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Low Pathogenicity Avian Influenza Subtypes Isolated from Wild Birds in the United States, 2006–2008

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SUMMARY. Due to concerns that high pathogenicity avian influenza would enter into the United States, an interagency strategic plan was developed to conduct surveillance in wild birds in order to address one of the possible pathways of entry. The USDA and state wildlife agencies participated in this effort by collecting samples from 145,055 wild birds from April 2006 through March 2008 in all 50 states. The majority (59%) of all wild bird samples was collected from dabbling ducks, and 91% of H5 detections using real-time reverse transcriptase polymerase chain reaction (rRT-PCR) were in dabbling ducks. Apparent prevalence of H5 by rRT-PCR in all birds sampled was 0.38%. Most (48%) H5 detections were found in mallards (*Anas platyrhynchos*). Thirty-three virus subtypes were identified; H5N2 was the most prevalent subtype and accounted for 40% of all virus isolations. We present the virus subtypes obtained from the national surveillance effort and compare them with research results published from various countries.

RESUMEN. Subtipos del virus de la influenza aviar de baja patogenicidad aislados de aves silvestres en los Estados Unidos.

Como respuesta a la preocupación de que el virus de la influenza aviar de alta patogenicidad pudiera aparecer en los Estados Unidos, se desarrolló un plan estratégico entre diferentes agencias para realizar muestreos de vigilancia en aves silvestres con el fin de estudiar una de las posibles vías de entrada. El Departamento de Agricultura de los Estados Unidos (con las siglas en inglés USDA) y las agencias estatales para la vida silvestre participaron en este proyecto mediante la recolección de muestras de 145,055 aves silvestres de Abril del 2006 a Marzo del 2008 en los 50 estados. De todas las muestras de aves silvestres, la mayoría (59%) se recolectaron de patos chapoteadotes y el 91% de los virus H5 detectados mediante la transcripción reversa y reacción en cadena de la polimerasa en tiempo real (rRT-PCR) fueron en patos chapoteadotes. La prevalencia aparente en todas las aves del subtipo H5 detectado por rRT-PCR fue del 0.38%. La mayoría de los virus H5 detectados (48%) se detectaron en patos de collar (*Anas platyrhynchos*). Treinta y tres subtipos fueron identificados, el subtipo H5N2 fue el más prevalente, ya que se detectó en el 40% de los aislamientos virales. En este estudio se presentan los subtipos virales obtenidos durante un muestreo de vigilancia a nivel nacional y se comparan estos resultados con los publicados en varios países.

Key words: avian influenza, mallards, subtypes, surveillance, virus isolation, wild birds

Abbreviations: BHI = brain heart infusion; HPAI = high pathogenicity avian influenza; LPAI = low pathogenicity avian influenza; NAHLN = National Animal Health Laboratory Network; NVSL = National Veterinary Services Laboratories; rRT-PCR = real-time reverse transcriptase–polymerase chain reaction; SPF = specific-pathogen-free; USDA = United States Department of Agriculture

An outbreak of high pathogenicity avian influenza (HPAI) H5N1 at Qinghai Lake in western China in 2005 was responsible for a large die-off of migratory waterbirds, primarily involving bar-headed geese (*Anser indicus*), in an area with no poultry (6). Prior to this event, it had been thought that transmission occurred only in wild birds within the vicinity of poultry. This event prompted the United States to assess potential impacts of an HPAI introduction to the U.S. economy and poultry industry and resulted in the development of an aggressive strategic plan to address the possibility. Additionally, a poultry outbreak of HPAI H5N1 was implicated in the death of a 3-year-old boy in Hong Kong in 1997 (7), adding attention and concern regarding the possibility of transmission of HPAI from birds to humans.

Low pathogenicity avian influenza (LPAI) viruses cycle naturally through waterfowl (Anseriformes) and shorebird (Charadriiformes) populations, and these populations of wild birds are considered to be natural reservoirs for the low pathogenicity form of avian influenza (1,38). LPAI is distinguishable from HPAI based on its ability to cause disease in chickens. HPAI infection in wild birds also usually results in morbidity or mortality (29); however, recent literature has documented mallards (*Anas platyrhynchos*) (18), northern pintails (*Anas acuta*), blue-winged teal (*Anas crecca*), redheads (*Aythya americana*) (4), Eurasian wigeon (*Anas penelope*), and gadwall (*Anas strepera*) (15) as being able to contract and shed HPAI virus without exhibiting clinical signs.

