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Michael J. Yabsley
University of Georgia, myabsley@uga.edu

SCWDS BRIEFS

A Quarterly Newsletter from the

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY
COLLEGE OF VETERINARY MEDICINE
THE UNIVERSITY OF GEORGIA
ATHENS, GEORGIA 30602

Phone (706) 542-1741
FAX (706) 542-5865

Gary L. Doster, Editor

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Avian Influenza Update - May 2006

Concern remains high for highly pathogenic avian influenza (HPAI) H5N1 virus and its potential impacts on human and animal health. As of early May 2006, the World Health Organization (WHO) reported a total of 207 human cases with 115 fatalities. A total of 62 human fatalities have occurred in 2006, with the highest number (14) in Indonesia. In Egypt, 13 human cases, resulting in 5 fatalities, have been reported since late March 2006. Human cases continue to be strongly associated with contact with diseased poultry.

Since our last update (SCWDS BRIEFS Vol 21, No. 4), HPAI H5N1 virus has been detected in wild birds in Denmark, France, Germany, Sweden, the United Kingdom, and other European countries. Affected birds primarily were waterfowl species, including swans (*Cygnus* spp.), tufted ducks (*Aythya fuliga*), and common pochards (*Aythya ferina*). In Germany, HPAI H5N1 virus was found in a clinically affected domestic cat and in a dead wild stone marten (*Martes foina*). Domestic poultry infections have been detected in France, Germany, Sweden, and other European countries and several African countries.

Preparations are underway in the United States for detection and response to HPAI H5N1 should it enter this country. Concerns continue to be raised regarding the potential introduction of HPAI H5N1 via migratory bird movements, as well as through smuggling of infected birds or products. A significant surveillance effort is being mounted for HPAI H5N1 in wild birds; the U.S. Department of Agriculture (USDA) and the U.S. Department of Interior (DOI) are conducting surveillance and providing support to state fish and wildlife management agencies to submit samples from wild birds for HPAI H5N1 testing.

Surveillance strategies are described in the *Early Detection System for HPAI H5N1 in Wild Migratory Birds* and include (1) investigation of wild bird morbidity/mortality events, (2) surveillance of live wild birds, (3) surveillance of hunter-killed birds, (4) sentinel species, and (5) environmental sampling. USDA-APHIS-Wildlife Services and the four North American flyway councils have prioritized states and wild bird species for sampling. States have been categorized in a 3-level system: state fish and wildlife management agencies in participating Level 1 states will collect 800-1,000 samples; Level 2 states will collect 650-750 samples; and Level 3 states will collect 400-500 samples. Similar numbers of samples in each state are to be collected by USDA-APHIS-Wildlife Services. Additional funding has been made available by the DOI for surveillance in Alaska and other Pacific Flyway States.

Updated information on avian influenza virus, including reports on the occurrence of HPAI H5N1, can be found at the websites of WHO (www.who.int), CDC (www.cdc.gov), USDA (www.aphis.usda.gov), and USGS (www.nwhc.usgs.gov). An updated version of the SCWDS fact sheet *Highly Pathogenic Avian Influenza Virus H5N1 and Wild Birds* can be found at www.scwds.org. (Prepared by John Fischer and David Stallknecht)

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SCWDS AI Virus Studies Continue

Field Studies

SCWDS remains actively involved in several field and laboratory studies investigating the ecology of avian influenza virus (AIV), including both high pathogenicity viruses (HPAI) and low pathogenicity viruses (LPAI). During 1999-2005, AIV surveillance of species in the order Charadriiformes (shorebirds and gulls) was conducted at multiple sites in the eastern half of the continental United States and Argentina, Bermuda, and Chile. Approximately 9,700 birds were sampled, and AIV was isolated from 311 birds. Although ruddy turnstones comprised just 24% of the birds sampled, they accounted for 86% of the isolates. Only nine AIV isolations were made from birds at three locations outside the Delaware Bay region. The H10 subtype predominated in the Delaware Bay isolates, but this subtype has not been found every year. These results suggest that AIV infection among shorebirds is localized and species-specific. At this time, Delaware Bay is the only site worldwide where a high prevalence of AIV has been reported from shorebirds and most isolates at this site have been collected from ruddy turnstones. SCWDS surveillance of Delaware Bay shorebird populations will continue in 2006, with an objective of identifying factors associated with the species-related prevalence differences at this site.

