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A new subtype of avian influenzavirus: antigenic characteristics of envelope antigens

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The haemagglutination and neuraminidase antigens of three influenza A isolates from ducks in the Ukraine were compared with those of a collection of reference strains of influenza A virus. Duck/Ukraine/1/60 virus contained haemagglutinin related to that of duck/England/56 while its neuraminidase was related to that of turkey/Wisconsin/68 virus and the human A/Hong Kong/1/68 virus. Duck/Ukraine/2/60 and duck/Ukraine/1/63 were themselves closely related. They contained haemagglutinin antigens unrelated to the six haemagglutinin subtypes previously described for avian influenzaviruses and it is suggested that they should be classified as belonging to haemagglutinin subtype Hav7. The neuraminidase antigens of these isolates were antigenically related to those of a number of other avian influenza viruses isolated in England, Canada, and Italy and to that of A/equine/Miami/63 virus.

The first isolations of type A influenzaviruses from ducks were reported from Czechoslovakia and England (Andrewes & Worthington, 1959; Koppel et al., 1956). Subsequently avian influenzaviruses were isolated in South Africa, northern Scotland, Italy, Canada, and in the USA (Wells, 1963; Lang et al., 1963; Roberts, 1964; Pereira et al., 1966, 1967). Antigenic characterization of these strains has revealed that some of them contain surface antigens related to those of type A influenzaviruses isolated from pigs, horses, and man (Pereira et al., 1967; Tumová & Pereira, 1968; Schild & Newman, 1969; Schild et al., 1969; Tumová & Easterday, 1969).

In 1960–63, nine myxoviruses were isolated in the USSR from cases of duck sinusitis; the disease was widespread among ducklings in the southern regions of the Ukraine (Prokof'eva & Tsimokh, 1966). Of these viruses, two were originally designated " Jalta " and " Borki " and were isolated in 1960. The previously unidentified strain " BVI " was isolated in the same area in 1963. In this paper these strains are designated duck/Ukraine/1/60, duck/Ukraine/2/60, and duck/Ukraine/1/63, respectively. The antigenic relationships of these strains to influenzaviruses isolated from several avian and mammalian species including human beings have been studied.

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MATERIALS AND METHODS

Viruses

The histories and characteristics of the strains employed in this study have been described previously (Tumová & Easterday, 1969; Easterday & Tumová, 1970).

Sera

Rat sera. 3-ml volumes of allantoic fluid containing 10^3 EID_{50} of virus were injected intraperitoneally (4 times at 3- or 4-day intervals). A fifth injection was given 6 weeks after the first. The antigen for the second to fifth injections had been purified by adsorption on chicken erythrocytes and elution into saline. The rats were bled 14 days after the fifth injection.

Antisera. Guineapig strain-specific V antisera were prepared by the technique described by Lief & Henle (1959).

Ferret sera. Ferrets were infected intranasally with 0.2-ml volumes of allantoic fluid containing 10^3 EID_{50} of virus and reinfected in the same manner 4 weeks after the primary infection. Serum specimens

were taken before the infection, after primary infection, before reinfection, and 16 days after reinfection.

Sera were inactivated at 56°C for 45 min for all serological tests and were treated with receptor-destroying enzyme before use in haemagglutination inhibition (HI) tests (WHO Expert Committee on Influenza, 1959).

Serological methods

HI tests were performed by microtitration in Linbro plastic trays using 0.025-ml volumes. Mixtures of serum and 4 haemagglutinin units of virus were incubated at room temperature for 1 h before adding erythrocytes (0.7% suspension).

Complement fixation (CF) tests were performed as described by Pereira et al. (1964). Neuraminidase inhibition (NI) tests were performed by the method described by Webster & Pereira (1968).

The results of serological tests were expressed as reciprocals of the serum dilution giving 50% inhibition.