An introduction of an HPAI virus into the United States could potentially decimate the $23.3 billion poultry industry by affecting international trade, consumer confidence, and costs associated with eradication. Although HPAI viruses do not currently exist in North America, the possibility that HPAI H5N1 could enter the country via poultry trade, illegal wild bird trade, or migrating birds stimulated an interest to develop a proactive plan for detection. To address the possibility of entry into the United States via wild birds, the U.S. Department of Agriculture (USDA) codeveloped an interagency strategic plan to standardize the collection, processing, and testing of wild bird samples (37). The primary objective of the national surveillance plan was to provide a framework for an early detection system in wild birds which could be modified and adjusted to particular flyway needs and state-specific plans. A secondary objective was to gain additional knowledge regarding LPAI viruses in wild birds in the United States to help better protect the U.S. poultry industry and economy.

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MATERIALS AND METHODS

Species prioritization. Waterfowl and shorebirds were targeted for surveillance. Other wild bird species considered at risk of contracting HPAI, based on species-specific migratory paths, were also considered and included in sampling strategies, when appropriate. During the second year of surveillance (April 2007 through March 2008), targeted surveillance was enhanced by placing a greater emphasis on species that tested positive for LPAI H5 and H7 subtypes during the first year of surveillance. Response to morbidity or mortality events, regardless of size or species, was a focus throughout both years.

Sample size determination. All reported morbidity–mortality events were investigated and sampled when possible. National sample sizes for all other techniques combined was determined based on detecting virus at a prevalence of 0.1% with a 99% confidence interval, a test sensitivity of 73.4%, and a specificity of 99.8% (35). It was calculated based on the North American population of ducks (approximately 41 million), and geese and swans (12 million), for a target sample size of 94,836 samples nationwide. Sample sizes were allocated to each state based on historic LPAI disease prevalence, species-specific migratory pathways, geographic size and location of the state, wetland habitat, linear distance of shoreline and, eventually, band recovery data. Once sample numbers were determined for each state, local expertise from wildlife veterinarians and waterfowl biologists was used to refine priority species and sampling locations within each state. The target number of samples for the first year was 75,000, which was decreased to 50,000 samples the following year. During the second year of surveillance, collectors were encouraged to increase target sampling of select species and to sample in areas considered the most likely places for introduction of the virus.

Sample collection. The USDA Wildlife Services, and state and Tribal wildlife agencies, collected samples using standardized protocols and procedures (36). Sample media and sampling kits were prepared and distributed by the USDA National Veterinary Services Laboratories (NVSL) in Ames, Iowa. The transport media, brain heart infusion (BHI; Becton Dickinson and Co., Sparks, MD), was distributed in glass vials with 3 ml of BHI. Media was stored in a refrigerator if it had melted during shipping, or in a freezer at −20 C if the media remained frozen during distribution. Once the media thawed, it was not refrozen.

During the first year of sample collection (April 2006 through March 2007), one cloacal sample was collected from each bird using sterile dacron-tipped swabs (Puritan®, Puritan Medical Products Company LLC, Guilford, ME) and placed in the transport media. For the second year of surveillance (April 2007 through March 2008), one cloacal and one oropharyngeal swab were collected from each bird and combined in the same vial of BHI media. Swabs were left in the sample vial after collection during both years.

Four collection strategies were identified for collecting samples directly from wild birds. The live wild bird collection strategy was used to refer to the use of rocket nets, cannon nets, mist nets, swim-in traps, or any other type of trap where a bird was captured, sampled, and released. Hunter harvest was used to refer to samples collected opportunistically from birds killed by hunters, or by other intentional means. Sentinel species were used in areas where they had the potential to commingle with infected wild birds (37). Investigation of morbidity–mortality events was the collection strategy used to collect samples from sick or dead birds. For morbidity–mortality events, one cloacal and one tracheal sample was collected from each bird and placed in separate vials.

