Tracking a Deadly Virus
HIGHLY PATHOGENIC AVIAN INFLUENZA IN WILD BIRDS

By Tom DeLiberto and Gail Keirn

For Dennis Kohler, the call was somewhat unexpected. On a cold, windy morning in December 2014, Kohler sat in his Colorado office reviewing research plans for upcoming disease studies. A few hours later, Koehler’s colleagues at the National Wildlife Health Center in Madison, Wis., called to notify him and other members of the U.S. Department of Agriculture’s National Wildlife Disease Program (NWDP) that recent samples collected from wild birds in Washington State had tested positive for highly pathogenic avian influenza (HPAI).

Earlier that month, a die-off of mallards (Anas platyrhynchos), American wigeon (A. Americana) and northern pintails (A. acuta) had occurred on Wiser Lake in Whatcom County, Wash., just south of the Canadian border near the site of HPAI outbreaks in British Columbia. State biologists had collected samples for testing, and the analysis revealed the birds likely died of aspergillosis, a common fungal infection. But what was worrisome is that they also tested positive for the Eurasian H5 avian influenza virus, marking the first time a highly pathogenic Eurasian strain of avian influenza had been detected in the United States (Ip et al. 2015).

Kohler knew an outbreak of HPAI in domestic turkey and chicken flocks in Canada had led the state to conduct enhanced surveillance but was still a little surprised to learn that HPAI had been discovered in wild birds. The NWDP had been monitoring and preparing for HPAI in wild birds since the 2005 HPAI H5N1 scare in Southeast Asia that caused officials to kill hundreds of thousands of domestic poultry. Now the virus was confirmed in the U.S. and Kohler needed to mobilize a team of experts to respond.

At Kohler’s disposal was information gathered from almost a decade of disease surveillance and research that would now help guide the HPAI emergency response and strategic planning efforts. Its potentially devastating impact on wild and domestic birds would soon launch the largest animal health emergency response in U.S. history.

HPAI Surveillance in Wild Birds
Nearly 10 years earlier, NWDP and its state and federal partners designed, developed and conducted the largest-ever national avian influenza surveillance effort in wild birds. NWDP wildlife disease biologists and their partners collected more than 500,000 wild bird and environmental samples from across the U.S. in order to determine if the HPAI H5N1 strain that had been decimating domestic poultry flocks in Asia and Europe was making its way to this country. HPAI viruses cause high mortality in chickens and other poultry, and occasionally high mortality in certain species of wild birds.

At the time, no one had ever attempted to conduct such a comprehensive wildlife disease surveillance program. Although neither the H5N1 strain nor...
any other HPAI strain was found, NWDP, the U.S. Geological Survey, the U.S. Fish and Wildlife Service and state wildlife agency employees set the gold standard for such efforts. Their work resulted in the largest database of avian influenza samples in the United States. In the following years, scientists with the Wildlife Services’ National Wildlife Research Center and others tapped into the database to learn more about the prevalence and ecology of avian influenza and to develop more strategic surveillance plans.

A wildlife disease biologist for the NWDP for nine years, Kohler also serves as the emergency response coordinator for the program — tracking and training wildlife disease biologists to respond to wildlife emergencies ranging from oil spills to natural disasters to disease outbreaks. NWDP is part of USDA’s Animal and Plant Health Inspection Service’s Wildlife Services program. Since its inception in 2003, the program has hired and trained wildlife disease biologists to serve as first responders in wildlife-related emergencies. It also coordinates a national disease surveillance system in wildlife, conducts disease surveillance at international borders and establishes global partnerships to enhance wildlife disease surveillance worldwide. Through these efforts, Wildlife Services can quickly respond to wildlife emergencies at local, state and regional levels.

Outbreak

Fast forward to 2015 and the value of these efforts is clearly apparent. The spread of three strains of HPAI — H5N2, H5N8 and H5N1 — viruses originating from the Eurasian H5 strain in Washington State to commercial U.S. poultry and backyard flocks that spring ended up affecting nearly 50 million domestic birds, costing over $800 million and requiring the expertise of more than 600 APHIS employees and 2,700 contractors and state and federal partners.

