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High Rates of Detection of Clade 2.3.4.4 Highly Pathogenic Avian Influenza H5 Viruses in Wild Birds in the Pacific Northwest During the Winter of 2014–15

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SUMMARY. In 2014, clade 2.3.4.4 H5N8 highly pathogenic avian influenza (HPAI) viruses spread across the Republic of Korea and ultimately were reported in China, Japan, Russia, and Europe. Mortality associated with a reassortant HPAI H5N2 virus was detected in poultry farms in western Canada at the end of November. The same strain (with identical genetic structure) was then detected in free-living wild birds that had died prior to December 8, 2014, of unrelated causes in Whatcom County, Washington, U. S. A., in an area contiguous with the index Canadian location. A gyrfalcon (*Falco rusticolus*) that had hunted and fed on an American wigeon (*Anas americana*) on December 6, 2014, in the same area, and died 2 days later, tested positive for the Eurasian-origin HPAI H5N8. Subsequently, an active surveillance program using hunter-harvested waterfowl in Washington and Oregon detected 10 HPAI H5 viruses, of three different subtypes (four H5N2, three H5N8, and three H5N1) with four segments in common (HA, PB2, NP, and MA). In addition, a mortality-based passive surveillance program detected 18 HPAI (14 H5N2 and four H5N8) cases from Idaho, Kansas, Oregon, Minnesota, Montana, Washington, and Wisconsin. Comparatively, mortality-based passive surveillance appears to have detected these HPAI infections at a higher rate than active surveillance during the period following initial introduction into the United States.

RESUMEN. Altas tasas de detección del virus de influenza aviar altamente patógeno H5 clado 2.3.4.4 en aves silvestres en la parte noroeste del Pacífico durante el invierno 2014-15.

En 2014, los virus de influenza aviar altamente patógenos H5N8 clado 2.3.4.4 se diseminaron a través de la República de Corea y posteriormente, se reportaron en China, Japón, Rusia y Europa. Se detectó mortalidad asociada con un virus reacomodado altamente patógeno de influenza aviar H5N2 en granjas avícolas en el oeste de Canadá a finales de noviembre. Se detectó entonces la misma cepa (con estructura genética idéntica) en aves silvestres de vida libre que habían muerto antes del 8 de diciembre del 2014 por causas no relacionadas en el Condado de Whatcom, Washington, en los Estados Unidos, en una zona contigua con la ubicación del caso índice en Canadá. Un halcón gerifalte (*Falco rusticolus*) que había cazado y se había alimentado de un silbón americano (*Anas americana*) el 6 de diciembre del 2014, en la misma zona, y que murió dos días después, resultó positivo a la presencia del virus de alta patogenicidad de origen euroasiático H5N8. Posteriormente, un programa de vigilancia activa basado en el muestreo de aves acuáticas cazadas y recolectadas en Washington y Oregón detectó diez virus de influenza aviar altamente patógena H5 de tres subtipos diferentes (cuatro del subtipo H5N2, tres del subtipo H5N8 y tres subtipo H5N1) con cuatro segmentos en común (HA, PB2, NP, y MA). Además, mediante un programa de vigilancia pasiva basado en el muestreo de aves muertas se detectaron 18 virus de influenza aviar de alta patogenicidad (catorce subtipo H5N2 y cuatro H5N8) en Idaho, Kansas, Oregón, Minnesota, Montana, Washington y Wisconsin. Comparativamente, la vigilancia pasiva basada en la mortalidad parece haber detectado estas infecciones del virus de influenza de alta patogenicidad en un porcentaje mayor en comparación con la vigilancia activa durante este período después de la introducción inicial en los Estados Unidos.