Laboratory procedures. Samples were stored in coolers with ice immediately after collection, transferred to refrigerators, and usually shipped to testing laboratories within 24 hr. Samples were sent for testing to one of 42 diagnostic laboratories that are part of the National Animal Health Laboratory Network (NAHLN). Each sample was screened for type A influenza at an NAHLN laboratory with the matrix real-time reverse transcriptase–polymerase chain reaction (rRT-PCR) assay (25). During the first year, cloacal samples were tested in pools of up to five samples, whereas during the second year, combined oropharyngeal–cloacal samples were tested individually. If the matrix assay was positive for type A influenza, subtyping was conducted with modified H5- and H7-specific rRT-PCR assays (25). No further testing was conducted on samples that tested negative with the matrix assay. Diagnostic testing at the NAHLN laboratories was completed within 48 hrs of receipt of samples. All samples screened as H5 or H7 positive were shipped to the NVSL for additional rRT-PCR testing, virus isolation, subtyping, and pathogencity testing.

Table 1. Distribution of wild bird samples collected from March 2006 through April 2008, by functional group (n = 145,055).

Functional group	Percent of total samples
Dabbling ducks	59
Diving ducks	7
Perching ducks	3
Geese and swans	15
Gulls, terns, and alcids	4
Shorebirds	9
Other	3

Virus isolation was also conducted on all samples sent to the NVSL by inoculating a suspension of each specimen into the embryos of specific-pathogen-free (SPF) chicken eggs (32). All isolated viruses were identified, subtyped, and characterized according to standard NVSL procedures. The hemagglutinin and neuraminidase subtypes were determined by classical subtyping procedures, the hemagglutination-inhibition and neuraminidase-inhibition tests. Hemagglutination-inhibition and neuraminidase-inhibition assays were conducted according to standard NVSL protocols and by procedures described by the American Association of Avian Pathologists (32).

RESULTS

From April 2006 through March 2008, 145,055 samples were collected from more than 200 species of wild birds in all 50 states; no samples tested positive for HPAI H5N1. Of the samples collected, 83% were collected from dabbling ducks, geese, swans, and shorebirds. The remaining 17% were collected from a variety of other species (Table 1).

The majority (67%) of the samples were collected using the hunter harvest collection strategy, followed by live wild bird (32%) and sentinel species (1%). Samples collected during morbidity and mortality events were not included in the analysis because two separate samples were collected from each bird. However, 2,469 total samples were collected during morbidity–mortality events, and only four of these were confirmed as H5 positive by rRT-PCR.

Of 145,055 samples submitted for testing, 780 screened H5 or H7 positive by rRT-PCR at an NAHLN laboratory. The NVSL confirmed 555 H5 positives by rRT-PCR from 25 different species of wild migratory birds. Only three samples screened positive for H7 at an NAHLN laboratory, but none of these were confirmed at the NVSL. Virus was isolated from 194 of 780 (25%) samples. Of these, H5 subtypes were isolated from 13 species of wild birds, and H7 subtypes were isolated from two species. Seven different H5 subtype combinations and two different H7 subtype combinations were identified by virus isolation.

A total of 194 virus isolates were identified by the NVSL. Hemagglutinin groups represented in these viruses were H1–H8, H10, and H11 (Table 2). All nine neuraminidase groups were represented in the viruses isolated.

Mallards were sampled more than any other species (33,793 of 145,055), and the number of H5 detections was equal proportionally to the number of samples collected (269 of 555; $z = -11.82$). The majority (59%) of the samples were collected from dabbling ducks (84,897), and the majority of H5 viruses detected by rRT-PCR were found in dabbling ducks (506 of 555, $z = -27.01$; Table 3).

Table 2. Subtypes of viruses isolated from wild bird samples sent for H5 or H7 confirmation from April 2006 through March 2008.

