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## Antigenic Characterization of H3 Subtypes of Avian Influenza A Viruses from North America

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**SUMMARY.** Besides humans, H3 subtypes of influenza A viruses (IAVs) can infect various animal hosts, including avian, swine, equine, canine, and sea mammal species. These H3 viruses are both antigenically and genetically diverse. Here, we characterized the antigenic diversity of contemporary H3 avian IAVs recovered from migratory birds in North America. Hemagglutination inhibition (HI) assays were performed on 37 H3 isolates of avian IAVs recovered from 2007 to 2011 using generated reference chicken sera. These isolates were recovered from samples taken in the Atlantic, Mississippi, Central, and Pacific waterfowl migration flyways. Antisera to all the tested H3 isolates cross-reacted with each other and, to a lesser extent, with those to H3 canine and H3 equine IAVs. Antigenic cartography showed that the largest antigenic distance among the 37 avian IAVs is about four units, and each unit corresponds to a 2 log<sub>2</sub> difference in the HI titer. However, none of the tested H3 IAVs cross-reacted with ferret sera derived from contemporary swine and human IAVs. Our results showed that the H3 avian IAVs we tested lacked significant antigenic diversity, and these viruses were antigenically different from those circulating in swine and human populations. This suggests that H3 avian IAVs in North American waterfowl are antigenically relatively stable.

**RESUMEN.** Caracterización antigénica de subtipos H3 de virus de la influenza aviar A de América del Norte.

Además de infectar a los seres humanos, los subtipos H3 del virus de la influenza A (IAVs) pueden infectar a varios huéspedes animales, incluyendo aves, porcinos, equinos, caninos, y especies de mamíferos marinos. Estos virus H3 son tanto antigénica y genéticamente diversos. En este estudio, se caracterizó la diversidad antigénica de virus H3 contemporáneos recuperados de aves migratorias en América del Norte. Se realizaron pruebas de inhibición de la hemaglutinación (HI) en 37 H3 aislamientos de origen aviar recuperados de 2007 a 2011 usando sueros de pollo de referencia. Estos aislamientos fueron recuperados de las muestras tomadas de las rutas migratorias de aves acuáticas del Atlántico, Mississippi, Centro y del Pacífico. Los antisueros de todos los aislamientos H3 analizaron mostraron reacciones cruzadas entre sí y en menor medida, con aquellos virus H3 de origen canino y equino. La cartografía antigénica demostró que la mayor distancia antigénica entre los 37 virus de este tipo de aves es de aproximadamente cuatro unidades, y cada unidad corresponde a una diferencia de dos logaritmos en el título de inhibición de la hemaglutinación. Sin embargo, ninguno de los virus H3 de este tipo mostró reacción cruzada con sueros de hurón específicos para virus de cerdos y humanos contemporáneos. Estos resultados mostraron que los virus H3 de origen aviar que se analizaron carecían de diversidad antigénica significativa y estos virus fueron antigénicamente diferentes de las que circulan en poblaciones de cerdos y de humanos. Esto sugiere que los virus H3 de aves acuáticas de América del Norte son relativamente estables antigénicamente.

**Key words:** influenza A virus, avian influenza virus, migratory waterfowl, H3, low pathogenic avian influenza virus, antigenic cartography, migratory flyway

**Abbreviations:** HA = hemagglutinin; HI = hemagglutination inhibition; IAV = influenza A virus; NA = neuraminidase; SPF = specific pathogen free

Specific strains of influenza A virus (IAV) can cause a pandemic disaster affecting human health on multiple continents and then persist causing seasonal influenza epidemics (1,23). Four documented influenza A pandemics occurred in the last hundred years: 1918, 1957, 1968, and 2009. About 50 million people died of the 1918 H1N1 influenza A pandemic (21,31). The annual inter-pandemic influenza season in the northern hemisphere, from October to April, results in over 200,000 hospitalizations and up to 49,000 deaths annually in the United States (8,46,47).

