

Influenza in the USSR in 1977: recurrence of influenzavirus A subtype H1N1*

L. JA. ZAKSTELSKAJA, M. A. YAKHNO, V. A. ISAČENKO, E. V. MOLIBOG, S. A. HLUSTOV,
I. V. ANTONOVA, N. V. KLITSUNOVA, G. K. VORKUNOVA, A. G. BUKRINSKAJA,
A. F. BYKOVSKY, G. G. HOHLOVA, V. T. IVANOVA, & V. M. ŽDANOV

Early in November 1977, several outbreaks of influenza were reported in the far eastern region of the USSR. The epidemic spread rapidly throughout the country affecting mainly people under the age of 20 years. Most of the strains of virus isolated were found to be influenza A subtype H1N1. The serological characterization of the strains is described in this paper.

In 1977 local outbreaks of influenza were reported in technical schools and other groups of teenagers early in November in the far eastern region of the USSR and in other cities of the country. During the last 10 days of November an increase in the number of cases of influenza was noted in some large cities of the Asian part of the USSR (Vladivostok, Habarovsk, Novosibirsk). In December the epidemic spread through the European part of the USSR (Leningrad, Murmansk, Moscow, Poltava, Harkov). The epidemic was of moderate severity, the level of morbidity reported being half that reported during epidemics caused by Asian (H2N2) and Hong Kong (H3N2) viruses, in 1957 and 1968 respectively. The majority of patients were in the age group of 14-20 years. Local laboratories connected with the Regional Influenza Centre of the Ivanovskij Institute noted that influenza viruses were easily isolated and were not inhibited by antiserum to H3N2 viruses in haemagglutination-inhibition tests. Therefore they were sent to this Centre for further study.

A preliminary paper on the epidemic has already been published (1). This paper describes the antigenic properties of the influenza virus A(H1N1) strains isolated during the outbreak. Strains similar to A/Victoria/75 (H3N2) and A/Texas/77 (H3N2) variants were also isolated in the USSR during November-December, 1977.

MATERIALS AND METHODS

Viruses

Fifty-four isolates of virus obtained from the epidemics in November and December 1977 in

* From the D. I. Ivanovskij Institute of Virology, Academy of Medical Sciences, Moscow, USSR.

Moscow, Vladivostok, Habarovsk, Novosibirsk, Belgorod, and Poltava were studied and compared with the reference human influenza virus strains A/New Jersey/8/76 (Hsw1N1), A/PR/8/34 (H0N1), A/FM/1/47 (H1N1), A/Singapore/1/57 (H2N2), A/Hong Kong/1/68 (H3N2), A/Port Chalmers/1/73 (H3N2), A/Victoria/3/75 (H3N2), A/Texas/1/77 (H3N2), and B/Hong Kong/7/73 and animal influenza viruses A/FPV/27 (Hav1Neq1), A/chicken/Germany N/49 (Hav2Neq1), A/duck/England/56 (Hav3Nav1), A/duck/Czechoslovakia/56 (Hav4Nav1), A/tern/South Africa/61 (Hav5Nav2), A/turkey/Massachusetts/65 (Hav6N2), and A/duck/Ukraine/1/63 (Hav7Neq2).

Sera

Immune sera prepared by the Leningrad Institute of Vaccines and Sera, postinfection ferret sera and immune chicken sera obtained from WHO, and sera prepared in our laboratory by immunization of rabbits and rats (2) to the reference viruses were used. We also used immune sera to recombinant strains A/equine/Prague/56 (Heq1)-A/New Jersey/8/76 (N1), A/equine/Prague/56 (Heq1)-PR/8/34 (N1), A/equine/Prague/56 (Heq1)-FM/1/47 (N1), A/equine/Prague/56 (Heq1)-Hong Kong/1/68 (N2), and A/equine/Prague/56 (Heq1)-Victoria/3/75 (N2), and immune sera to purified haemagglutinin subtypes of influenza A human and animal viruses (the latter were kindly given to us by Dr R. Webster.^a

Serological reactions

Immuno-double-diffusion reactions (IDD) in agarose gel were conducted according to the method of Schild & Pereira (3) using immune serum against

^a St Jude's Hospital, Memphis, TN, USA.

ribonucleoprotein. Haemagglutination-inhibition (HI) tests were carried out with 1% chicken red blood cells and 4 agglutinating units of viral antigens. Neuraminidase-inhibition reactions (NI) were performed by the method described by Aymard-Henry et al. (4) using fetuin as a substrate for the enzyme.

Electron microscopy

Viruses were purified from the allantoic fluid of infected chick embryos by centrifugation in sucrose density gradients and studied in the electron microscope (JEOL 100B) by the standard method of negative contrasting.

