

## TYPE A INFLUENZA VIRUS STRAINS ISOLATED FROM FREE LIVING DUCKS IN CZECHOSLOVAKIA DURING 1978—1981

R. TUREK, \*B. TŮMOVÁ, V. MUCHA, \*A. ŠTUMPA

Institute of Virology, Slovak Academy of Sciences, 817 03 Bratislava, Czechoslovakia; and  
\*Institute of Hygiene and Epidemiology, Czechoslovak Influenza Centre, 100 42 Prague, Czechoslovakia

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*Summary.* — Eight influenza virus A strains were isolated from 269 cloacal swabs taken from wild ducks (*Anas platyrhynchos*), shot during their autumn migrations in the years 1978—1981. One strain was identified as subtype A-H3N8N6 (Hav7Neq2Nav1), the remaining seven as subtype A-H4N6 (Hav4Nav1).

*Key words:* influenza A virus; isolation; ecology; free living ducks

### Introduction

In the course of recent studies on influenza virus ecology, a great many avian influenza A viruses have been isolated from aquatic birds caught in different parts of the world (Easterday and Tůmová, 1978; Lvov, 1978; Hinshaw *et al.*, 1980; 1981; Shortridge, 1982). The present paper summarizes the results of long-term studies on the occurrence of influenza A viruses in free living ducks in south Slovakia, extending previous investigations in this and other regions of Czechoslovakia (Grešíková *et al.*, 1978; Janout *et al.*, 1979).

### Materials and Methods

*Isolation experiments.* Cloacal swabs were obtained from free living ducks shot near two villages, Jakubov and Bohelov, during the autumn hunting seasons in 1978—1981. The swabs were collected and examined as described by Grešíková *et al.* (1978).

*Virus identification.* Serological identification of the strains isolated was done with a battery of the following anti-influenza sera:

A/duck/Hong Kong/29/76	A-H3N8 (Hav7Neq2)*
A/duck/Hong Kong/22B/76	A-H3N6 (Hav7Nav1)
A/duck/Slovakia/9/77	A-H3N6 (Hav7Nav1)
A/turkey/Alberta/66	A-H4N6 (Hav4Nav1)
A/duck/Czechoslovakia/56	A-H4N6 (Hav4Nav1)
A/duck/England/56	A-H11N6(Hav3Nav1)
A/quail/Italy/1117/65	A-H10N8(Hav2Neq2)

\*For the nomenclature see WHO Report (1980).

*Serological tests.* The haemagglutination-inhibition microtest (HIT) and the neuraminidase-inhibition test (NIT) were carried out according to the method of Palmer *et al.* (1975).

**Table 1. Isolations of type A influenza viruses from wild ducks (*Anas platyrhynchos*) during 1978—1981; results obtained from cloacal swabs**

Locality	Swabs/isolations/year	Prototype	Isolation rate
Jakubov	37/1/1978	H3N8 (H?N6)*	2.70%
	29/2/1980	H4N6	6.89%
	75/3/1981	H4N6	4.00%
Bohelov	17/1/1978	H4N6	5.88%
	111/1/1981	H4N6	0.90%

\* mixture of two strains

*Immune sera* were prepared in rats and guinea pigs by intratracheal and intramuscular inoculation of  $10^4$  EID<sub>50</sub>/0.1 ml of virus, and an equal booster dose given 3 weeks later. The sera were treated with RDE before use.

*Electron microscopy.* Concentrated virus was placed onto a formvar carbon-coated copper grid, negatively stained by 2% phosphotungstic acid (1 min, pH 6.5) and examined in a Philips 300 electron microscope.

## Results

### *Isolation of virus strains*

In 1978—1981 eight type A influenza virus strains were isolated from cloacal swabs collected from wild ducks shot in southern Slovakia during their autumn migrations. Isolation rates for the individual virus subtypes in relation to locality and year are given in Table 1.

All isolation attempts from tracheal swabs in aquatic avian species including wild ducks were negative. Similarly, no influenza virus was recovered from cloacal swabs of other aquatic bird species, viz. *Fulica atra*, *Aythya ferina*, *Anas strepera*, *Anas querquedula*, that are regularly seen together with *Anas platyrhynchos* during the autumn migrations. Because an organized shooting of free living ducks is permitted only in autumn, no data are available for other seasons of the year.

### *Identification of the viruses*

Results of identification of the isolated influenza A virus strains in terms of their envelope haemagglutinin and neuraminidase antigens are presented in Table 2. Most of the isolated influenza A strains could be unambiguously included in the A-H4N6 subtype. However, as HIT results indicated, the new isolates showed different degrees of antigenic difference from the prototype strains dk/Czech/56 and ty/Alberta/66. Strain A/anas/CS/2/78 was the only isolate with haemagglutinin of the H3/Hav7/subtype. On the other hand, test for neuraminidase gave cross-reactions with subtype N8/Neq2/sera and antigens and moreover a clear one-directional reaction of the serum of this strain with antigen N6/Nav1/ (Table 2 bottom).

