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Avian Influenza Viruses in Minnesota Ducks During 1998–2000

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SUMMARY. Although wild ducks are known to be a major reservoir for avian influenza viruses (AIV), there are few recent published reports of surveillance directed at this group. Predominant AIV hemagglutinin (HA) subtypes reported in previous studies of ducks in North America include H3, H4, and H6, with the H5, H7, and H9 subtypes not well represented in these host populations. The objective of this study was to determine whether these subtype patterns have persisted. Each September from 1998 to 2000, cloacal swabs were collected from wild ducks banded in Roseau and Marshall counties, MN. Mallards (*Anas platyrhynchos*) were sampled all years, and northern pintails (*A. acuta*) were sampled only in 1999. Influenza viruses were isolated from 11%, 14%, and 8% of birds during 1998, 1999, and 2000, respectively. Prevalence, as expected, was highest in juveniles, ranging from 11% to 23% in mallards. Viruses representative of the HA subtypes 2, 3, 4, 5, 6, 7, 9, 10, 11, and 12 were isolated. Viruses in the H5, H7, and H9 subtypes, which are associated with high-pathogenicity influenza in poultry or recent infections in humans, were not uncommon, and each of these subtypes was isolated in 2 out of the 3 years of surveillance.

RESUMEN. Virus de Influenza Aviar en Patos en Minnesota 1998–2000.

A pesar de que los patos salvajes son el mayor reservorio de los virus de influenza aviar, pocos reportes recientes sobre la vigilancia epidemiológica dirigida hacia dicho grupo se han publicado. Los subtipos de hemoaglutinina del virus de influenza aviar predominantes en patos de América del Norte reportados en estudios anteriores incluyen los subtipos H3, H4 y H6, mientras los subtipos H5, H7 y H9 no se encuentran representados adecuadamente en estas poblaciones huéspedes. El objetivo de este estudio fue determinar si los patrones de estos subtipos han persistido. Durante el mes de Septiembre desde el año 1998 hasta el 2000, se tomaron hisopos cloacales a partir de patos salvajes obtenidos en los condados de Roseau y Marshall en MN. Todos los años se tomaron muestras en patos ánade azulones (*Anas platyrhynchos*) y durante 1999 en ánades de cola larga (*A. acuta*). Se aislaron virus de influenza a partir del 11%, 14% y 8% de las aves durante los años 1998, 1999 y 2000, respectivamente. La mayor prevalencia, como se esperaba, se observó en patos jóvenes, en un rango del 11 al 23% en patos ánades azulones. Se aislaron virus representativos de los subtipos de hemoaglutinina 2, 3, 4, 5, 6, 7, 9, 10, 11 y 12. Los virus de los subtipos H5, H7 y H9, los cuales se encuentran asociados con influenza de alta patogenicidad en aves o con infecciones recientes en humanos, fueron comunes, y cada uno de estos subtipos fue aislado en 2 de los 3 años de la vigilancia epidemiológica.

Key words: avian influenza, mallard, Minnesota, pintail, poultry

Abbreviations: AIV = avian influenza virus; BHI = brain heart infusion; HA = hemagglutinin; MEM = minimal essential media; NA = neuraminidase; NVSL = National Veterinary Services Laboratory; SPF = specific pathogen free; WMA = wildlife management area

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Table 1. Prevalence of avian influenza virus in migratory ducks, Roseau and Marshall counties, MN, September 1998–00.