As part of the response effort, NWDP and its state and federal collaborators leveraged their previous avian influenza experience and collected over 45,000 samples from outwardly healthy wild birds in targeted areas throughout the United States from July 2015 to March 2016. The researchers hypothesized that wild birds would serve as an early warning system for the detection of HPAI in commercial flocks. Their work was based on guidance provided by the Interagency Steering Committee for Surveillance for HPAI in Wild Birds, which included experts from APHIS’ Wildlife Services and Veterinary Services programs, USGS, USFWS, the Centers for Disease Control and Prevention and the National Flyway Council.

In order to make the 2015 effort as efficient and informative as possible, the team first analyzed waterfowl movement patterns and information from the 2006 – 2011 surveillance effort to determine the most appropriate species and sampling locations (Farnsworth et al. 2011). For instance, they looked at records assembled by NWRC researchers and partners that analyzed samples collected from 13,574 blue-winged teal (Anas discors) from 2007 – 2010 (Nallar et al. 2015). The results showed that during late summer (July – August) and fall migration (September – October), birds less than 1-year old were more likely to be infected.

Avian Influenza Primer

Avian influenza is a virus that infects wild birds such as ducks, gulls and shorebirds, and domestic poultry, including chickens, turkeys, ducks and geese. The viruses are classified by a combination of two groups of proteins: the hemagglutinin (H) proteins, of which there are 16 (H1–H16), and the neuraminidase (N) proteins, of which there are 9 (N1–N9). Strains also are divided into two groups based upon the ability of the virus to produce disease in poultry: low pathogenic avian influenza (LPAI) and highly pathogenic avian influenza (HPAI).

In 2015, USDA scientists identified HPAI Eurasian H5N8 and mixed-origin viruses, H5N2 and a novel H5N1, in birds of the Pacific Flyway. Another strain, the HPAI H5N2 virus, was confirmed in several states along three of the four North American Flyways: Pacific, Central and Mississippi. The novel HPAI H5N1 virus is not the same virus as the H5N1 virus previously found in Asia, Europe and Africa that has caused some human illness. Instead, it is a new mixed-origin virus.

With several different viruses circulating in wild birds, it is not unexpected that the birds harbored a new mixed-origin virus. Viruses continually mutate and form new combinations with genetic material from similar viruses. It is a main reason that continued surveillance efforts are necessary.

For more information, please see USDA Q&A: Biology of Avian Influenza and Recent Outbreaks, 2015.
with type A influenza than older birds. However, there was no difference between age groups for the remainder of the year — winter, spring migration and breeding period — likely due to younger birds’ maturing immune systems.

Another analysis of the 2006 – 2011 surveillance effort showed that dabbling ducks accounted for 92 percent of all avian influenza detections (Bevins et al. 2014). Because of these and other findings, the 2015 – 2016 effort focused mostly on sampling live-captured and hunter-harvested dabbling ducks, such as American black ducks (A. rubripes), American green-winged teal (A. carolinensis), blue-winged teal, mallards, American wigeon, northern shovelers (A. clypeata) and northern pintails. In addition, NWDP and its state and federal partners collected environmental fecal samples from waterfowl and samples from wild bird morbidity and mortality events. These surveillance results are currently being incorporated into national risk assessments and preparedness and response planning efforts to reduce HPAI risks in commercial and backyard poultry, wild and falconry birds, game-bird farms, wild bird rehabilitation facilities and captive bird collections at zoos and aviaries.

More HPAI Research
Avian influenza viruses are found naturally in waterfowl and other wild bird species. Research has also shown that several mammal species, such as the house mouse (Mus musculus), eastern cottontail rabbit (Sylvilagus floridanus) and raccoon (Procyon lotor) can be infected with avian influenza (Shriner et al. 2012, Root et al. 2014). Most of these viruses are native North American strains, which cause either no signs or only minor clinical signs of infection in birds and mammals. Unfortunately, low pathogenic H5 and H7 strains can mutate into high pathogenic viruses, so wildlife managers need to closely monitor these viruses.

The HPAI Eurasian H5 virus is a good example of the virus’ pathogenicity. It causes severe illness and death in domestic birds, raptors and commercial poultry. Because of the disease’s devastating impacts on both domestic and wild birds, NWRC researchers are studying how the HPAI viruses spread among species and the environment.

