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INFLUENZA A VIRUSES ISOLATED FROM MIGRATING DUCKS IN OKLAHOMA[1]

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Abstract: Nine type A influenza viruses were isolated from migrating and wintering ducks in Oklahoma in 1976-77. Antigenic classification of the viruses isolated revealed three different subtypes: Hav1 Nav2, Hws N1, and Hav6 N2. Transmission of influenza viruses from the wild ducks to sentinel birds (McGraw mallards) on the same lakes was not detected.

INTRODUCTION

Migratory waterfowl harbor a large variety of influenza A viruses.[4,5] These influenza viruses in free-ranging birds have been suggested as the source of virus responsible for outbreaks of influenza in domestic species.[3] It also has been postulated that these avian viruses play a role in the origin of new human pandemic strains of influenza.[12] Surveillance of influenza viruses in free-ranging birds has been undertaken to better understand the ecology of these viruses in the birds, as well as their relationship to viruses appearing in other species.[7]

Studies on free-ranging ducks in Canada just prior to migration have shown a high prevalence of diverse influenza A viruses in these birds.[5] Other studies have shown that influenza A viruses continue to circulate in waterfowl outside of the breeding area and along the migratory route although the prevalence of infection is noticeably lower.[5,13] The viruses detected in these studies included strains with surface antigens (i.e., hemagglutinin and neuraminidase) antigenically related to viruses previously isolated during domestic avian disease outbreaks, as well as viruses isolated from humans.[5]

The studies presented here were conducted to determine the circulation of these viruses in ducks along the migration route and during the wintering period and to study transmission in nature by keeping sentinel ducks on a lake where migrating ducks were trapped.

MATERIALS AND METHODS

Trapping Wild Waterfowl

In the fall of 1976, and the spring of 1977, free-ranging waterfowl were trapped with the aid of a rocket net at a Soil Conservation Service Impoundment, (100 surface ha) known as Ham's Lake in Payne County, Oklahoma. Wild Birds were transient on Ham's Lake although mallard ducks *(Anas platyrhynchos)* are known to winter in Central Oklahoma. All birds were bled from the brachial vein and serum was collected and stored at -70 C until analysis.

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"Non-migrating" Waterfowl

Mallard ducks used as sentinel birds were obtained from the Max McGraw Wildlife Foundation. All McGraw ducks were laboratory reared, pinioned, and pretested prior to release in the study area. These birds were allowed free access to the entire lake and mixed freely with wild birds. McGraw birds were sampled along with wild ducks.

Virus Detection

Samples of the trachea of wild and McGraw waterfowl were taken with sterile aluminum shafted nasopharyngeal Calgiswabs. Cloacal samples were taken with standard sterile cotton-tipped applicator sticks. The swabs were placed into a two ml solution of cold tryptose phosphate broth with 0.5% gelatin and antibiotics (Penicillin G-10,000 units/ml,[4] Streptomycin - 10 mg/ml,[5] Amphotericin B-25,000 mg/ml[4]) in 10 ml screwcapped glass tubes. The tubes were immediately placed on ice, then transported to the laboratory where they were frozen at -70 C.

Swab samples were thawed at room temperature and then placed in a refrigerator at 4 C for 1.5 h. Three nine to twelve-day old embryonated chicken eggs were injected with 0.3 ml of sample: 0.2 ml was injected into the chorioallantoic cavity of each egg and 0.1 ml, into the amniotic sac of each egg. Inoculated eggs were candled twice daily to detect embryo death. Allantoic and amniotic fluids were collected from each egg when death was detected at four days after inoculation. Allantoic fluids containing hemagglutinating agents were tested for bacterial and fungal contamination by streaking on heart infusion blood agar and IPPLO agar plates and by inoculation of thiol broth. These media were incubated both aerobically and anaerobically.

Serologic Tests

Hemagglutination (HA) titration and hemagglutination inhibition (HI) tests were performed in microtiter plates with receptor-destroying enzyme-treated sera.[1] Neuraminidase (NA) titrations and neuraminidase inhibition (NI) tests have been fully described.[17] Type A ribonucleoprotein (A-RNP) was identified as previously described[4] in a single-radial immunodiffusion test with goat antisera specific for type A-RNP.[11] In HI and NI tests, antisera specific for the isolated hemagglutinin subunits and for most of the neuraminidase subunits of the reference strains of influenza A viruses[13,14,16] were prepared in goats.[11] Antisera to other neuraminidase antigens were prepared in rabbits or goats with antigenic hybrid viruses possessing an irrelevant hemagglutinin subunit.[11]

RESULTS

Three-hundred forty-six free-ranging waterfowl were sampled during the course of this study. Species sampled, sampling dates and virus detection is shown in Table 1. An additional 93 samples were taken from the McGraw mallards over the same time period.