RESULTS

The viruses under study share the type-specific ribonucleoprotein antigen, common to all type A influenzaviruses. The relationships of the surface antigens, haemagglutinin, and neuraminidase were determined by means of the HI test, the strain-specific CF test, and the NI test. The results are summarized in Tables 1 and 2.

It is evident from the results of the HI tests with hyperimmune rat sera that duck/Ukraine/1/60 is antigenically related to duck/England/56 by its haemagglutinin antigen and to turkey/Wisconsin/68 by its neuraminidase antigen (Table 1). There were no cross-reactions in HI or NI tests with other avian, equine, porcine, and human type A influenzaviruses. The duck/Ukraine/1/60 virus was found to be antigenically distinct from the duck/Ukraine 2/60 and duck/Ukraine 1/63 viruses.

The duck/Ukraine/2/60 and 1/63 viruses are mutually related and are distinct from previously isolated avian influenzaviruses with respect to their haemagglutinin content (Table 2). However, both are related by their neuraminidase antigens to other avian influenzavirus strains, isolated at different times in different countries, belonging to different avian subtypes with regard to their haemagglutinin. No relationship was demonstrated to porcine (Hsw1N1), equine (Heq1Neq1), and human (H0N1 and H1N1) influenzaviruses.

The relationship of the envelope antigens of duck/Ukraine/2/60 and duck/Ukraine/1/63 with those of equine/Miami/63 and equine/Detroit/63, and the human A/Hong Kong/1/68 viruses have been reported previously (Tumová & Easterday, 1969) and were confirmed in the present study by HI and NI tests using hyperimmune rat sera. Also, experiments with sera from ferrets experimentally infected with duck/Ukraine/2/60 and duck/Ukraine/1/63 indicated a rise in antibody to the Heq2 haemagglutinin and to that of A/Hong/Kong/1/68 virus suggesting an antigenic relationship between the envelope antigens of these viruses and those of the duck/Ukraine isolates.

DISCUSSION

Until now, avian influenzaviruses have been divided into 6 subtypes based on the results of the HI, CF, and virus neutralization tests (Pereira et al., 1965, 1966, 1967). The antigenic character of the haemagglutinins of avian influenzaviruses has been used as the basis for their separation into antigenic groups and the present studies suggest that duck/Ukraine/1/60 has the same haemagglutinin subtype (Hav3) as duck/England/56.

The duck/Ukraine/1/63 and duck/Ukraine/2/60 viruses, antigenically similar in both envelope antigens, contain haemagglutinin that is different from any of the 6 proposed avian haemagglutinin subtypes and forms a new subtype Hav7; their neuraminidase antigens are similar to those of some strains with Hav2 or Hav6 haemagglutinin. There is, however, no strict dividing line between subtypes. Antigenic overlapping occasionally occurs between strains when a virus antigenically "bridges" two different subtypes because of a similar haemagglutinin or neuraminidase; "antigenic bridges" also occur between influenzaviruses of different animal species, as is demonstrated by the relationship between duck/Ukraine/2/60, 1/63, equine/Miami/63, A/Hong Kong/68 and other viruses (Webster & Pereira, 1968; Tumová & Pereira, 1968; Schild & Newman, 1969; Tumová & Easterday, 1969). Antigenic characterization of new influenzavirus isolates becomes even more complicated when both of the known envelope antigens are taken into consideration.

The present studies have shown that duck/Ukraine/1/60 is not related to duck/Ukraine/2/60 and duck/Ukraine/1/63 even though they were isolated from the same species in the same area. Similar observations of the isolation of antigenically different influenza A viruses from the same avian or mammal-

Table 1. Haemagglutinin and neuraminidase antigens of duck/Ukraine/1/60 virus

Sera [a]	Antigens					
	duck/Ukraine/1/60		duck/England/56		turkey/ Wisconsin/68	
	HI	NI	HI	NI	HI	NI
duck/Ukraine/1/60	160	90	320	[c]	[b]	10
duck/England/1/56 (Hav3 Nav1)	20	[b]	640	[c]	[b]	[b]
turkey/Wisconsin/68 (Hav5N?)	[b]	60	[b]	[c]	240	40

[a] Hyperimmune rat sera.
[b] Results were negative in the initial serum dilution (1 : 10).
[c] Not tested.