IAVs have been recovered from at least 105 wild bird species from 26 different families (2,29). Among these birds, those living in

wetland and aquatic environments (e.g., *Anseriformes* spp., particularly ducks, geese, and swans, and *Charadriiformes* spp., particularly gulls, terns, and waders) are by far the major source of these isolates and are generally accepted as major natural IAV reservoirs (25,50). Infected wild birds, especially migratory waterfowl, are believed to serve as a vector facilitating the spread of avian IAVs among wild bird species and different geographic locations and are one source for viral transmission into domestic poultry (34,50), swine (19), and humans (16).

The IAV has eight genomic segments (segment 1–8) encoding for at least 11 proteins: PB2 by segment 1, PB1 and PB1-F2 by segment 2, PA by segment 3, hemagglutinin (HA) by segment 4, nucleoprotein by segment 5, neuraminidase (NA) by segment 6, matrix proteins

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M1 and M2 by segment 7, and nonstructural protein NS1 and NS2 by segment 8. To date, 18 HA (H1-H18) and 11 NA subtypes (N1-N11) have been reported in IAVs (48), although no isolates have been recovered for H17 and H18 subtypes. HA and NA are commonly used in combination to identify viral subtypes (i.e., H1N1). Antigenic changes in HA and NA, the two surface glycoproteins, are mainly due to accumulating point mutations, resulting in antigenic drift or genomic reassortment. These changes in the HA are especially important because they allow an IAV to evade the population immunity established from previous influenza infections or vaccination (26).

The H3 IAVs have very diverse host ranges in addition to infecting many species of birds. After the emergence of the pandemic virus A/Hong Kong/1968 (H3N2), this strain of IAV became endemic after the first year and has since been causing yearly seasonal epidemics in humans. The H3 IAVs circulate in swine, equine, canine, and avian species and cause sporadic outbreaks in sea mammals. H3N2 is one of the predominant IAVs infecting both domestic and feral swine (14,45,49), H3N8 was shown to infect seals (22), H3N8 is endemic in domestic dogs in North America and equine worldwide (9,17), and the avian origin H3N2 infected domestic dogs in Asia (24,40).

Previous studies documented substantial antigenic diversity in the H3 IAV in mammals (14). Since 1968, antigenic drift has led to more than 28 updates of the H3N2 components in the seasonal influenza vaccines (37,43). In swine, the H3N2 IAVs are genetically and antigenically diverse. Four genetic clusters of H3N2 IAVs (clusters I–IV) have been identified in U.S. swine populations (19,30,33,43), and the viruses in these four H3N2 clusters are antigenically different from each other. Additionally, Cluster IV, which has become predominant among the U.S. swine population, has further evolved into two antigenic clusters: H3N2SIV- $\alpha$  and H3N2SIV- $\beta$  (14). Additionally, both H3N2 and H3N8 canine IAVs are antigenically distinct from contemporary human influenza viruses (54).

In wild migratory birds, H3 is one of the more frequent HA subtypes recovered in IAV surveillance projects (27). However, the antigenic diversity of these H3 viruses is not well characterized. This study aims to characterize the antigenic diversity of contemporary H3 IAVs recovered from migratory birds in North America. This information helps us understand the natural history of influenza evolution and may also help improve knowledge on influenza prevention and control.

## MATERIALS AND METHODS

**Viruses.** A total of 36 H3 isolates (H3N1, H3N2, H3N6, H3N7, H3N8, or H3N9) recovered from migratory birds in North America from 2007 to 2011 were included in this study (Table 1). These viruses were recovered from American black duck (*Anas rubripes*), mallard (*Anas platyrhynchos*), ring-necked duck (*Aythya collaris*), hooded merganser (*Lophodytes cucullatus*), northern pintail (*Anas acuta*), snow goose (*Chen caerulescens*), blue-winged teal (*Anas discors*), and long-tailed duck (*Clangula hyemalis*). These H3 isolates were recovered from samples collected in Canadian locations in Nova Scotia, New Brunswick, Nunavut, and Prince Edward Island, as well as the U.S. states of Maine, New Hampshire, North Dakota, Colorado, Oregon, Washington, Wisconsin, Iowa, Maryland, and New York, encompassing regions across the Atlantic, Mississippi, Central, and Pacific Bird Migratory Flyways in North America. Additionally, one H3N8 isolate from a harbor seal (*Phoca vitulina*), A/harbor seal/New Hampshire/179629/2011 (H3N8), was also included in this study. These viruses were recovered and propagated in 9–11-day-old specific pathogen-free (SPF) embryonated chicken eggs before serological characterization (28).

