

SURVEILLANCE OF PELAGIC BIRDS FOR INFLUENZA A VIRUSES

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Summary. — Within a 4-year surveillance period for influenza A virus in pelagic birds, 351 influenza A strains were isolated from the trachea or cloaca of 3344 apparently healthy ducks, gulls, swans, terns and geese. The isolated influenza A viruses represent 14 subtypes. Their haemagglutinins (HA) were mainly related to avian HA, but also to the human HA H2 and to the swine HA Hsw1. The neuraminidases (NA) were identified as avian, equine and human NA. The isolated influenza A strains include fowl plague-like viruses Hav1 Neq1, strains with the surface antigen Hsw1 Nav4 and the subtype Hav7 Nav1 isolated from unconcentrated water samples. A subtype unknown to date, with the antigen formula H2 Nav4, was isolated from ducks. 8.2% of pekin ducks showed dual infections.

Key words: influenza A virus; avian influenza; mixed infection; water isolation

Introduction

Influenza A viruses with a variety of haemagglutinin (HA) and neuraminidase (NA) antigens have been isolated from different species, especially from birds (Hinshaw *et al.*, 1979a). These viruses are of interest as a possible source of new human strains either through direct transfer or through recombination of human and animal influenza viruses and also, as possible pathogens of domestic fowl (Webster and Laver, 1975). We are presenting the results of a 4-year period of surveillance of pelagic birds for influenza A viruses aimed at elucidating the ecology of these viruses.

Materials and Methods

Collection of material. Tracheal and cloacal swabs were collected during 1977 and from 1979 to 1981 from 1283 ducks (578 feral ducks, 705 white pekin ducks), 966 gulls, 503 swans, 442 terns and 150 geese. The species sampled are listed in Table 1. Samples were coming from apparently healthy adult birds. Water specimens for isolation were taken from the pond of a duck farm. The swabs were placed into 1.0 ml of phosphate buffered saline (pH 7.2), containing 50% glycerol and antibiotics. The samples were kept at 4 °C for 3-5 hr and then at -80 °C until use.

Virus isolation and identification. For virus isolation, the samples were processed in a building used only for processing field specimens. A 0.1 ml volume of the sample was inoculated into

Table 1. Species of waterfowl sampled in 1977 and from 1979 to 1981

Species	D	Year				
		1977	1979	1980	1981	
Ducks	<i>Anas platyrhynchos</i>	1	8*	2	110	210
	<i>Anas platyrh. domest.</i>	2	—	—	413	292
	<i>Clangula hyemalis</i>	4	9	—	10	138
	<i>Melanitta fusca</i>	5	1	—	—	37
	<i>Penelope strepera</i>	—	—	2	19	22
	<i>Anas crecca</i>	3	10	—	—	—
Geese	<i>Anser anser</i>	10	41	16	—	38
	<i>Anser fabalis</i>	—	—	55	—	—
Gulls	<i>Larus argentatus</i>	—	—	—	109	—
	<i>Larus ridibundus</i>	6	17	94	19	186
	<i>Larus canus</i>	—	74	206	22	239
Terns	<i>Sterna paradisaea</i>	8	—	—	—	28
	<i>Sterna sandvicensis</i>	9	—	75	87	189
	<i>Sterna hirundo</i>	—	—	—	50	13
Swans	<i>Cygnus olor</i>	7	—	126	69	308
Total			160	576	908	1700

D = designation of the species for Table 2

* number of samples collected

the allantoic cavities of 11-day-old chick embryos. The eggs were incubated at 36 °C for 48 hr and the allantoic fluids tested for HA activity with chicken red blood cells. For virus identification hyperimmune sera prepared in rabbits and onedose immune sera prepared in ferrets against the following reference strains except for Hav10 were used:

A/PR/8/34 (H0N1), A/FM/1/47 (H1N1), A/Singapore/1/57 (H2N2), A/Hong Kong/1/68 (H3N2), A/Port Chalmers/1/73 (H3N2), A/Texas/77 (H3N2), A/swine/Iowa/15/30 (HswN1), A/New Jersey/8/76 (HswN1), A/FVP/Rostock/34 (Hav1N1), A/chicken/Germany/N/49, (Hav2Neq1), A/duck/England/56 (Hav3Nav1), A/duck/Czech./56 (Hav4Nav1), A/tern/South Africa/61 (Hav5Nav2), A/turkey/Mass./65 (Hav6N2), A/shearwater/Austral./1/72 (Hav6Nav5)/A/duck/Ukraine/1/63 (Hav7Neq2), A/turkey/Ontario/6118/68 (Hav8Nav4), A/turkey/Wisconsin/1/66 (Hav9N2), A/duck/GDR/72 (H2Nav6), A/equine/Prague/56 (Heq1Neq1) and A/equine/Miami/63 (Heq2Neq2).

