

**2006 Report  
to Western Association of Fish and Wildlife Agencies  
from the  
USGS National Wildlife Health Center (NWHC)**

**Mortality Investigations, June 2005 – June 2006**

**Seabird Mortality along the Pacific Coast:** From January through August 2005 unusually high numbers of beached seabird carcasses were reported along the California and Oregon coasts. The species most affected were common murre and Brandt's cormorants. Based on counts made by trained biologists from federal, state, and nongovernmental agencies that conduct bird surveillance along predetermined segments of coastal beaches in the affected area, the known mortality exceeds 1,150 common murre and 375 Brandt's cormorants. Necropsies of freshly dead birds by the California Department of Fish and Game, the Marine Wildlife Veterinary Care and Research Center, and the USGS National Wildlife Health Center have consistently documented the predominant pathological condition in the birds to be emaciation. These diagnostic findings are consistent with observations reported by marine biologists and oceanographers that abnormal ocean conditions, extending from Washington to central California, have resulted in a breakdown of the marine food web, from phytoplankton to the top of the food chain. This disruption has resulted in starvation of higher trophic level marine species including seabirds.

In March and April 2006, dead rhinoceros auklets were washed up along much of the Oregon coast. The total estimate of dead carcasses based on counts conducted by state, federal and non-governmental agencies exceeded 400 birds. Specimens of dead auklets were submitted to the NWHC. Diagnostic evaluation of the submitted birds led to a diagnosis of emaciation resulting from probable starvation.

**Nevada White Pelican Mortality due to West Nile Virus:** In late July 2005, wildlife biologists from the Nevada Department of Wildlife and the U.S. Fish and Wildlife Service began observing sick and dead American white pelicans at saline wetlands in western Nevada. A total of 45 affected pelicans were picked up at the wetlands through early September. Over 90% of the pelicans observed were adults. Sick birds exhibited signs of lethargy and loss of muscle coordination. Oral swabs from seven birds were collected and submitted for West Nile virus (WNV) testing through the State of Nevada WNV surveillance system. All seven samples were positive. Three carcasses of adult pelicans were submitted to the NWHC for diagnostic evaluation. Two of these birds were positive for WNV by virus isolation. Testing of blood from the birds for botulism type C toxin was negative. Significant mortality of juvenile American white pelicans has been consistently reported from colonies in the northern Great Plains since 2002.

**Newcastle Disease in Nevada Double-crested Cormorants:** During routine colonial nesting bird surveys at Anaho Island NWR on August 16, 2005, U.S. Fish and Wildlife Service biologists discovered one dead American white pelican chick and 10 dead and 3 sick double-crested cormorants fledglings at a nesting site with total populations of about

30 pelicans and 2,000 cormorants. The sick cormorant fledglings all showed some degree of flaccid paralysis and hemorrhage from the mouth. The sick cormorants were euthanized and submitted to the NWHC, along with the dead pelican chick for diagnostic evaluation. A diagnosis of botulism type C intoxication was made in the pelican chick. Virus isolation attempts in cell culture on brain and other organs from the cormorant chicks produced cytopathic effects suggestive of Newcastle Disease virus (NDV) in two chicks. Hemagglutination inhibition tests identified the virus isolates as NDV and samples of the viruses were sent to the USDA National Veterinary Services Lab (NVSL), which confirmed the virus to be NDV and determined that they were highly pathogenic for chickens. NVSL conducted genetic analysis of the virus isolates and reported that they were different from the NDV strain that affected domestic poultry in southern California, Nevada, and Texas in 2002 and 2003. However, the viruses were determined to have a high genetic homology with strains isolated from double-crested cormorants in the United States since 1992.

**Botulism type C at Bear River Migratory Bird Refuge:** From late July to mid-September 2005, U.S. Fish and Wildlife personnel picked up the carcasses of about 6,000 waterbirds from a 1,800-acre wetland management unit at Bear River Migratory Bird Refuge (MBR) at the Northeast edge of the Great Salt Lake. The primary species involved were dabbling ducks and small wading birds. Carcasses of dead ducks were submitted to the NWHC for diagnostic evaluation in early August. Necropsy of the specimens showed no significant gross pathological lesions. The birds were negative for liver lead and West Nile virus by virus isolation. The birds were positive for botulism type C toxin in mouse protection tests. Bear River MBR has a long history of major botulism die-offs, tracing back to the early part of the 20th century. Significant mortality due to botulism type C has not been documented at the refuge since the late 1990s, when it was estimated that over 500,000 birds were found dead in 1997 and 10,627 birds were picked up in 1998.

