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Occurrence of Hsw1N1 Subtype Influenza A Viruses in Wild Ducks in Europe

By

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Summary

Two identical strains of influenza A viruses antigenically related to swine influenza (Hsw1N1) have been isolated from adult mallard ducks *(Anas platyrhynchos)* in Southern Germany. They were designated A/Duck/Bavaria/1/77 and A/Duck/Bavaria/2/77. Serologic tests revealed a close antigenic relationship to the strain A/Duck/Alberta/35/76.

Experimental infections of piglets with strain A/Duck/Bavaria/1/77 demonstrated the susceptibility of swine to this virus strain. The virus was isolated from nasal swabs of infected piglets up to 8 days p. inf. and from contact animals up to 9 days. No seroconversion was detected during an observation period of 30 days.

Introduction

Influenza A viruses have frequently been isolated from wild and domestic birds. Under the sponsorship of the WHO Influenza Ecology Programme, studies on the occurrence of these viruses have been intensified during recent years.

In water fowl avian influenza virus strains have commonly been recovered from the cloaca and sometimes also from the trachea as well as from droppings and unconcentrated lake water (2, 6, 8, 11, 13, 16). Typing of the isolates revealed a great heterogeneity of strains (7).

Of special interest are subtypes isolated from water fowl that also occur in mammals. Isolates with haemagglutinins antigenically related to human H2 strains have been reported (14). Recently influenza viruses related to swine influenza viruses (Hsw1N1) were recovered from ducks in Canada, and the USA (7) and Hong Kong (3) in areas with a history of frequent outbreaks of influenza in the swine populations (4, 5).

In the present paper we report the isolation of viruses antigenically related to swine influenza viruses (Hsw1N1) from wild ducks in Europe, and experimental infection of domestic pigs with one of these isolates.

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Materials and Methods

Collection of Samples and Virus Isolation

On December 7, 1977 three mallard ducks *(Anas platyrhynchos)* were shot near Munich, Bavaria. Cloacal and tracheal swabs were taken and placed in vials containing 1.5 ml of phosphate-buffered saline (pH 7.2) with 40 per cent glycerol and the following antibiotics: penicillin 5000 units/ml, streptomycin 1000 μg/ml, polymyxin B 1000 units/ml, gentamycin 250 μg/ml.

All samples were stored frozen at −70° C until assayed. A 0.2 ml portion of each sample was inoculated into the allantoic cavity of four embryonated chicken eggs (10 days old). After incubation of eggs at 35° C for 72 hours allantoic fluids were tested individually for haemagglutinating activity.

Serological Tests

Allantoic fluids showing haemagglutinating activity were tested for type specificity by double immunodiffusion test using type A ribonucleoprotein and matrix protein antisera (18). Diffusion was carried out on plastic Petri dishes (30 mm) prepared with 1.5 per cent agarose in phosphate-buffered saline (pH 7.2) containing 0.1 per cent sarkosyl NL-97 (Ciba-Geigy) and 0.1 per cent sodium azide. All haemagglutinating agents were screened in routine haemagglutination inhibition tests (HI) against influenza B and Newcastle disease reference antisera.

Haemagglutination inhibition (HI) tests were performed in microtiter plates after treatment of sera with receptor-destroying enzyme (RDE) and absorption with chicken red blood cells (18). HI titers represent reciprocals of highest dilutions causing inhibition of 4 haemagglutinating units. Neuraminidase (NA) titrations and neuraminidase inhibition (NI) tests were done according to recommended standard procedures (1).

Antisera to selected reference strains were supplied by the WHO Collaborating Centre for Influenza, CDC, Atlanta, Georgia, U.S.A. Antiserum production was carried out in goats using isolated and purified haemagglutinins of the respective antigens. Antiserum to the isolated haemagglutinin of influenza A/Duck/Alberta/35/76 produced in rabbits and to whole virus in ferrets against U.S.S.R./90/77, NJ/8/76 and Sw/Iowa/15/30 were kindly supplied by Dr. Virginia S. Hinshaw, St. Jude Children's Hospital, Memphis, Tennessee, U.S.A.

