Archives of Virology 86, 347—351 (1985)

Archives of Virology

An Avian Influenza A Virus Killing a Mammalian Species — the Mink

Brief Report

By

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With 1 Figure

Accepted August 26, 1985

Summary

During October of 1984 an influenza epidemic occurred on mink farms in the coastal region of South Sweden. Six strains of an influenza A virus were isolated. All six isolates were of the H10 subtype in combination with N4. The H10 subtype in combination with various N subtypes was hitherto only known to occur in avian strains, the prototype being the A/chicken/Germany/N/49 (H10N7) virus.

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Influenza A viruses which cause infections in humans, lower mammals and birds, are subdivided into 13 haemagglutinin (H) subtypes. All these subtypes have been isolated from birds, while up to now only the subtypes H1, H2, H3, H4 and H7 have been found in mammals including man, horse, swine and seal. Similarly from the 9 known neuraminidase subtypes only 5 could be detected in mammalian viruses. Although there is considerable antigenic and genetic relatedness between influenza A viruses of avian and mammalian origin, only the seal virus raises the possibility that mammalian influenza viruses may be directly derived from avian strains in nature (2, 8). We now report the isolation of an influenza A virus possessing avian H10N4 surface antigens from mink that died from pneumonia in several farms on

the South coast of Sweden. This could represent a further example of the potential role of an avian influenza virus as the causative agent of a severe outbreak of mammalian influenza.

The outbreak occurred during October 1984 in 33 mink farms, containing 100,000 animals, situated close to each other within an area of the South coast of Sweden (Fig. 1). There was almost 100 per cent morbidity and 3000 mink died. The most pronounced signs of the disease were anorexia, sneezing and coughing and nasal and ocular discharges. On post mortem examination an acute interstitial pneumonia with alveolar involvement was diagnosed.

Suspensions of lungs from six dead mink from 5 different farms were inoculated into the amniotic sack of 11 day old chicken embryos. Amniotic fluids of all the inoculated eggs taken 48 hours after infection caused a stable agglutination of chicken erythrocytes. The virus grows in chicken embryo cells and forms plaques in the presence of trypsin in the agar overlay. Complement fixation tests with antisera to the influenza types A and B revealed that the mink virus belongs to the influenza A viruses. An antiserum prepared by intranasal inoculation of ferrets with one of the isolates (No. 3900) as well as a serum from a convalescent mink, inhibited haemagglutination of the six isolates by equally high titers. Haemagglutination-inhibition (HI) and neuraminidase-inhibition (NI) tests with reference antisera against each of the 13H and 9N subtypes revealed that the isolate belongs to the serotype H10N4. An antiserum against A/chicken/Germany/N/49 (H10N7) reduced the infectivity of isolate 3900 by more than 10^5 plaque forming units. The isolate from mink is therefore designated A/mink/Sweden/84 (H10N4).

In order to confirm the spread of the same virus through the farms involved, sera were collected from 30 mink that had recovered from the disease, 10 mink per farm. Additionally, 15 samples were collected from two farms not involved in the outbreak. All the sera were tested by HI against two of the six mink isolates (Nrs. 3900 and 3849) as well as against various other influenza virus subtypes. The results in Table 1 show that the subtype H10N4 was spread through at least three of the farms involved. When tested against the two human H3N2 viruses all the 45 sera proved to be positive. This is in accord with the results reported by OKAZAKI *et al.* (5), who in mink farms noted silent infections with the human subtypes H3N2 and H1N1 by serology.

In order to learn about the infectivity and pathogenicity of the mink virus, five mink were each inoculated with 1280 HA units intranasally. The virus was from the first passage of the isolate 3900 in the allantoic sack. On day 1 after infection 5 mink were alternatively put into contact with an inoculated mink by housing the noninoculated mink in a cage neighbouring that of an inoculated mink; the fence between them being wire netting. Serum samples were collected every third day until day 16 post infection.

Table 1. *Spread of the H10N4 virus in the farms involved in the outbreak of influenza*

Subtype/isolate	HI test: number of sera positive/tested[h]	
	From 3 farms involved	From 2 farms not involved
H10N4/3900	24/30	0/15
H10N4/3849	24/30	0/15
H10N7/Virus N	24/30	0/15
H3N2[a]	30/30	15/15
H3N2[b]	30/30	15/15
H3N8[c]	0/30	0/15
H7N7[d]	0/30	0/15
H2N2[e]	0/30	0/15
H1N1[f]	0/30	0/15
H1N1[g]	0/30	0/15

[a] A/Hong Kong/1/68
[b] A/Aichi/2/68
[c] A/equine/Solvalla/79
[d] A/equine/Prague/56
[e] A/Singapore/57
[f] A/porcine/Sweden/83
[g] A/PR/8/34
[h] HI-titers of 1:8 or >1:8 considered as positive

After incubation periods of 2 to 5 days for the inoculated mink and 5 to 7 days for the noninoculated mink, the first sign of disease was anorexia followed by similar signs to those seen in the field, i.e. sneezing, pertussis-like attacks and dyspnoea accompanied by nasal and ocular discharges. Two mink were sacrificed on day 6 and 9 post infection when moribund. The virus was recovered from the lungs and liver and in one mink from the brain too. One of the inoculated mink also showed ataxia and the virus was recovered on day 9 after infection only from the brain.