All these reference strains were checked for specificity with a set of antisera obtained from Dr. R. Webster before used for immunization. Haemagglutination inhibition (HI) tests were carried out as described by Palmer *et al.* (1975). All sera were treated with a receptor destroying enzyme before testing. The neuraminidase (NI) tests were performed according to Palmer *et al.* (1975) but the periodate and the sodium arsenite reagents were modified according to Aminoff (1961).

Results

A total of 351 influenza A viruses were isolated from 3344 samples taken from ducks, gulls, swans, terns, geese and from water in 1977, 1979, 1980 and 1981 (Table 2). All isolation attempts were performed contemporarily from tracheal and cloacal swabs of the same birds. The overall isolation

Table 2. Influenza A virus isolations from waterfowl during 1977-1981

Year	Influenza virus subtype	Ducks					Gulls		Swans	Terns		Geese	Water sample	Total
		1*	2	3	4	5	6	7	8	9	10			
1977	Hav7Neq2			4										4
	Hav6N2	1												1
	Hav6Nav4										1			1
1979	Hav1Neq1								2					2
	? Nav2						1			1				2
1980	H2 Nav2	8												8
	Hav1Neq1	3												3
	Hav1Nav1						6							6
	Hav4Nav1		4											4
	Hav6N2	13	207											220
	Hav6Neq2	1	38											39
	Hav6Nav1		1											1
	Hav6Nav4		2											2
	Hav6Nav5		8											8
	Hav7Neq2		5											5
	Hsw1Nav4		1											1
	1981	H2 Nav4	2											
H2 Nav2		14			2	3		1						20
Hav1Nav2								5						5
Hav4Nav1		10												10
Hav7Nav1			2									2		4
Hav7N2			2											2
Hsw1Nav4		1												1
Total		53	270	4	2	3	7	6	2	1	1	2		351

* Species listed in Table 1

rates were 10.7% in feral ducks, about 1% in other feral birds and 38% in pekin ducks. The isolation ratio was a little higher from cloacal (75.0%) than from tracheal swabs (70%). Twenty-two of the pekin ducks (8.2%) showed dual infections: either with two different influenza subtypes or with an influenza strain and with Newcastle disease virus.

Serological results of cross reactions (HI and NI tests) showed, that the influenza A isolates belong to 14 subtypes: H2 Nav2, H2 Nav4, Hav1 Neq1, Hav1 Nav2, Hav4 Nav1, Hav6 N2, Hav6 Neq2, Hav6 Nav1, Hav6 Nav4, Hav6 Nav5, Hav7 N2, Hav7 Neq2, Hav7 Nav1, Hsw1 Nav4. The HA subtype Hav6 was found in 5, Hav7 in 3 combinations with NA antigens. Two subtypes of Hav1 were isolated in 1979 from terns (Hav1 Neq1), in 1980 from mallards (Hav1 Neq1) and gulls (Hav1 Nav2), and in 1981 from swans (Hav1 Nav2). The combination of Hsw1 Nav4 was isolated from a pekin duck in 1980 and from a mallard in 1981. Two strains originating from water samples had the same subtype as isolations from ducks of this farm, namely Hav7 Nav1. Two influenza virus isolates with the NA Nav2 could not be determined in their HA subtype.

Discussion

A total of 351 influenza virus A strains were isolated from feral and domestic birds in 1977 and from 1979 to 1981. Most of the isolates came from ducks, which is in agreement with a previous report (Webster *et al.*, 1976) and supports the importance of ducks in the ecology of influenza viruses. The isolates represented 14 different subtypes. The HA were mainly identified as avian HA, but the human H2 and swine haemagglutinin Hsw1 were included. The NA of the isolates were related to avian, equine and human NA. A similar diversity of different influenza subtypes in wild birds has been reported previously (Easterday, 1975).

The high isolation rate in ducks and the great number of dual infections in one duck is obviously due to the very dense contact between animals under farm conditions and makes recombinations obviously more often possible. So the surface antigen Hav6 was found in combination with the NA N2, Neq2, Nav1, Nav4 and Nav5. Similar results were reported in a study of domestic poultry in Hongkong (Shortridge *et al.*, 1979). Isolations of two different subtypes from one animal has also been described (Shortridge *et al.*, 1977). The influenza A isolations from unconcentrated water with the same surface antigens as strains from ducks of this farm supports the view that transmission of viruses is possible by this route. Avian influenza viruses are excreted by the faeces of ducks in high titres (Webster *et al.*, 1978; Hinshaw *et al.*, 1979b).

Five isolations with the antigenic combination of the virulent reference strain A/FPV/Dutch/27 — Hav1 Neq1 — were isolated from terns (*Sterna paradisica*) and mallards (*Anas platyrhynchos*) which showed no signs of illness. Similar results are published in studies of free living (Lipkind *et al.*, 1979) as well as domestic birds (Alexander *et al.*, 1979). In 1980 and 1981 we identified two isolations from ducks as subtypes Hsw1 Nav4. This antigenic configuration has been reported only once. It was found in feral ducks in Japan in 1979 (Yamane *et al.*, 1979). The isolation H2 Nav4 possessed a previously unknown combination of HA and NA.

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