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Influenza A Viruses from Overwintering and Spring-Migrating Waterfowl in the Lake Erie Basin, United States

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SUMMARY.

Influenza A virus (IAV) surveillance in migratory waterfowl in the United States has primarily occurred during late summer and the autumn southern migration. Data concerning the presence and ecology of IAVs in waterfowl during winter and spring seasons in the U.S. northern latitudes have been limited, mainly due to limited access to waterfowl for sampling. The southwestern Lake Erie Basin is an important stopover site for waterfowl during migration periods, and over the past 28 years, 8.72% of waterfowl sampled in this geographic location have been positive for IAV recovery during summer and autumn (June–December). To gain a better understanding of influenza A viral dynamics in waterfowl populations during winter and spring migration (February through April), cloacal swabs were collected from overwintering and spring-migrating waterfowl in Ohio and Michigan in 2006, 2007, 2013, and 2014. A total of 740 cloacal swabs were collected and tested using virus isolation in embryonating chicken eggs, resulting in the recovery of 33 (4.5%) IAV isolates. The influenza A isolates were recovered from eight waterfowl species in the order Anseriformes. Antigenically, the IAV isolates represent 15 distinct hemagglutinin (HA) and neuraminidase (NA) combinations, with seven (21%) of the isolates reported as mixed infections based on antigenic HA subtyping, NA subtyping, or both. This effort demonstrates the presence of antigenically diverse IAV in waterfowl during overwintering and spring migration at northern latitudes in the United States, thereby contributing to the understanding of the maintenance of diversity among waterfowl-origin IAVs.

RESUMEN.

Nota de investigación- Los virus de la influenza A partir de aves acuáticas migratorias durante el invierno y primavera en la cuenca del lago Erie, en los Estados Unidos.

La vigilancia de los virus de la influenza A en las aves acuáticas migratorias en los Estados Unidos se ha llevado a cabo especialmente durante finales de verano y durante la migración hacia el sur en el otoño. Los datos relativos a la presencia y la ecología de los virus de este tipo de aves acuáticas durante las temporadas de invierno y primavera en las latitudes del norte de Estados Unidos han sido limitados, debido principalmente a un acceso limitado a las aves acuáticas para el muestreo. El sudoeste de la cuenca del lago Erie es un sitio de parada importante para las aves acuáticas

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durante los periodos de migración y en los últimos 28 años, 8.72% de las aves acuáticas en la muestra en esta ubicación geográfica han sido positivos en la recuperación del virus de influenza aviar durante el verano y el otoño (Junio a Diciembre). Para obtener una mejor comprensión de la dinámica viral de la influenza A en las poblaciones de aves acuáticas durante el invierno y la migración de primavera (Febrero a Abril), se recolectaron hisopos cloacales de aves acuáticas durante el invierno y durante la migración de primavera en Ohio y Michigan en 2006, 2007, 2013, y 2014. Se recolectaron un total de 740 hisopos cloacales se analizaron mediante aislamiento del virus en huevos embrionados, dando como resultado la recuperación de 33 (4.5%) aislamientos de influenza aviar. Los aislamientos de influenza aviar fueron recuperados de ocho especies de aves acuáticas del orden Anseriformes. Antigénicamente, los aislamientos del virus de la influenza aviar representaron 15 combinaciones distintas de hemaglutinina (HA) y de neuraminidasa (NA), con siete (21%) de los aislamientos reportados como infecciones mixtas con base en los subtipos de HA, de NA o de ambos. Este esfuerzo demuestra la presencia diversa antigénicamente del virus de influenza aviar en las aves acuáticas durante el invierno y durante la migración de primavera en latitudes septentrionales en los Estados Unidos, lo que contribuye a la comprensión de la conservación de la diversidad entre los virus originados en este tipo de aves acuáticas.

