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INCIDENCE OF INFLUENZA C VIRUS IN CZECHOSLOVAKIA AND GERMAN DEMOCRATIC REPUBLIC

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Summary. — Out of 1091 and 2275 influenza virus strains isolated in Czechoslovakia (C.S.S.R.) and German Democratic Republic (G.D.R.) from 1969 to 1981, six and two were type C strains. All type C strains were isolated in the course of type A or B influenza epidemics. Double-diffusion tests indicated a relationship between the type C strains isolated in both countries in 1981 and the prototype strain C/Taylor/49; however, haemagglutination inhibition test (HIT) showed a heterogeneity within the 1981 group of viruses and their antigenic distinctness from the strain C/Taylor/49. The low number of type C isolates was in a sharp contrast with the antibody titres found in most adult sera. Only 10—30% of 275 and 2750 serum samples collected from persons aged 6—50 years in Czechoslovakia and G.D.R., respectively, were negative. The percentage of positive sera rose steadily in children 2—4 years of age. These serological findings suggest that influenza C virus was more or less permanently circulating among population, but the disease caused by it tends to take an inapparent or very mild course, so that the virus was isolated only exceptionally.

Key words: influenza C; epidemic incidence; antigenic properties; antibodies

Introduction

Owing to its high cultivation requirements, type C influenza virus is isolated very rarely. Moreover, it is not generally considered to be an epidemiologically important agent, although its epidemiology is not quite clear. However, because of frequency of antibodies the prevalence of type C influenza virus in human population is relatively high, and the virus undergoes similar slow antigenic changes as type B strains (Czelakowski and Prasad, 1973; El-Rai *et al.*, 1977; Chakraverty, 1978; Dykes *et al.*, 1980; O'Callaghan *et al.*, 1980; Palese and Young, 1982).

This paper reports the occurrence of type C influenza virus and corresponding antibodies in different age categories of the populations in two European countries where systematical long-term influenza surveillance has been carried out.

Materials and Methods

Isolation of virus was carried out on chick embryos inoculated intraamniotically with pharyngeal washings or swabbed material collected from patients with respiratory infection. The washings were done with phosphate buffered saline (PBS) solution containing 0.1% Hefeextract (Difco), or veal extract (Veal Infusion Broth, Difco), and 5% bovine albumin. Antibiotics were added immediately on collection of the washings.

Type C influenza virus strains, both standard and newly isolated, were propagated in the amnion of 8 to 10-day-old chick embryos at 33.0—35.5 °C. The amniotic fluid was used as antigen in serological studies and for immunization of animals.

Immune sera were prepared in hamsters inoculated intranasally 2—4 times at 3-week interval and in chickens receiving intravenously a single 5 ml injection of freshly propagated virus. All animals were bled 10 days after the last injection.

Human sera for immunological studies were collected in October 1981 in 2 localities in C.S.S.R. and 5 in G.D.R. The sets of 275 (C.S.S.R.) and 2750 (G.D.R.) sera represented 7 groups: 0—3, 4—5, 6—14, 15—19, 20—29, 30—50 and $\geq$ 50 years of age. During the same time 111 sera from 1—4-year-old children were collected separately in two areas in C.S.S.R. The sera were stored at —20 °C (Prague) or at +4 °C (Berlin). Before use, they were treated with receptor destroying enzyme (RDE) and inactivated.

Haemagglutination-inhibition test (HIT) was performed according to the WHO method in micropanels (Palmer *et al.*, 1975). The titres were expressed as the reciprocal values of the end dilutions that gave 100% haemagglutination inhibition.

Double diffusion test in gel (DDT) was done by the method of Palmer *et al.* (1975). Virus concentrated by high-speed centrifugation (75 000 × g) for 3 hr was cleft by sodium sacrosylsulphate and used as antigen.

Results

Virus isolations

Isolations of type C influenza virus were done from pharyngeal washings or swabbed material collected in surveillance programmes run systematically in both countries (Strnad *et al.*, 1976). These programmes include epidemiological and virological investigation of acute respiratory infections among all age groups of the population. Data on the type C influenza virus strains isolated in the years 1969—1981 in both countries are presented in Table 1. Striking are the very small numbers of these strains as compared with the 1093 (C.S.S.R.) and 2275 (G.D.R.) type A and B strains isolated during this period. The actual difference in frequency is still greater, because during 1969—1981, 12 influenza epidemics had occurred but type C virus was isolated only from 4 of them in C.S.S.R. and 1 in G.D.R.

All type C strains were isolated in the course of type A or B influenza epidemics, from cases of acute respiratory disease, most of them mild, in children 2—3 years or adults 22—41 years of age. The strains were detected on chick embryos already in the first or second passages. In the Prague study, virus isolations were also attempted on monkey- and dog-kidney cell cultures,

Table 1. C influenza isolations in C.S.S.R. and G.D.R.