**Table 2. Identification of envelope antigens in influenza virus strains newly isolated from *Anas platyrhynchos***

Antigens	HIT			NIT		
	dk/CS/56 (H4N6)	ty/Alb/66 (H4N6)	anas/42/78	dk/E/56 (H11N6)	dk/HK/22B (H3N6)	dk/S1/9/77 (H3N6)
Anas 5/80	80	20	160	600	1 280	1 280
Anas 238/80	80	80	160	500	1 280	1 280
Anas 42/78	40	160	640	640	NT*	NT
Anas 6/81	40	20	160	620	2 400	1 400
Anas 12/81	40	40	80	640	2 560	1 500
Anas 74/81	40	40	80	600	2 560	1 450
Anas 83/81	20	20	80	620	2 560	1 600
Homologous titres	80	320	640	640	2 560	1 000

  

	Guinea pig/rat sera (H3)			Guinea pig/rat sera (N6, N8)		
	anas/2/78	dk/S1/9/77 (H3N6)	dk/HK/22B (H3N6)	anas/2/78	dk/HK/29 (H3N8)	dk/E/56 (H11N6)
Anas 2/78	<b>1 280</b>	640	20	1 280	> 640	10
dk/S1/9/77	320	<b>1 280</b>	80	NT	NT	NT
dk/HK/22B	1 280	1 280	<b>160</b>	NT	NT	NT
dk/HK/29/76	NT	NT	NT	640	> <b>640</b>	0
q/It/1117	NT	NT	NT	720	> 640	0
dk/E/56	NT	NT	NT	600	0	<b>640</b>

\* not tested

The morphology of two of the influenza isolates, one subtype A-H3N8N6 and the other subtype A-H4N6, is shown in Figs. 1 and 2. Electron microscopy of negatively stained virus preparations revealed differences between populations of the two virus subtypes. Subtype A-H3N8N6 consisted of complete pleomorphic particles containing a dense nucleoid and well-discernible glycoprotein spikes on the viral envelope. Particles of subtype A-H4N6 were for the most part damaged and possessed thin glycoprotein spikes.

#### Discussion

The autumn migration routes of wild ducks over Slovakia are orientated approximately from northeast to the southwest (Hudec, 1967), traversing the selected study area of Jakubov and Bohelov. Adult birds ringed in Czechoslovakia have been trapped in southern Europe (Italy, France, Spain) as well as in the Middle East. To the north, ringed wild ducks from Czechoslovakia have been found in the southern part of Scandinavia, in Poland and in the European part of the Soviet Union. However, the ecology of influenza viruses is complicated by a great number of factors, such as the

free migration of wild ducks and other aquatic avian species, not excluding their occasional wintering on Czechoslovak territory in mild winters. The low average isolation rate for type A influenza strains in our study is comparable with the data reported in Austria (Stünzner *et al.* 1980), but is much lower than the results obtained from large nesting (or assembling) areas of domestic and wild water birds in Asia and in Canada (Shortridge, 1982; Hinshaw *et al.*, 1980; 1981). This difference can probably be explained by the low nesting density of birds in our localities or possibly by other factor as well.

Influenza virus subtype A-H4N6, the most frequently detected subtype in our study, had originally been isolated from domestic ducklings in eastern Slovakia in 1956 (Koppel *et al.*, 1956). Repeated isolations of this subtype during the present 5-year period suggest its permanent circulation in wildlife. The antigenic differences found between the newly isolated and the prototype strains confirm a heterogeneity in avian influenza viruses even within the same subtype.

The isolation of virus A/anas/Slovakia/2/78 possessing H3 and two neuraminidase subtypes N8 and N6, is most likely a result of mixed infection of the duck with two influenza viruses with an identical H but different N antigens. Verification of this conclusion by further serological methods in progress. Both N8 and N6 antigens have been demonstrated in some viruses isolated from wild ducks in this or a close-by area (Grešíková *et al.*, 1978; Janout *et al.*, 1979; Stünzner *et al.*, 1980; Sinnecker *et al.*, 1983). Mixed infection in waterfowls is apparently quite frequent as a consequence of waterborne infection but the demonstration of mixed strains, however, is less common (King and Shortridge, 1982). Successful virus isolations from wild ducks and negative results in other aquatic avian species confirm the observations from Asia and N. America that wild ducks can be considered a natural reservoir of influenza virus.

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*Explanation of Electron Micrographs (Plate LIV):*

- Fig. 1.* Influenza A-H3N8N6 (Hav7Neq2Nav1) virus particles negatively stained by phosphotungstic acid. The surface glycoproteins are well-discernible. Magn.  $\times$  200 000
- Fig. 2.* Influenza A-H4N6 (Hav4Nav1) virus particles negatively stained by phosphotungstic acid. The particles are mostly damaged, only some of them being enveloped by glycoprotein point formations (spikes). Magn.  $\times$  160 000