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Isolation of an Influenza Virus, Similar to A/Port Chalmers/1/73 (H3N2) from a Common Murre at Sakhalin Island in U.S.S.R (Strain A/CommonMurre/Sakhalin/1/74)

By

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With 1 Figure

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Summary

An influenza A virus isolated from the cloaca of a common murre was characterized antigenically as H3N2 and was most closely related to the A/Port Chalmers/1/73 strain. Serological studies of sera collected from common murres in the area of virus isolation showed that 21 per cent of the birds had antibodies to Hong Kong influenza virus.

Introduction

The importance of influenza A viruses in the animal and bird populations of the world have not been fully elucidated. The number of influenza viruses in the lower orders is not known, nor is their importance in the origin of new pandemic strains of human influenza viruses. The number of influenza viruses isolated from birds has increased rapidly during the past few years with an awareness of the importance in understanding the ecology of influenza viruses (2, 4, 5, 8—18, 22). In the present study, an influenza A virus similar to Port Chalmers/1/73 which was isolated from a common murre in the Far East of the U.S.S.R. is reported.

Materials and Methods

Study Area

Samples for serological and virological studies were collected from common murres *(Uria aalge)* nesting on Tuleni Island in August, 1974. Tuleni Island is located 20 km from the eastern coastline of Sakhalin Island in Terpenia Bay, U.S.S.R. The island (600 m long and 100 m wide) is a rocky plateau; the beaches are occupied by fur seals *(Callorhinchus ursinus L.)* and sea lions *(Eumethias jubatus Sch)* from April to November. The top of the plateau is occupied from May—August by common murres and by

kittewakes *(Rissa tridactyla)*. The common murres and fur seals mix, providing biocenotic connections between these species.

For virologic examinations tracheal and cloacal swabs were collected from 100 common murres. Swabs were taken into plastic tubes containing tissue culture media 199 and 1 per cent bovine serum albumin with 2000 units of penicillin and 500 μg of streptomycin. Samples were frozen in liquid nitrogen for transport to the laboratory.

For virus isolation studies 10-day-old chick embryos were inoculated by the amniotic and allantoic route and incubated at 35° C for 48 hours.

To minimize the possibility of laboratory contamination, the initial isolation and the following 3 passages were done in the virology laboratory at Sakhalinsk Sanitary-Epidemiologic Station, later passages were done at the Department of Ecology of Viruses of the Ivanovsky Institute of Virology. All samples were passaged 3 times before they were considered negative.

Virus isolates were concentrated and partially purified by adsorption and elution from chick erythrocytes and finally sedimented through a sucrose gradient (10—60 per cent sucrose in phosphate-buffered saline, pH 7.2).

Antisera

Immune antisera to the isolated haemagglutinin and neuraminidase subunits of the reference strains of influenza A viruses were made with antigen emulsified in Freund's complete adjuvant (20).

The antigen mixture was injected into goats—into the tail as well as intramuscularly. The animals received a second dose of antigen in adjuvant plus an intravenous injection of saline 40 days later. Blood samples were collected 7 days after the second injection and serum stored at —20° C. Antisera to antigenic hybrid viruses were prepared in rabbits as previously described (20).

Serological Tests

Haemagglutination titrations (HA) and haemagglutination inhibition (HI) tests were performed in plastic trays with receptor-destroying enzyme (RDE)-treated sera as previously described (6). Neuraminidase titrations (NA) were done by the method of WARREN (19) and AMINOFF (3). Neuraminidase inhibition (NI) tests were performed as previously described (21) using preincubation of virus-antibody and substrate (fetuin) at 37° C for 30 minutes prior to assay for free sialic acid. Intact influenza viruses were used in all tests.

Immunodiffusion tests were performed in 1.5 per cent agarose (A 37) dissolved in phosphate-buffered saline (PBS) (pH 7.2) containing 0.1 per cent sarkosyl NL 97 and 0.1 per cent sodium azide (20). Purified virus (HA 6.0 $\log_{10}$ units/ml) was disrupted with 0.1 per cent sarkosyl NL 97 and the same concentration was added to the antisera before addition to the plates to prevent non-specific precipitation bands. Single radial immunodiffusion plates (SRID) (1) containing disrupted A/chicken/Germany/N/49 (Hav 2 Neq 1) influenza virus were used to detect antibodies to influenza A ribonucleoprotein; other plates containing intact A/Hong Kong/68 (X-31) were used to detect antibodies to the surface antigens of A/Hong Kong/68 influenza virus. 5 μl of untreated sera were added to SRID plates. Positive and negative control sera were added to each plate.

