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FURTHER EVIDENCE OF THE CIRCULATION OF PMV-4 AND INFLUENZA VIRUSES WITH N2 — 1957 ENZYME IN THE MIGRATORY WATERFOWLS

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Summary. — In the years 1980—1984, one paramyxovirus type 4 and 11 influenza viruses were isolated from cloacal swabs collected from migratory waterfowls in Fed. Rep. Germany. One influenza virus of H4N8 subtype was isolated from swabs of commercial ducks collected at an abbatoir. Seven of 10 influenza strains, isolated from mallard ducks and coot were identified as a mixture of 2—3 strains of H1, H4, and H5 subtype; 3 virus strains from the same locality relate antigenically to subtype H4 with enzyme serologically identical with N2 — Singapore/57 as demonstrated by means of polyclonal and monoclonal antibody.

Key words: *avian influenza*; *paramyxoviruses*; *waterfowls*; *influenza ecology*; *neuraminidase*

Migratory waterfowls are known to harbour most of the subtype of influenza viruses and paramyxoviruses (PMV) type 4, 6, 8, and 9. The first PMV of these types were isolated in South-East Asia and North America, however, since 1977 PMV-3, PMV-4, and PMV-6 have been also demonstrated in Europe (Ottis and Bachmann, 1983; Tůmová *et al.*, 1984; Tůmová, 1986). Contrary to PMV, influenza viruses are more spread in the avian population worldwide; the predominance of one or two subtypes of haemagglutinin and neuraminidase in various mutual combinations is characteristic for viruses of a certain period and locality. Although most avian influenza subtypes are apathogenic for feral birds and have been isolated from healthy birds, the same subtype may become lethal for animals kept for commercial purposes. This has been confirmed quite recently during destructive epizootics in pig herds in Europe (Pensaert *et al.*, 1981) and in poultry industry in the U.S.A. (Nettles *et al.*, 1985).

The possible role of avian influenza viruses in human influenza shift variants have extensively been discussed and elucidated by antigenic analysis on the molecular level. Therefore the effort devoted to influenza virus ecology, namely in avian population is important and well substantiated.

Table 1. Results of neuraminidase inhibition test with polyclonal N2 antibodies represented by immune sera to different influenza strains and with two N2 specific monoclonal antibodies

Immune sera	Homologous titre	Munich antigens 523	851	522
*/ Recomb. X15 (H7 N2-Sing/57)	2100	1700	1700	2560
***/ A/Singapore/57 (H2N2)	3000	2000	2000	—
**/ A/Ostrava/59 (H2N2)	1000	1250	900	—
*/ Recomb. 64b (H0N2-Hong Kong/68)	1300	20	20	15
**/ Ty/Mass/65 (H6N2)	2100	1300	1250	—
**/ Ty/Engl/1/66 (H6N2)	750	700	850	—
**/ Ty/Wiscon/66 (H9N2)	2000	2600	2560	—
**/ Ty/Wash/67 (H6N2)	1800	1600	1800	—
***/ DK/Hong Kong/34/76 (H3N2)	2000	1600	2000	—
***/ DK/Munich/9/79 (H2N2)	2560	2560	2800	—

Ascitic fluid	Antigens 64b	X-15			
monoclonal 11	0	220	3800	2800	3900
monoclonal 31	0	3400	300	4000	3000

Explanation: */ rabbit sera **/ white rat sera ***/ guinea pig sera; — not tested

Immune sera and antigens were prepared as described previously (Tůmová *et al.* 1984). Monoclonals were received by courtesy of dr. R. G. Webster, St. Jude Children's hospital, Memphis, Tenn. U.S.A.

In the period 1980—1984, during an extended surveillance programme of influenza in wild population in Germany, cloacal swabs were collected from free-flying waterfowls on Hemmelmarker See near Eckermforde (Schleswig-Holstein) from September to December and from 7 weeks old ducklings at abbatoir in Erlangen (March 1984).

All cloacal swabs were placed immediately in transport medium containing antibiotics and shipped to the laboratory where they were inoculated in the allantois of 10 days old embryos (Ottis and Bachmann, 1983). Isolated haemagglutinating viruses were identified by means of haemagglutination-inhibition (HIT), neuraminidase-inhibition (NIT) and immunodiffusion tests (IDT) (Palmer *et al.*, 1975) using a broad pannel of immune sera of white rats, guinea pigs, rooster and rabbit immune sera (Tůmová *et al.*, 1984).

Of 12 viruses included in these tests only one — Duck/Munich/431/81 was identified as paramyxovirus type 4. This type of PMV seems to be quite domesticated in some western and central European countries since 1977. PMV-4-related strains were detected in Germany and Czechoslovakia in the same species of migratory waterfowls almost at the same time. In Germany in the years 1977—1980 they represented 27 % of total PMV isolates (Ottis and Bachmann, 1983).

Of 11 influenza viruses, only the strain Duck/Munich/35/84-antigenically related to H4N8 subtype was isolated from commercial mallard duck; other 10 viruses were recovered from migratory ducks (*Anas platyrhynchos*) and

coot (*Fulica atra*). Seven of these strains represented a very heterogeneous group, reacting in HIT with H4 and/or H1, H3, and H5 antibody. Similar isolates mixed of 2 or 3 H or N antigens are fairly common among waterfowls in localities with a large number of persistently infected — nevertheless healthy — birds migrating from various geographic areas. Strains sharing H4, H3, H1 haemagglutinin were already described in previous studies in Germany and also in other parts of Europe (Ottis and Bachmann, 1983; Alexander *et al.*, 1986; Meulemans, 1986; Tůmová, 1986). Subtype H5 however, was frequently demonstrated only in Ireland and on the British Islands during the last 10 years (Alexander *et al.*, 1986). There it has occurred in feral birds and later on farms as a highly pathogenic agent of ducks and turkeys. This example together with experience of the U.S. in 1980—83 suggests that any evidence of H5 in free living birds must be viewed as a potential pathogen for domestic or commercial poultry.

Another 3 strains included in this study — Duck/Munich/522/80, Duck/Munich/523/81, and Coot/Munich 851/81 — were shown to share related envelope antigens of H4N2 subtype. Crossreactions were demonstrated among isolated strains and the viruses of the H4 subtype: Duck/Czechoslovakia/56, Turkey/Alberta 6962/66, Duck/Hong Kong/11/77, and Duck/Slovakia 42/78. The N2 enzyme was identified and specified by means of NIT with polyclonal antibodies of 3 different animal sera as A/Singapore/57-like (Table 1); the results have proved its relatedness to N2 of two human and 6 avian influenza strains which had been isolated in various countries in the period from 1965 to 1979. These results were confirmed by the test employing two monoclonal antibodies capable to discriminate specific N2/57 from N2/58 neuraminidase.

The isolation of these strains adds further support to the suggestion that the influenza viruses bearing the old type N2/57 neuraminidase continue to circulate unchanged for over 20 years in avian population. Analogically to other outdated antigens such as H2 and H3, as analysed by Kida *et al.* (1987) these findings indicate that haemagglutinin and neuraminidase genes remain conserved as there is no selective pressure of specific antibody in feral birds which would force mutational changes in a sense of drift variants of human viruses (Webster *et al.*, 1982). Therefore, avian population may represent a genetic pool of influenza virus, which deserves systematic attention.

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