

Characterization of the haemagglutinin and neuraminidase antigens of some recent avian type A influenzavirus isolates from Hong Kong

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During 1969 type A influenzaviruses were isolated from three outbreaks of disease among domestic ducks in Hong Kong. The isolates were characterized in haemagglutination inhibition and neuraminidase inhibition tests with antisera to prototype avian and human influenza strains. A/duck/Hong Kong/46/69 and 120/69 contained haemagglutinin and neuraminidase antigens related to those of A/turkey/Wisconsin/66. The neuraminidases of these Hong Kong isolates, like that of turkey/Wisconsin/66, were antigenically closely related to those of human Asian influenzaviruses. A/duck/Hong Kong/826/69 contained haemagglutinin and neuraminidase antigens related to those of chicken/Scotland/59 and tern/South Africa/61, respectively. The duck influenza A isolates represent the first viruses of their antigenic variety to be isolated in South-East Asia. The possible epidemiological significance of the duck/Hong Kong/69 strains is discussed.

Avian type A influenzaviruses have been isolated in many parts of the world and form a large and antigenically heterogeneous group of agents. Although their natural history is incompletely understood, serological surveys have suggested that exposure of wild and domesticated birds to type A influenzaviruses is more frequent than is reflected in records of clinical disease (Easterday et al., 1968). Although South-East Asia is of considerable interest in relation to the emergence of human influenza epidemics, little is known of the ecology of type A influenzaviruses in domestic and wild animal and bird populations in the area. During 1969, Higgins (1971) isolated type A influenzaviruses from three outbreaks of severe clinical disease affecting domestic ducks in Hong Kong. In the present paper we describe the antigenic characteristics of these isolates. It is of particular interest that two of the virus strains were found to contain neuraminidase of the same antigenic variety as that contained in human Asian influenzaviruses.

MATERIALS AND METHODS

Viruses

The origins of the Hong Kong isolates, A/duck/Hong Kong/46/69, 120/69, and 826/69 have been described by Higgins (1971). The prototype influenzaviruses shown in Tables 1 and 2 have been described previously (Pereira et al., 1966; Pereira et al., 1967a; Lang et al., 1972). Viruses were propagated in fertile hen eggs and the allantoic fluids were used as a source of antigen for serological investigations.

Sera

Convalescent chicken sera (for avian type A influenzaviruses) and ferret sera (for other influenza A strains) were used in haemagglutination inhibition (HI) tests. Sera were treated with receptor-destroying enzyme according to standard methods (WHO Expert Committee on Respiratory Viruses, 1959). Hyperimmune rabbit sera (Schild & Newman, 1969) were used in neuraminidase inhibition (NI) tests.

Immunological tests

HI tests were performed by conventional methods (WHO Expert Committee on Respiratory Viruses, 1959) and NI tests as described by Webster & Pereira (1968).

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Table 1. Comparison of influenza A/duck/Hong Kong/69 viruses with other influenzaviruses in haemagglutination inhibition tests

HA subtype	Virus	HI titres ^a with:		
		Homologous virus	Duck/HK/46/69	Duck/HK/120/69
Avian influenzavirus strains				
Hav1	A/FPV/Dutch/27 (Hav1Neq1)	320		
	A/turkey/Eng/63 (Hav1Nav3)	160		
Hav2	A/chicken/Germany 'N'/49 (Hav2Neq1)	640		
	quail/Italy/1117/65 (Hav2Neq2)	320		
	pheasant/Italy/647/66 (Hav2N7)	640		
Hav3	duck/England/56 (Hav3Nav1)	120		
Hav4	duck/Czech/56 (Nav4Nav1)	240		
	duck/England/62 (Hav4Nav1)	120		
Hav5	chicken/Scotland/59 (Hav5N1)	320		160
	tern/South Africa/61 (Hav5Nav2)	160		20
Hav6	turkey/Canada/63 (Hav6Neq2)	80		
	turkey/California/64 (Hav6N2)	160		
	turkey/England/66/(Hav6N?)	240		
	turkey/Massachusetts/65 (Hav6N2)	320	20	
	turkey/Wisconsin/66 (Hav6N2)	640	640	80
Hav7	duck/Ukraine/1/63 (Hav7Neq2)	160		
Hav8	turkey/Ontario/6118/68 (Hav8Nav4)	320		
Newcastle disease virus				
	Newcastle disease virus	320		
Human influenza A virus strains				
	A/BEL/42 (HON1)	640		
	A/FM1/47/(H1N1)	640		
	A/Singapore/1/57 (H2N2)	1 280		
	A/Hong Kong/1/68 (H3N2)	5 120		