Keywords

influenza A virus; avian influenza virus; wild birds; northern migration

Migratory waterfowl, (order Anseriformes), are a major reservoir for influenza A viruses (IAVs). Waterfowl play an important role in the natural history of IAVs, contributing to the dispersal of diverse IAVs to naïve populations, thereby perpetuating the transmission of IAVs in waterfowl populations (1,3,14,17). Low pathogenic (LP) IAVs infect migratory waterfowl mainly in the digestive tract and are shed in feces for up to 28 days, providing ample opportunity for fecal-oral viral transmission between individual birds (5,14). More recent studies have shown peak vial shedding in experimentally infected waterfowl occurs during the first 5 to 7 days postinfection, with intermittent shedding after 7 to 10 days (4). Most of the IAV surveillance in the United States has primarily been conducted in late summer and early autumn during the premigration staging season and during southern migration. Less is known about the presence and ecology of IAVs during the northerly spring migrations because access to waterfowl is more limited during spring when they are not hunted (3,9,10,11,17). Two previous studies have shown decreased viral shedding in waterfowl during spring migration compared with autumn migration; however, spring sampling efforts are much less expansive (15,17). Therefore, the current knowledge base regarding the diversity of IAVs in the waterfowl reservoir in the United States is largely based on late summer–autumn surveillance efforts. The movement of diverse IAVs from southern wintering grounds to northern breeding grounds has been reported in shorebirds; however, little information is available regarding similar IAV movement in waterfowl (8). This project was conducted to examine the frequency of IAV infections in wild waterfowl present in Ohio and Michigan during overwintering and spring migration.

MATERIALS AND METHODS

Cloacal swabs were collected from live-trapped, overwintering, resident, and northern-migrating waterfowl in Ohio and Michigan in 2006, 2007, 2013, and 2014 during the months of February through April, as previously described (13). Waterfowl were not accessible 2008–2012; thus, no samples were obtained. All bird handling and sample collection procedures were approved and accomplished under animal use protocol number 2007A0148 of The Ohio State University. Virus isolation attempts on each sample were done using 10-day-old embryonating chicken eggs by using a previously described protocol (12,13). All samples that demonstrated hemagglutinating activity via a hemagglutination test with 0.7% chicken red blood cells were tested for the presence of IAV by using the Avian Influenza Virus Type A Antigen Test Kit (Synbiotics Corporation, San Diego, CA). All IAV isolates were submitted to the National Veterinary Service Laboratory (NVSL) in Ames, Iowa, for antigenic hemagglutinin (HA) and neuraminidase (NA) subtyping using traditional methods, and all H7 isolates were pathotyped (16).

Selected IAV isolates were submitted to the J. Craig Venter Institute (Rockville, MD) or the University of Minnesota (St. Paul, MN) for full length genomic sequencing by using next-generation sequencing technologies, as previously described (3).

RESULTS

A total of 740 cloacal swabs were collected from eight species of Anseriformes from February to April during 2006, 2007, 2013, and 2014, resulting in the recovery of 33 IAV isolates (4.5%; Table 1). Isolates were recovered from 11 mallards (*Anas platyrhynchos*), two blue-winged teal (*Anas discors*), four ring-necked ducks *(Aythya collaris*), 11 redhead ducks (*Aythya americana*), one canvasback (*Aythya valisineria*), two American black ducks (*Anas rubripes*), one lesser scaup (*Aythya affinis*), and one northern pintail (*Anas acuta*). Antigenically, these isolates represent 15 distinct HA-NA combinations, with 7 (21%) isolates reported as mixed infections (Table 1). All H7 IAV isolates were determined to be LP by amplification of the cleavage site at the NVSL.

Full-length genomic sequencing was completed for 13 of the 33 isolates. The genomes of these isolates were found to be most similar to North American waterfowl–origin IAV isolates by using the Basic Local Alignment Search Tool in GenBank (data not shown). Genomic sequencing identified one IAV isolate (A/ring-necked duck/Ohio/06OS588/2006) that had multiple genomes for HA and NA genes that were not in agreement with the antigenic HA-NA subtyping (Table 2). These mismatches have been demonstrated in previous surveillance efforts, but a clear explanation is not available (2). The remaining 12 isolates had consistent HA-NA subtypes between the two testing methods (Table 2).