Experimental Infections of Pigs

Three weanling piglets (5½ weeks old) without serological evidence of swine influenza virus infection were inoculated intranasally with 3 ml (1 piglet) and 1.5 ml each (2 piglets) of crude allantoic fluid of influenza A/Duck/Bavaria/1/77 (2nd egg passage, HA titer 1:32). Two more piglets of the same age were added as contacts, one on day 2 post infection (p.i.) and one on day 4 p.i.

Nasal and rectal swabs were taken and temperature recorded daily for 24 days p.i. Blood samples were obtained prior to inoculation of the virus as well as on days 22 and 30 p.i.

Results

Isolation and Identification of Influenza A Viruses

The cloacal samples of two adult mallard ducks of different sex showed HA activity after one passage in chicken embryos. All other samples were negative.

The first passage of the isolated strains formed precipitin lines in double-immunodiffusion tests against antiserum to influenza A virus ribonucleoprotein (NP) and matrixprotein (M). The samples were also tested for the presence of influenza B and Newcastle disease antigens using HI tests, and results were negative.

Haemagglutinin Antigen

The results of HI tests with antisera to either whole virions or the purified haemagglutinin of reference strains, or newly isolated viruses are shown in Table 1. The two virus isolates A/Duck/Bavaria/1/77 and A/Duck/Bavaria/2/77 reacted with antisera against four reference influenza A strains as follows: low titer reactions with PR/8/34 (HON1), Hongkong/1/68 (H3N2) and Sw/Wis/1/67 (Hsw1N1), and a titer of 1:320 with Dk/Alb/35/76 (Hsw1N1). No reactions were detected with reference sera against other subtypes except for FM/1/47 (H1N1) and Tern/S.A./1/61 (Hav5Nav2) which showed insignificant levels of inhibition (HI titer $<1:20$). Antiserum obtained by i.v. inoculation of chickens with the A/Duck/Bavaria/1/77 virus inhibited haemagglutination by the homologous virus and the A/Duck/Alberta/35/76 strain in the same dilution (Table 2). The isolated viruses also reveal a close relationship to the swine influenza virus strains like Sw/Wis/1/67.

Table 1. *Identification of the haemagglutinin antigen of A/Dk/Bav/1/77 using reference antisera against the presently known haemagglutinin types for influenza A viruses*

Antisera to	HI titers to		
	Dk/Bav/1/77	Dk/Bav/2/77	Homologous strain
H0 PR/8/34	40	40	10,240
H1 FM/1/47	20	20	10,240
H1 U.S.S.R./90/77	<20	<20	320
H2 Sing/1/57	20	<20	2,560
H3 Hkg/1/68	40	40	8,000
Heq1 Eq/Pr/1/56	<20	<20	8,000
Heq2 Eq/Miami/63	<20	<20	5,120
Hsw1 Sw/Iowa/15/30	<20	<20	1,280
Hsw1 N.J./8/76	<20	<20	640
Hsw1 Sw/Wis/1/67	80	80	320
Hsw1 Dk/Alb/35/76[a]	320	320	320
Hav1 FPV/Durch/27	<20	<20	1,000
Hav2 Chick/Ger/"N/49	<20	<20	1,280
Hav3 Dk/Eng/56	<20	<20	640
Hav4 Dk/Czech/56	<20	<20	160
Hav5 Tern/S.A./61	20	<20	640
Hav6 Turk/Mass/65	<20	<20	1,280
Hav7 Dk/Ukr/1/63	<20	<20	1,280
Hav8 Turk/Ont/6118/68	<20	<20	320
Hav9 Turk/Wis/1/66	<20	<20	1,800

[a] Dk/Alb/35/76: Antiserum to isolated haemagglutinin

Table 2. *Haemagglutination inhibition (HI) reactions using Influenza A/Dk/Alb/35/76 and A/Dk/Bav/1/77 antigens and antisera*