^a Expressed as reciprocal of titre; blank spaces indicate titres of <10.

RESULTS

The results of HI and NI tests on the duck/Hong Kong/69 viruses are shown in Tables 1 and 2 respectively.

A/duck/Hong Kong/46/69 and 120/69 both cross-reacted with antiserum to A/turkey/Wisconsin/66 (Hav6N2) in HI tests and both also contained neuraminidase that was inhibited by antiserum to turkey/Wisconsin/66 virus (Smithies et al., 1969). The neuraminidase of these two duck viruses was also inhibited by antisera to human A/Singapore/1/57 (H2N2) and A/Hong Kong/1/68 (H3N2) viruses, indicating that, in common with turkey/Wisconsin/66 virus (Webster & Pereira, 1968; Schild & Newman, 1969), they contained neuraminidases antigenically closely related to those of certain human viruses.

A/duck/Hong Kong/826/69 virus was strongly inhibited in HI tests by antiserum to chicken/Scot-

land/59 (Hav5N1) virus (Andrewes & Worthington, 1959) and slightly by antiserum to tern/South Africa/61 (Hav5Nav2) (Becker, 1966). However, in NI tests the neuraminidase of 826/69 was strongly inhibited by antiserum to tern/South Africa/61 and was not inhibited by potent serum to chicken/Scotland/59. These results were taken to indicate that the isolate 826/69 contained neuraminidase closely related to that of tern/South Africa/61 and haemagglutinin more closely related to that of chicken/Scotland/59.

DISCUSSION

It is of interest that the duck type A influenza-viruses from Hong Kong contained envelope antigens (haemagglutinin and neuraminidase) related to those of avian type A influenzaviruses isolated on other continents (i.e., A/turkey/Wisconsin/66, A/tern/South

Table 2. Comparison of duck/Hong Kong/69 influenza A isolates with prototype strains in neuraminidase inhibition tests

Hyperimmune rabbit serum	Neuraminidase inhibition titre ^a with:			
	Homologous virus	Duck/HK/46/69	Duck/HK/120/69	Duck/HK/826/69
Avian strains ^b				
1. A/FPV/Rostock/34 (Hav1N1)	300			
chicken/Scotland/59 (Hav5N1)	500			
duck/Germany/210/67 (Hav4N1)	>1 000			
duck/Germany/1868/68 (Hav6N1)	300			
2. turkey/Massachusetts/65 (Hav6N2)	300	250	300	
turkey/Wisconsin/66 (Hav6N2)	>1 000	>1 000	450	
3. A/FPV/Dutch/27 (Hav1Neq1)	300	20	20	
chicken/Germany 'N'/49 (Hav2Neq1)	>1 000			
4. quail/Italy/1117/65 (Hav2Neq2)	500			20
turkey/Canada/63 (Hav6Neq2)	>1 000			20
duck/Ukraine/63 (Hav7Neq2)	200			
5. duck/England/56 (Hav3Nav1)	>1 000			
duck/Czech/56 (Hav4Nav1)	>1 000			
duck/England/62 (Hav4Nav1)	300			
6. turkey/England/63 (Hav1Nav3)	300			
7. tern/South Africa/61 (Hav5Nav2)	1 000			50
8. turkey/Ontario/6118/68 (Hav8Nav4)	800			800
Human, swine, and equine strains				
A/BEL/42 (HON1)	>1 000			
A/FM1/47 (H1N1)	300			
A/Singapore/1/57 (H2N2)	>1 000	1 000	500	
purified A/57 neuraminidase ^c	3 000	1 000	800	
A/Hong Kong/1/68 (H3N2)	>1 000	200	100	
A/swine/Iowa/15/30 (Hsw1N1)	>1 000			
A/equine/Prague/56 (Heq1Neq1)	500			
A/equine/Miami/63 (Heq2Neq2)	500			