**Sera.** Two-week-old broiler chicks hatched from SPF eggs were group housed in HEPA-filtered, ventilated caging in ABSL-2-approved

facilities. Six birds were housed per isolator and used for each challenge virus. Chicks were provided commercial poultry ration and water *ad libitum* and were maintained on a 12-hr light cycle at a room temperature of 20–22 C. Each bird was inoculated nasally with  $10^6$ , 50% tissue culture infective dose in a 100- $\mu$ L volume of a selected H3 isolate and boosted 14 days later with the same amount of virus. Six birds were used for each isolate, and the sera with at least a hemagglutination inhibition (HI) assay titer of 1:40 were pooled. The sera were stored at  $-20$  C until characterization. Reference sera were raised using isolates selected to cover two genetic clusters of North America origin H3 avian IAVs in the phylogenetic trees (Fig. 1). Additionally, different years, locations, and host species were also considered to maximize the potential coverage. Consequently, 11 viruses were selected, including A/snow goose/Nunavut/03438/2010 (H3N8), A/hooded merganser/New Brunswick/03749/2009 (H3N8), A/blue-winged teal/Nova Scotia/00069/2010 (H3N8), A/American black duck/Nova Scotia/02213/2007 (H3N8), A/American black duck/New Brunswick/03451/2009 (H3N9), A/American black duck/New Brunswick/02650/2007 (H3N2), A/long-tailed duck/Maine/295/2011 (H3N8), A/harbor seal/New Hampshire/179629/2011 (H3N8), A/ring-necked duck/Nova Scotia/03378/2009 (H3N8), A/mallard/New Brunswick/00593/2010 (H3N7), and A/American black duck/New Brunswick/00618/2010 (H3N6) (Table 1).

To assess cross-reactivity with contemporary human and swine H3 IAVs, we also included a set of ferret sera (14) specifically against 20 H3 IAVs, including eight swine and 12 human IAVs [A/swine/Ohio/09SW0964/2009 (H3N2), A/swine/Ohio/09SW96/2009 (H3N2), A/swine/Ohio/10SW130/2010 (H3N2), A/swine/Ohio/10SW156/2010 (H3N2), A/swine/Ohio/10SW215/2010 (H3N2), A/swine/Ohio/11SW111/2011 (H3N2), A/swine/Ohio/11SW208/2011 (H3N2), A/swine/Ohio/11SW347/2011 (H3N2), A/Bangkok/1/1979 (H3N2), A/Philippines/2/82 (H3N2), A/Caen/1/1984 (H3N2), A/Mississippi/1/1985 (H3N2), A/Leningrad/360/1986 (H3N2), A/Sichuan/02/1987 (H3N2), A/Sichuan/60/1989 (H3N2), A/Ann Arbor/03/1993 (H3N2), A/Johannesburg/33/1994 (H3N2), A/Nanchang/933/1995 (H3N2), A/Sydney/05/1997 (H3N2), and A/Wisconsin/67/2005 (H3N2)].

**HA and HI assays.** The HA and HI assays were performed according to World Organisation for Animal Health (OIE) guidelines (13) using 1% chicken red blood cells (Lampire Labs, Pipersville, PA).

**Antigenic cartography.** Antigenic cartography projects influenza viruses into a two- or three-dimensional map based on antigenic relationships embedded in the serological data. Antigenic cartography has been used as an effective method of characterizing antigenic diversity for IAVs (7,38). In this study, AntigenMap (4,7) was used to assess antigenic variability among the H3 IAVs we selected. A threshold of 1:10 was used to define the low reactor.

**Phylogenetic analysis and molecular characterization.** Multiple sequence alignments of the HA genes of H3 subtypes were created by using the MUSCLE software package (12). Phylogenetic analyses were performed using maximum likelihood by the GARLI version (5), and bootstrap resampling analyses were conducted using PAUP\* 4.0 Beta (52) with a neighbor-joining method, as previously described (6).