Virus subtype	April 1, 2006 through March 31, 2007	April 1, 2007 through March 31, 2008	Total
H1N1	2	1	3
H2N2	1	–	1
H3N1	2	2	4
H3N2	2	2	4
H3N3	–	1	1
H3N4	1	–	1
H3N6	2	2	4
H3N8	6	4	10
H3N9	1	2	3
H4N2	3	–	3
H4N3	1	–	1
H4N6	26	3	29
H4N8	–	2	2
H5N1	6	2	8
H5N2	55	23	78
H5N3	5	3	8
H5N4	1	–	1
H5N7	–	1	1
H5N8	–	1	1
H5N9	1	1	2
H6N1	3	1	4
H6N2	4	1	5
H6N4	3	–	3
H6N5	2	–	2
H6N8	–	1	1
H6N9	1	–	1
H7N2	–	1	1
H7N3	–	3	3
H8N4	–	1	1
H10N2	–	1	1
H10N3	–	1	1
H10N7	3	1	4
H11N9	–	2	2

There were 13 states in which no isolates were identified over 2 yr of surveillance (Table 4). All of the remaining states had between 1 and 10 isolates except for Nevada, Minnesota, Michigan, and Washington with 22, 17, 14, and 14 isolates, respectively. More virus isolates were detected in 2006 ($n = 132$) than in 2007 ($n = 62$). H5N2 was detected more frequently than any other subtype (78 of 194).

DISCUSSION

Sampling was designed to target groups of wild birds that were at greater risk of infection with avian influenza viruses and, specifically, with HPAI H5N1 (37). Dabbling ducks were the primary focus for sampling based on the U.S. Interagency Strategic Plan and on literature that suggests that these species are the primary reservoirs of avian influenza (20,27). The prevalence of avian influenza is less common in diving ducks, presumably because of their feeding behavior (20). While geese and swans usually have a lower prevalence than ducks, they were targeted in this effort because of their potential to be infected with HPAI H5N1 from endemic areas in Asia and Europe (37).

Collection of samples from hunter-harvested birds was one of the easiest and most reliable forms of sampling compared to other sampling techniques such as the live bird captures. Consequently, a large percentage of samples (67%) were collected using the hunter-harvested collection strategy, and most samples (69%) were collected from various duck species that are most likely to be hunted. The advantage of using the hunter harvest strategy to collect samples is that it focuses on waterfowl species at a specific time of the year (e.g., October through December) when the historic prevalence of avian influenza viruses are relatively high in these species (13,26). However, the hunter harvest strategy is not effective for use in shorebirds because most of these species cannot be legally hunted in the United States. Most shorebird samples were obtained through live-bird captures, which are more expensive and difficult to conduct, resulting in the limited sample size (Table 1).

Table 3. Frequency of type A influenza virus recovery by species and year.

Species of duck	April 2006 through March 2007	April 2007 through March 2008	Total (%)
Dabbling ducks			
Mallard	88/18,769	24/15,024	112/33,793 (0.33%)
American black duck	0/521	2/1,116	2/1,637 (0.12%)
Northern pintail	7/6,512	5/3,845	12/10,357 (0.12%)
American wigeon	5/3,494	2/2,632	7/6,126 (0.11%)
American green-winged teal	16/7,916	11/6,202	27/14,118 (0.19%)
Blue-winged teal	1/2,134	4/3,484	5/5,618 (0.09%)
Northern shoveler	4/3,361	8/2,745	12/6,106 (0.20%)
Perching ducks			
Wood duck	0/1,355	1/3,347	1/3,482 (0.03%)
Diving ducks			
Ring-necked duck	1/893	2/1,229	3/2,122 (0.14%)
Geese and swans			
Mute swan	2/740	1/496	2/1,236 (0.16%)
Canada goose	7/6,954	1/5,374	8/12,328 (0.06%)
Cackling goose	1/439	0/351	1/790 (0.13%)
Lesser snow goose	1/1,154	0/656	1/1,810 (0.06%)
Shorebirds			
Ruddy turnstone	1/348	0/255	1/603 (0.17%)

Table 4. Total samples collected and virus isolation results, by state, 2006–2008.