Results

Virus Isolation

One haemagglutinating agent was isolated from the cloacal sample of a common murre (Table 1). The agent was first detected in the second passage in the allantoic cavity and subsequently grew to a high titer, both in the allantoic and amniotic cavities. Viruses were not isolated from the tracheal swabs.

Initial studies in HI tests using specific antisera to the isolated haemagglutinin of all of the reference strains of influenza A viruses showed that the agent isolated

from the common murre was either of the H3 (Hong Kong) or Hav7 (Duck/Ukraine/63) subtypes. In order to further characterize this virus, it was studied in HI tests with antisera to the variants of Hong Kong influenza virus.

Table 1. *Isolation of a hemagglutinating agent from the cloaca of a common murre in chick embryos*

Route of inoculation	Passage numbers						
	Initial	I	II	III	IV	V	VI
Amniotic	0	0	0	+[a]	+ 1:16[b]	+ 1:128	+ 1:512
Allantoic	0	0	+	+	+ 1:32	NT[c]	NT

[a] Shows detectable hemagglutination
[b] Hemagglutinin titer
[c] Not tested

Characterization of the Haemagglutinin Antigen

In HI tests the A/common murre/74 influenza virus is most closely related to A/Port Chalmers/1/73 influenza virus (Table 2) although it also reacts with antisera to Hong Kong/68 and England/42/72 to lower titers. In gel diffusion tests, the A/common murre/74 influenza virus shows a line of patrial identity with Hong Kong/68 (Fig. 1a) and a line of identity with Port Chalmers/73 (Fig. 1b). The multiple lines of precipitation with specific antisera to the haemagglutinin of influenza viruses has been reported previously (7) and may reflect the multiple antigenic determinants on the haemagglutinin subunits.

Table 2. *Characterization of the haemagglutinin on the A/common murre/1/74 influenza virus*

Antisera to	HI titers to the following influenza viruses			
	Hong Kong/68	England/72	Port Chalmers/73	Common murre/74
Aichi/2/68	*1800*[a]	600	200	280
England/42/72	1200	*3000*	1600	1800
Port Chalmers/1/73	400	2200	*3000*	4000

[a] Figures give the reciprocal of the dilution inhibiting three out of four haemagglutinating doses of virus. Antisera were prepared in chickens to the intact influenza viruses and were bled 14 days later

Characterization of the Neuraminidase Antigens

Neuraminidase inhibition tests with specific antisera to all of the prototype influenza A virus neuraminidases showed that the only antisera that neutralized the enzyme of the virus isolated from a common murre was the N2 type (results not shown). The neuraminidase antigen was further characterized using antisera to different variants of Hong Kong influenza virus (Table 3). The neuraminidase on the A/Common murre/Sakhalin/1/74 influenza virus appears to be most closely related to Port Chalmers/1/73 influenza virus.

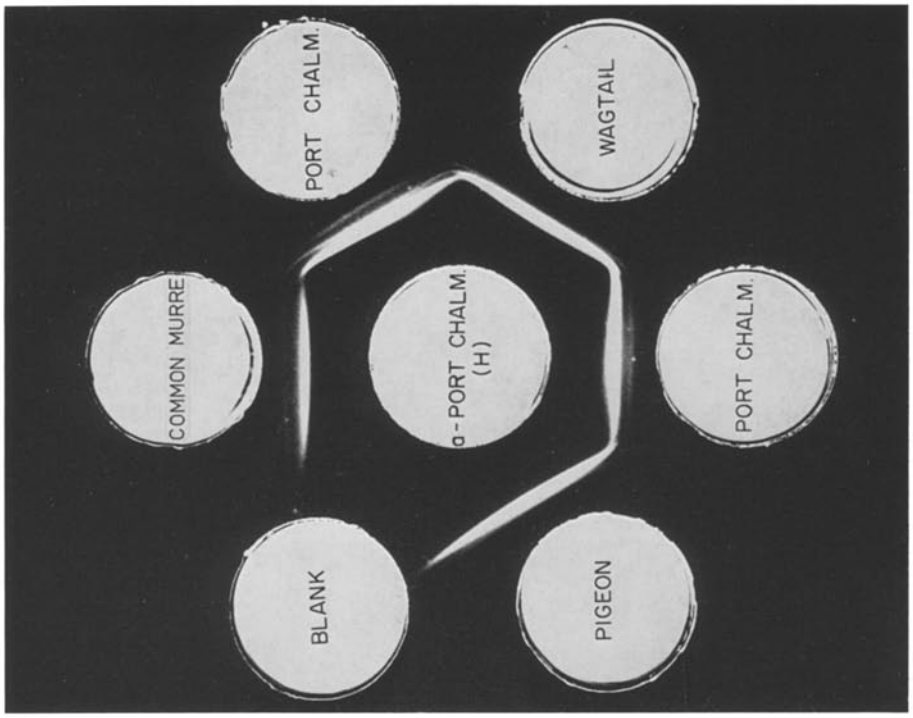
COMMON MURRE
HONG KONG
CHICK/GERM./N
α-HK (H)
WAGTAIL
PIGEON
HONG KONG

Fig. 1 a

COMMON MURRE
PORT CHALM.
BLANK
α-PORT CHALM. (H)
WAGTAIL
PIGEON
PORT CHALM.