**Analysis of antibody binding sites.** To analyze sequence diversity among the antibody binding sites of hemagglutinin protein, sequence diversity profiles were generated for antibody binding sites in the viruses we studied using a WebLogo 3 web server (10). Antibody binding sites were annotated based on previous studies (53), and they were mapped in a three-dimensional structure of hemagglutinin protein using the Protein Data Bank entry 1MQL (18) as the template. Additionally, a total of 1265 hemagglutinin protein sequences for North America avian origin H3 IAVs were retrieved from the National Center for Biotechnology Information Influenza Virus Database (3), and their sequence logos for antibody binding sites were also analyzed.

## RESULTS

**Genetic diversity in H3 avian IAVs is large in North America.** Phylogenetic analysis showed that the H3 genes of avian IAVs in North America formed two genetic clusters, and both were genetically

Table 1. Summary of H3 avian IAVs in North America used in this study.

Virus <sup>A</sup>	County <sup>B</sup>	State/Province	Date collected	Flyway <sup>C</sup>	Sex	Age class
A/American black duck/Nova Scotia/02213/2007 (H3N8)	Cumberland	Nova Scotia	NA	ATLA	Female	Local
A/American black duck/New Brunswick/03451/2009 (H3N9)	Westmorland	New Brunswick	Aug. 12, 2009	ATLA	Male	Hatch year
A/American black duck/New Brunswick/03398/2009 (H3N8)	Westmorland	New Brunswick	Aug. 9, 2009	ATLA	Male	Hatch year
A/mallard/New Brunswick/03396/2009 (H3N8)	Westmorland	New Brunswick	Aug. 9, 2009	ATLA	Female	Hatch year
A/American black duck/New Brunswick/02650/2007 (H3N2)	Gloucester	New Brunswick	Sep. 24, 2007	ATLA	Female	Hatch year
A/American black duck/New Brunswick/02525/2007 (H3N8)	Gloucester	New Brunswick	Sep. 24, 2007	ATLA	Male	Hatch year
A/American black duck/New Brunswick/02651/2007 (H3N2)	Gloucester	New Brunswick	Sep. 24, 2007	ATLA	Male	Hatch year
A/hooded merganser/New Brunswick/03749/2009 (H3N8)	Sunbury	New Brunswick	Sep. 14, 2009	ATLA	Male	Hatch year
A/ring-necked duck/Nova Scotia/03378/2009 (H3N8)	Cumberland	Nova Scotia	Aug. 8, 2009	ATLA	Female	After hatch year
A/northern pintail/New Brunswick/03547/2009 (H3N8)	Gloucester	New Brunswick	Sep. 11, 2009	ATLA	Female	After hatch year
A/snow goose/Nunavut/03438/2010 (H3N8)	Kivalliq	Nunavut	Jul. 20, 2010	ATLA	Male	Adult
A/blue-winged teal/Nova Scotia/00069/2010 (H3N8)	Cumberland	Nova Scotia	Sep. 7, 2010	ATLA	Male	Hatch year