Year	Species	Age	Total
1998	Mallard	Juvenile	41/375 (10.9)
1999	Mallard	Adult	2/87 (2.3)
		Juvenile	58/247 (23.4)
	Pintail	Adult	1/96 (1.0)
		Juvenile	3/37 (8.1)
2000	Mallard	Adult	8/380 (2.1)
		Juvenile	41/201 (20.3)
Total			154/1423 (10.8)

Ducks, geese, and swans of the order Anseriformes are the primary reservoir for avian influenza viruses (AIV) and have been implicated in the spread of influenza to domestic poultry (8,13). In the 1990s, at least 21 countries reported isolating AIV from wild or domestic birds (20), and highly pathogenic strains affected more than 14 million domestic poultry (28). These high-pathogenicity influenza outbreaks all have been associated with AIVs of the hemagglutinin (HA) subtypes H5 and H7, which represent the only subtypes linked to highly pathogenic influenza outbreaks in poultry (28). In 1997, an H5 AIV (H5N1) also represented the first suspected direct transmission of AIV from poultry to humans and was responsible for six deaths in Hong Kong (1). More recently in 1999, an H9N2 virus was isolated from two patients in Hong Kong and was also suspected of direct avian-to-human transmission (17). The threat of continued introductions of these viruses to humans or poultry populations and the recognition of wild birds as the reservoir for these viruses reaffirms the need for surveillance of these reservoir populations to understand the potential for the emergence of pathogenic human and avian strains.

Considerable surveillance of waterfowl in the 1970s and 1980s forms the base of our current knowledge of avian influenza ecology in North America (14,23,26,27). Although all 15 hemagglutinin (HA) subtypes have been isolated from wild waterfowl (18,21), the H3, H4, or H6 subtypes have been isolated most frequently (22). In contrast, the H5, H7, and H9 subtypes have been poorly represented in these reported isolations. For example, in surveys of AIV in North American waterfowl, the H5, H7, and H9 subtypes represent only 0.4%, 0.7%, and 0.4% of over 3100 isolates, respectively (2,6,9,10,11,14,15,19,23,24,27,30). This has led some researchers to suggest that waterfowl may not represent reservoirs for all HA subtypes (22). Reports of recent virus isolation attempts from waterfowl in North America are few (12,16), and surveillance of migrating waterfowl in the Atlantic and Mississippi flyways has not been reported since 1991. The objective of this study is to provide current information on the AIV subtype diversity present in migrating North American wild ducks from sites previously sampled during 1973–76 (4,5).

MATERIALS AND METHODS

In mid-September 1998–2000, cloacal swabs were collected from ducks captured on Thief Lake Wildlife Management Area (WMA), Roseau River WMA, or Agassiz National Wildlife Refuge in Roseau and Marshall counties, MN, during annual waterfowl banding programs conducted by the Minnesota Department of Natural Resources and the U.S. Fish and Wildlife Service. Adult and juvenile mallards (*Anas platyrhynchos*) were sampled all 3 yr, but northern pintail (*A. acuta*) was sampled in 1999 only. An emphasis on sampling of juvenile mallards was based on previous reports of high isolation rates from this species and age class (11,29) and the abundance of this species at the sites. Ducks were sexed, aged, and banded by wildlife agency personnel. Cloacal swabs were collected using sterile cotton-tipped applicators (Puritan®, Hardwood Products Company, Guilford, MN) and placed in sterile polypropylene tubes (Corning Inc., Corning, NY) containing 4 ml of transport media supplemented with penicillin G (10,000 μ/ml), streptomycin (2 mg/ml), kanamycin (0.6 mg/ml), gentamicin (1 mg/ml), and amphotericin B (0.02 mg/ml) (Sigma Chemical Company, St. Louis, MO). Either minimal essential media (MEM) (Sigma Chemical Company) or brain heart infusion media (BHI) (Becton Dickinson, Sparks, MD) were used in 1998–99, while only BHI was used in 2000. Samples were stored on ice in the field and were shipped overnight (approximately 24–48 hr on ice total) to the laboratory where they were frozen at −70 C until processed.

Samples were thawed, vortexed, and centrifuged at $1{,}500 \times g$ for 15 min. The supernatant was inoculated (0.25 ml/egg) via the allantoic route into four 9-day-old specific pathogen free (SPF) embryonated chicken eggs. Hemagglutination testing was completed as previously described (27), except only two eggs were used during the second egg passage for samples that were negative on the first egg passage. All isolates were serotyped using hemagglutinating inhibition and neuraminidase inhibition tests at the National Veterinary Services Laboratories (NVSL), Veterinary Services, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, Ames, Iowa.