DISCUSSION

Active surveillance for IAVs in waterfowl populations using the Lake Erie Basin in Ohio has been ongoing since 1986 during the autumn southern migration. These sampling efforts have demonstrated, on average, 8.75% of the tested individuals were shedding IAVs at the time of sampling. Lebarbenchon *et al.* reported an average frequency of IAV viral shedding

of 28% in waterfowl populations in Minnesota during the southerly migrations of 2010 and 2011 (7). In contrast, a previous report described approximately 1% IAV recovery in North American waterfowl during the spring migration period in the northern latitudes of the United States (17). Wallensten *et al.* reported 3.4% frequency of IAV recovery during the spring of 2003 from mallards during April–June, with 6.5% recovery in May, with additional species having had a <1% viral recovery rate in Sweden (15). The reduced frequency of IAV viral recovery during the spring migration as compared with the autumn migration at the Lake Erie Basin is consistent with these previous reports.

Interestingly, the HA-NA subtype diversity shifted during the spring migration to subtypes that are less common during autumn migration. The IAV HA-NA subtypes detected in the autumn have been diverse, although the most frequently isolated subtype combinations are isolated each year and include H4N6 (16.5%), H4N8 (12.4%), and H3N8 (8.1%; Slemons, unpubl. data). In contrast, the most commonly isolated HA-NA subtypes during the spring migration were H11N2 (12.1%), H10N7 (9%), and H7N3 (9%). This phenomenon may be important in the maintenance cycle of IAV viral diversity; however, additional effort is needed to better understand this dynamic. As is the case in shorebirds, northern migrating waterfowl may be carrying viruses from the south, perpetuating the continuation of viral lineages; however, the differences in the dominant strain being shed in the autumn *vs.* spring indicate additional factors are involved (6).

Differences in the level and diversity of viral recovery between individual years could be a result of the variation of species sampled during each year. In 2006 and 2007, samples were obtained from birds collected as part of a study of foraging ecology of spring-migrating waterfowl mallards, gadwall (*Anas strepera*), blue-winged teal, lesser scaup, and ring-necked ducks in Ohio and Michigan. In 2013 and 2014, samples were obtained waterfowl captured during trap-release banding of waterfowl that were using isolated open water sources during the otherwise frozen winter and spring months. The bird populations varied year to year, with redheads being the predominant species in 2013 and mallards and gadwalls being the predominant species in 2014. Variation in rates of viral shedding among waterfowl host species using a given geographic location at varying population densities across years would lead to a better understanding of the natural history of IAVs in reservoir species during winter and spring migration.

Although this effort demonstrates the presence of antigenically diverse IAVs in waterfowl populations during overwintering and spring migration in the northern United States, it was limited in size and scope and should not be overinterpreted. Viral diversity could be grossly underestimated due to the limited sample size and variations in host species. A more systematic approach, targeting the same species temporally, should be employed to better understand the viral diversity within migratory waterfowl populations during the spring migration.

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Abbreviations:

HA	hemagglutinin
IAV	influenza A virus
LP	low pathogenic
NA	neuraminidase
NVSL	National Veterinary Services Laboratory

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Table 1.

Total number of samples collected, IAV isolates recovered, and their antigenic subtypes by year.