Fig. 1 b

Serological Studies on Sera from Common Murres

Sera collected from 142 common murres on Tuleni Island in August 1974 were examined for antibodies to influenza viruses in HI tests. 21 per cent of the sera contained antibodies to A/Hong Kong/1/68 influenza virus. The majority of the sera (92) were also examined in SRID tests for antibodies to Hong Kong/68 influenza virus and for antibodies to the ribonucleoprotein of influenza A viruses; 21 per cent of the sera reacted to A/Hong Kong/68 and 23 per cent reacted with the RNP of influenza A viruses.

Table 3. *Identification of the neuraminidase antigen on an influenza A virus isolated from a common murre in U.S.S.R.*

	Antisera			
Viruses	A/Japan/57 (isolated N2)	Hong Kong/68 [Equine 1 (H)-Hong Kong/68 (N)]	England/42/72 [Equine 1 (H)-England/72 (N)]	Port Chalmers/1/73 [Equine 1 (H)-Port Chalmers/73 (N)]
A/Japan/305/57	*200*	80	<10	<10
A/Hong Kong/1/68	30	*200*	<10	<10
A/England/42/72	30	30	*600*	100
A/Port Chalmers/73	<10	<10	50	*100*
A/common murre/74	20	20	50	100

Antisera to A/Japan/57 were prepared with isolated neuraminidase
Antisera to Hong Kong/1/68, England/42/72 and Port Chalmers/1/73 neuraminidase were prepared against antigenic hybrids possessing equine/Prague/1/56 hemagglutinin and the appropriate neuraminidase. Values represent the reciprocal of the dilution causing 50 per cent inhibition of virus neuraminidase giving an approximate O.D. value of 0.50

Discussion

An influenza A virus isolated from the cloaca of a common murre caught on Tuleni Island off the east coast of U.S.S.R. was shown to possess H3N2 antigens. The haemagglutinin and neuraminidase subunits were most closely related to the A/Port Chalmers/1/73 variant of Hong Kong influenza virus. Serological studies of sera from common murres collected at Tuleni Island showed that 21 per cent of the birds had antibodies to Hong Kong influenza virus.

Hong Kong influenza virus and its variants are ubiquitous throughout the world in their ability to infect many domestic and feral species. These viruses have been isolated from many species, including pigs, calves, chickens, dogs, cats, monkeys, gibbons, baboons, and feral sea birds [for review see EASTERDAY

Fig. 1a (left). Double Immunodiffusion. Center: Antiserum to the isolated hemagglutinin of A/Hong Kong/1/68 influenza virus

Fig. 1b (right). Center: Antiserum to the isolated hemagglutinin of A/Port Chalmers/1/73. Outer: Chick/Germ/N A/chick/Germany/"N"/49, Common murre A/common murre/Sakhalin/1/74, Hong Kong A/Hong Kong/1/68, Wagtail A/wagtail/Ukraine/107/74, Hong Kong A/Hong Kong/1/68, Pigeon A/pigeon/Ukraine/111/74, Port Chalmers A/Port Chalmers/1/73, Blank Saline
Gel diffusion tests were done as described in Materials and Methods

(5)]. The isolation of a human influenza virus (A/Port Chalmers/73) from common murres raises the question of whether common murres contact this virus from man or whether viruses of the A/Port Chalmers/73 type have been in bird populations for a long period of time. The latter possibility seems unlikely, so we must assume that common murres contact these viruses either directly or indirectly from man. It is not known whether the A/common murre/74 influenza virus will persist in the common murre population; serological studies suggest that 21 per cent of the population had experienced this virus before August 1974. Studies on Hong Kong influenza viruses in chickens (8, 22) have shown that this virus is capable of circulating in an avian population with periodic epizootics. Other studies on Hong Kong influenza viruses in pigs (Schild, G. C., personal communication) suggest that this virus has not established itself in the porcine population but is reintroduced at intervals from the human population.

The importance of pelagic birds or the various domestic and feral animals from which Hong Kong influenza viruses have been isolated and their potential as reservoirs for Hong Kong influenza viruses remains to be established. To date, transmission studies within or between feral species, have not been done. The possibility remains, however, that a wide range of animals and birds could serve as potential reservoirs of Hong Kong influenza viruses.

Acknowledgments

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