Characterization of a New Avian Influenza Virus Subtype and Proposed Designation of this Haemagglutinin as Hav10

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SUMMARY

The haemagglutinin of A/Dk/Alb/60/76, an influenza A virus isolated from feral ducks in Canada, possesses no antigenic relatedness to any of the 16 reference haemagglutinin subtypes. Results of serological tests (HI and double immuno-diffusion) with monospecific antisera to the haemagglutinin of this virus indicate that it represents a new avian haemagglutinin subtype. We propose that this haemagglutinin be designated as Hav10 under the current system of nomenclature.

Diverse influenza A viruses concurrently circulate in the avian species, particularly in waterfowl (Easterday, 1975; Hinshaw et al. 1978, 1979). According to the current system of nomenclature, avian influenza A viruses are divided into nine distinct haemagglutinin (HA) subtypes (Hav1 to Hav9; WHO Report, 1971; Webster et al. 1976b). Some HA antigens in avian viruses are closely related to haemagglutinins detected in mammalian viruses, including those such as Hsw1, H2 and H3 (Webster & Laver, 1975; Hinsenh et al. 1978). These findings have contributed to the hypothesis that these avian viruses may play a role in the evolution of new human pandemic strains of influenza (Webster & Laver, 1975).

Studies on feral ducks in Canada over the past 3 years (Hinshaw et al. 1979) have revealed a high incidence of antigenically diverse influenza viruses concurrently circulating in this bird population. In 1976, the antigenic classification of 106 influenza A viruses isolated from ducks (Hinshaw et al. 1978) yielded one isolate A/Dk/Alb/60/76 [Dk/76] with a haemagglutinin which could not be classified. Briefly, Dk/76 was isolated from a cloacal swab of a healthy juvenile mallard duck in Alberta, Canada during August, 1976. The virus was identified as an influenza A virus morphologically by electron microscopy and serologically by double immunodiffusion (DID) tests with hyperimmune goat antisera to influenza A RNP. Haemagglutination inhibition (HI; Palmer et al. 1975) tests with hyperimmune goat antisera prepared to the isolated haemagglutinins of 16 reference subtypes showed no significant reaction (i.e. HI titre of < 1:40) with Dk/76. Neuraminidase inhibition (NI; WHO Report, 1973) tests with hyperimmune goat antisera to the isolated NA of the 10 reference subtypes showed that the NA of Dk/76 was Nav5, antigenically related to the NA of A/Shearwater/Australia/1/72 (Hav6Nav5; Downie & Laver, 1973). The virus was cloned by two limit dilution passages in embryonated chicken eggs; HI and NI tests on the cloned virus yielded the same results as the initial isolate.

Since both surface antigens (HA and NA) of Dk/76 were stable in SDS, these two antigens could not be separated electrophoretically (Laver & Webster, 1973). Therefore, a recombinant, A/Dk/Alb/60/76 (H)-A/Bel/42(N) (H?N1) [Dk/76(H)-Bel (N)] with a labile NA, was prepared (Webster, 1970). The recombinant Dk/76 (H)-Bel (N) and cloned parental Dk/76 were purified from allantoic fluid by adsorption to and elution from chicken erythrocytes followed by differential centrifugation and sedimentation through a sucrose gradient (10 to 40% sucrose in 0.15 M-NaCl), as previously described (Laver, 1969). The HA of Dk/76 (H)-Bel (N) [H?N1] was isolated by disruption of purified virus with SDS and electrophoresis on cellulose acetate strips (Laver & Webster, 1973). Hyperimmune antiserum to the isolated HA from the recombinant Dk/76 (H)-Bel (N) was prepared in rabbits. The use of
Fig. 1. Double immunodiffusion test showing that A/Dk/Alb/60/76 is an influenza A virus and that antiserum to the haemagglutinin of this virus reacts specifically with the haemagglutinin of A/Dk/Alb/60/76. Immunodiffusion tests were performed in 1.5% agarose A37 in phosphate-buffered saline containing 0.1% sodium lauroylsarcosinate NL97 and 0.1% sodium azide. The purified viruses (HA 6.0 log10 units/ml) were disrupted with sodium lauroylsarcosinate NL97 and precipitin lines were photographed without staining.
antisera to the isolated HA avoids: (a) steric interference in HI tests due to antibodies to the NA and (b) cross-reactions in gel diffusion due to antibodies to NA and internal antigens.

HI tests with antiserum to the isolated HA of Dk/76 (homologous HI titre of 1:2560) showed no reaction (HI titre of < 1:40) with influenza A viruses possessing the 16 different reference HA subtypes. Reciprocal DID tests were done: (a) purified Dk/76 was tested against the 16 monospecific HA antisera and (b) antiserum to the HA of Dk/76 was tested against purified reference viruses for each of the 16 HA subtypes. No lines of precipitation were observed in the gels except between Dk/76 and homologous antiserum (Fig. 1), indicating that the antiserum was specific for the HA of Dk/76 and that this HA was unrelated to the 16 reference HA subtypes (gels not shown). Since neither DID nor HI showed any cross-reactions with the reference subtypes of influenza A, HA of Dk/76 is antigenically unique and represents an additional subtype of avian viruses. In the current nomenclature system the virus would be designated Hav10.

To determine the infectivity of Dk/76 for other avian species, 2 day-old domestic chickens and domestic Peking ducks were inoculated intratracheally and orally with 0.5 ml of allantoic fluid containing approx. 10^7 EID_{50} of Dk/76. Dk/76 was isolated from tracheal and cloacal samples of both chickens and ducks for 6 days p.i., at which time the birds were exsanguinated. Neither the ducks nor the chickens exhibited any disease symptoms. These results suggested that the replication of Dk/76 was similar in feral and domestic ducks and chickens, indicating that the viruses from wild birds represent a source of influenza viruses for transmission to domestic species.

Other influenza A viruses which possess an HA antigenically related to the HA of Dk/76 have been detected in avian species – from a mallard duck in Canada in 1977 (Hinshaw et al. 1978) and from two mallard ducks in Wisconsin in 1976 (Dr B. C. Easterday, personal communication). Of the large number (approx. 1200) of influenza A viruses isolated from Canadian feral ducks in our studies during the past several years, the frequency of isolates (two) with the HA of Dk/76 has been low. Studies on the Canadian feral ducks in 1978 indicate that although many different viruses concurrently and continually circulate in these birds, the predominant subtypes in this population do change from year-to-year, as for example from Hav7Neq2 in 1977 to Hav6N2 in 1978 (V. S. Hinshaw, unpublished data).

An interesting finding from the surveillance studies to date is that relatively few new subtypes have been detected. Many new combinations of HA and NA subtypes have been described but, since 1971, when the present system of nomenclature was introduced, only one new HA subtype, Hav9 (Webster et al. 1976b), and two new NA subtypes, Nav5 and Nav6 (Downie & Laver, 1973; Webster et al. 1976a), have been proposed. The available information thus suggests that there are a finite number of HA and NA subtypes which exist in nature. The host range of these different subtypes has not been fully elucidated nor has the role of these viruses in the disease outbreaks in domestic species and the origin of pandemic strains of influenza in humans. A better understanding of the ecology of these viruses in birds will contribute to our understanding of the appearance and circulation of antigenically related viruses appearing in different species, including man.

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