	2006	2007	2013	2014
No. of samples collected	185	240	141	174
No. of IAV isolates (%)	3 (1.6)	4 (1.7)	11 (7.8)	15 (8.6)
Antigenic subtypes	H7N9 (1)	H3N1 (1)	H1N2 (1)	H1N1,4 (1)
	H11N7 (1)	H3N? (1)	H3N6 (2)	H4N4,8 (1)
	H11N9 (1)	H7N3 (2)	H3N8 (1)	H6N2 (1)
	—	—	H3,4N6,8 (1)	H7N1,7 (1)
	—	—	H4N6 (1)	H10N3 (1)
	—	—	H6N2 (1)	H10N8 (1)
	—	—	H7N3 (1)	H10N1,4 (2)
	—	—	H10N7 (3)	H10N2 (1)
	—	—	—	H10N3,7 (1)
	—	—	—	H11N2 (5)

Table 2.

IAV isolate names and antigenic and genomic HA-NA subtype combinations. In addition, GenBank accession numbers are listed for sequenced IAV isolates.

Isolate name	Antigenic HA-NA subtype	Genomic HA-NA subtype	GenBank accession no.
A/blue-winged teal/Ohio/06OS566/2006	H7N9	H7N9	CY24818–CY24825
A/ring-necked duck/Ohio/06OS588/2006	H11N7	H3,10N3,7,8	CY081324–CY081331
A/ring-necked duck/Michigan/06OS429/2006	H11N9	—	NA[A]
A/mallard/Ohio/07NR002/2007	H7N3	—	NA
A/blue-winged teal/Ohio/07NR302/2007	H7N3	—	NA
A/mallard/Michigan/07NR214/2007	H3N1	—	NA
A/ring-necked duck/Michigan/07NR240/2007	H?N1	—	NA
A/redhead/Ohio/13OS0367/2013	H10N7	H10N7	KR077961–KR077968
A/redhead/Ohio/13OS0373/2013	H3N6	H3N6	KR077977–KR077984
A/redhead/Ohio/13OS0376/2013	H4N6	H4N6	KR077953–KR077960
A/redhead/Ohio/13OS0377/2013	H3,4N6,8	H3,4N6,8	KR077993–KR077997
A/redhead/Ohio/13OS0391/2013	H10N7	H10N7	KR077946–KR077952
A/redhead/Ohio/13OS0428/2013	H6N2	H6N2	CY187053–CY187060
A/redhead/Ohio/13OS0431/2013	H1N2	H1N2	CY186600–CY186607
A/redhead/Ohio/13OS0507/2013	H10N7	H10N7	CY187061–CY187068
A/redhead/Ohio/13OS0363/2013	H3N8	H3N8	KR077985–KR077992
A/redhead/Ohio/13OS0399/2013	H3N6	H3N6	KR077969–KR77976
A/redhead/Ohio/13OS0406/2013	H7N3	H7N3	KR077937–KR07797
A/canvasback/Ohio/14OS0600/2014	H10N3	—	NA
A/American black duck/Ohio/14OS0611/2014	H4N4,8	—	NA
A/mallard/Ohio/14OS0634/2014	H11N2,9	—	NA
A/mallard/Ohio/14OS0635/2014	H11N2	—	NA
A/mallard/Ohio/14OS0636/2014	H11N2	—	NA
A/mallard/Ohio/14OS0639/2014	H11N2	—	NA
A/mallard/Ohio/14OS0650/2014	H10N8	—	NA
A/lesser scaup/Ohio/14OS0652/2014	H1N1,4	—	NA
A/mallard/Ohio/14OS0654/2014	H10N1,4	—	NA

Isolate name	Antigenic HA-NA subtype	Genomic HA-NA subtype	GenBank accession no.
A/ring-necked duck/Ohio/14OS0656/2014	H10N2	—	NA
A/mallard/Ohio/14OS0665/2014	H7N1,7	—	NA
A/northern pintail/Ohio/14OS0677/2014	H6N2	—	NA
A/American black duck/Ohio/14OS0681/2014	H10N1,4	—	NA
A/mallard/Ohio/14OS0695/2014	H10N3,7	—	NA
A/mallard/Ohio/14OS0700/2014	H11N2	—	NA

[A]NA = not applicable.