^a NI titres are expressed as the serum dilution inhibiting 50 % of neuraminidase activity; blank spaces indicate titres of <20.

^b Avian viruses within each group (1–8) contain immunologically closely related neuraminidases.

^c See Schild & Newman (1969).

Africa/61, and A/chicken/Scotland/59). Since they are the first influenzavirus isolates from nonhuman hosts in Hong Kong, there is no indication whether these viruses represent recent movements of existing strains into South-East Asia, or whether they had existed in the area for some time.

The neuraminidase antigens of two of the duck

influenzavirus isolates described in the present paper are closely related to those of human Asian influenza-viruses. Such relationships of neuraminidase antigens of avian, animal, and human influenzaviruses have been described previously (Webster & Pereira, 1968; Tumová & Easterday, 1969; Schild & Newman, 1969) but their epidemiological significance in relation to

the animal origins of the human type A influenza-viruses remains uncertain. However, in the laboratory, recombinant viruses that have "exchanged" antigens can readily be created both in mixed cultures (Easterday et al., 1969) and mixed infections (Webster et al., 1971) between viruses of two distinct antigenic types. The work of Webster et al. (1971) suggests that this is a method of species adaptation, for when it occurs between two viruses each normally pathogenic for a distinct host species, the recombinants are stable and pathogenic within the host in which recombination took place. It is

interesting in the present context to isolate avian viruses that could have a genetic relationship with recent human pandemic strains originating in the same area. It seems likely that genetic interaction in nature between human and avian influenzaviruses can give rise to human pathogens possessing antigens of avian strains (Pereira et al., 1967b; Schild et al., 1969; Tumová & Easterday, 1969; Easterday et al., 1969). Equally, however, the human virus strains may have existed first and the avian strains arisen from them (Webster & Pereira, 1968; Dasen & Laver, 1970).

RÉSUMÉ

CARACTÉRISATION DES ANTIGÈNES (HÉMAGGLUTININE ET NEURAMINIDASE) DE QUELQUES VIRUS GRIPPAUX A AVIAIRES RÉCEMMENT ISOLÉS À HONG KONG

En 1969, 3 virus grippaux A ont été isolés au cours d'épizooties survenues chez des canards domestiques à Hong Kong. Les caractéristiques antigéniques de leurs hémagglutinines et de leurs neuraminidases ont été étudiées en épreuves d'inhibition à l'aide d'antisérums spécifiques de diverses souches grippales aviaires et humaines.

Les virus A/duck/Hong Kong/46/69 et A/duck/Hong Kong/120/69 renfermaient une hémagglutinine et une neuraminidase apparentées à celles du virus A/turkey/Wisconsin/66. Les propriétés antigéniques des neurami-

nidases étaient très voisines de celles caractérisant les virus A de la grippe asiatique humaine. L'hémagglutinine du virus A/duck/Hong Kong/826/69 était proche de celle du virus A/chicken/Scotland/59 alors que sa neuraminidase s'apparentait à celle du virus A/tern/South/Africa/61.

Ces virus A isolés chez le canard en 1969 sont les premiers présentant de telles caractéristiques antigéniques à être identifiés en Asie du Sud-Est. Leur importance épidémiologique éventuelle est discutée.

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