RESULTS

A preliminary study of the newly isolated viruses by means of HI tests showed that they are not inhibited by immune sera to the H3N2 virus strains that have been in circulation since 1968, including immune sera to A/Victoria/3/75 and to A/Texas/1/77. All newly isolated viruses reacted in the IDD test with the immune serum to ribonucleoprotein of influenza A virus and therefore are classified as influenza A viruses.

To determine the specificity of the haemagglutinin, HI tests were conducted with all 54 isolates obtained from various parts of the country using a set of immune sera to the subtypes of human influenza haemagglutinins. Five of the most typical strains, 3 from Moscow and 2 from Novosibirsk, were selected for further study. It is seen from Table 1 that none of the newly isolated viruses reacted with immune sera to haemagglutinin subtypes H0, H2, and H3, whereas all were inhibited by immune sera to haemagglutinin H1. It should be noted that haemagglutination by the newly isolated viruses was inhibited by reference antiserum to A/FM/1/47 (H1N1) virus obtained from WHO, by diagnostic serum from a similar virus strain prepared by the Leningrad Institute for Production of Vaccines and Sera, and by immune serum to purified haemagglutinin H1 obtained from Dr R. Webster. The five selected strains were tested by HI also against antisera to the following haemagglutinins: Hsw1, Heq1, Heq2, Hav1, Hav2, Hav3, Hav4, Hav5, Hav6, and Hav7. None of the five strains reacted with these antisera, whereas they reacted with antiserum to the HI of A/FM/1/47 to the same titre as the homologous virus. Thus the haemagglutinins of all these newly isolated viruses were classified as subtype H1.

The next step was to determine the subtype of the neuraminidase. For this purpose a set of immune

Table 1. Identification of newly isolated viruses in HI tests

Antisera	Number of strains studied	Number positive
A/PR/8/34 (H0N1)	54	0
A/WS/33 (H0N1)	54	0
A/FM/1/47 (H1N1) ^a	54	54
A/FM/1/47 (H1) ^b	6	6
A (H1N1) ^c	54	54
A/USSR/Klim/49 (H1N1)	8	8
A/USSR/Pan/52 (H1N1)	8	8
A/Singapore/57 (H2N2)	54	0
A/Port Chalmers/1/73 (H3N2)	54	0
A/Victoria/3/75 (H3N2)	54	0
A/Texas/1/77 (H3N2)	54	0
B/Hong Kong/7/75	54	0

^a Obtained from WHO.

^b Obtained from Dr R. Webster.

^c Diagnostic serum to A(H1N1) prepared by the Leningrad Institute for Production of Vaccines and Sera.

sera was used, including those obtained by immunization of rabbits with recombinant strains that had Heq1 haemagglutinin and N1 or N2 neuraminidase. As is seen from Table 2, the neuraminidase of all strains studied was markedly inhibited by immune sera to the N1 subtype of neuraminidase, and none of them was inhibited by immune sera to the N2 subtype. However, these results were not so uniform as those in experiments with the haemagglutinins. Whereas strains isolated in Moscow were more strongly inhibited by immune serum to the neuraminidase (N1) of A/PR/8/34 virus, some strains were inhibited only with sera of the neuraminidases of A/NJ/8/76 or A/FM/1/47 viruses. One of the strains tested was also slightly inhibited by immune serum to N2.

An electron micrograph of one of the newly isolated viruses is presented in Fig. 1. Both thread-like and spherical virions are present.

DISCUSSION

From the time influenza A virus was first detected up to the present, the antigenic character of the prevalent strains has undergone several major changes and each of these "shifts" has been followed by the disappearance of the formerly prevalent viruses. Although there has been some speculation