Key words: avian influenza, intercontinental transmission, wild, migratory birds, surveillance strategy, HPAIV, H5Nx

Abbreviations: ASP = active surveillance program; HPAI = highly pathogenic avian influenza; NWHC = U.S. Geological Survey National Wildlife Health Center; PSP = passive surveillance program

Clade 2.3.4.4 H5N8 (hereafter H5N8) highly pathogenic avian influenza (HPAI) viruses first emerged from reassortment with HPAI A/goose/Guangdong/1/1996 H5N1 lineage viruses in China in 2010 (20). Reassortants were detected in China in 2013 (3) and in the Republic of Korea in 2014 (11). In 2014, two additional reassortants (both of the subtype H5N8) were identified in the area around Donglim Reservoir, Gochang Province, Republic of Korea, one of which was associated with significant mortalities in Baikal teal (*Anas formosa*) and with outbreaks at nearby poultry farms (8). Subsequently, the virus spread to over 200 farms in six provinces and was found in eight species of wild birds (8). This same virus was also detected in Japan in April 2014 (9), and in Liaoning, China, and Sakha (Yakutia), Russia, in September 2014 (12). Beginning in November 2014, the H5N8 virus was detected during poultry outbreaks in Germany, Hungary, Italy, the Netherlands, United Kingdom, Sweden, and again in Japan and South Korea (1,4,5,10,12). The virus was also recovered from apparently healthy wild birds (or their feces) in Germany and the Netherlands, and in sick or dead mallards (*Anas platyrhynchos*), white-naped cranes (*Grus vipio*), and hooded (*Grus monacha*) cranes in Japan (19).

The long-distance spread of H5N8 from Asia into Central Asia and Europe raised the possibility of transmission by migratory birds (18) and elevated this possibility when a reassortant HPAI H5N2 (hereafter H5N2) virus was detected in poultry from two farms in the Fraser

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Valley of British Columbia, Canada (14). Increased surveillance was conducted in the United States, initially in the area adjacent to the Fraser Valley (Whatcom County, Washington). A summary of the initial discovery of H5N2 in free-living wild birds that died and the first North American detection of H5N8 from captive raptors that had fed on an American wigeon (*Anas americana*) carcass has already been described (7).

The detection of H5N2/H5N8 in wild birds in Washington and the detection of H5N2 in poultry in adjacent British Columbia raised questions as to the threat posed by the introduction of these HPAI viruses. Immediately following these detections, we initiated an active surveillance program (ASP) consisting of the sampling of hunter-harvested wild birds in northern Oregon and Washington, as well as requested increased submissions of dead birds nationwide for diagnostic evaluation and HPAI testing (especially raptors and waterfowl) to our diagnostic laboratory at the U.S. Geological Survey National Wildlife Health Center (NWHC; passive surveillance program [PSP]). In this study, we report results from these separate surveillance efforts and compare results from the ASP and PSP. We also compare ASP results for H5 positives obtained in 2014–15 with results obtained from previous surveillance conducted in the western United States.

MATERIALS AND METHODS

ASP sampling was performed using opportunistically sampled hunter-harvested wild birds in selected areas in the states of Washington and Oregon. Heavily hunted waterfowl areas were selected for sampling. Combined oropharyngeal and cloacal swab samples were collected from ASP birds and submitted for virologic testing (6). All samples were from birds shot between December 22, 2014, and January 11, 2015.

PSP surveillance was conducted using carcasses from avian mortality events submitted to the NWHC from December 1, 2014, to April 30, 2015. All birds were tested for the presence of avian influenza regardless of postmortem findings. Submission criteria included receiving specimens from: mortality events of any size involving waterfowl, other water birds, raptors, or avian scavengers; mortality events involving any avian species with an aggregate total of more than 500 dead birds; mortality events in any avian species that occurred in close proximity to poultry operations; or mortality events associated with captive birds that were imported from countries where HPAI viruses were known to occur. In additional to these, captive birds, particularly raptors, that died following being fed meat obtained from wild hunted birds were also included in the PSP. Cloacal and tracheal swabs were collected from each submitted carcass and tested separately (6).

Samples were tested for avian influenza using the current National Animal Health Laboratory Network procedures. RNA was extracted using the MagMax™ Viral AI/ND RNA isolation kit (Ambion, Carlsbad, CA) and tested for presence of the influenza A virus matrix gene, and matrix reverse-transcriptase-polymerase chain reaction (RT-PCR)–positive samples were tested for H5 according to the methods of Spackman *et al.* (16). H5-positive samples were sent to National Veterinary Services Laboratories in Ames, IA, for confirmation and pathogenicity characterization by sequencing of the protease cleavage site or experimental infection as per World Organisation for Animal Health (OIE) guidelines (13).