Mink virus specific antibodies as determined by ELISA appeared on day 4 after infection in inoculated mink and on day 6 to 9 in contact mink; the titres increased to reach high levels on day 16.

The characteristics of the virus that could be determined suggest that at least the genes coding for the surface antigens are of avian origin. A virus of the subtype H10N7, then called virus N (A/chicken/Germany/N/49), was first isolated from a dead chicken in 1949 in Bavaria by DINTER (1) and has been subsequently found in different avian species in various countries. In the original combination with N7 the serotype H10 has been encountered as an agent of influenza outbreaks in turkeys in 1979 and 1980 in Minnesota, U.S.A. Feral ducks were suspected to be silent carriers (3). Together with some other neuraminidase (N) antigens the serotype H10 was found on coots in Israel (4), in feral birds in Europe (7), and in ducks in China (9). Thus, the virus was considered to be a strictly avian subtype.

At present it is unknown how the virus was introduced into the mink farms, and how the virus spread between the farms. The mink farms which have been involved in the outbreak are situated close to the sea (Fig. 1),

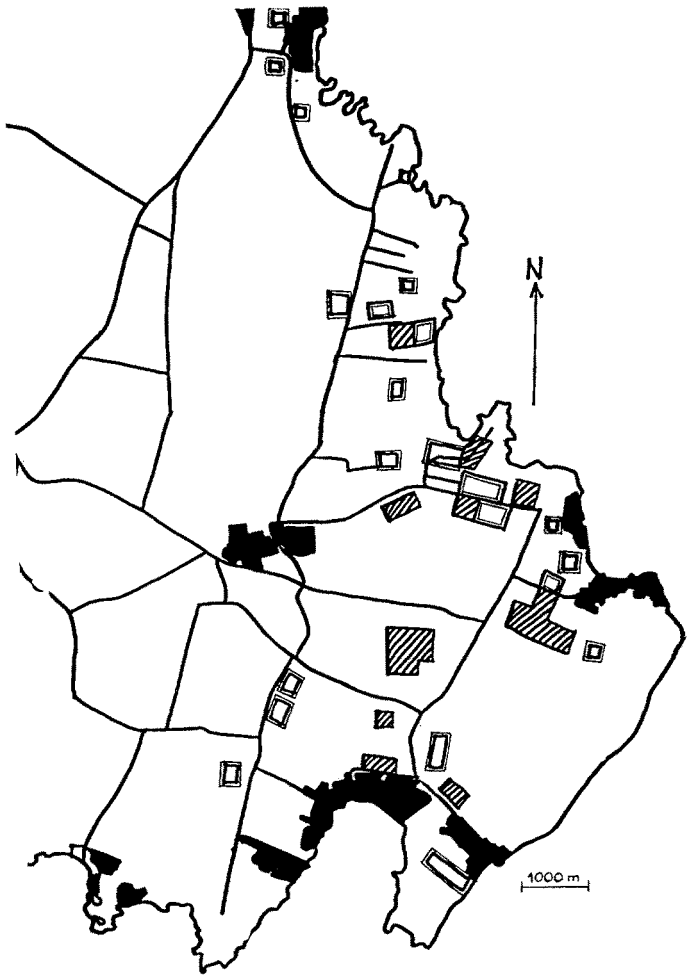

Fig. 1. Map of the Listerland peninsula of the South coast of Sweden indicating location of mink farms involved and not involved in the outbreak of influenza. The mink farms are visited all seasons by crows *(Corvus curone)* and gulls *(Laridae* spp.). In October also migrating birds, predominantly eider ducks *(Somateria molissima)* will pass the area. Mink farms involved (▨) and not involved (▭); Village (■)

and are often invaded by seabirds, particularly gulls, and during October, by other birds migrating to the South. Since the mink cages are kept outside, the birds become attracted to the mink food, which is placed on the cages.

It is obvious that the mink is susceptible to several influenza A viruses, including human subtypes H3N2 and H1N1 as shown by OKAZAKI *et al.* (5, 6), and in Table 1. In all these cases the mink never became ill. It is tempting to speculate that avian influenza viruses of the subtype H10

have the capacity to be transmitted to mink and may acquire virulence by the selection of virus variants by adaption to the novel host.

Acknowledgements

We thank Miss H. Isaksson and Miss M. Orlich for excellent technical assistance.

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Received August 26, 1985

Verleger: Springer-Verlag KG, Mölkerbastei 5, A-1010 Wien. — Herausgeber: Dr. F. A. Murphy, Division of Viral Diseases, Centers for Disease Control, Atlanta, GA 30333, U.S.A. — Redaktion: Mölkerbastei 5, A-1010 Wien. — Hersteller: R. Spies & Co., Straußengasse 16, A-1050 Wien. — Verlagsort: Wien. — Herstellungsort: Wien. — Printed in Austria.