Table 2. Antigenic relationships between envelope antigens of avian influenzaviruses *

Strain (immune sera)	Homologous HI titre	Antigens							
		duck/Ukraine/1/60		duck/Ukraine/2/60			duck/Ukraine/1/63		
		HI [a]	NI [a]	HI [a]	CF [b]	NI [a]	HI [a]	CF [b]	NI [a]
turkey/England/63 (Hav1 Nav3)	640								
duck/Canada/53 (Hav2 N?)	640								
chick/Germany/N/49 (Hav2 Neq2)	1 280								
quail/Italy/1117/65 (Hav2 Neq2)	2 560				15	>100			>100
quail/Italy/544/66 (Hav2 Neq2)	1 280				40	>100		5	>100
pheasant/Italy/647/66 (Hav2 N?)	7 280					40			50
duck/England/56 (Hav3 Nav1)	640	20							
duck/Ukraine/1/60	160	160	90						
duck/Czechoslovakia/56 (Hav4 Nav1)	120								
	320					>100			>100
chicken/Scotland/59 (Hav5 N1)	320								
turkey/Ontario/6213/66 (Hav5 N1)	320								
turkey/Wisconsin/68 (Hav5 N?)	640		60						
turkey/Canada/63 (Hav6 Neq2)	240				40	80		10	100
turkey/Massachusetts/65 (Hav6 N2)	640								
turkey/Wisconsin/66 (Hav6 N2)	2 560								
turkey/England/66 (Hav6 N?)	1 280								
duck/Ukraine/2/60	80			80	160	350	80	20	2 230
duck/Ukraine/1/63	640			80	80	310	640	40	1 000

* Blank spaces denote negative results in the initial 1 : 10 serum dilution.
[a] Hyperimmmune rat sera were used for HI and NI tests.
[b] CF tests were carried out using V antigens and V antisera.

ian species in the same locality or even the same epidemic have been made previously (Pereira et al., 1967; Smithies et al., 1969).

Special attention is directed to the fact that the duck/Ukraine/2/60 virus was circulating in an avian population in the Ukraine 3 years prior to the isolation of the equine/Miami/63 virus from horses in the USA and 8 years prior to the isolation of the A/Hong Kong/68 virus from human beings of Hong Kong.

RÉSUMÉ

UN NOUVEAU SOUS-TYPE DE VIRUS DE LA GRIPPE AVIAIRE: CARACTÉRISTIQUES ANTIGÉNIQUES DES ANTIGÈNES D'ENVELOPPE

On a comparé l'hémagglutinine et la neuraminidase de trois souches de virus grippaux A isolées chez des canards en Ukraine avec celles de diverses souches de référence de virus grippaux A. Le virus duck/Ukraine/1/60 renferme une hémagglutinine apparentée à l'hémagglutinine du virus duck/England/56, alors que sa neuraminidase est antigéniquement proche de celles des virus turkey/Wisconsin/68 et A/Hong Kong/1/68. Il existe une étroite parenté antigénique entre les virus duck/Ukraine/2/60 et duck/Ukraine/1/63. Leurs hémagglutinines ne présentent aucun lien antigénique avec les six sous-types d'hémagglutinines de virus aviaires précédemment décrits et on propose de les classer dans un nouveau sous-type d'hémagglutinines, le sous-type Hav7. Les neuraminidases de ces virus offrent des affinités antigéniques avec celles d'un certain nombre de virus de la grippe aviaire isolés en Angleterre, au Canada et en Italie, ainsi qu'avec la neuraminidase du virus A/equine/Miami/63.

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