Reaction in SRIO, examination by electron microscopy, and antigenic characterization in HI and NI tests indicated that the nine viruses recovered from the free-ranging ducks were influenza A viruses of three different antigenic combinations: Hsw1 Na, Hav1, Nav2 and Hav6 N2 (Table 2). The two viruses isolated from the sentinel birds were identified as paramyxoviruses but showed no reaction with reference antiserum to NDV. No further characterization of the paramyxoviruses was attempted.

[4] E. R. Squibb & Sons, Inc., Princeton, New Jersey 08540, USA.

[5] Pfizer Laboratory Division, New York, New York 10017, USA.

TABLE 1. Virus detection from free-ranging and non-flying waterfowl in Oklahoma.

Species	No. Examined	No. of viruses detected	Sampling Date
Mallard *(Anas platyrhynchos)*	91	7	Oct, Nov, Dec 1976 Jan, Feb, Mar 1977
American Wigeon *(Anas americana)*	22	1	Nov 1976
Pintail *(Anas acuta)*	4	0	Nov 1976 Feb 1977
American Green-winged Teal *(Anas crecca)*	126	1	Mar, Apr, May 1977
Blue-winged Teal *(Anas discors)*	98		Mar, Apr, May 1977
Wood Duck *(Aix sponsa)*	5	0	Apr, May 1977
McGraw Mallards*	93	2	Oct, Nov, Dec 1976 Jan, Feb, Mar 1977

*The total number of samples is greater than the total number of birds released due to repeat sampling.

DISCUSSION

The isolation of nine influenza A viruses from migrating ducks (primarily from the cloacae) in Oklahoma would agree with other studies[3,5,9,10,13] from other flyways demonstrating that diverse influenza viruses do circulate in these birds and can be isolated from them at various points along their migration route. Hinshaw *et al.*[5] reported the isolation of Hav1 Nav2 virus (which has a hemagglutinin antigenically related to that of a classical fowl plague virus) and Hsw1 N1 virus (antigenically related to swine and human viruses) from ducks in Canada prior to migration during 1977; these studies indicate that the viruses were still circulating after the birds left the marshalling areas in Canada. The antigenic subtypes, i.e., Hav1 Nav2 and Hav6 N2, previously have been detected in birds during disease outbreaks in domestic turkeys in North America.[2,8] The presence of high titers of infectious influenza viruses in the feces of ducks[15] and the isolation of viruses from lake water[6] have led to the suggestion that in nature these viruses could be transmitted to other birds via fecally contaminated water supplies.[13] In this study, no transmission to sentinal birds was detected. The results indicate, however, that viruses were still circulating and/or are transmitted among free-ranging birds during a great portion of the migratory and wintering period within the central flyway.

The surveillance of influenza A viruses in birds continues to offer the opportunity to study the ecology of these viruses in the ducks and to determine their role in the appearance of antigenically related viruses in other species, including man.

TABLE 2. Isolation and antigenic classification of hemagglutinating viruses isolated from ducks in Oklahoma in 1976-77.

Sample No.	Type of Duck	Date of Isolation	Source of Sample*	Influenza A-RNP	Antigenic Configuration	Morphological Identification
	Free-ranging ducks					
20	Mallard *(Anas platyrhynchos)*	Oct. 1976	C	+	Hsw1 N1	Influenza
24	American Wigeon *(Anas americana)*	Nov. 1976	C	+	Hav6 N2	Influenza
42	Mallard *(Anas platyrhynchos)*	Nov. 1976	C,T	+	Hsw1 N1	Influenza
44	Mallard *(Anas platyrhynchos)*	Nov. 1976	C	+	Hsw1 N1	Influenza
49	Mallard *(Anas platyrhynchos)*	Dec. 1976	T	+	Hws1 N1	Influenza
57	Mallard *(Anas platyrhynchos)*	Dec. 1976	C	+	Hsw1 N1	Infuenza
188	Green-winged Teal *(Anas crecca)*	Apr. 1977	C	+	Hav1 Nav2	Influenza
25	Mallard *(Anas platyrhynchos)*	Dec. 1977	C	+	Hsw1 N1	Influenza
28	Mallard *(Anas platyrhynchos)*	Dec. 1977	C	+	Hsw1 N1	Influenza
	Sentinel Ducks					
M50	McGraw Mallard	Oct. 1976	C	—	—	Paramyxovirus
M11	McGraw Mallard	Oct. 1976	C	—	—	Paramyxovirus
M209	McGraw Mallard	Dec. 1976	C	—	—	Paramyxovirus
M35	McGraw Mallard	Dec. 1976	C	—	—	Paramyxovirus

*Swabs were taken from the trachea (T) and/or the cloaca (C).

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