that the causative agent was present in the gastrointestinal (GI) contents, but not in the brain, fat, kidney, liver or muscle of the chickens (Lewis-Weis, et al., submitted to JWD). During recent work, we demonstrated that ingestion of several samples of *Hydrilla* (but not all) from lakes with ongoing outbreaks of AVM resulted in brain lesions (in mallards) indicative of AVM. These results support the hypothesis that the causative agent of AVM is ingested by waterbirds while consuming aquatic vegetation at affected sites. At two sites with AVM, *Hydrilla* is the dominant aquatic vegetation, however, it is not present in all AVM-affected lakes.

Although we don't have definitive data, we suspect the disease is associated with other aquatic vegetation that is dominant in other affected lakes. Based on results of our previous work with sentinel mallards and coots at WL that demonstrated that the exposure to AVM is site-specific and seasonal (Rocke et al, 2002), we hypothesize the agent is either seasonally accumulated by aquatic vegetation, such as *Hydrilla*, or seasonally produced by one or more organisms associated with aquatic vegetation at affected sites. Also, upon ingestion of some *Hydrilla* samples collected during an AVM outbreak, several coots in our studies became sick and died with neurologic signs similar to those seen in wild birds, but lacking the characteristic brain lesions of AVM. We are currently in the process of conducting a site characterization of lakes with AVM in comparison with paired control lakes and additional animal trials to determine the etiologic agent.

Plague (*Yersinia pestis; Sylvatic Plague*)

Prairie Dogs

Prairie dogs (*Cynomys* sp.) and their most dependent predator, the endangered black-footed ferret (*Mustela nigripes*), are highly susceptible to sylvatic plague (*Yersinia pestis*) and have experienced significant declines in the last century, in part due to this disease. Prairie dogs are also significant reservoirs of plague for humans in the western United States. We have been conducting studies to determine if protective immunity against plague could be induced in black-tailed prairie dogs (*C. ludovicianus*) by voluntary consumption of a novel plague vaccine and in black-footed ferrets by inoculation.

A recombinant raccoon poxvirus that expresses the F1 antigen of *Y. pestis* (designated RCN-F1) was incorporated into a palatable gelatin-based carrier bait and offered to 18 fasted prairie dogs for voluntary consumption; 18 negative control animals received placebo baits. Baits were given to prairie dogs at weeks 1 and 4, and at week 7, all animals were challenged with virulent *Y. pestis*. Survival rates differed significantly between the two groups ($P<0.01$); 10 of 18 (55.6%) vaccinates survived compared to two of 17 (11.8%) negative controls. Serum IgG antibody titers against *Y. pestis* F1 antigen increased significantly between baseline and post-prime samples ($P<0.01$) and between post-prime and post-boost samples ($P<0.02$) in the vaccinated animals. The results of this study suggest that a protective immune response to *Y. pestis* infection can be elicited through voluntary consumption of palatable baits laden with the RCN-F1 vaccine. This strategy may prove useful in controlling plague epizootics in free-ranging prairie dog colonies.

Plague
Black-footed Ferrets

Black-footed ferrets (*Mustela nigripes)* depend primarily on prairie dogs for both food and shelter and thus may be exposed to the bacteria either by consumption of plague-infected prey or by flea-bite. Once thought to be extinct, a captive breeding and recovery program was established for black-footed ferrets in 1987 after an outbreak of canine distemper nearly decimated the last known wild colony that was discovered 6 years earlier. The occurrence of plague in prairie dog populations and its potentially devastating effect on black-footed ferret re-establishment is a major impediment to the captive breeding and recovery program of this federally listed endangered species. We conducted further experiments to assess the feasibility of vaccinating black-footed ferrets (BFF) against plague using a recombinant fusion protein consisting of F1 and V antigens from *Y. pestis.* On days 0 and 28, post-reproductive BFFs were immunized with the fusion protein by subcutaneous (s.c.) injection. Control animals received a placebo by the same route. Two weeks after the second immunization, mean antibody titers to *Y. pestis* F1 antigen were measured and found to be significantly higher in vaccinates than their pre-immunization value ($P<0.001$) and significantly higher than the control value ($P < 0.001$). Six months post-immunization, 16 vaccinates and 8 controls were challenged with approximately 8,000 colony forming units (cfu) of virulent *Y. pestis* by s.c. injection. Eleven of 16 vaccinates survived challenge with no ill effects; their survival rate was significantly different (P=0.02) from the eight control animals, all of which died within 3-6 days. Two months later, the 11 surviving vaccinates were challenged again by ingestion of a plague-infected mouse. None of the animals showed any ill effects and all survived. In contrast, seven control animals fed infected mice died of plague within 2-4 days, including one animal that did not actually ingest the mouse, but likely sniffed or licked it. This study demonstrates that immunization of black-footed ferrets with the recombinant F1-V fusion protein can induce significant antibody responses and reduce their susceptibility to plague infection. Until other methods of plague control are developed, the F1-V vaccine might be useful in protecting black-footed ferrets in captive-breeding facilities and animals intended for reintroduction programs. Based on these results, this year we are immunizing groups of captive-reared black-footed ferrets with F1-V at the National Black-footed Ferret Conservation Center. Vaccinated animals and an equal number of unvaccinated animals will be released in several states (Colorado, Arizona, and Montana) this fall to determine if vaccination improves survival.