State	No. of samples collected	Virus subtypes
Alaska	7144	na[A]
Alabama	2177	na
Arkansas	3436	H5N2
Arizona	2038	H5N2
California	3828	H5N2
Colorado	2500	H2N2, H5N2, H6N1, H7N3
Connecticut	3130	na
Delaware	5364	H1N1, H4N6, H5N1, H5N2, H6N4, H10N7
Florida	2310	H5N2
Georgia	2679	na
Hawaii	1251	na
Iowa	2576	H3N2, H4N6, H5N2, H6N1, H6N5
Idaho	2573	H4N2, H5N2, H8N4
Illinois	2913	H5N1, H5N3, H5N9, H6N1
Indiana	1964	H5N2
Kansas	2821	H5N2
Kentucky	1984	H5N2
Louisiana	4316	H3N9, H5N2
Maine	1984	H4N6
Maryland	3135	H5N2, H5N8
Massachusetts	1741	na
Michigan	3484	H3N1, H5N1, H5N2, H6N2, H6N5
Minnesota	3556	H3N1, H3N6, H3N8, H3N9, H4N6, H4N8, H5N2, H6N1, H6N4, H6N8, H10N3, H11N9
Missouri	2542	H1N1, H5N2, H5N3
Mississippi	2458	H5N2
Montana	3537	H4N6, H5N1, H5N2, H5N3, H6N2
North Carolina	2541	H5N2
North Dakota	3474	H5N2, H6N9
Nebraska	3488	na
Nevada	2419	H3N6, H3N8, H4N2, H4N6, H5N2
New Hampshire	1835	na
New Jersey	3098	H3N3, H3N8
New Mexico	2274	na
New York	3134	H5N2, H11N9
Ohio	2549	H3N2, H5N2, H6N2
Oklahoma	2609	H3N1, H6N1
Oregon	3990	H3N8, H4N6, H4N8, H5N2, H7N2
Pennsylvania	1891	H3N2, H3N6, H4N6, H5N1, H5N4, H6N4
Rhode Island	1903	na
South Carolina	2494	na
South Dakota	3590	H5N2, H5N7, H6N2
Tennessee	2007	H10N7
Texas	4576	H4N6, H5N2
Utah	3339	H5N2, H5N3, H7N3, H10N2, H10N7
Vermont	1872	H3N6, H4N2, H4N6
Virginia	3258	na
Washington	4848	H3N8, H4N3, H4N6, H5N2, H5N3, H10N7
West Virginia	1363	na
Wisconsin	3029	H1N1, H3N2, H3N4, H3N9, H5N2
Wyoming	2033	H5N2

[A]na = not applicable in state.

The majority (91%) of virus subtypes identified during the 2 yr of surveillance were detected in wild ducks, which agrees with previous reports (12,31,38). Viral subtypes reported here are likely not representative of all the subtypes circulating in wild birds during the sampling period because sampling was targeted to species most often associated with avian influenza viruses. Additionally, only samples that screened positive for H5 or H7 subtypes were subjected to virus isolation. The most common subtype isolated was H5N2, and it was detected predominately in mallards, which is consistent with the results of a concurrent sampling effort focused in the Pacific Flyway of the United States (8).

The majority of the samples (99.6%) forwarded to the NVSL for virus isolation initially screened positive for the H5 subtype; only three screened positive for the H7 subtype. Subsequent virus isolation and testing determined that H5 viruses were the most-commonly identified subtypes. This result is due, in part, to our targeted sampling and testing approach; sampling was focused on species most likely to introduce HPAI H5N1, and virus isolation and subtyping was only conducted on samples that screened positive for the H5 or H7 subtype by rRT-PCR. However, Dusek *et al.* (8) found similar results in a concurrent sampling effort in the Pacific Flyway of the United States. While Dusek *et al.* (8) also conducted targeted surveillance, a subset of their samples was subjected to virus isolation regardless of the rRT-PCR results. H7 detections were lower than expected, likely because of poor specificity and as a result of the assay being changed after the first 2 yr of surveillance.

Prior to implementation of the U.S. Early Detection System for Highly Pathogenic H5N1 Avian Influenza, monitoring of avian influenza viruses in the United States was primarily conducted as a part of specific research projects that were temporally or spatially restricted. Consequently, insights regarding avian influenza viruses in wild waterfowl populations at landscape and continental scales relied on extrapolating results obtained through those restricted studies. For example, until this effort, it was assumed that H5 subtype viruses were relatively uncommon in waterfowl (14,20,22,24,26,38). After finding no H5 or H7 subtypes in a limited sample of wild waterfowl on the eastern shore of Maryland, Slemons *et al.* (23) questioned whether wild birds served as maintenance hosts for these viruses in the Northeastern United States and recommended further evaluation.