A/blue-winged teal/New Brunswick/00283/2010 (H3N8)	Queens	New Brunswick	Sep. 14, 2010	ATLA	Female	Hatch year
A/blue-winged teal/New Brunswick/00597/2010 (H3N7)	Albert	New Brunswick	Aug. 15, 2010	ATLA	Male	Hatch year
A/blue-winged teal/Prince Edward Island/00831/2010 (H3N7)	Queens	Prince Edward Island	Sep. 2, 2010	ATLA	Male	Hatch year
A/mallard/New Brunswick/00593/2010 (H3N7)	Albert	New Brunswick	Aug. 15, 2010	ATLA	Male	Hatch year
A/American black duck/New Brunswick/00618/2010 (H3N6)	Albert	New Brunswick	Aug. 16, 2010	ATLA	Male	Hatch year
A/long-tailed duck/Maine/295/2011 (H3N8)	Hancock	Maine	Dec. 5, 2011	ATLA	Female	After hatch year
A/harbor seal/New Hampshire/179629/2011 (H3N8)	NA	New Hampshire	NA	ATLA	NA	NA
A/mallard/North Dakota/A00094822/2007 (H3N6)	Ramsey	North Dakota	Oct. 10, 2007	CENT	Female	After hatch year
A/mallard/Colorado/A00170366/2006 (H3N8)	Saguache	Colorado	Sep. 2, 2006	CENT	Female	Hatch year
A/mallard/Oregon/A00282268/2007 (H3N8)	Klamath	Oregon	Aug. 7, 2007	PAC	Female	Hatch year
A/mallard/Oregon/A00282275/2007 (H3N8)	Klamath	Oregon	Aug. 7, 2007	PAC	Female	Hatch year
A/mallard/Washington/A00461816/2008 (H3)	Whatcom	Washington	Aug. 15, 2008	PAC	Male	After hatch year
A/mallard/Washington/A00466471/2008 (H3N8)	Whatcom	Washington	Oct. 9, 2008	PAC	Female	Hatch year
A/mallard/Oregon/A00571121/2007 (H3N8)	Lake	Oregon	Sep. 11, 2007	PAC	Male	Hatch year
A/mallard/Wisconsin/A00661712/2009 (H3N2)	Dodge	Wisconsin	Oct. 3, 2009	MISS	Female	Hatch year
A/mallard/Iowa/A00683081/2008 (H3N8)	Tama	Indiana	Oct. 18, 2008	MISS	Female	Hatch year
A/mallard/Maryland/A00713446/2009 (H3N6)	Dorchester	Maryland	Nov. 27, 2009	ATLA	Female	After hatch year
A/mallard/Wisconsin/A00713769/2009 (H3N1)	Marathon	Wisconsin	Aug. 15, 2009	MISS	Male	After hatch year
A/mallard/Wisconsin/A00714818/2009 (H3N2)	Manitowoc	Wisconsin	Aug. 13, 2009	MISS	Female	Hatch year
A/mallard/New York/A00723440/2009 (H3N6)	Jefferson	New York	Nov. 8, 2009	ATLA	Female	After hatch year
A/mallard/Wisconsin/A00751345/2009 (H3N8)	Manitowoc	Wisconsin	Aug. 13, 2009	MISS	Male	Undetermined
A/mallard/Wisconsin/A00751351/2009 (H3N6)	Manitowoc	Wisconsin	Aug. 13, 2009	MISS	Male	Hatch year
A/mallard/New York/A00755144/2009 (H3N6)	Montgomery	New York	Dec. 6, 2009	ATLA	Male	Hatch year
A/mallard/Maryland/A00871428/2009 (H3N6)	Dorchester	Maryland	Nov. 27, 2009	ATLA	NA	NA
					Male	After hatch year