Two years of AIV surveillance have been completed among wintering waterfowl populations in collaboration with the North Carolina Wildlife Resources Commission. Results were consistent with previous estimates of less than 2% prevalence, suggesting that risk of infection with all AIV subtypes generally is very low in wintering ducks in the southeastern United States. LPAI viruses were isolated from black ducks, northern shovelers, and a tundra swan. There was no evidence of AIV infection in resident mallards and wood ducks prior to the arrival of migrants.

Experimental Studies

These trials are part of a larger study to evaluate the potential for HPAI H5N1 viruses to be transported long distances by wild birds or become established in North American Anseriformes (ducks, geese, and swans) and Charadriiformes populations if the virus is introduced. Wild birds in these orders are the natural reservoirs for LPAI viruses. The experimental studies are a collaborative effort between SCWDS and the USDA-ARS-Southeast Poultry Research Laboratory (SEPRL) and are funded by the United States Poultry and Egg Association, the Morris Animal Foundation, and other SCWDS supporters.

In an initial experimental study we assessed the clinical response, as well as the volume and duration of viral shedding, in laughing gulls and five species of North American ducks inoculated with two Asian HPAI H5N1 virus strains. Birds were challenged at approximately 2-4 months of age, which is consistent with observed temporal peaks in AIV prevalence and fall migration. All species became infected, but wood ducks and laughing gulls were the only species that exhibited morbidity or mortality. Infected mallards, northern pintails, blue-winged teal, and redheads did not exhibit clinical signs. The highest viral titers were detected in swabs of the oropharyngeal cavity. Viral shedding ranged from 1 to 10 days and correlated with clinical response. Viral shedding of the longest duration was observed in wood ducks and laughing gulls. The species-related responses in this study indicate that susceptibility studies of additional wild birds are warranted, and experimental infections of several species with HPAI H5N1 viruses are planned.

Because LPAI virus transmission occurs via a fecal/oral route in contaminated water, it is important to understand the potential for HPAI H5N1 viruses to remain infective in water. In recent trials, we compared two Asian HPAI H5N1 viruses with eight LPAI viruses (H5 and H7 subtypes) isolated from waterfowl and shorebirds in North America. All of these viruses

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water for extended periods of time (weeks and months) at 17 C. In all cases, the ability of these viruses to remain infective in water was lower at higher temperatures and higher salinities. Of all viruses tested, the two HPAI H5N1 viruses demonstrated the shortest persistence in water, and results suggest they are not as hardy as naturally occurring LPAI viruses in water. This does not imply that these viruses cannot be transmitted within ecosystems utilized by wild birds, but it does suggest that transmission and maintenance within these populations would not be as efficient as with naturally occurring LPAI viruses. Several studies are planned to further investigate the role of the environment in the ecology of AIV. The results of this study recently were presented at the 6th International Symposium on Avian Influenza held in Cambridge, England, and will be published in a special issue of *Avian Diseases* comprising papers presented at the symposium. (Prepared by Justin Brown and Dave Stallknecht)

Canadian Wild Bird Survey for Influenza Viruses

A plan to conduct a survey of avian influenza (AI) viruses in wild birds in Canada was developed late in 2004 by the Canadian Cooperative Wildlife Health Centre (CCWHC) in consultation with a wide range of federal and provincial government agencies with responsibilities for wildlife management, domestic animal health, and public health. The purpose of the survey was to obtain information on the range of influenza virus strains in wild birds throughout Canada.

The survey was implemented in August-December 2005, and cloacal swabs were collected from wild ducks caught at banding stations operated by personnel with the Canadian Wildlife Service, the Ontario Ministry of Natural Resources, and Ducks Unlimited Canada. A target of 800 samples was set for each of six general regions representing different migration corridors in Alberta, British Columbia, Manitoba, Ontario, Quebec, and the Atlantic Region. Five hundred samples were to be collected from mallards and 300 from other duck species, with juvenile birds preferred whenever possible. Initial analysis of the samples was carried out at a regional laboratory in each of the six regions as an in-kind contribution to the survey by provincial governments. These laboratories did polymerase chain reaction (PCR) analysis for influenza A matrix protein. Positive samples then were tested by PCR for the presence of H5 and H7 protein classes. Cloacal swab samples positive for H5 or H7 were shipped immediately to Canada's National Centre for Foreign Animal Diseases (NCFAD) of the Canadian Food Inspection Agency in Winnipeg, where PCR results were verified and the viruses were further characterized to determine origin and potential to cause disease in chickens. Regional laboratories isolated viruses from the AI-positive samples from which H5 or H7 genes were not detected. Isolated viruses were sent to the NCFAD and to the co-located National Microbiology Laboratory of the Public Health Agency of Canada, both in Winnipeg, for H and N serotyping, genetic sequence analysis, and placement in a national archive of virus strains.