Results from this surveillance effort and from Dusek *et al.* (8) provide convincing evidence to support the Slemons *et al.* (24) concern for the appearance of H5N2. Of the 194 viruses isolated here, 99 (51%) were of H5 subtypes, and 78 (40%) were specifically H5N2. Because mutations to HPAI of an LPAI form of H5N2 (by introduction of basic amino acid residues into the HA0 cleavage site) are possible (9), the presence of H5N2 in wild waterfowl may increase risks to commercial poultry. Of the three HPAI outbreaks reported in the United States, the last two were caused by H5N2 subtypes (5,21). During this study, four LPAI H5N2 detections were reported in commercial poultry flocks in Canada and the United States. Additionally, LPAI H5N2 was detected in three of 139 submissions and in 38 of 39 submissions from U.S. live bird markets in 2006 and 2007, respectively (33,34).

Three additional LPAI detections occurred in domestic flocks during 2007–2008; an H5N1 subtype was identified in Virginia turkeys, an H7N3 was identified in Arkansas chickens, and an H5N8 was identified in farm-raised game birds in Idaho. All three of these subtypes were detected in wild birds. In previous outbreaks and in these specific events, testing of the wild bird population immediately surrounding the premises have been sampled, but no definitive establishment of transmission between poultry and wild

birds has been established (19). While genetic sequencing of viruses in this study has not been completed, such analyses would be required for adequate determination of the relationship of the wild bird LPAI viruses reported here to the HPAI and LPAI detections in poultry (30). Future studies are warranted to identify the genetic sequencing of the isolates identified during surveillance efforts.

Mallards accounted for a large percentage of our samples (23%) and resulted in a large proportion of the total detections (48%). This is not surprising, considering that more isolations have been reported from mallards than from any other species (26). Mallards are important carriers of H5 and H7 subtypes that are closely related to viruses responsible for previous high pathogenicity outbreaks in poultry (18). We detected several of these low pathogenicity viruses in our surveillance including H5N2, H5N3, H5N6, H5N9, and H7N3.

A number of subtypes that we detected (i.e., H3N8, H4N6, H6N1, and H6N2) have been previously reported in wild ducks in the eastern hemisphere (2). Subtypes H2N3 and H12N5 were identified in Louisiana in a previous study (28), but were not identified during our 2 yr of surveillance. This may support the theory that avian influenza subtypes cycle in nature (17), or it may be a limitation of our methodology. We also detected H8N4, which is considered to be a rare subtype in ducks in North America, in a mallard (17,26). Consistent with Webster *et al.* (39), we did not detect H14, H15, or H16 in any of the wild bird samples tested.

Implementation of the U.S. Interagency Early Detection System for Highly Pathogenic H5N1 Avian Influenza in Wild Migratory Birds provided a unique opportunity to increase our understanding of LPAI viruses in their natural reservoir. Various studies in the United States have provided valuable information about subtypes of avian influenza virus on a relatively small spatial scale (3,10,11,16,23,28). However, regional and continental surveillance efforts such as this one provide important insights on the ecology of avian influenza viruses in wild birds, and the information gained through this work has improved our knowledge of the spatial and temporal distribution of LPAI viruses. Continued surveillance through this and similar efforts, combined with genetic sequencing of all avian influenza viruses, will significantly improve our understanding of their ecology in wild reservoirs and domestic birds.

REFERENCES

1. Alexander, D. J. A review of avian influenza in different bird species. Vet. Microbiol. 74:3–13. 2000.

2. Alexander, D. J. Report on avian influenza in the eastern hemisphere during 1997–2002. Avian Dis. 47:792–797. 2003.

3. Alfonso, C. P., B. S. Cowen, and H. Van Campen. Influenza A viruses isolated from waterfowl in two wildlife management areas of Pennsylvania. J. Wildl. Dis. 31:179–185. 1995.

4. Brown, J. D., D. E. Stallknecht, J. R. Beck, D. L. Suarez, and D. E. Swayne. Susceptibility of North American ducks and gulls to H5N1 highly pathogenic avian influenza viruses. Emerg. Infect. Dis. 12:1663–1670. 2006.

5. Buisch, W. W., A. E. Hall, and H. A. McDaniel. 1983–1984 lethal avian influenza outbreak. In: Proc. United States Animal Health Association, Fort Worth, TX. pp. 430–446. Oct. 21–26, 1984.