During the 2015 outbreak, NWRC researchers collected more than 2,600 samples from 426 birds and mammals found on 10 poultry farms in Iowa. Five of the farms were known to have HPAI.

Sampling focused on common species found on farms, such as house sparrows (Passer domesticus), European starlings (Sturnus vulgaris), rock pigeons (Columba livia), and house mice. But other species caught included barn swallows (Hirundo rustica), cliff swallows (Petrochelidon pyrrhonota), deer mice (Peromyscus spp.), eastern cottontail rabbits, northern short-tailed shrews (Blarina brevicauda) and raccoons. The researchers collected oral and external body swabs and/or cloacal or nasal swabs from each species and tested them for avian influenza virus. They also collected blood and tissue samples from a few select specimens for further analysis.

Of the 2,184 screened tissue samples and oral, cloacal and external swabs, only one tested positive for avian influenza viral RNA. The positive sample was from the lung tissue of a juvenile European starling captured on an infected farm using a mist net that targeted a cavity nest built on a walkway between two poultry barns. Of the 252 blood samples taken from birds, only seven tested positive for exposure to influenza A virus in initial screenings and were sent for further testing at the USDA’s National Veterinary Services Laboratories in Ames, Iowa. This additional testing indicated that five samples — two American robins (Turdus migratorius), two European starlings and one house sparrow — were antibody positive for the HPAI Eurasian H5 virus.
Guided by the emergency response priorities set by USDA’s HPAI Incident Coordination Team, NWRC Research Biologist Susan Shiner and others conducted the majority of the sampling on the infected farms after the poultry had been removed, making the likelihood of detecting the virus much lower than sampling conducted around live poultry. Evidence of exposure to HPAI Eurasian H5 virus in American robins, European starlings and house sparrows suggests the need for further studies with these species.

The low number of positive results does not exclude wildlife as a potential biosecurity threat. Scientists continue to study how the virus is spreading from farm to farm. Hopefully, results from other studies will shed more light on this complex disease issue.

Although it is possible for domestic poultry to become infected with avian influenza from direct contact with wild birds and possibly other wildlife, it is more likely that the viruses are spread indirectly to poultry on contaminated food, clothing and equipment. Consequently, the USDA emphasizes that exercising thorough and consistent biosecurity practices across the entire poultry industry is crucial to preventing the spread of avian influenza viruses to poultry farms.

**Jumping to Other Species?**

For the past several years, NWRC scientists also have conducted studies with captive animals to learn more about the spread of avian influenza within and between species. For instance, studies have investigated how long and how much virus waterfowl shed (VanDalen et al. 2010) and whether previous infections affect viral shedding if birds are reinfected (Pepin et al. 2012). Others have examined the ecology and prevalence of native North American avian influenza viruses across the United States (Piaggio et al. 2012). This information is being used to identify “hotspots” where the virus has the highest chance of spreading from the environment into domestic poultry.

More recent studies have shown that experimentally infected striped skunks and eastern cottontail rabbits shed large amounts of native avian influenza viral RNA (Root et al. 2015a) and can indirectly transmit LPAI to mallards (Root et al. 2015b). Scientists also have found that captive raccoons are more likely to become infected with avian influenza from contaminated water versus eating contaminated animals or eggs (Root et al. 2014b). Insights about how wild animals carry and move the viruses are helpful for developing better biosecurity methods for domestic poultry facilities.

**Next Steps**

In May 2015, NWDP wildlife disease biologists responded to an outbreak of LPAI in domestic poultry in Missouri. As with the Washington State effort, they are conducting enhanced wild bird surveillance in the outbreak area. The program also plans to continue wild bird surveillance throughout the country until at least the spring of 2017.

Research is also ongoing at the National Wildlife Resource Center. Scientists are focusing on understanding primary influenza virus transmission routes in natural hosts, identifying wildlife species that might contribute to intercontinental HPAI spread and evaluating the role that wildlife species living near people play in moving HPAI viruses within and between farms.

Today, Kohler is no longer surprised when a call comes in about the discovery of avian influenza in wild birds. Each new sample and discovery helps the NWDP team and its collaborators not only protect domestic poultry and people, but also wild birds.