RESULTS

During the ASP, 1231 hunter-harvested wild bird samples from the states of Washington and Oregon were collected between December 22, 2014, and January 11, 2015. Samples originated from 26 different species, primarily ducks. Mallards (45.2%), American wigeon (17.5%), and northern pintail (*Anas acuta*; 11%) made up 73.7% of the collection (Table 1). The overall prevalence of avian influenza as indicated by matrix RT-PCR was 14.0%, and individual viral prevalence among these three species was 14.9%, 16.7%, and 11.9%, respectively. Twenty-one of 172 matrix-positive samples were also

Table 1. Species distribution and avian influenza status of ASP samples by species common name (scientific name); species in bold indicate HPAI H5 detected.

Species	M[A]	H5[B]	H7[C]	Neg[D]	Total[E]	% MA pos[F]
American coot (*Fulica americana*)	1			1	2	50.0
American wigeon (*Anas americana*)	**36**	**6**		**179**	**215**	**16.7**
Bufflehead (*Bucephala albeola*)	2			26	28	7.1
Cackling goose (*Branta hutchinsii*)	3			30	33	9.1
Canada goose (*Branta canadensis*)	0			26	26	—
Canvasback (*Aythya valisineria*)	1			7	8	12.5
Cinnamon teal (*Anas cyanoptera*)	1			3	4	25.0
Common goldeneye (*Bucephala clangula*)	0			3	3	—
Crow, unspecified	1			2	3	33.3
Gadwall (*Anas strepera*)	0			16	16	—
Glaucous-winged gull (*Larus glaucescens*)	0			1	1	—
Green-winged teal (*Anas crecca*)	**8**	**1**		**48**	**56**	**14.3**
Hooded merganser (*Lophodytes cucullatus*)	0			2	2	—
Lesser scaup (*Aythya affinis*)	2			8	10	20.0
Mallard (*Anas platyrhynchos*)	**83**	**11**		**474**	**557**	**14.9**
Northern pintail (*Anas acuta*)	**16**	**3**		**119**	**135**	**11.9**
Northern shoveler (*Anas clypeata*)	13		3	39	52	25.0
Pine-billed grebe (*Podilymbus podiceps*)	0			1	1	—
Red-breasted merganser (*Mergus serrator*)	0			1	1	—
Redhead (*Aythya americana*)	0			3	3	—
Ring-necked duck (*Aythya collaris*)	4			41	45	8.9
Ruddy duck (*Oxyura jamaicensis*)	0			2	2	—
Snow goose (*Chen caerulescens*)	0			16	16	—
Surf scoter (*Melanitta perspicillata*)	0			2	2	—
White-winged scoter (*Melanitta fusca*)	0			3	3	—
Wood duck (*Aix sponsa*)	1			6	7	14.3
Summary	172	21	3	1059	1231	14.0

[A]M = number of matrix RT-PCR positives.
[B]H5 = number of H5 RT-PCR positives.
[C]H7 = number of H7 RT-PCR positives.
[D]Neg = number of matrix RT-PCR negatives.
[E]Total = total number of birds of each species sampled.
[F]% MA pos = the percentage of samples positive by matrix RT-PCR.

Table 2. The species distribution and avian influenza status of PSP samples by species common name (scientific name); species in bold indicate species found to be positive for HPAI H5.