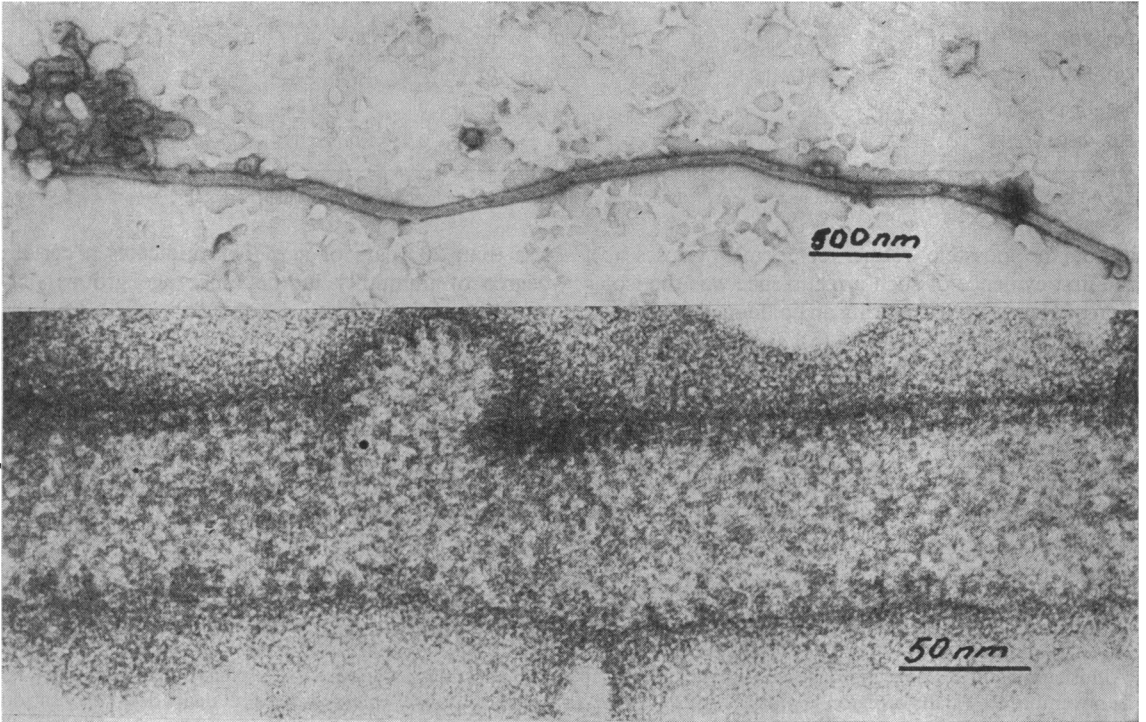


Fig. 1. Electron micrograph of one of the newly isolated H1N1 viruses.

Table 2. NI tests on newly isolated strains with immune sera to neuraminidases N1 and N2 ^a

Immune sera to virus recombinants	Neurami- nidase type	Virus strains											
		A/NJ/8/76	A/PR/8/34	A/FM/1/47	A/Victoria/3/75	Moscow 675	Moscow 687	Moscow 837	Habarovsk 33574	Habarovsk 34457	Novosibirsk 2158	Novosibirsk 2279	Novosibirsk 2277
Eq1-New Jersey/8/76	N1	270	0	30	0	180	90	60	0	0	90	90	0
Eq1-PR/8/34	N1	0	270	0	0	160	120	90	0	0	0	270	0
Eq1-FM/1/47	N1	60	60	270	0	60	90	60	90	90	0	90	90
Eq1-Hong Kong/1/68	N2	0	0	0	60	0	0	0	0	0	0	0	0
Eq2-Victoria/3/75	N2	0	0	0	540	0	0	0	0	0	0	60	0

^a The figures represent titres of immune sera that inhibited neuraminidase (average from three parallel experiments).

about the possible return of former viruses (5), the first evidence of such a recurrence was the isolation of Hsw1N1 virus during an epidemic of influenza in New Jersey, USA in 1976 (6). However that virus did not spread throughout the country as had been anticipated and the outbreak remained localized. The reappearance after 20 years of H1N1 virus in the USSR was followed by epidemic spread of the virus. The epidemic caused by this virus was of moderate severity, and it affected mainly people

less than 20 years of age. This indicates a certain degree of immunity in the older age groups that had been infected with a related virus 20-30 years earlier.

The next months will show whether the return of H1N1 virus will be followed by the disappearance of the H3N2 virus, and the next years will show whether the H1N1 virus will undergo antigenic drift as has occurred previously with all other influenza A subtypes.

RÉSUMÉ

LA GRIPPE EN URSS EN 1977: RÉAPPARITION DU VIRUS GRIPPAL A, SOUS-TYPE H1N1

Plusieurs flambées de grippe ont été signalées au début de novembre 1977 dans la région la plus à l'est de l'URSS. L'épidémie s'est ensuite propagée rapidement au reste du pays, frappant principalement les groupes d'âge au-dessous de 20 ans. L'article est consacré aux travaux de caractérisation sérologique des 54 souches de virus grippal isolées, parmi lesquelles 5 des plus typiques ont

été sélectionnées pour étude plus poussée. Toutes les souches nouvellement isolées étaient des souches du virus grippal A et, en ce qui concerne leur spécificité antigénique, les épreuves pratiquées ont permis d'établir qu'elles se rattachaient au sous-type H1 (hémagglutinine) N1(neuraminidase).

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