<sup>A</sup>Viruses in bold are those selected to generate chicken antiserum.

<sup>B</sup>NA = not available.

<sup>C</sup>Flyways are as follows: PAC = Pacific, CENT = Central, MISS = Mississippi, ATLA = Atlantic.



Fig. 1. Phylogenetic analysis of HA genes of H3 IAV isolates from migratory waterfowl in North America (2007–2011). The H3 IAV isolates included in antigenic characterization are marked with a star. The phylogenetic analyses were performed using maximum likelihood by the GARLI version (5), and bootstrap resampling analyses were conducted using PAUP\* 4.0 Beta (52) with a neighbor-joining method, as previously described (6).

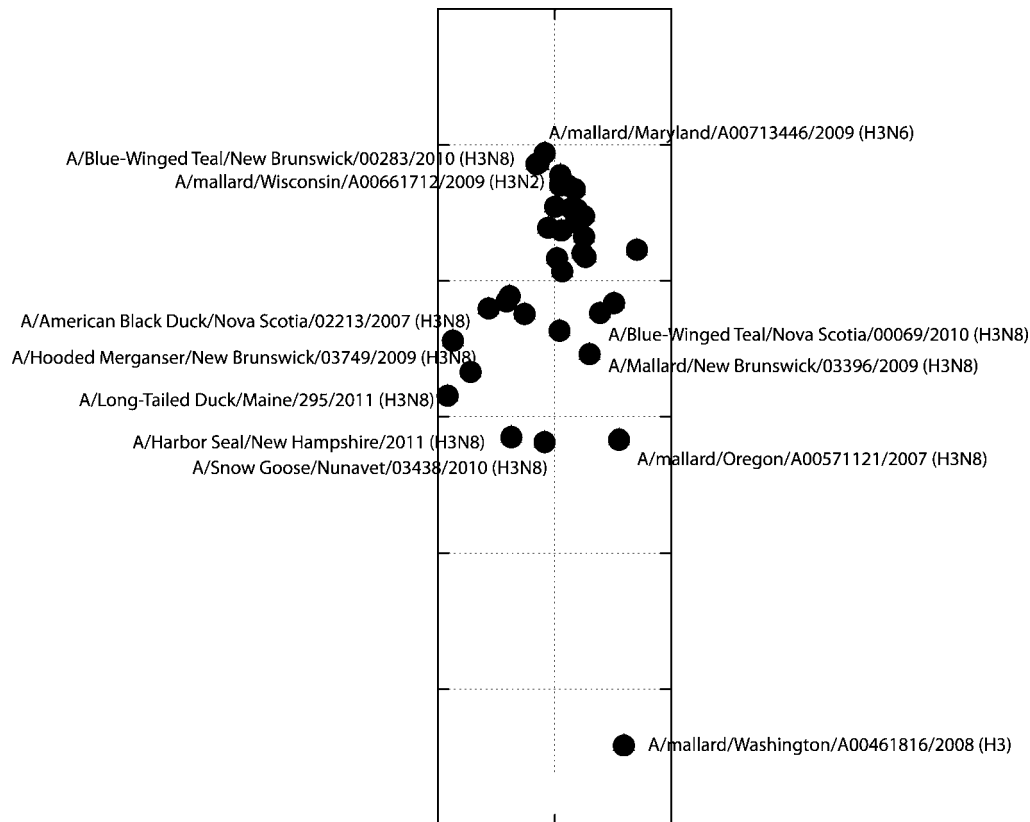


Fig. 2. Antigenic cartography for H3 avian IAVs in North America (2007–2011) based on HI data from chicken sera. Antigenic cartography was constructed using AntigenMap (<http://sysbio.cvm.msstate.edu/AntigenMap>) (4,7).

distinct from Eurasian H3 avian IAVs from wild birds (Fig. 1). The HA genes of H3 avian IAVs from North America we tested were distributed throughout the branch containing other IAVs from public databases, indicating the viruses we selected were representative strains for contemporary H3 avian IAVs in North America. The nucleotide sequence identities among these H3 avian influenza viruses from 2007 to 2010 in North America varied from 81.94% to 100%. These viruses were genetically distinct from the human, equine, and swine H3 IAVs (Fig. 1). The isolate from the harbor seal was genetically close to Lineage I of North American H3 avian IAVs.

**H3 avian IAVs lack antigenic diversity in North America.** Our results demonstrated that all sera raised to the avian H3 IAVs tested were highly cross-reactive with each other (Supplemental Table S1). Antigenic cartography showed that all 36 viruses were antigenically similar to each other (Fig. 2). The average antigenic distance in the cartography was estimated to be 0.99 units (SD = 0.62 units). The maximum antigenic distance observed among these viruses was 3.62 units and occurred between virus A/mallard/Washington/A00461816/2008 (H3N?) and A/mallard/Oregon/A00282275/2007 (H3N8) (Fig. 2). The antigenic distances for all 35 isolates except A/mallard/Washington/A00461816/2008 (H3N?) were approximately 2 units.

**H3 avian IAVs are antigenically distinct from human, swine, and canine H3 subtypes.** To test the antigenic divergence of H3 avian IAVs from North American migratory birds and from other hosts, we performed HI experiments on the ferret sera raised to H3N2 viruses, representing human IAV antigenic clusters from 1979 to 2002, eight H3N2 influenza viruses representing both H3N2-alpha and -beta circulating in swine populations, and H3N2 and H3N8 canine influenza viruses. None of the H3 avian IAVs cross-reacted

with ferret sera against H3N2 human viruses (data not shown) or the ferret sera against H3N2 swine viruses (Supplemental Table S3). The ferret sera against H3N8 canine influenza virus had a HI titer of 1:40 against 3 of the 22 tested isolates, whereas all these viruses were negative against the ferret sera against H3N2 avian origin canine influenza viruses (Supplemental Table S2). The ferret sera against two H3N2 avian influenza viruses showed that the one against the virus from 1999 had a HI titer of 1:40 against 15 of the tested 22 isolates, whereas the one against the virus from 2011 had a HI titer of 1:40 against 14 of the tested 22 isolates.