West Nile Virus
Humans

As of September 22, 2004, the current tallies for WNV from CDC (to this point in 2004):

# of states reporting WNV =	47 (all continental states except WA)
# of human cases reported =	1,604
# of states reporting human cases =	39
# deaths =	48 (2%)

At this time in 2003, 45 states (as opposed to 47) had reported West Nile virus. Of the 1,541 cases in humans so far this year, 34% are neuroinvasive (versus 29% at this time in 2003) with median age of 57 years old. The median age for 45 of the 38 deaths reported is 75 years old. In addition, blood banks across the country have reported to CDC 143 presumptive viremic donars, with 3 who have gone on to develop neuroinvasive disease and 32 who developed WN fever. There is one suspected transfusion-related case in a human in Arizona.

West Nile Virus
Wildlife

With regard to surveillance since 1999, only AK and HI have not yet detected WNV activity in their states. WA state was positive in 2002, but not since – lots of testing has been going on in many states, academic institutions and laboratories. So far this year, hotspots in 2004 primarily are in AZ (Phoenix) & Southern CA.

To date, with regard to wildlife, WNV has now been detected in over 275 avian species, 22 mammalian and 1 reptilian species. Collaborative efforts with state/federal/public health/academic/wildlife agency interdisciplinary activities are ongoing. For surveillance, there is Department of Defense WNV surveillance in 20 states (CA, DC, GA, ID, IL, KS, KY, MD, ME, MI, NJ, NY, OH, OK, PA, SD, TX, VA, WA, WI), extensive state surveillance in WA, NJ, NC (Cabarrus & Mecklenberg Co.), and TX. Laboratory research involves inoculation trials to determine susceptibility of waterfowl and chukar partridge to WNV. Field research is investigating WNV impacts on wild bird populations, including the American white pelican populations in MT & SD, where WNV was reported in 2002 and 2003, with significant 2004 mortalities due to WNV in juvenile pelicans. Recently, a field serosurvey in kestrel populations in CO is being conducted in 2004 and 2005 by two USGS facilities (USGS NWHC and FORT). There is a sagebrush ecosystem/greater sage-grouse study ongoing (USGS NWHC and FRESC in OR) to determine the prevalence of WNV in greater sage-grouse and other birds and small mammals in sage habitat in NV, CA and OR, as well as looking at the prevalence of WNV or antibodies in hunter-killed birds in NV; this information will be correlated with on-the-ground geospatial ecosystem features to try to predict where the next outbreaks may occur. Additionally, there are ongoing studies examining the impacts of WNV on ruffed grouse and woodcock populations in north-central MN, and in 5 species of waterfowl (ducks) collected in TN, SC, ME and ND.

White Pelican Investigation

Early this summer, in the Intermountain West area there were concerns expressed in newspaper articles regarding the health of white pelicans in the area. In response to these concerns, looking at one possibility, USGS's National Wildlife Health (NWHC) and Northern Prairie Research Centers (NPRC) are working cooperatively with some National Wildlife Refuges and State Waterfowl Management Areas on a two year study titled: *Impact of West Nile virus on white pelican colonies in northern Montana, North Dakota and South Dakota.*

The first field season of this two-year study is completed. White pelican colonies in Montana and South Dakota were successfully banded, monitored and dead birds were collected for examination and West Nile virus testing. At the NWHC, we received a total of 115 pelicans; necropsies and West Nile virus testing was accomplished on all these birds. West Nile virus has been isolated and confirmed by RT-PCR in a significant number of the juvenile pelicans. Microscopic examination of tissues and tests for Botulism type C and Salmonella are near completion. A third study colony in North Dakota was not used in this year's investigation, due to an unexplained mysterious departure of nearly 30,000 adult breeding pelicans prior to onset of the study and prior to breeding season.