Species	M[A]	H5[B]	Neg[C]	Total[D]	% MA pos[E]
Aleutian Canada goose (*Branta hutchinsii leucopareia*)	0		1	1	—
American coot (*Fulica americana*)	7		21	28	25.0
American crow (*Corvus brachyrhynchos*)	0		8	8	—
American robin (*Turdus migratorius*)	0		2	2	—
American white pelican (*Pelecanus erythrorhynchos*)	0		1	1	—
American wigeon (*Anas americana*)	**5**	**2**	22	**27**	**18.5**
Bald eagle (*Haliaeetus leucocephalus*)	**3**	**1**	31	**34**	**8.8**
Barn owl (*Tyto alba*)	0		4	4	—
Barred owl (*Strix varia*)	1		3	4	50.0
Barrow's goldeneye (*Bucephala islandica*)	0		1	1	—
Black vulture (*Coragyps atratus*)	0		4	4	—
Bufflehead (*Bucephala albeola*)	0		3	3	—
Cackling goose (*Branta hutchinsii*)	0		2	2	—
California gull (*Larus californicus*)	0		3	3	—
Canada goose (*Branta canadensis*)	**3**	**2**	7	**10**	**30.0**
Canvasback (*Aythya valisineria*)	0		3	3	—
Cassin's auklet (*Ptychoramphus aleuticus*)	0		7	7	—
Common gallinule (*Gallinula galeata*)	0		5	5	—
Common goldeneye (*Bucephala clangula*)	0		8	8	—
Common grackle (*Quiscalus quiscula*)	0		1	1	—
Common loon (*Gavia immer*)	0		4	4	—
Common murre (*Uria aalge*)	0		6	6	—
Common poorwill (*Phalaenoptilus nuttallii*)	0		3	3	—
Cooper's hawk (*Accipiter cooperii*)	**4**	**2**	6	**10**	**40.0**
Crow, unspecified	0		1	1	—
Double-crested cormorant (*Phalacrocorax auritus*)	0		6	6	—
Eared grebe (*Podiceps nigricollis*)	0		2	2	—
Eurasian collared-dove (*Streptopelia decaocto*)	0		1	1	—
European starling (*Sturnus vulgaris*)	0		1	1	—
Ferruginous hawk (*Buteo regalis*)	0		1	1	—
Gadwall (*Anas strepera*)	0		6	6	—
Glaucous-winged gull (*Larus glaucescens*)	0		2	2	—
Golden eagle (*Aquila chrysaetos*)	0		5	5	—
Great horned owl (*Bubo virginianus*)	**1**	**1**	4	**5**	**20.0**
Greater white-fronted goose (*Anser albifrons*)	0		3	3	—
Green-winged teal (*Anas crecca*)	1		1	2	50.0
Gyrfalcon (*Falco rusticolus*)	**2**	**2**	0	**2**	**100.0**
Gyrfalcon hybrid	**1**	**1**	0	**1**	**100.0**
Hooded merganser (*Lophodytes cucullatus*)	0		1	1	—
Horned grebe (*Podiceps auritus*)	0		1	1	—
Lesser scaup (*Aythya affinis*)	0		11	11	—
Lesser snow goose (*Chen caerulescens caerulescens*)	0		8	8	—
Long-eared owl (*Asio otus*)	0		4	4	—
Mallard (*Anas platyrhynchos*)	**12**	**4**	82	**94**	**12.8**
Marbled murrelet (*Brachyramphus marmoratus*)	0		2	2	—
Mew gull (*Larus canus*)	0		2	2	—
Mississippi sandhill crane (*Grus canadensis pulla*)	0		1	1	—
Mourning dove (*Zenaida macroura*)	0		2	2	—
Mute swan (*Cygnus olor*)	1		4	5	20.0
Northern goshawk (*Accipiter gentilis*)	0		1	1	—
Northern pintail (*Anas acuta*)	**2**	**1**	14	**16**	**12.5**
Northern shoveler (*Anas clypeata*)	1		3	4	25.0
Peregrine falcon (*Falco peregrinus*)	**2**	**2**	1	**3**	**66.7**
Red-breasted merganser (*Mergus serrator*)	0		1	1	—
Red-tailed hawk (*Buteo jamaicensis*)	**3**	**2**	9	**12**	**25.0**
Redhead (*Aythya americana*)	0		1	1	—
Ring-necked duck (*Aythya collaris*)	0		3	3	—
Ring-necked pheasant (*Phasianus colchicus*)	1		1	2	50.0
Ross's goose (*Chen rossii*)	2		9	11	18.2
Ruddy duck (*Oxyura jamaicensis*)	0		14	14	—
Rusty blackbird (*Euphagus carolinus*)	0		3	3	—
Sandhill crane (*Grus canadensis*)	1		2	3	33.3
Sharp-shinned hawk (*Accipiter striatus*)	0		6	6	—
Snow goose (*Chen caerulescens*)	0		30	30	—
Snowy owl (*Bubo scandiacus*)	**1**	**1**	2	**3**	**33.3**
Steller's jay (*Cyanocitta stelleri*)	0		2	2	—
Surf scoter (*Melanitta perspicillata*)	0		1	1	—
Trumpeter swan (*Cygnus buccinator*)	10		38	48	21.3
Tundra swan (*Cygnus columbianus columbianus*)	0		8	8	—
Varied thrush (*Ixoreus naevius*)	2		3	5	40.0
Whooping crane (*Grus americana*)	0		2	2	—
Wild turkey (*Meleagris gallopavo*)	0		3	3	—
Summary	66	21	464	530	4.0