HI analyses showed that the sera to the seal isolate cross-reacted well with 4 of the 35 avian H3 IAVs tested, with a titer  $\geq$  1:40 and a maximum titer of 1:160 (Supplemental Table S1).

**Antibody binding sites of H3 avian IAVs lack divergence.** To identify the potential variations of antibody binding sites of H3 avian IAVs, we compared the reported five antibody binding sites A, B, C, D, and E (53), for all H3 avian IAVs from North America in the public database (Fig. 3). There was a lack of divergence of these residues in the five sites (Fig. 3A,C). We further characterized the profiles of the antibody binding sites of the 37 IAVs evaluated here and compared them with those from the public database. The antibody binding sites of the viruses we tested are similar to those from the public database (Fig. 3B,C).

## DISCUSSION

The evolution of IAVs in waterfowl was proposed to be static (41,42). However, other studies have shown that the H5N1 highly pathogenic IAVs circulating in ducks have evolved rapidly (20,41). A recent serological surveillance of wild birds suggested that wild

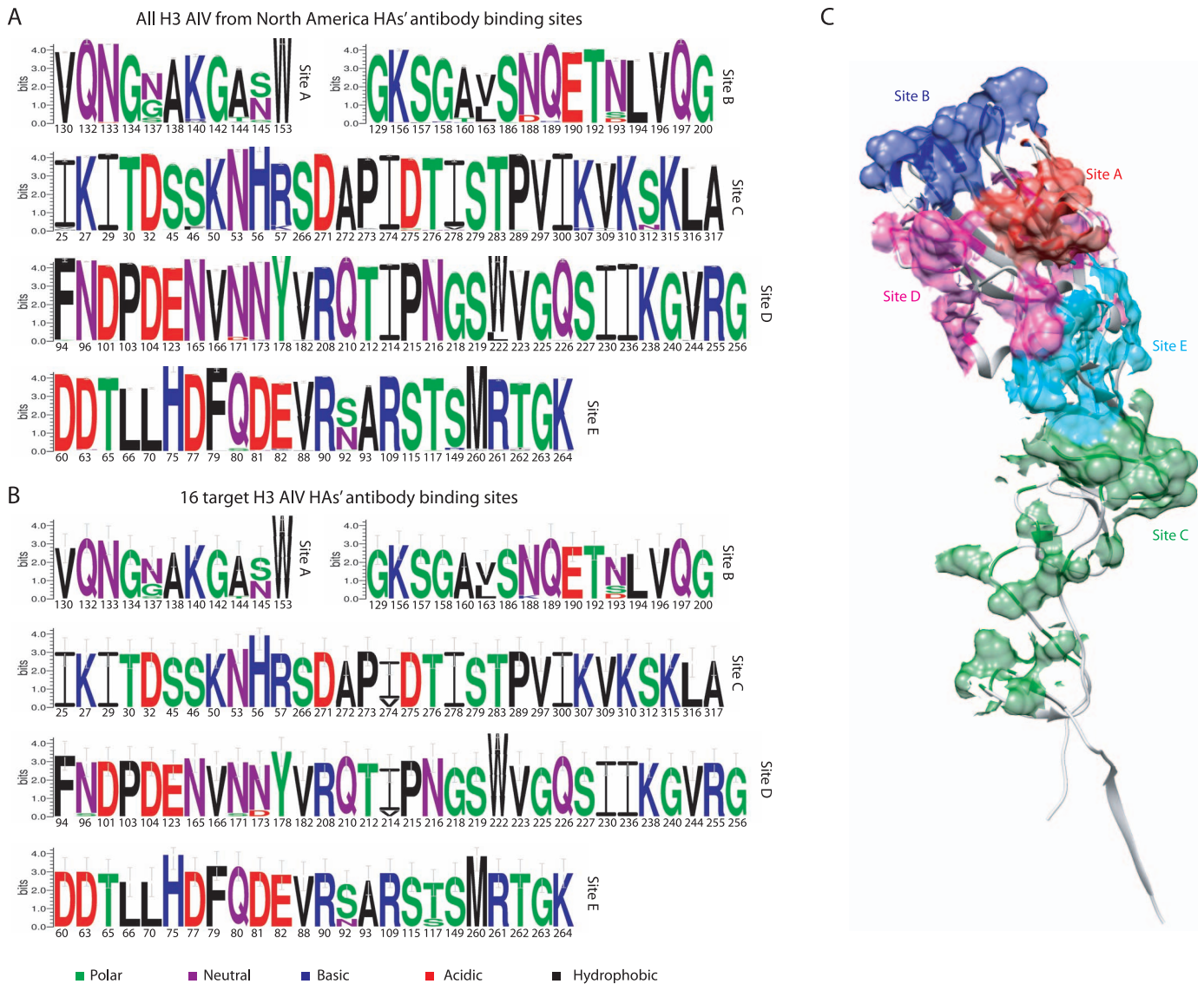


Fig. 3. Conservation among the reported antibody binding sites in the HA protein of H3 avian influenza viruses from North America: (A) amino acid variations of antibody binding sites on all H3 IAVs from North America; (B) amino acid variations of antibody binding sites on 16 of the 37 target H3 IAV HAs; and (C) a structural model of H3 HA with antibody binding sites mapped. The antibody binding sites were annotated by Wilson and Cox (53). The conservation analyses were characterized using a WebLogo 3 web server (10). The locations of these antibody binding sites were visualized using Chimera (32) based on the template of Protein Data Bank entry 1MQL (18).

birds may have been exposed to multiple, antigenically diverse H5 IAVs (15). Thus, understanding the antigenic diversity of avian IAVs in migratory waterfowl is critical for understanding the natural history of these viruses.

This study evaluated H3 viruses from 2007 to 2011 and those from all four migratory flyways in North America. Thus, we considered these viruses to be representative of the antigenic diversity of H3 IAVs in migratory birds. Antigenic characterization of the 37 IAVs in this study demonstrated that there is little antigenic diversity for contemporary H3 IAVs in migratory birds. Such results suggest that H3 IAVs probably had been exposed to little selective pressure for antigenic evolution, supporting the previous concept of the static evolution of H3 viruses in migratory waterfowl (41).

