

## **Type A Influenza Virus Surveillance in Free-Flying, Nonmigratory Ducks Residing on the Eastern Shore of Maryland**

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**SUMMARY.** Virus surveillance in free-flying, nonmigratory ducks living on the eastern shore of Maryland indicated that influenza A viruses were introduced into the area or that the prevalence of endemic infections increased between July 15 and August 27, 1998. Cloacal swabs collected between May 28 and July 15, 1998, were negative for influenza A virus recovery (0/233), whereas 13.9% (29/209) of swabs collected between August 27 and September 2, 1998, were positive for influenza A virus recovery. Five hemagglutinin subtypes (H2, H3, H6, H9, and H12), six neuraminidase subtypes (N1, N2, N4, N5, N6, and N8), and nine HA-NA combinations were identified among 29 influenza A isolates. Interestingly, 18 of the 29 isolates initially appeared to contain two or more HA and/or NA subtypes. The free-flying, nonmigratory ducks served as excellent sentinels for the early detection of type A influenza viruses in the southern half of the Atlantic Migratory Waterfowl Flyway during the earliest phase of the yearly southern migration.

**RESUMEN.** Vigilancia epidemiológica del virus de influenza tipo A en patos salvajes no migratorios residentes en la costa este de Maryland.

La vigilancia epidemiológica del virus de influenza tipo A en patos salvajes no migratorios residentes en la costa este del estado de Maryland en los Estados Unidos indicó que los virus de influenza tipo A fueron introducidos a la zona o que la prevalencia de las infecciones endémicas se incrementó entre Julio 15 y Agosto 27 de 1998. Los hisopos cloacales tomados entre Mayo 28 y Julio 15 de 1998 fueron negativos al virus de influenza tipo A (0/233) mientras que el 13.9% (29/209) de los hisopos tomados entre Agosto 27 y Septiembre 2 de 1998 fueron positivos al virus de influenza aviar tipo A. Se identificaron 5 subtipos de hemoaglutinina (H2, H3, H6, H9 y H12), 6 subtipos de neuraminidasa (N1, N2, N4, N5, N6 y N8) y 9 combinaciones de hemoaglutinina-neuraminidasa entre 29 aislamientos de influenza tipo A. Inicialmente, 18 de los 29 aislamientos parecían contener dos o más subtipos de hemoaglutinina y neuraminidasa. Los patos salvajes no migratorios sirvieron como unos excelentes centinelas para la detección temprana de los virus de influenza tipo A en la zona media sur de la ruta migratoria de las aves acuáticas en el Atlántico durante la fase más temprana de la migración anual hacia el sur.

**Key words:** Atlantic Migratory Waterfowl Flyway, avian influenza, ducks, epidemiology

**Abbreviations:** HA = hemagglutinin; NA = neuraminidase; RSAs = regulated shooting areas

Chesapeake Bay and the Delmarva Peninsula are important to both waterfowl populations and

domestic poultry production. For waterfowl, this region is located at the last major convergence point on the Atlantic Migratory Waterfowl Flyway (3). Waterfowl reaching this convergence point originate from as far north as the Arctic Ocean, as far northwest as the Northwest Territory of Canada, and as far northeast as Greenland. From the Chesapeake Bay/Delmarva Peninsula convergence point, the Atlantic flyway extends south in a relatively narrow

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band along the east coast of the United States into Florida and then on to Cuba and Hispaniola. Therefore, migratory waterfowl using the Atlantic flyway and originating from widely dispersed locations in Canada, Greenland, and the United States come in contact with each other during their fall and spring migrations and during the winter season. The Delmarva Peninsula is also important to poultry production in the United States, with a large commercial broiler industry plus smaller producers raising multiple species of domestic poultry that are marketed locally and in live bird markets in northeastern United States. Therefore, understanding the dynamics of influenza A virus infections in waterfowl and domestic poultry residing in the Chesapeake Bay/Delmarva Peninsula region is important for both wild waterfowl populations and domestic poultry production.

This basic epidemiologic investigation had three objectives. The first objective was to document the occurrence of influenza A virus infections in non-migratory waterfowl on the eastern shore of Maryland. The second objective was to obtain a current collection of waterfowl-origin influenza A viruses circulating in ducks residing on the eastern side of the Chesapeake Bay for comparison to influenza A viruses circulating in domestic poultry in the northeastern region of the United States. The third objective was to gain insight into the dynamics of influenza A virus infections in nonmigratory ducks living in the southern half of the Atlantic Migratory Waterfowl Flyway.

## MATERIALS AND METHODS

The sample population consisted of wild, free-flying, nonmigratory and raised-and-released, free-flying, nonmigratory ducks residing on the eastern shore of Maryland. This area is immediately east of the Chesapeake Bay and is part of the Delmarva Peninsula that includes Delaware and parts of Maryland and Virginia. The wild ducks were captured in swim-in traps by federal and state waterfowl experts at various wildlife management areas as part of ongoing waterfowl population studies. Capturing of the raised-and-released mallards was conducted on various Regulated Shooting Areas (RSAs). The cooperation of these progressive RSAs was critical to the success of the investigation and clearly demonstrated their support and interest in furthering the health of waterfowl populations.