As of May 2006, 4,306 samples had been analyzed by PCR in regional laboratories. Of these, 1,580 (36%) contained influenza A viruses. Of the positive samples, 1,148 were collected from 2,600 mallards tested, 107 positive samples were from 437 blue-winged teal, 98 positive samples were from 297 American black ducks, and 66 positive samples came from 104 wood ducks. Strains of H5 virus were detected in 208 of the influenza-positive samples (5% of all samples, 13% of influenza-positive samples). All H5 viruses, including one H5N1 strain from a mallard, proved to be North American strains of low/no pathogenicity. Strains of H7 virus were not found in any samples. Numerous samples contained two, and in some cases three, different strains of influenza A virus.

In November 2005, Canada also initiated regular testing for Influenza A of all dead birds submitted to its national wildlife disease surveillance

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program, which is operated by the CCWHC. As of May 2006, 367 birds representing more than 60 species had been tested. Influenza A viruses were detected in 25 of these birds: 15/124 from gulls, ducks, geese, swans, grebes, and loons; 4/51 American crows; 2/32 rock doves; 1/6 mourning doves; 1/7 peregrine falcons; 1/2 sharp-shinned hawks; and 1/7 red-tailed hawks. Strains of H5 and H7 viruses were not detected in any of the birds.

Results of this survey are highly consistent with previous work that indicates that wild birds are the reservoirs of AI viruses of low pathogenicity and that these viruses will be detected in future surveys for HPAI H5N1 strains currently circulating in Africa, Asia, and Europe. Complete information on AI surveillance in wild birds in Canada can be found at the CCWHC website: http://wildlife1.usask.ca/en/aiv/index.php
(Prepared by Ted Leighton, CCWHC)

Avian Flu Virus in Mammals

The highly pathogenic avian influenza (HPAI) H5N1 virus of current concern not only affects birds and humans, but also has caused illness and death in a variety of other mammals. The potential for HPAI H5N1 virus to infect mammals was first seen in 2003 with the deaths of tigers (*Panthera tigris*) and leopards (*Panthera pardus*) at zoos in Thailand. The mode of transmission in these cases is believed to be feeding the cats raw chicken carcasses from a local slaughterhouse. This was the first documented instance of influenza virus infection in non-domestic cats. HPAI H5N1 virus also caused the deaths of several captive Owston's palm civets (*Chrotogale owstoni*) in Vietnam in 2005. In March 2006, the virus was detected in a free-ranging stone marten (*Martes foina*) in Germany.

Domestic animals also can be affected by HPAI H5N1 virus. The virus has been isolated from dead domestic cats in Germany and Thailand. An unpublished survey in Thailand identified dogs with antibodies to H5N1 virus, but whether the virus causes disease in dogs is unknown. Of greater concern with domestic mammals is the finding of H5N1 virus-infected pigs in southeast China and Indonesia. Pigs carry swine influenza viruses and are susceptible to infection by both human and avian influenza viruses and can become "mixing vessels" to produce a reassorted virus that could cause a pandemic in the human population.

Experimental inoculations have been conducted to study HPAI H5N1 virus and its effects on a variety of mammals. Experimental infection of cats has shown that transmission of the virus to cats may occur through contact with other cats, as well as by eating infected birds. HPAI H5N1 avian influenza virus caused disease or death when inoculated into domestic cats (*Felis silvestris catus*), domestic ferrets (*Mustela putorius furo*), and cynomolgus macaques (*Macaca fascicularis*). No morbidity or mortality was observed in laboratory rats or New Zealand white rabbits (*Oryctolagus cuniculus*) experimentally infected with HPAI H5N1 virus.

The results of natural and experimental infections of mammals with HPAI H5N1 are indicators of the broad species susceptibility to this virus and the need for vigilance in monitoring HPAI H5N1 virus.
(Prepared by Sam Gibbs)

WNV - 2005 in Review

It=s that time of the year again - to start thinking about West Nile virus (WNV). Since the virus was first detected in New York in 1999, 19,655 human cases have been reported in the United States, resulting in 782 deaths. From these data, experts at the Centers for Disease control and Prevention (CDC) estimate that there probably were at least 200,000 cases that were not reported. West Nile fever/encephalitis is now the dominant vector-borne disease in North America.