6. Chen, H., G. J. D. Smith, S. Y. Zhang, K. Qin, J. Wang, K. S. Li, R. G. Webster, J. S. M. Peiris, and Y. Guan. H5N1 virus outbreak in migratory waterfowl. Nature 436:191–192. 2005.

7. Claas, E. C. J., A. D. M. E. Osterhaus, R. van Beek, J. C. De Jong, G. F. Rimmerlzwaan, D. A. Senne, S. Krauss, K. F. Shortridge, and R. G. Webster. Human influenza A H5N1 virus related to highly pathogenic virus. Lancet 351:472–477. 1998.

8. Dusek, R. J., J. B. Bortner, T. J. DeLiberto, J. Hoskins, J. C. Franson, B. B. Bales, D. Yparraguirre, S. R. Swafford, and H. S. Ip. Surveillance for highly pathogenic avian influenza virus in wild birds in the pacific flyway of the United States, 2006–2007. Avian Dis. 53:222–230. 2009.

9. Fouchier, R. A. M., V. J. Munster, J. Keawcharoen, A. D. M. E. Osterhaus, and T. Kuiken. Virology of avian influenza in relation to wild birds. J. Wildl. Dis. 43:S7–S14. 2007.

10. Hanson, B. A., D. E. Stallknecht, D. E. Swayne, L. A. Lewis, and D. A. Senne. Avian influenza viruses in Minnesota ducks during 1998–2000. Avian Dis. 47:867–871. 2003.

11. Hanson, B. A., D. E. Swayne, D. A. Senne, D. S. Lobpries, J. Hurst, and D. E. Stallknecht. Avian influenza viruses and paramyxoviruses in wintering and resident ducks in Texas. J. Wildl. Dis. 41:624–628. 2005.

12. Hinshaw, V. S., R. G. Webster, and B. Turner. The perpetuation of orthomyxoviruses and paramyxoviruses in Canadian waterfowl. Can. J. Microbiol. 26:622–629. 1980.

13. Hinshaw, V. S., J. M. Wood, R. G. Webster, R. Deibel, and B. Turner. Circulation of influenza viruses and paramyxoviruses in waterfowl originating from two different areas of North America. Bull. World Health Organ. 63:711–719. 1985.

14. Kawaoka, Y., T. M. Chambers, W. L. Sladen, and R. G. Webster. Is the gene pool of influenza viruses in shorebirds and gulls different from that in wild ducks? Virology 163:247–250. 1988.

15. Keawcharoen, J., D. van Riel, G. van Amerongen, T. Bestebroer, W. E. Beyer, R. van Lavieren, A. D. M. E. Osterhaus, R. A. M. Fouchier, and T. Kuiken. Wild ducks as long-distance vectors of highly pathogenic avian influenza virus (H5N1). Emerg. Infect. Dis. 14:600–607. 2008.

16. Kocan, A. A., V. S. Hinshaw, and G. A. Daubney. Influenza A viruses isolated from migrating ducks in Oklahoma. J. Wildl. Dis. 16:281–285. 1980.

17. Krauss, S., D. Walker, S. P. Pryor, L. Niles, L. Chenhong, V. S. Hinshaw, and R. G. Webster. Influenza A viruses of migrating wild aquatic birds in North America. Vector Borne Zoonotic Dis. 4:177–189. 2004.

18. Munster, V. J., A. Wallensten, C. Baas, G. F. Rimmelzwaan, M. Schutten, B. Olsen, A. D. M. E. Osterhaus, and R. A. M. Fouchier. Mallards and highly pathogenic avian influenza ancestral viruses, northern Europe. Emerg. Infect. Dis. 11:1545–1551. 2005.

19. Nettles, V. F., J. M. Wood, and R. G. Webster. Wildlife surveillance associated with an outbreak of lethal H5N2 avian influenza in domestic poultry. Avian Dis. 29:733–741. 1985.

20. Olsen, B., V. J. Munster, A. Wallensten, J. Waldenstrom, A. D. M. E. Osterhaus, and R. A. M. Fouchier. Global patterns of influenza A virus in wild birds. Science 312:384–388. 2006.

21. Pelzel, A. M., B. J. McCluskey, and A. E. Scott. Review of the highly pathogenic avian influenza outbreak in Texas, 2004. J. Am. Vet. Med. Assoc. 228:1869–1875. 2006.