Antisera to	HI titers to	
	Dk/Alb/35/76	Dk/Bav/1/77
Dk/Alb/35/76	320	160
Dk/Bav/1/77	160	320

Neuraminidase Antigen

The neuraminidase activity of the two isolates was inhibited by antiserum to the recombinant virus A/eq/Pr/1/56 (H)-N.J./8/76 (N) (Heq1N1) and, to a lesser degree, by the antiserum to the recombinant strain eq-Bel which possesses a "human" N1 neuraminidase, thus indicating that the isolated strains possess a N1 neuraminidase.

These results suggest that the isolated virus strains are Hsw1N1 viruses closely related to A/Duck/Alberta/35/76 isolated in Canada in 1976 (7), and to the swine influenza virus isolated in Wisconsin from pigs in 1967.

Experimental Infection of Piglets

Virus was recovered from nasal swabs one to eight days p.i. from infected animals, and one to nine days from contact animals. Virus isolation was also successful from a rectal sample of one piglet on day 4 p.i. at the height of its virus excretion, but since this was the only isolation from a rectal swab it is not clear whether this isolate originates from an intestinal infection or comes from an external contamination through nasorectal contact with companion animals. No overt clinicial symptoms were observed except for two animals which showed a moderate rise in temperature to 40.5° C and 40.7° C 6 to 8 and 3 to 4 days p.i. respectively. Coughing was noticed very occasionally. All animals failed to produce detectable levels of antibodies to A/Duck/Bavaria/1/77 (HI titer $< 1:10$) during the observation period of 30 days.

Discussion

During the autumn and winter of 1977 several influenza viruses were isolated from different species of ducks in Southern Germany. All these viruses were classified as Hav6Neq2 and Hav7N2 (Ottis and Bachmann, unpublished results), except for two which belonged to the HO/H1/Hsw1 group of viruses. These two isolates were closely related to the Hsw1-subtype and share a close antigenic relationship with the haemagglutinin of recent swine influenza viruses, in particular that of the Hsw1N1 strain of A/swine/Wisconsin/1/67.

While there have been several swine influenza isolations from humans and pigs (5, 9, 12), there have been no Hsw1N1 virus isolations from wild birds until 1976, when isolates were reported from Canada, Hong Kong and the United States of America (3, 7). Comparing our serological results it is apparent that these Hsw1N1 viruses are antigenically closely related. The Hav5 haemagglutinin of the A/tern/South Africa/1/61 virus has been shown to react to some extent with the HO/H1/Hsw1-group (15). However, only the A/Duck/Bavaria/1/77 virus demonstrated a minor cross-reaction with the reference serum to Hav5.

The Hsw1N1 viruses collected from wild ducks replicate in both the trachea and the cloaca (7), while recent Hsw1N1 viruses from swine (A/swine/Tennessee/1/75) apparently replicate only in the respiratory tract of ducks (17). These findings, and the fact that there was actually no detectable antibody response in pigs exposed to the A/Duck/Bavaria/1/77 virus, seem to indicate that the Hsw1N1 viruses from ducks have a somewhat different host range compared to the recent

Hsw1N1 viruses from humans and pigs. This suggestion is supported by the "avian type" nucleoprotein demonstrated for the A/Duck/Bavaria/1/77 strain by SCHILD (pers. communication, 1979; 10).

Wild and domestic ducks may serve as a reservoir for swine influenza viruses that may have the potential of being pathogenic for man and swine (3, 8). The A/Duck/Bavaria/1/77 virus demonstrated clearly its infectivity and contagiousness for pigs in the first passage. The virus was recovered for more than one week from nasal swabs indicating that virus replication is actually taking place in the respiratory tract, although replication in the digestive tract cannot be excluded.

Interspecies transmissions of Hsw1N1 viruses between humans and pigs have occurred. Similar transmission from wild ducks to domestic ducks and to pigs seems possible which is of special interest to those countries which are free of swine influenza.

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