During the past 4 years, between 2,000 and 10,000 human cases have occurred in North America annually. Human cases have been reported in every state except Alaska, Hawaii,

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Maine, and Washington. During 2005, 2,949 human cases were reported from 42 states and Washington, D.C., causing at least 116 deaths.

During surveillance efforts among wild birds from 1999 through 2005, WNV was detected in 53,267 dead birds, representing 308 species. Nationwide surveillance in 2005, disclosed 21,496 birds that died from various causes. Of these, 8,653 were tested and 5,344 were positive for WNV. The positive birds were from 45 states and Washington, D.C., and the majority (81%) were corvids (primarily American crows and blue jays).

Between 1999 and 2005, 45 states and Washington, D.C., reported 35,569 WNV-positive mosquito pools, involving more than 60 mosquito species. In 2005, 2.3 million mosquitoes were pooled and tested for WNV, and 11,485 mosquito pools from 42 states and Washington, D.C. were positive.

Between 1999 and 2005, WNV was detected in 23,117 equines in 45 states. During 2005, 1,139 WNV-infected equines were detected in 36 states.

WNV transmission to humans has been documented by five routes: mosquito bites, blood transfusions, organ transplantation, transplacental transfer, and breastfeeding. Of these, being fed upon by a WNV-infected mosquito is considered the most important transmission route. As in previous years, CDC suggests that the risk for human and domestic animal infection with WNV may be minimized by increased surveillance geared toward early viral detection, mosquito control and avoidance, and activities that interrupt amplification cycles. Prevention activities delineated by CDC continue to include: (1) public education programs urging reduction of mosquito breeding sites around residential areas and personal protective measures to reduce mosquito exposure; (2) development of sustained, community□level integrated mosquito surveillance and management programs; and (3) high-priority emphasis on the control of urban mosquitoes in the genus *Culex*. (Prepared by Danny Mead)

National Fish and Wildlife Health Initiative

At its annual meeting in September 2005, the Association of Fish and Wildlife Agencies (AFWA) (formerly known as the International Association of Fish and Wildlife Agencies) passed a resolution and adopted guiding principles for the development of a national fish and wildlife health initiative under AWFA leadership (SCWDS BRIEFS Vol. 21, No. 3). Following completion of the national initiative, a North American Initiative to protect fish and wildlife health throughout the continent will be developed in cooperation with Canada and Mexico. In November 2005 the United States Animal Health Association also resolved to support the development and implementation of the initiative.

In January 2006 a working group from several state fish and wildlife management agencies met in Lansing, Michigan, and drafted an outline for the initiative. The primary goals of the initiative are to enhance state fish and wildlife agency capacity and develop national strategies to effectively address fish and wildlife health issues. In March 2006 the group met with partners in federal animal health and natural resource agencies and associations to further develop the plan. Meetings currently are being held with the four regional fish and wildlife agency associations, and personnel with state and federal natural resource, animal health, and public health agencies are invited to participate and to provide input on the initiative. Meetings have been completed with the Northeastern Association of Fish and Wildlife Agencies and the Midwestern Associations of Fish and Wildlife Agencies and are scheduled to be conducted with the Western Association in July 2006 and with the Southeastern Association in August 2006. Also, in August 2006, appropriate non-governmental organizations will be invited to attend and provide input for the development and implementation of the initiative. This timetable was developed in order to deliver the initiative to AWFA at its next annual meeting in

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September 2006. In addition to hosting meetings, the working group is making the draft available to organizations and agencies to seek their assistance and input on the initiative. Individuals and agencies who are called upon to participate in this important and worthwhile effort are encouraged to answer the call, become involved, and help ensure its success. (Prepared by John Fischer)

Raccoon Parvovirus

Parvovirus was confirmed as the cause of mortality in a young raccoon submitted to SCWDS in January. This raccoon was one of two raised by a wildlife rehabilitator in coastal Georgia and released locally. Both raccoons returned to the home of the rehabilitator and both had severe diarrhea. One responded to fluid therapy and antibiotics, but the other raccoon died despite intensive therapy. The veterinarian who treated the animals performed a necropsy and submitted tissue samples to SCWDS.

The most severe lesions were in the small and large intestines, which had necrosis of epithelial cells in the crypts and an overgrowth of bacteria on the ulcerated surface. Bacterial culture revealed that the bacteria were primarily *Streptotococcus* species. Immunohistochemistry demonstrated parvovirus antigen in the crypt epithelium and in adjacent inflammatory cells. The bacterial proliferation probably was secondary to the viral enteritis. Other lesions included acute thrombosis that could have been related to severe dehydration, and a moderately fatty liver.