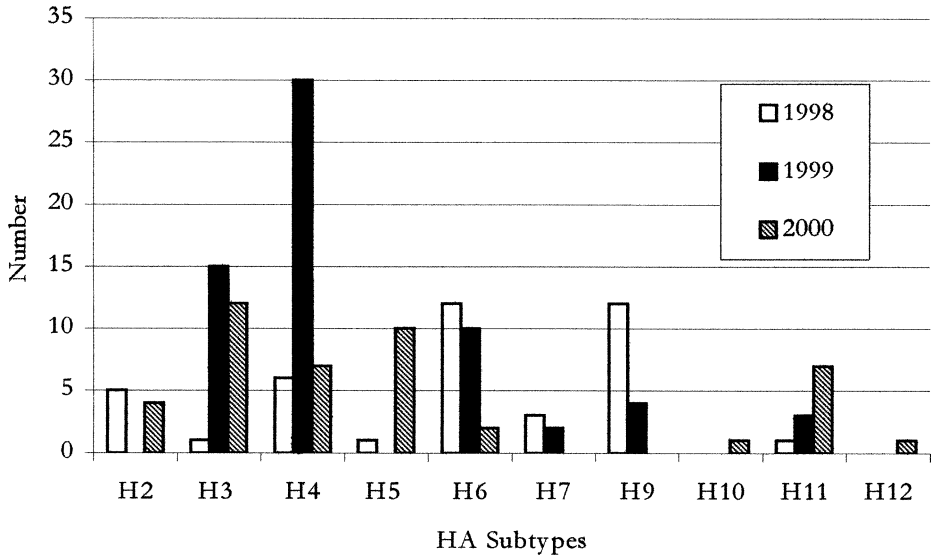

Fig. 1. Number of HA subtypes in 149 avian influenza viruses isolated from waterfowl from Roseau and Marshall counties, MN, September 1998–2000.

Differences in prevalence estimates between species, age class, and transport media were tested using chi-square analysis (25).

RESULTS

Avian influenza viruses were detected during all 3 years with 154 AIVs isolated from 1423 (10.8%) sampled ducks (Table 1). Prevalence of infection in mallards (18%) sampled during 1999 was significantly higher than observed in northern pintails (2.9%) ($P < 0.0001$). A significant difference in prevalence between juveniles (16.6%) versus adults (2%) was detected ($P < 0.0001$). Prevalence of AIV also was higher in mallard juveniles (17%) than in mallard adults (2.1%) ($P < 0.0001$). No significant difference in isolation rates between pintail adults and juveniles was detected ($P = 0.11$), probably due to the low sample size of juveniles.

As expected, the H3, H4, and H6 subtypes predominated, representing 18.5%, 28.5%, and 16%, respectively, of all AIV isolations (Fig. 1). These subtypes and the H11 subtype were detected each year. Thirty-two viruses of the H5, H7, and H9 subtypes were isolated, representing 7.4%, 3.4%, and 10.7% of all isolates, respectively. Each was identified in 2 of the 3 years in mallards and one H7 AIV was isolated from a juvenile pintail. All nine neuraminidase (NA) subtypes were represented, and 32 HA NA subtype combinations were detected (Table 2). More than one HA and/or NA were identified in 15 isolates (1.2% of total). These were interpreted as dual infections, which are not uncommon in waterfowl (21).

To avoid species- and age-related variation, comparison of AIV isolation rates between samples stored in BHI (22.0%) *vs.* MEM (11.2%) were restricted to juvenile mallards. Virus isolation attempts were more successful from samples stored in BHI ($P = 0.0005$).

