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SURVEY OF INFLUENZA VIRUSES IN FERAL BIRDS IN 1979 AND ISOLATION OF A STRAIN POSSESSING Hav6Nav5 FROM CLOACA OF AN EASTERN DUNLIN

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In the course of a survey in 1979 of influenza virus in feral birds, including 255 water fowls of 19 species, 719 small migrating birds of 32 species and 126 sea birds of 6 species, an influenza virus was isolated from a cloacal swab of one of the 38 eastern dunlins (*Calidris alpina sakhalina*) examined. No influenza virus was isolated from the other birds. The isolate possessed Hav6 hemagglutinin and Nav5 neuraminidase and was designated as A/eastern dunlin/Hokkaido/101/79 (Hav6Nav5).

INTRODUCTION

Numerous influenza viruses of various subtypes have been isolated from apparently healthy wild free-flying brids.[4,5,14,15,17] These viruses have most frequently been isolated from ducks and not from other birds. Although influenza viruses have been isolated from many species of birds[17], few have been isolated from snipes (WHO, The Ecology of Influenza Viruses; Report of Meeting of Investigation, Geneva, 1976).

Influenza viruses isolated from birds such as shearwaters[3], budgerigars[10], mynahs[11] and other domestic species were from the respiratory tracts, while those from ducks[5,8,13,16] were from cloacal swabs. A concept of intestinal influenza has been presented[16]. Experiments have shown the replication sites of influenza viruses to be the respiratory tract in budgerigars[6] and the intestine in ducks.[8,13,16] It is not known what other species of birds are susceptible to intestinal influenza.

In the course of a survey in 1979 of influenza virus from wild free-flying birds,

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we isolated an influenza virus from a cloacal swab taken from an eastern dunlin. The results are described below.

MATERIALS AND METHODS

Collection of materials: Cloacal and tracheal swabs were collected from 255 water fowls of 19 species, which were comprised predominantly of 38 eastern dunlins (*Calidris alpina*), 90 wigeons (*Anas penelope*), 31 eastern scanp-ducks (*Aythya marila*), 25 tufted ducks (*Aythya fuligula*) and 21 teals (*Anas crecca*). Also, cloacal swabs were collected from 719 small, mostly migratory birds of 32 species, comprised predominantly of 207 Japanese buntings (*Emberiza spodocephala*), 157 Japanese bushwarblers (*Cettia diphone*), 76 Kamchatkan rustic bundings (*Emberiza rustica*), 47 Japanese green finches (*Carduelis sinica*), 46 dusky thrushes (*Turdus naumanni*) and 38 eastern dunlins (*Calidris alpina sakhalina*). The birds were netted for banding research on the Pacific Flyway at Hamatonbetsu in Hokkaido, Japan in October and November of 1979. The swabs were put into a broth containing antibiotics (10,000 units penicillin and 10 mg streptomycin per ml) and frozen in a dry-ice-methanol bath for 6 hours and then stored at 80°C until assayed. Tracheas, lungs and lower intestines were collected from 126 sea birds representing 6 species and comprised mainly of 65 slender-billed shearwaters (*Puffinus tenuirostris*) and 49 sooty shearwaters (*Puffinus griseus*) which were netted with fish which drowned in the North Ocean (40° 40′S–55° 56′N latitude; 167° 53′E–177° 31′W longitude) in June, September, October and November of 1979. Each sample was ground with sterile sand and suspended in the broth at a concentration of 20%. The suspension was then centrifuged at 2500 rpm for 30 minutes and the supernatant was used for virus isolation.

Virus isolation: Virus isolation was performed by intraamniotic and intraallamtoic inoculation into 9 to 11-day-old chick embryos and incubated at 35°C. Two eggs were used for each sample. The inoculated eggs were candled twice daily to detect embryo's deaths. When death was detected or at the end of 5 days, the amniotic and allantoic fluids were collected from each egg and checked for hemagglutinating activity. Each negative primary passage was followed by a blind passage.

Viruses: The following reference strains of influenza viruses were used in these experiments. A/PR/8/34 (HON1), A/FM/1/47 (H1N1), A/Singapore/1/57 (H2N2), A/Aichi/2/68 (H3N2), A/equine/Prague/1/56 (Heq1Neq1), A/swine/Iowa/15/30 (Hsw1N1), A/duck/England/56 (Hav3Nav1), A/duck/Czech/56 (Hav4Nav1), A/tern/S. Africa/61 (Hav5 Nav2), A/turkey/Mass./65 (Hav6N2), A/duck/Ukraine/1/63 (Hav7Neq2), A/turkey/Ontario/6118/68 (Hav8Nav4), A/turkey/Wisconsin/66 (Hav9N2) and A/shearwater/E. Aust./1/72 (Hav6Nav5).