Parvoviruses are among the smallest of the DNA viruses. Feline panleukopenia virus of the feline parvovirus subgroup was among the first to be discovered. Raccoon parvovirus and mink enteritis virus, also a parvovirus, are both members of this subgroup. Experimentally, raccoons are susceptible to both feline panleukopenia and mink enteritis virus infections but do not develop disease when inoculated with canine parvovirus-2. Canine parvovirus-2, a well known cause of diarrhea in dogs, is related to feline panleukopenia virus and probably was derived by mutations of panleukopenia virus or a closely related virus. However, canine parvovirus is distinguishable from the feline parvovirus subgroup by a number of physical characteristics.

The diagnosis of parvovirus in raccoons at SCWDS is a rare event. A review of SCWDS clinical records revealed only 12 other cases of parvovirus in 655 raccoon submissions received since 1976. All but one of the affected animals had been held in captivity for varying lengths of time. Seven were raccoons that were in rehabilitation centers and four had been captured and translocated to another site, at which time they developed fatal diarrhea. In all cases of parvovirus in captive raccoons, extensive morbidity and mortality occurred within the captive populations. Only one of the raccoons did not have a history of captivity for any length of time. That raccoon was killed by a hunter who submitted it to personnel of the Georgia Department of Natural Resources because it was in poor physical condition. (Prepared by Kevin Keel).

Yabsley New Assistant Professor; 3 Others Promoted

We are pleased to announce that Dr. Michael J. Yabsley has accepted an offer to fill the vacancy created by the recent retirement of Dr. William Randolph Davidson. Michael was one of Randy's graduate students and received his PhD degree in 2004. He has been on the SCWDS faculty as Assistant Research Scientist since September 2004. The position is a joint appointment from the University of Georgia's Daniel B. Warnell School of Forestry and Natural Resources (WSFNR) and the College of Veterinary Medicine. It is a tenure-track position with the title Assistant Professor of Wildlife Disease Ecology. Michael will be teaching multiple classes on wildlife diseases, including one for undergraduate students in the Forestry School. Michael will have his office at SCWDS

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and will continue to conduct most of his research in the SCWDS building. Michael has received numerous awards and other recognition during his academic career and is a competent, conscientious, diligent worker, and we are fortunate to have him on board. He will be an excellent asset to our organization. Congratulations Michael.

Along these same lines, we also are proud to report that three other SCWDS staff members recently were promoted. Our director Dr. John Fischer was promoted from Associate Professor to Professor and was awarded tenure; Dr. Joseph Corn was elevated from Public Service Assistant to Public Service Associate; and Dr. Danny Mead was advanced from Assistant Research Scientist to Associate Research Scientist. Heartiest congratulations to these three hardworking, deserving individuals. (Prepared by Gary Doster).

Gift From a Friend of SCWDS

We are very excited because an anonymous donor recently contributed $100,000 to the Southeastern Wildlife Health Development Fund! This fund was started several years ago to establish a funding base to help SCWDS achieve its long-term goals.

SCWDS is constantly vulnerable to budget upheavals in both state and federal governments, because we depend primarily on annual contracts from supporting state and federal agencies. This potential instability makes long-term scientific studies and personnel continuity more difficult. Large gifts can be placed in trust to provide sustained support. For example, the principal from our recent generous gift will be preserved, and interest will be accumulated and used to supplement the salary of a graduate student or faculty member or to support pilot research projects.

Our donor offers this gift as a challenge for other friends of SCWDS to contribute to the fund as we prepare to observe our 50th anniversary in 2007. Such contributions are vital to help SCWDS continue to serve wildlife resources and the agencies and individuals that manage them. Our ultimate goal is to use gifts to endow permanent SCWDS faculty and graduate student positions.

We thank our generous donor and encourage other SCWDS friends to consider responding to the challenge. We know that few can afford a gift of this size, but all contributions are appreciated. And, of course, all gifts are tax deductible. For more information please contact SCWDS Director Dr. John Fischer.

Information presented in this Newsletter is not intended for citation as scientific literature. Please contact the Southeastern Cooperative Wildlife Disease Study if citable information is needed.

Information on SCWDS and recent back issues of SCWDS BRIEFS can be accessed on the internet at www.scwds.org. The BRIEFS are posted on the web cite at least 10 days before copies are available via snail mail. If you prefer to read the BRIEFS on line, just send an email to Gary Doster (gdoster@vet.uga.edu) or Michael Yabsley (myabsley@uga.edu) and you will be informed each quarter when the latest issue is available.