**Suspect Aflatoxicosis in Waterfowl in Southeastern Idaho:** In March and April 2006, a cooperative disease investigation effort involving personnel from the U.S. Fish and Wildlife Service (USFWS), USGS National Wildlife Health Center, and Idaho Department of Fish and Game documented the mortality of at least 437 Ross' geese, lesser snow geese, coot, Canada geese and 1 trumpeter swan at Camas NWR, Market Lake and Mud Lake in southeast Idaho. Analysis of snow and Ross' geese submitted to the NWHC showed microscopic lesions consistent with exposure to aflatoxin, a fungal toxin commonly associated with ingestion of moldy grain. Tests for infectious and parasitic disease agents produced no significant findings.

**Avian cholera at Upper Klamath Lake:** Beginning in mid-April 2006, mortality of waterfowl was reported by biologists from the USFWS and Oregon Department of Fish and Wildlife on Upper Klamath Lake in southern Oregon. Ruddy ducks, lesser scaup and bufflehead were most affected, but coots, gulls and a few shorebirds were also involved. Dead birds continued to be collected until mid-May when most of the migratory birds left the area to migrate north. Dead birds were submitted to the NWHC for diagnostic evaluation and a wildlife disease specialist from the NWHC conducted a field

investigation at the site. *Pasteurella multocida*, the cause of avian cholera, was cultured from tissues of the majority of birds tested. Avian influenza tests were negative on the submitted birds. It was estimated that 1,000 birds died during this event.

**Avian Botulism at Kern National Wildlife Refuge:** Mortality of ducks, shorebirds, and coots began at Kern NWR in early June 2006. By the end of June the number of dead birds collected and disposed had reached 480. The Refuge has had a history of avian botulism mortality and blood samples from dead birds submitted to the NWHC were positive for botulism type C toxin. Avian influenza tests and analysis of brain cholinesterase activity for evidence of exposure to organophosphate or carbamate pesticides were all negative.

### **Disease Investigations 2005 - 2006**

**H5N1 Highly Pathogenic Avian Influenza:** In response to the continuing threat of H5N1 Highly Pathogenic Avian Influenza (HPAI) being introduced to North America and American-associated states and territories in the Pacific, the U.S. Department of Interior (DOI), U.S. Department of Agriculture (USDA), and the U.S. Department of Health and Human Services, along with other federal and state partners, developed a U.S. Interagency Strategic Plan for “An Early Detection System for H5N1 Highly Pathogenic Avian Influenza in Wild Migratory Birds,” at the request of the President’s Council on Homeland Security Policy Coordinating Committee. This strategic interagency plan is posted at <http://www.nwhc.usgs.gov> and at <http://pandemicflu.gov>. This plan was funded through a FY2006 Supplemental Congressional appropriation, and surveillance began in April 2006. The Plan includes five strategies for detecting HPAI H5N1 in migratory birds: 1) investigation of avian morbidity and mortality events; 2) targeted sampling of live-captured migratory birds; 3) sampling of sport and subsistence hunter-killed birds; 4) environmental sampling; and 5) sentinels.

The USGS National Wildlife Health Center (NWHC) has developed capacity to process large volumes of samples integral to this surveillance. As of July 12, 2006, NWHC has received and processed samples from more than 3,770 migratory birds. The majority of these samples so far have been from subsistence hunting in Alaska and mortality events nationwide. To date, H5N1 HPAI has not been detected in any of the samples. Results of testing are posted as they become available on the HPAI Early Detection Data System (HEDDS) at <http://wildlifedisease.nhii.gov/ai/> and will be available to the public in the near future. Plans for sampling hunter-killed birds during fall migration are well underway with the Fish and Wildlife Service, state agencies, and USDA. A total of 75,000-100,000 samples are expected to be collected as part of the strategic plan during the 2006 season. The NWHC conducted multiple disease workshops in the western region. Specifically, there were several workshops in Alaska, Oregon and California. These workshops were attended by state and federal partners. Subject matter included disease investigation techniques, PPE (personal protective equipment), contingency planning, and information on other diseases in addition to avian influenza.

The NWHC Web site at <http://www.nwhc.usgs.gov> continues to be updated frequently with current information about H5N1 HPAI occurring around the world, as well as information and developments in surveillance and detection activities in the U.S., including four avian influenza Wildlife Health Bulletins, Fact Sheets, maps, a referenced List of Species Affected by H5N1, summaries, and links to other sites with HPAI H5N1 information.

Surveillance activities for HPAI H5N1 are also occurring in Canada. Current information on results of their sampling and testing can be found at the Canadian Cooperative Wildlife Health Center's Web site: <http://wildlife1usask.ca/en/aiv/index.php>.