A cloacal swab collected from each duck was placed in an individual vial containing virus-transport me-

dium. The vials were temporarily stored in a cooler in the field and then placed in a  $-70$  C freezer within 4 days. Sampling was conducted between May 28 and June 11; between July 1 and July 15, and between August 27 and September 2, 1998.

Standard virus isolation procedures were accomplished using 10-day-old embryonating chicken eggs (1). Hemagglutinating agents were identified as type A influenza viruses using a commercially available diagnostic assay to identify the type A antigen of influenza A viruses (Directigen<sup>®</sup> Flu A Assay, Becton Dickinson Microbiology Systems, Cockeysville, MD). Hemagglutinin (HA) and neuraminidase (NA) subtyping of the type A influenza isolates were accomplished using standard procedures at the U.S. Department of Agriculture National Veterinary Services Laboratories (6).

## RESULTS

Twenty-nine type A influenza isolates were recovered from a total of 442 cloacal swabs (6.6%). However, all of the isolates were recovered from swabs collected between August 27 and September 2, resulting in a frequency of virus recovery of 13.9% ( $n = 209$ ) for that sampling period. During this period, the frequency of virus recovery by species ranged from 17% to 11.9% to 8.3% for raised-and-released mallards, wild wood ducks, and wild mallards, respectively.

Subtyping of the 29 influenza A virus isolates identified five HA subtypes (H2, H3, H6, H9, and H12), six NA subtypes (N1, N2, N4, N5, N6, and N8), and nine HA-NA combinations. Initial HA and NA subtyping results indicated that 18 of the 29 isolates initially contained viruses representing more than one HA and/or NA subtype. These mixed virus isolates were subjected to two sequential limiting dilutions and then subtyped a second time.

## DISCUSSION

Many of the findings from this investigation were expected, but additional insight did emerge on the natural history of influenza A viruses in ducks. Isolating 29 type A influenza isolates from free-flying, nonmigratory waterfowl on the eastern shore of Maryland was not a surprise. The recovery of waterfowl-origin influenza A viruses from ducks and geese was previously reported during the fall hunting season in nearby Delaware (4). Identifying five HA subtypes, six NA subtypes, and nine HA-

NA combinations among the 29 isolates was consistent with many reports for influenza A virus surveillance in waterfowl (2,4). Not detecting influenza A viruses in resident ducks prior to July 15 and then recovering influenza A viruses this far south (in the Atlantic Migratory Waterfowl Flyway) in addition to the relatively high frequency of virus recovery so early in the year were interesting findings. Possible explanations for these observations are that early migrating birds reintroduced multiple strains of influenza A virus into 3- to 5-mo-old, influenza A-naïve, free-flying, nonmigratory ducks between July 15 and August 27 or that the prevalence of endemic infections was below the detection level of the surveillance protocol.

The absence of the H5 and H7 HA subtypes among the duck-origin influenza A isolates was a welcome finding. Waterfowl are commonly believed to serve as a source of the avian influenza viruses that infect domestic poultry, and the H5 and H7 subtypes have historically been of most concern to the world's poultry industry. Since 1983, these subtypes have been associated with sporadic avian influenza outbreaks and subclinical infections in poultry in the northeastern United States. The absence of H5 and H7 HA subtypes in this admittedly limited sampling raised the question of whether commercial poultry, or other wild birds, serve as maintenance hosts for the H5 and H7 influenza A viruses sporadically appearing in the northeastern United States. Further evaluation of this possibility appears justified.

In Minnesota, penned and/or pinioned influenza A-naïve mallards were shown to be effective sentinels in influenza A virus surveillance and epidemiologic investigations (2). In our investigation, free-flying, nonmigratory ducks also proved to be effective sentinels. Their use as sentinels resulted in the early detection of influenza A viruses on the Delmarva Peninsula in late August, when relatively few birds have migrated into the area and well before most migratory waterfowl arrive in the area.

Detecting influenza A viruses this far south this early in the yearly southern waterfowl migration season raised the question as to the source of the viruses. Shorebirds and early-arriving ducks would be one possibility. Shorebirds generally begin arriving in the area around the first or second week of August. Blue-winged teal and a few pintail ducks generally begin arriving during the middle of August. In the Mississippi Migratory Waterfowl

Flyway, influenza A viruses have been recovered from blue-winged teal as early as September in coastal Louisiana (5). Therefore, if shorebirds and/or blue-winged teal were the sources of the viruses, the viruses would have been introduced into the local nonmigratory ducks less than 4 wk prior to August 27. The relatively high frequency of virus isolations and the relatively high percent of mixed infections are consistent with recent introductions of influenza A viruses. The other most likely source of the viruses would be the occurrence of undetected endemic infections early in the summer, followed by a situation in which host or environmental factor changes triggered an increase in the incidence of infections during the late summer.

Recovery of type A influenza viruses from resident (nonmigratory) and migratory ducks sampled in November in coastal Louisiana supported the premise that influenza A viruses could winter-over and persist in wild duck populations (5). This report provides additional evidence that nonmigratory ducks (resident ducks) can play an active role in the maintenance of antigenically diverse type A influenza viruses in migratory waterfowl.

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