Chronic Wasting Disease

RISK FACTORS WORKSHOP

Risk analysis tools have been successfully used to determine the potential hazard associated with disease introductions and have facilitated management decisions designed to limit the potential for disease introduction. Chronic Wasting Disease (CWD) poses significant challenges for resource managers due to an incomplete understanding of disease etiology and epidemiology and the complexity of management and political jurisdictions. Tools designed specifically to assess the risk of CWD introduction would be of great value to policy makers in areas where CWD has not yet been detected.

To this end, the USGS created a steering committee representing states, native communities, federal, academic, and non-governmental entities. This committee formulated a collaborative process for the development of CWD risk assessment tools applicable to both free-ranging and captive populations. The committee recommended a workshop be held on the topic and suggested the format, content, and potential participants. Identified objectives of the workshop included:

1. Identify and discuss the needs of various government and non-government groups involved with assessing, managing, and/or preventing CWD.
2. Identify current gaps in CWD research specifically in relation to information applicable to the risk analysis process.
3. Construct a general, consensual, framework model that incorporates all factors identified as potentially associated with the presence or absence of CWD.

The resulting CWD Risk Analysis Workshop was held May 11-13, 2004 in Fort Collins, Colorado. The workshop was attended by 28 individuals representing a cross-section of management, research, and non-government organizations. Experts with experience in a variety of risk analysis approaches and representatives from public and private user groups, presented in the plenary session. The remainder of the workshop consisted of facilitated breakout sessions and all-group discussions.

A summary report of the Workshop has been produced, review by the participants, and is available upon request. It contains summaries of speaker presentations, group discussions, a list of identified risk factors, and the framework model. Further funding for this project is not available. Nevertheless, we will try to work with existing resources to create additional products that will make limited risk analysis information tools available for managers.

CWD DATA CLEARINGHOUSE PROTOTYPE

Production of a prototype for the Chronic Wasting Disease Data Clearinghouse(CWDDC) began in March, 2004 as a collaborative project of the National Biological Information Infrastructure (NBII), the NBII Wildlife Disease Information Node (WDIN), and the USGS National Wildlife Heath Center (NWHC). Following initial development, the Nebraska Game and Parks Commission, the Tennessee Wildlife Resources Agency, and the Wisconsin Department of Natural Resources joined the partnership, contributing a subset of their existing CWD data for testing purposes. The Maryland Department of Natural Resources offered their support as a test bed for the data entry process. When the prototype became functional, partner representatives, as well as those from USDA, USGS and non-governmental organizations, were invited to participate in multiple on-line “virtual workshops”, to demonstrate the system, and offer feedback for improvements. Following the workshops, all participants were given the opportunity to trial the CWDDC at their own desks. These comments were then reviewed and changes were made to the prototype. This second version of the prototype will be demonstrated during the 2004 IAFWA meeting, and be available for testing and comments. Additional comments will be reviewed and incorporated before a working system is made available. The CWDDC will continually be modified to ensure that it meets agency needs.

RESEARCH UPDATE

CW-Positive Tissue Bank

This research projects objective is to collect and maintain significant amounts of CWD-positive tissues and make them available for valid research projects as reference materials. Twelve elk, 12 mule deer and 12 white-tailed deer have been captured and transferred to Sybille Wildlife Facility in Wyoming. All 36 will be orally-inoculated with CWD in the near future. Animals will be serially harvested and tissues collected at ~ 6, 12 and 18 months post-inoculation. This will provide a time series of tissues that may provide useful for testing various existing and new assays. Collaborators on this project include Wyoming Fish and Game, University of Wyoming and the National Wildlife Health Center.

Strain Identification Assay for CWD in Cervids:

This ongoing project will identify and monitor strains of CWD in wild and captive cervids and establish a strain identification assay for CWD. Preliminary results indicate that a western blot fingerprinting technique may be useful for identifying specific CWD strains. Collaborators for this project are Dr. Richard Bessen at Montana State University and Dr. Tonie Rocke at the Naitonal Wildlife Health Center.