22. Sharp, G. B., Y. Kawaoka, S. M. Wright, B. Turner, V. Hinshaw, and R. G. Webster. Wild ducks are the reservoir for only a limited number of influenza A subtypes. Epidemiol. Infect. 110:161–176. 1993.

23. Slemons, R. D., W. R. Hansen, K. A. Converse, and D. A. Senne. Type A influenza virus surveillance in free-flying, nonmigratory ducks residing on the eastern shore of Maryland. Avian Dis. 47:1107–1110. 2003.

24. Slemons, R. D., M. C. Shieldcastle, L. D. Heyman, K. E. Bednarik, and D. A. Senne. Type A influenza viruses in waterfowl in Ohio and implications for domestic turkeys. Avian Dis. 35:165–173. 1991.

25. Spackman, E., D. A. Senne, T. J. Myers, L. L. Bulaga, L. P. Garber, M. L. Perdue, K. Lohman, L. T. Daum, and D. L. Suarez. Development of real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. J. Clin. Microbiol. 40:3256–3260. 2002.

26. Stallknecht, D. E., and J. D. Brown. Wild birds and the epidemiology of avian influenza. J. Wildl. Dis. 43:S15–S20. 2007.

27. Stallknecht, D. E., and J. D. Brown. Ecology of avian influenza in wild birds. In: Avian influenza. D. E. Swayne, ed. Blackwell Publishing, Ames, IA. pp. 43–58. 2008.

28. Stallknecht, D. E., S. M. Shane, P. J. Zwank, D. A. Senne, and M. T. Kearney. Avian influenza viruses from migratory and resident ducks of coastal Louisiana. Avian Dis. 34:398–405. 1990.

29. Sturm-Ramirez, K. M., T. Ellis, B. Bousfield, L. Bissett, K. Dyrting, J. E. Rehg, L. Poon, Y. Guan, M. Peiris, and R. G. Webster. Reemerging

H5N1 influenza viruses in Hong Kong in 2002 are highly pathogenic to ducks. J. Virol. 78:4892–4901. 2004.

30. Suarez, D. L. Evolution of avian influenza viruses. Vet. Microbiol. 74:15–27. 2000.

31. Suss, J., J. Schafer, H. Sinnecker, and R. G. Webster. Influenza virus subtypes in aquatic birds of eastern Germany. Arch. Virol. 135:101–114. 1994.

32. Swayne, D. E., D. A. Senne, and C. W. Beard. Avian influenza. In: A laboratory manual for the isolation and identification of avian pathogens. D. E. Swayne, ed. American Association of Avian Pathologists, Kennett Square, PA. pp. 150–155. 1998.

33. United States Department of Agriculture, Animal and Plant Health Inspection Service. 2006 United States animal health report. Agricultural Information Bulletin No. 801. Washington, DC. 2007.

34. United States Department of Agriculture, Animal and Plant Health Inspection Service. 2006 United States animal health report. Agricultural Information Bulletin No. 803. Washington, DC. 2008.

35. United States Department of Agriculture, Veterinary Services. Assessment of notifiable avian influenza surveillance in the United States. National Surveillance Unit, Fort Collins, CO. pp. 1–46. 2008.

36. United States Department of Agriculture, Wildlife Services. Wildlife Services and State/Tribal cooperator avian influenza surveillance procedure manual. Fort Collins, CO. pp. 1–89. Unpublished field manual. 2006.

37. U.S. Interagency Working Group. An early detection system for highly pathogenic H5N1 avian influenza in wild migratory birds: U.S. Interagency Strategic Plan. [Internet]. Washington, DC; 2006. [cited 2006 March 16]. Available from: http://www.usda.gov/documents/wildbirdstrategicplanpdf_seg0.pdf

38. Webster, R. G., W. J. Bean, O. T. Gorman, T. M. Chambers, and Y. Kawaoka. Evolution and ecology of influenza A viruses. Microbiol. Rev. 56:152–179. 1992.

39. Webster, R. G., S. Krauss, D. Hulse-Post, and K. Sturm-Ramirez. Evolution of influenza A viruses in wild birds. J. Wildl. Dis. 43:S1–S6. 2007.

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