**Chronic Wasting Disease:** The USGS, in collaboration with the University of Wyoming and the Wyoming Game and Fish Department, is developing a CWD-positive tissue bank to gain a better understanding of this disease. Thirty six wild-caught cervids (12 elk, 12 mule deer, and 12 white-tailed deer) were orally inoculated with CWD at the Tom Thorne/Beth Williams Wildlife Research Center (formerly the Sybille Wildlife Research Center) in Wheatland, Wyoming. Animals have been euthanized and tissues harvested at 6-month intervals, post-inoculation. The final tissue collection is scheduled for November 2006. Harvested tissues will be made available for reviewed research projects and for testing/validation of CWD assays.

USGS researchers are collaborating with the Colorado Division of Wildlife, the University of South Carolina, and the Wildlife Conservation Society to apply modern statistical epidemiological theory to long-term CWD data sets collected by the States of Colorado and Wisconsin. In this effort to rapidly advance our understanding of the factors driving the dynamics of CWD epidemics in space and time, researchers will draw upon analytical theory from epidemiology and spatial analysis to develop new approaches to analyze geo-referenced CWD data. The goal of the analysis is to determine the extent to which CWD distribution can be explained by landscape and geographic features.

**Measuring the Effects of West Nile Virus on American Kestrel Populations in Colorado:** USGS scientists are monitoring kestrels along the Front Range of Colorado to assess the annual inception and level of occurrence of West Nile virus (WNV) among these birds, and compare survival and reproduction parameters between infected and non-infected individuals within the population. Beginning in 2004, scientists have monitored about 75 active nests resulting in the capture and sampling of more than 100 adults and 200 chicks each year through 2006. Of adults sampled, 97.4% tested positive for specific WNV neutralizing antibody in 2004, while in 2005, 79% of adult female and 56% of adult male kestrels had detectable antibody. Just 1.2% (2004) and 5.4% (2005) of blood-sampled chicks tested positive for specific WNV neutralizing antibody, possibly from maternal antibody transfer. Collection and analysis of 2006 samples is ongoing. In 2004, clutch size, hatching success, and fledging success did not differ from what has been previously reported for this species.

**Occurrence of West Nile virus in selected avian species at the Salton Sea, California:**

USGS scientists investigated the prevalence of specific West Nile virus neutralizing antibodies and frequency of overwinter transmission of WNV in American kestrels, burrowing owls, and least and western sandpipers in and around the Salton Sea National Wildlife Refuge and Calipatria, California. In January and March 2006, 208 kestrel, 118 owl, and 494 shorebird samples were obtained for WNV analysis. Lab analysis of samples is currently underway.

**West Nile Virus and Other Avian Pathogens in Greater Sage-grouse:** USGS is evaluating the exposure of greater sage-grouse and other vertebrate species within sagebrush habitat to WNV and other pathogens to determine if they are affected by WNV and what role they may play in the transmission of the disease. Field research has been conducted at study sites in Nevada, Oregon, and California. To date, WNV has been found in 1 of 13 greater sage-grouse carcasses examined, but samples from over 500 live greater sage-grouse, 1,500 passerines, and 700 and feral horses have not shown evidence of exposure to the virus. Laboratory studies indicate that chukar partridge may amplify the virus making them a useful sentinel for WNV in the field.

**Risk Assessment for West Nile Virus in Hawaii:** USGS is collaborating with Hawaii Department of Health (HIDOH) to conduct serosurveys (serologic tests on populations) for flaviviruses in Hawaii in an attempt to detect WNV as early as possible, if it is transported to the islands from the mainland by air. Scientists have collected blood samples from 405 birds including java sparrows, laceneck doves, house finches, house sparrows, zebra doves, and red-crested cardinals from the Honolulu International Airport. This wild bird surveillance supplements mosquito surveillance and abatement activities being done by HIDOH. USGS is also conducting laboratory studies, in partnership with the State of Hawaii and the U.S. Fish and Wildlife Service, to determine the suitability of non-native Hawaiian birds, such as java sparrows, laceneck and zebra doves, house finches, Japanese white-eye, and house sparrows, to serve as amplifying, reservoir hosts or sentinels for WNV in Hawaii. All species were susceptible to infection with WNV by needle inoculation; however, morbidity and mortality were observed only in Java sparrows, house finches, Japanese white-eye, and house sparrows. While culture of blood was the most sensitive method of detecting WNV in these species, culture of oral swabs was nearly as sensitive with the exception of the common mynah, in which it was less sensitive. We found RT-PCR on oral swabs to be nearly as sensitive as culture in all species except mynahs and zebra doves, in which it was less sensitive. The WNV epitope blocking assay is sensitive in detection of antibody to WNV in those species that do not experience mortality due to the virus. However, in species that experience mortality the test is less sensitive. For WNV surveillance in Java sparrows, Japanese white-eye, house finches, and house sparrows both serology and RT-PCR of oral swabs, rather than just serology alone, will increase the likelihood of detecting WNV infection. Based on the results of this study, a dead bird surveillance program should be based on discovering and testing house sparrows, house finches, and Japanese white-eye. Another laboratory study is involved with investigating the susceptibility of native honeycreepers to WNV by using the amakihi. Amakihi were found to be susceptible to WNV by either needle inoculation or through the feeding of a WNV-infected Hawaiian *Culex quinquefasciatus*