[A]M = number of matrix RT-PCR positives.
[B]H5 = number of H5 RT-PCR positives.
[C]Neg = number of matrix RT-PCR negatives.
[D]Total = total number of birds of each noted species sampled.
[E]% MA pos = the percentage of samples positive by matrix RT-PCR.

positive for H5 by RT-PCR (12.2%). H5-positive species included the same three species that constituted the majority of the sample but also included one green-winged teal (*Anas crecca*). The prevalence of H5 among matrix-positive samples was: northern pintail, 18.8% (3/16); American wigeon, 16.7% (6/36); mallard, 13.3% (11/83); and green-winged teal, 12.5% (1/8). H7 was detected in just three northern shovelers (*Anas clypeata*). We were only able to characterize

11 of the 21 H5-positive samples. One, from a northern pintail, had a predicted amino-acid sequence at the hemagglutinin protease cleavage site characteristic of low pathogenic avian H5. Ten H5 viruses belonging to HPAI H5 clade 2.3.4.4 were identified and included three H5N8, four H5N2, and three H5N1 (17). Taken together, the ASP identified three different HPAI viruses cocirculating in four species of apparently healthy waterfowl and provided early evidence of the spread of the viruses in Washington and adjacent Oregon beyond Whatcom County, Washington.

The PSP resulted in a total of 536 dead birds submitted to the NWHC for avian influenza testing between December 2014 and April 2015. These submissions represented 72 species from 33 states, and 66 (12.5%) of the carcasses were positive for avian influenza as indicated by matrix RT-PCR (Table 2). Twenty-one of 66 (31.8%) matrix-positive samples were H5 positive, and 18 HPAI H5 viruses were further characterized as H5N8 ($n = 4$) and H5N2 ($n = 14$). All were genetically similar to the viruses identified by ASP, and the viruses identified by PSP stemmed from 11 species (Table 2) that originated from seven states, including Idaho, Kansas, Missouri, Minnesota, Montana, Washington, and Wisconsin (data not shown). Consistent with the initial detection of HPAI in gyrfalcons (*Falco rusticolus*), 7 of 11 species determined to harbor HPAI by PSP were raptors, including a bald eagle (*Haliaeetus leucocephalus*), a Cooper's hawk (*Accipiter cooperii*), three captive gyrfalcon/gyrfalcon hybrids, one wild and one captive peregrine falcon (*Falco peregrinus*), two red-tailed hawks (*Buteo jamaicensis*), and a snowy owl (*Bubo scandiacus*). A captive great horned owl (*Bubo virginianus*), which was a long-time resident in a rehabilitation facility, was also infected and had been fed part of a hunter-shot mallard carcass according to treatment records. In contrast to the lack of clinical signs in Anseriformes, the raptors that were examined all exhibited multifocal hemorrhage and necrosis in multiple tissues, similar to the index gryfalcon (7). Overall, PSP facilitated the first detections of H5Nx in two states, in nine counties, as well as the earliest wild-bird detections of H5Nx in Kansas, Missouri, Minnesota, and Wisconsin.

Table 3. Summary of ASP and PSP RT-PCR results and HPAI virus identification.