Waterfowl serve as reservoirs for IAVs, and, consequently, IAVs emerging from these hosts could threaten human health. Genetic reassortments can lead to antigenic shift and the generation of

epidemic and pandemic influenza strains. At least two of four documented pandemic viruses had HA genes of avian origin (35,36,51). The 1957 H2N2 pandemic strain probably emerged from the avian H2N2 IAV and H1N1 human influenza IAV. The 1968 H3N2 pandemic strain was likely a reassortment of the avian H3 IAV and the human H2N2 IAV. Thus, antigenically distinct HA genes of avian IAVs can contribute to generating a novel pandemic virus through antigenic shift. This study demonstrated that the contemporary H3 avian IAVs in migratory birds are antigenically distinct from those causing seasonal outbreaks in humans as well as swine; thus, there may be lack of immunological protection in the human populations against these H3 avian IAVs. These H3 avian IAVs could potentially lead to future outbreaks either through direct or indirect spillover transmission to humans.

Additionally, IAVs from wild birds are genetically diverse, and characterization of the transmissibility of these viruses in mammals



is very limited. Although some studies showed that avian IAVs have little transmissibility in mammals (11), a more recent study demonstrated that an H3N8 avian IAV isolated from a harbor seal could be transmitted among ferrets through aerosol droplets (22) and infect pigs without previous adaptation (39). This same strain was shown to have antigenic properties similar to those from migratory waterfowl but distinct from human viruses (Supplemental Table S2). Further studies are needed to compare the antigenic divergence of H3 IAVs in North America to those from other regions of the world, especially Eurasia.

In summary, this study showed that H3 IAVs from migratory waterfowl in North America lack antigenic diversity and that these viruses are antigenically distinct from those contemporary H3 IAVs in humans, swine, and canine. Although we included H3 IAVs with diverse geographic and temporal coverage, it is likely some H3 antigenic variants in migratory birds could have been missed. Additional studies are needed to compare antigenic diversity of waterfowl origin IAVs to those from domestic poultry, especially those in the areas where H3 vaccination is used in turkeys (44).

Supplementary data associated with this article can be found at <http://dx.doi.org/10.1637/11086-041015-RegR.s1>.

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