mosquito. Morbidity and mortality developed among infected amakihi and the amakihi developed a sufficient viremia to infect feeding mosquitoes.

**Impact of West Nile Virus on White Pelican Colonies in Northern Montana, North Dakota and South Dakota:** The USGS, in cooperation with the U.S. Fish and Wildlife Service, is in the third year of a project that assesses the affects of West Nile virus (WNV) on three pelican nesting colonies in North Dakota, South Dakota, and Montana. Between June 29 and August 16, 2004, 119 pelican carcasses were collected in South Dakota and Montana for examination, West Nile virus sample collection, and analysis. Breeding adult pelicans at the third site in North Dakota abandoned their nesting colony. The first positive WNV cultures and serology were from birds collected in South Dakota and in Montana in July 2004. After the initial positive WNV sample was confirmed at each site, a high frequency of the remaining birds collected were WNV positive (89 percent in SD and 71 percent in MT). Between June 17 and August 25, 2005, 134 pelican carcasses were collected in North Dakota, South Dakota and Montana for examination, West Nile virus sample collection, and analysis. The first positive WNV cultures were from birds collected in mid-July 2005. After the initial positive WNV sample was confirmed at each site, a high frequency of the remaining birds collected were WNV positive (70 percent in ND, 87 percent in SD, and 87 percent in MT).

**Sylvatic Plague in Prairie Dogs and Black-footed Ferrets:** The NWHC is continuing research and development of vaccines against sylvatic plague in prairie dogs and black-footed ferrets. An injectable vaccine has been shown to be successful in preventing plague experimentally in endangered black-footed ferrets, and was deployed 2 years ago in a field experiment in collaboration with the U.S. Fish and Wildlife Service. In this experiment, half of captive-bred ferrets released to the wild at several locations were vaccinated, and the other half served as unvaccinated controls. Survival rates of vaccinated and unvaccinated ferrets are being compared. Preliminary analyses of results at one site are promising with an increased rate of survival of vaccinates. This vaccine was also used to immunize wild black-footed ferrets in the Conata Basin in South Dakota, the site of the largest breeding colony of ferrets. A plague outbreak in prairie dogs about 10 miles south of the basin has raised concern the disease will spread to ferrets at this site. A different recombinant vaccine that can be delivered orally to prairie dogs has been shown to protect about 50% of vaccinates challenged in experimental studies. Another plague antigen has been incorporated into the vaccine and experimental studies on this improved vaccine will be conducted in prairie dogs, ground squirrels, and other rodents this summer.

**Investigation of Mortality of Western Snowy Plovers and Gulls in San Diego Bay:** The NWHC and the U.S. Fish and Wildlife Service Carlsbad Field Office in southern California received a grant to study the cause of sickness and death in populations of endangered western snowy plovers and gulls in San Diego Bay in 2006. The San Diego Bay population contained over a third of the entire southern California population before the recent series of die-offs of adults began in 2002. The cumulative mortality since 2002 represented over half of the breeding population in the area. No other area in the range of the species has experienced this level of mortality, or these particular clinical symptoms.

The birds were unable to fly, generally unable to stand, had normal body weight, but had no interest in eating. Some showed severe paralysis of their legs and wings. Similar symptoms have also been found in gulls collected in San Diego Bay. The objectives of the study are to: 1) determine the cause of disease of adult western snowy plovers from the San Diego Bay, Tijuana River Estuary, and adjacent coastal areas during one breeding and nesting season; 2) determine the cause of disease in western gulls, California gulls, or ring-billed gulls from the study area showing the same clinical signs as sick western snowy plovers; 3) develop background information on disease agents present in adult western snowy plovers that may have effects on population health and reproductive success; and 4) provide recommendations for future research on diseases impacting adult western snowy plovers.