Antisera: Specific antisera against the viruses were prepared in 3 to 6-month-old chickens by two successive intramuscular injections with an adjuvant and an intravenous

injection of purified and formalin-inactivated viruses at intervals of two weeks as described[7].

Serological test: Hemagglutination (HA) titrations and HA-inhibition (HI) tests were performed by microtitration in plastic trays using total volumes of 0.10 ml[12].

Neuraminidase (NA) titrations and neuraminidase-inhibition (NI) tests were carried out according to the method described by AYMARD-HENRY et al.[1] The antisera to the original reference viruses were used.

RESULTS

An avian influenza virus was isolated from a cloacal swab of one of the 38 eastern dunlins, which was apparently healthy. No influenza virus isolation was made from the other birds examined.

Hemagglutinating activity was found in the amniotic fluid of the egg which was inoculated with the material from the bird. Electron microscopically, long pleomorphic filamentous particles and round forms covered with spikes, 100–500 nm in diameter, were found in the amniotic fluid which showed hemagglutinating activity.

In HI tests, the isolated virus was closely related to A/turkey/Mass./65 (Hav6N2) (tab. 1). The isolated virus showed no relation with A/PR/8/34 (H0N1), A/FM/1/47 (H1N1), A/Singapore/1/57 (H2N2), A/Aichi/2/68 (H3N2), A/equine/Prague/1/56 (Heq1 Neq1), A/swine/Iowa/15/30 (Hsw1N1), A/duck/England/56 (Hav3Nav1), A/duck/Czech/56 (Hav4Nav1), A/tern/S. Africa/61 (Hav5Nav2), A/duck/Ukraine/63 (Hav7Neq2), A/turkey/

TABLE 1 *Cross hemagglutination-inhibition tests*

ANTIGENS	ANTISERA	
	Isolate	Ty/Mass.
Isolate	2048*1	1024
A/turkey/Mass./65 (Hav6N2)	1024	1024

*1 Reciprocal of dilution yielding inhibition of 4 hemagglutinating units of virus

TABLE 2 *Cross neuraminidase-inhibition tests*

ANTIGENS	ANTISERA		
	Isolate	Shearwater	Ty/Mass.
Isolate	3550*1	1413	—*2
A/shearwater/E. Aust./1/72 (Hav6Nav5)	3160	4470	—
A/turkey/Mass./65 (Hav6N2)	200	—	4470

*1 Reciprocal dilution of antiserum yielding 50% inhibition of neuraminidase
*2 Less than 10

Ontario/6118/68 (Hav8Nav4), A/turkey/Wisconsin/66 (Hav9Nav5) (data are not shown in the table).

In NI tests, the isolate cross-reacted with A/shearwater/E. Aust./1/72 (Hav6Nav5) but not with A/turkey/Mass./65 (Hav6N2) (tab. 2). These results show that subtype of the isolate is Hav6Nav5.

Discussion

A total of 1100 birds including water fowls, small migrating birds and sea birds was examined in 1979 for influenza virus distribution, and the virus designated A/dunlin/Hokkaido/101/79 (Hav6Nav5) was isolated from one of the 38 dunlins examined, this was considered to be one of the rare isolations of the virus from a snipe. The isolation of influenza virus from a snipe was reported once from the USSR (WHO, The Ecology of Influenza Viruses; Report of Meeting of Investigation, Geneva, 1976).

The influenza virus from the eastern dunlin was isolated from a cloacal swab, which indicates that the influenza virus may replicate in the intestine of the eastern dunlin, as it does in ducks. As there is no complete listing of bird species which are susceptible to intestinal influenza, the addition of the snipe is interesting.

Webster et al. showed that duck influenza virus was relatively stable at pH 4.0, whereas human strains tested were destroyed at this pH, and that the relative stability of duck viruses probably enables these strains to pass through the digestive tract of the duck[16]. The factors which determine replication of influenza virus in the intestine of some particular species of birds need further investigation and will be an interesting and important problem especially from the viewpoint of epidemiology of avian influenza and ecology of the virus. Wide distribution of influenza viruses in feral brids has been shown, leading to the hypothesis that a new pandemic human influenza virus will be produced by genetic reassortment of human and avian influenza viruses[2,9] in some unknown hosts. This possibility warrants that more studies be made on the nature of influenza virus infection in various species of birds.

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