Categories	Active[D]	Active[E] (%)	Passive[F]	Passive[G] (%)
M[A]	172	14.0	66	12.5
H5[B]	21	1.7	21	4.0
HPAI H5[C]	10	0.8	18	3.4
Total	1231		530	

[A]M = number of matrix RT-PCR positives.
[B]H5 = number of H5 RT-PCR positives.
[C]HPAI H5 = number of highly pathogenic H5 viruses identified.
[D]Active = active surveillance program.
[E]Active (%) = proportion of positives in the ASP in the same categories.
[F]Passive = passive surveillance program.
[G]Passive (%) = proportion of positives in the PSP in the same categories.

DISCUSSION

In late 2014, the first-ever introduction of HPAI occurred in North America. Surprisingly, the initial detection in Canada was a reassortant H5N2 virus that retained its ability to be pathogenic to poultry (14). In the United States, the reassortant H5N2 virus and the entirely Eurasian-origin H5N8 virus were detected in birds submitted as part of a national PSP (15). Upon discovery of the introduction of HPAI and the possible role of migratory birds in its introduction and spread, an ASP was conducted in Washington and Oregon, and the PSP was enhanced nationwide. The ASP identified an additional reassortant virus, H5N1, that was cocirculating in wild birds (18). Through initial PSP and ASP, at least four species of apparently healthy waterfowl were found to be infected with H5Nx, providing early evidence of spread beyond the area adjacent to the Canadian affected zone, including into adjacent Oregon.

Since that time, the viruses, especially H5N2, have spread into multiple flyways. The PSP, which covers a broad geographic area and is species neutral, has identified 12 additional H5Nx viruses and provided the first detections of HPAI in two states and in nine counties. Moreover, the PSP highlighted that raptors, as a group, appear to be particularly susceptible to H5Nx, as 11 of 18 of the isolates originated from hawks, eagles, and owls. Overall, raptors accounted for 17.7% (94/530) of the PSP sample but 52.4% (11/21) of the H5 positives; all H5 detections in raptors were HPAI. Of note, two Canada geese (*Branta canadensis*) that were reported to have exhibited neurologic signs before death were analyzed through the PSP, and H5N2 was isolated from both of these birds, indicating that Canada goose may be one of the few Anseriformes species that exhibits clinical signs of HPAI infection. In contrast to the PSP, the ASP identified HPAI H5Nx in apparently healthy hunter-harvested waterfowl, providing evidence that wild ducks can survive infection with these viruses. By sampling large numbers of birds, a better understanding of the prevalence of these viruses across the landscape can be achieved. In addition, this strategy also identified a novel reassortant not seen in the PSP.

In contrast to the spread of H5N8 and several reassortants following introduction to North America, the introduction of H5N8 to Europe was associated with very few wild birds (5,19). Sampling of over 4018 wild birds in the Netherlands detected only two examples of H5N8 (19). In contrast, the ASP in Washington and northern Oregon described here revealed an H5 prevalence rate of 1.7% (Table 3). This is a much higher proportion of H5 infection in wild birds when compared to the 2006–07 surveillance program in the Pacific Flyway, in which 0.41% (83/20371) of wild bird samples were H5 positive, all of which were low pathogenic viruses (2). In contrast, the prevalence of HPAI H5Nx viruses as identified by the 2014–15 ASP (0.8%) was approximately double the H5 prevalence rate in the same region in 2006–07.

Finally, we propose that the substantially higher detection rate of HPAI H5 from the PSP (3.4%, $n = 18/530$) compared to the ASP (0.8%, $n = 10/1231$) indicates that PSP can facilitate efficient and cost-effective detection of HPAI in wild birds (Table 3). We acknowledge that a successful PSP requires adequate reporting of sick and dead wild birds, and, in the present case, a sampling period that coincided (in hindsight) with active HPAI transmission in wild birds. Furthermore, while the majority of waterfowl do not exhibit overt field signs of HPAI infection, the PSP program suggests that sufficient numbers of susceptible apex predators (i.e., raptors) succumb to infection so as to compensate and facilitate detection. In addition to the utility of the PSP in the timely detection of HPAI in wild birds, national wild-bird morbidity and mortality surveillance provides a geographic- and species-independent strategy for early detection of other disease introduction events where *a priori* knowledge of species susceptibility may be lacking. However, with either strategy, some information will be missed, and a full understanding of the goals of surveillance along with the strategies to meet those goal should be thoroughly evaluated prior to the start of any program.

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