DISCUSSION

Variation in prevalence by age and year of sampling is consistent with previous AIV surveys. The higher isolation rate observed in juveniles *vs.* adults agrees with other studies (2,10,11). Yearly prevalence ranged from 10.9% to 23.4% in juvenile mallards. However, very high prevalence estimates, exceeding 60%, have been reported in other years and locations (11).

In 1999, the difference between isolation rates of pintails and mallards is more extreme than reported in other studies when AIVs were isolated from both species at similar rates (6,10,23). Although our sample size of juvenile pintails was small, only one AIV was detected among 96 adults. Hinshaw *et al.* detected AIV in adult pintails 5%–29% of the time (10). One possible explanation for this study's lower detection rate is the pintail's population fluctuations

Table 2. Hemagglutinin and neuraminidase antigenic classification of avian influenza subtypes isolated from ducks in Roseau and Marshall counties, MN, September 1998–00.

	H2	H3	H4	H5	H6	H7	H9	H10	H11	H12
N1		1								
N2	3	4	2	7	1		16		2	
N3	3		1	2	1	4				
N4	1	2			1					
N5		1		1	1					1
N6		4	21		1					
N7						1		1		
N8		7	17		12					
N9	2	2	1						9	

Mixed infections were observed for some samples including H2,5N2,6 (1), H3N4,8 (7), H4N4,8 (1), H5N1,4 (1), H5,6N6 (1), H6N1,4 (2), H6N4,8 (1), H?N2 (2), H?N4,8.

over the last two decades (7). The resulting age structures of the current population could be radically different than birds sampled 20 years ago, and, thus, it may be possible that the majority of adult pintails sampled have already acquired immunity to AIV.

The predominance of the H3, H4, and H6 virus subtypes, which represented 63.8% of all isolates, coincides with more than 30 years of surveillance data (2,6,9,10,11,14,15,19,23,24,27,30), suggesting that this serotype predominance may be a stable characteristic of AIVs in North American waterfowl. Likewise, all NA subtypes were detected, and the high prevalence of the N2, N6, and N8 subtypes (24%, 17.5%, and 23.4%, respectively) was consistent with other studies (22). Unlike other studies, however, the isolation rates of the H5, H7, and H9 subtypes (21.5% combined) were higher, and in no other study were so many of these particular subtypes isolated within a 3-yr time span. In fact, the isolation of all three subtypes has only been reported in Alberta during a 15-yr study (11,22), and these subtypes were not detected at all in several studies (2,24,27,30). From individual survey results, AIVs of the H5, H7, and H9 subtypes have never exceeded 8% (23), 1.6% (14), and 2.6% (14) of the total isolates, respectively. It is interesting that in a previous survey within the same region of Minnesota during 1973–76, the H5 virus subtype, as in this study, was isolated during 2 years, but the H7 and H9 subtypes were not detected in ducks (3). At least two H5 and one H9 subtypes were detected in domesticated turkeys in the state during 1972–79 (3).

Although previous studies suggest the H5, H7, and H9 virus subtypes are rare in North American waterfowl (14,22,29), the present study isolated these subtypes more than 20% of the time. It is interesting to note that in more recent work in Brazoria County, TX, AIVs were isolated from 11 of 96 ducks sampled in February and that the H7 subtype represented five of these isolates (unpubl. data). Whether this is due to the location of sampling, a real change over time, or some other factor is unknown, but these results suggest that spatial and temporal variation related to AIV in waterfowl populations may deserve additional attention. In North America, AIV isolations with serotyping results from waterfowl have been reported only from approximately 30 locations over the past 30 yr. Surveillance has not been reported in British Columbia, Saskatchewan, or Manitoba in Canada and has been completed in less than 20 states in the United States. Surveillance of ducks migrating along the Atlantic and Mississippi flyways has not been reported for more than 10 yr. Perhaps our knowledge of AIV ecology needs to be supplemented with new surveillance efforts from a broad range of locations, species, and seasons.

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