Pre-clinical Biomarkers

This objective of this project is to identify biomarkers indicative of chronic wasting disease (CWD) infection in cervids. Evidence of infection has been noted in the pituitary glands of (intracerebrally) scrapie–infected rodents. This result suggests that endocrine hormones are promising biomarkers that may be useful for detecting CWD before the onset of clinical disease. Collaborators here are Montana State University and the USGS National Wildlife Health Center.

Surveillance Design

Effective disease detection in free-ranging cervids presents some unique statistical challenges. It is impossible to obtain a statistically random sample from free-ranging wildlife Therefore, standard theory used for human and domestic disease detection cannot be applied. Computer simulation techniques are being utilized to design more effective surveillance designs for wildlife. Collaborators include Iowa State Cooperative Fish and Wildlife Research Unit and the USGS National Wildlife Health Center.

CWD Transmission to Small Mammals

A variety of small mammals scavenge deer carcasses and could therefore potentially come in contact with infectious material. It is critical to understand whether CWD can jump the species barrier and become established in other wildlife species. We are initiating challenge studies in the NWHC isolation facility to examine whether small rodents can contract CWD and whether CWD can adapt to a rodent host.

New/Upcoming CWD Activities

The 2nd International CWD Conference will be held July 12-14, 2005 at the Monona Terrace Conference Center in Madison, Wisconsin. Wisconsin Department of Natural Resources is primary sponsor, with assistance from USGS-NWHC and USDA-APHIS. Program planning is in early stages.

<u>Common Tern Mortalities in Massachusetts and Maine</u>

In July and August 2004, staff and researchers at Seal Island National Wildlife Refuge (NWR) in Maine and staff at Monomoy NWR and Cape Cod National Seashore (NS) in Massachusetts reported sick and dead fledgling common terns. Seal Island terns (Maine) exhibited signs of not being able to raise one wing and were unable to extend their wings, while those at Monomoy (Massachusetts) were unable to fly, and were observed circling, star-gazing, and with no ability to maintain balance. At necropsy, the primary

consistent finding was atrophy of immune system organs (thymus, bursa of Fabricius, spleen). Although no viruses were isolated, terns from both Maine and Massachusetts had intranuclear inclusion bodies in bursal cells, which are most often indicative of viral infections. Testing is underway to determine what virus may be present. Many specimens from Massachusetts tested positive for salmonella; these bacterial infections may be opportunistic in light of immune system impairment. Total mortality was over 1,600 common terns in Massachusetts, making this the largest tern mortality event in the NWHC Epizoo database.

- Fledgling common terns, some arctic terns
- Late July – late August 2004
- Wing or leg impairment
- Neurological signs: circling, star-gazing, twitching, imbalance
- Atrophy of immune system organs
- No viruses isolated
- Intranuclear inclusions seen histologically in some terns
- Systemic salmonella infections, likely secondary
- Over 1,600 dead, largest tern mortality in Epizoo

Winter Mortalities in Bald Eagles Along the Wisconsin River

Between early January and mid-March 1995, 16 American bald eagles died in three counties (Sauk, Columbia, Adams) along the Wisconsin River from Spring Green north to Wisconsin Dells—the Sauk Prairie area. Birds initially recovered live had neurological signs, including seizures, tremors, and impaired mobility. Thirteen of the eagles had hepatic lipidosis (fatty livers), and some had cerebral (brain) lesions; extensive testing found no toxic or infectious agents. Since the winter of 2000-2001 and continuing through last winter, similar mortality events occurred, with 3 to16 eagles each year diagnosed with "Wisconsin River Syndrome". A retrospective analysis of almost 600 case records of Bald Eagles from Wisconsin uncovered 7 additional cases beginning in 1990, for a total of 53 cases altogether and adding six more WI counties (Dane, Iowa, Grant (southwest of Sauk Prairie) and Marquette, Juneau, Wood (north of Sauk Prairie) in an area along the Wisconsin River farther north to Wisconsin Rapds. Despite continued extensive testing, no cause has been identified.

- American bald eagles
- Events began in January 1995 or earlier
- Neurological signs
- Hepatic lipidosis - all
- Cerebral lesions – some
- Extensive toxicological and pathogen screening conducted
- Annual recurrence beginning in 2000-2001
- No cause identified as yet
- 50 of 100 bald eagles submitted from 9 counties along the river
- 3 from outlying counties within 1 day flight distance of central area