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ISOLATION OF INFLUENZA A VIRUSES FROM MIGRATORY WATERFOWLS IN SAN-IN DISTRICT, WESTERN JAPAN, IN THE WINTER OF 1982—1983

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Summary. — From November 1982 to March 1983, winter migratory waterfowls of some species staying in San-in District, Western Japan, were surveyed for influenza virus at five stations. A total of eight influenza A viruses were isolated from 354 faeces samples of whistling swans; in contrast, no virus was isolated from any sample of 261 black-tailed gulls, of 113 pintails and of 10 mallards. Five of eight isolates belonged to human pandemic subtype H2N2, two isolates belonged to fowl plague subtype H7N7, and the remaining one to subtype H4N6.

Key words: influenza A virus; migratory waterfowls; H2N2 and H7N7 subtypes

Introduction

In the present time, free-living migratory waterfowls especially ducks are regarded for important reservoirs of influenza virus (Alexander, 1982; Webster *et al.*, 1974). Influenza A viruses isolated from animals have been implicated as a source of new human pandemic strains (Hinshaw *et al.*, 1981).

From December 1979 we have surveyed migratory waterfowls such as whistling swans (*Cygnus columbianus*), tufted ducks (*Aythya fuligula*), pintails (*Anas acuta*) and black-tailed gulls (*Larus crassirostris*) in Shimane and Tottori Prefectures, Western Japan, for influenza virus. During their stay in this district, we have regularly collected their faeces. At some stations novel influenza viruses have been isolated (Otsuki *et al.*, 1986; Tsubokura *et al.*, 1981). In this paper we describe the results of the surveillance in the winter of 1982—1983.

Materials and Methods

Faeces from whistling swan, pintail, mallard (*Anas platyrhynchos*) and black-tailed gull were collected regularly from November 1982 to March 1983 at five stations where we had been sampling since December 1979 (Otsuki *et al.*, 1986; Tsubokura *et al.*, 1981) (Table 1). We collected a total of 738 faeces, 354 of whistling swans, 261 of black-tailed gulls, 113 of pintails, and 10 of

Table 1. Detailed list of migratory waterfowls examined

Bird	Location	Date	Number of samples	Number of isolates
Whistling swan	Rice field, suburb of Yasugi City (Shimane Prefecture)	Nov/82—Feb/83	290	8
	Bank of Sendai River, Tottori City (Tottori Pref.)	Nov/82—Jan/83	64	0
Black-tailed gull	Karo, coast of the Japan Sea Tottori City	Dec/82—Mar/83	200	0
	Iwado, coast of the Japan Sea, Tottori City	Nov/82—Feb/83	61	0
Pintail	Coast of Lake Nakanoumi (Shimane Pref.)	Nov/82—Feb/83	113	0
Mallard	Bank of the Sendai River, Tottori City	Nov/82	10	0
		Total	738	8

mallards. Each faecal specimen was suspended to a concentration of 30% in phosphate buffered saline (pH 7.2) containing penicillin (8.000 units per ml) and streptomycin (8.000 μg per ml). The subsequent procedures were the same as those reported previously (Tsubokura *et al.*, 1981).

The reference viruses and hyperimmune reference sera used were the same as reported previously (Otsuki *et al.*, 1986).

Haemagglutination (*HA*) and haemagglutination-inhibition tests with reference strains and isolates were performed as described by Salk (1944) in reduced micromethod volumes.

Neuraminidase (*NA*) and neuraminidase-inhibition tests were made according to the method of Aymard-Henry *et al.* (1973).

Results and Discussion

Results summarized in Table 1 show that eight influenza A virus strains were isolated in this winter from faeces of whistling swans only. No influenza virus was isolated from any other species of the waterfowls examined.

Relationships between the date and place of sampling on one hand, isolation of influenza viruses and their antigenic subtype on the other hand are shown in Table 2. Collection of faecal samples was made ten times from November 1982 to March 1983. All viruses except one were isolated in December. No influenza virus was isolated from any faeces sample of whistling swans staying in the Sendai River, Tottori City. Both the HA and NA activities of five out of eight isolates were strongly inhibited by the antiserum to A/ /Singapore/1/57 (H2N2), therefore, these ioslates were classified as H2N2 subtype. HA activity of two isolates was inhibited by the antiserum to A/turkey/England/63 (H7N3), while their NA activity was inhibited by that to A/equine/Prague/1/56 (H7H7), respectively, thus both latter isolates belong to H7N7 subtype. The remaining virus was bound of H4N6 subtype.

Table 2. Surveillance of whistling swans for inuflenza A viruses

Year	1982							1983			
Month	Nov			Dec				Jan		Feb	
Date	2	16	26	3	7	24	25	21	31	24	
Place	A	A	B	B	A	B	A	B	A	B	Total
Number of samples	8	13	41	66	40	65	1	56	5	39	354
Number of isolates	0	0	0	4*	0	3**	0	1***	0	0	8

Place A: Bank of the Sendai River, Tottori City
B: Rice field, Yasugi City

* 3 H2N2 ** 2 H2N2 *** H7N7
1 H4N6 1 H7N7

In this surveillance, interesting influenza viruses were isolated from the faeces of whistling swans. Five of eight isolates possess surface antigens related to human pandemic occurring influenza virus H2N2. This is the first isolation of avian influenza virus possessing surface antigens related to human virus in Japan. Since 1972, H2 influenza viruses including H2N2 subtype have been isolated from birds of a few species such as domestic and free-living ducks in various countries (Boudreault and Lecompte, 1981; Shortridge, 1980; Sinnecker *et al.*, 1983; Tůmova *et al.*, 1975). Hinshaw *et al.* (1981) examined their replication in mammals and reported that infected pigs, ferrets or cats had no significant disease signs but they replicated in those mammals. Murphy *et al.* (1982) reported that H2N2 virus isolated from mallard, A/mallard/NY/6870/78, multiplied poorly in hamsters, ferrets and squirrel monkeys and caused no mortality; the same strain, however, replicated well in and caused death of BALB/c mice (Lu *et al.*, 1982). Our H2N2 isolates replicate in ddY mice but did not cause disease (data were not shown). These results suggest that the host range of H2N2 virus isolated from birds is broad and includes both birds and mammals. The potential exists that humans may be involved in the circulation of avian H2N2 influenza viruses in nature. Thus, H2N2 virus was noticed to be harbored by various migratory birds even when pandemic with H2N2 virus did not occur. H2N2 influenza virus has not been isolated from any species of birds in recent years in the U.S.S.R. (personal communication of Dr. Y. Z. Ghendon).

H7N7 influenza viruses were again isolated from whistling swans in two winters (Tsubokura *et al.*, 1981). These viruses do not form any plaques on chick embryo fibroblast cells in the absence of trypsin and are low pathogenic for young chicks (data were not shown). Thus these H7N7 viruses seemed not to have severe virulence for birds, resembling to same antigens possessing viruses isolated from whistling swans and black-tailed gulls in January 1980

in this region (Otsuki *et al.*, 1982). A few flocks of some migratory waterfowls flying to this district may successively harbor H7N7 virus since the winter of 1979—1980.

It remained obscure why only a few virus strains were isolated from free-living ducks such as pintails, tufted ducks and mallards in our surveillance, while numerous influenza viruses have been isolated from domestic and free-living ducks, as ducks are regarded for an important reservoir of influenza virus (Alexander, 1982; Webster *et al.*, 1974).

It seems interesting to continue further surveillance for influenza virus of migratory waterfowls flying to this district.

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