Year	Month	Number of C type	from total strains	Season	Patients
1969	II	2	428	epidemic A (H3N2)	adults — seroconversion to C infl. only
1972	XII	1	453	epidemic A (H3N2)	age 2 — serology not done
1974	IV	2	97	epidemic B type	adults (age 22 and 30) — only seroconversion to C infl.
1981	III	1	115	epidemic A (H1N1) + local epidemics B	age 3 — seroconversion to B + C
1981 (G.D.R)	II	2	19	epidemic B type sporadic A (H3N2) and A (H1N1)	adults (age 33 and 41) seroconversion to C

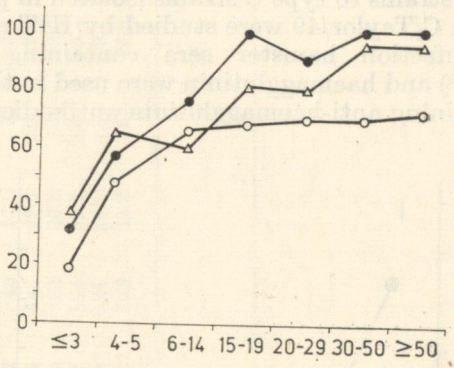

Fig. 2.
Antibodies to 3 type C influenza strains in human sera according to age groups
Serum sets from C.S.S.R. and G.D.R.
Strains: ●——● C/Taylor/49; △——△ C/Praha 33/81; ○——○ C/Berlin/2/81
Abscissa: age groups (years); ordinate: per cent of positive sera (titre ≥ 20).

but type C virus was detected only one (strain C/Praha 1/81) in monkey kidney cells and because of failure of further passaging in cell cultures, it had to be transferred to chick embryo.

Properties of the virus

In the course of passaging and identification of the type C strains we were able to confirm the selective agglutination of chick erythrocytes by type C virus and their very rapid elution evidencing the enzymatic activity of our strains. The phenomenon of virus inhibition by normal rat serum was demonstrated for all strains. Antigenic relationship of the newly isolated

Table 2. Antigenic relationships of C type inuflenza strains as demonstrated by HIT with hamster and chicken sera

Sera	Antigens							
	Taylor 49	Brat	Čs 1	Čs 2	Paris	Praha 74	Praha 81	Berlin 81
C/Taylor/49	**640**	80	80	80	640	160	80	80
C/Bratislava 17/65	80	**160**	40	80	40	80	80	80
			(320)			(160)		(40)
C/Čs 1/69	40	40	**640**	80	80	80	80	80
C/Čs 2/69	80	160	80	**640**	80	80	80	80
C/Paris/67	80	40	20	40	**80**	80	40	40
			(20)			(320)		(40)
C/Praha 1/74	80	40	40	20	80	**160**	40	40
C/Praha 33/81	80	160	160	80	80	160	**320**	320
			(320)			(640)		(640)
C/Berlin 1/81	40	40	40	40	40	40	80	**80**
Control rat serum inhibitors	10	80	20	40	10	10	40	20

Results with chicken sera are given in brackets.

virus strains to type C strains isolated in previous years and to the prototype strain C/Taylor/49 were studied by HIT and DDT. In most cases, boostered postinfection hamster sera containing antibodies to ribonucleoprotein (RNP) and haemagglutinin were used in these tests; in HIT also chicken sera containing anti-haemagglutinin antibodies were employed.

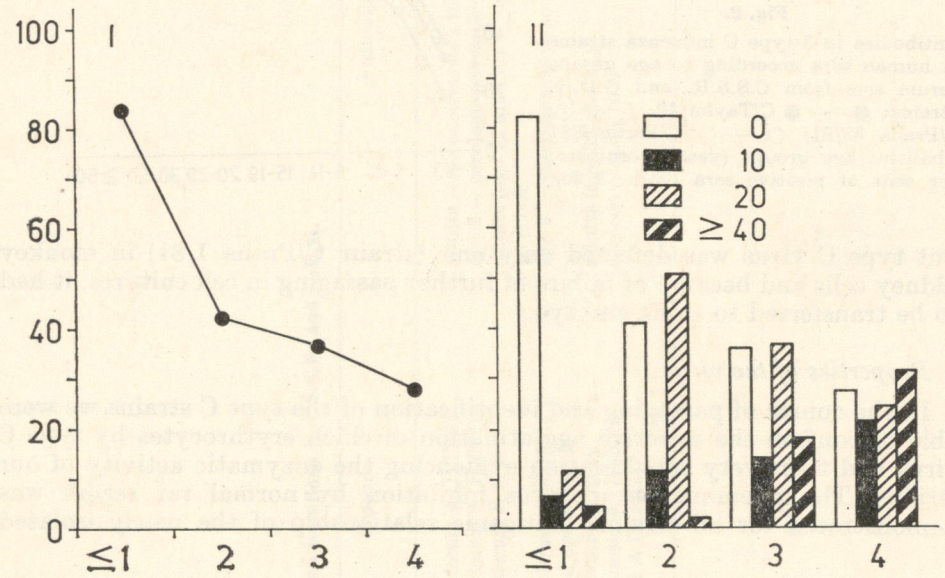

Fig. 3.

Decrease of negative (I) and increase of positive (II) antibody titres in sera of 1 to 4-year-old children
Serum set from C.S.S.R. Columns: HI antibody titres
Abscissa: age groups (years); ordinate: percentage of the sera.

In DDT (Fig. 1), clear precipitation lines appeared between antigens of strains C/Taylor/49, C/Paris/67, C/Czechoslovakia/69 and hamster sera against the Prague and Berlin isolates of 1981. The results of HIT for antigenic relationship with all the type C strains used are summarized in Table 2. Antigenic differences are evident between strains C/Taylor/49, C/Czechoslovakia 1/69, C/Praha 1/74 and the 1981 isolates. The cross-reaction values obtained with chicken sera in HIT suggest a heterogeneity between the strains isolated in 1969, 1974 and 1981.

Serological examination of human sera

Antibodies to type C influenza virus were tested by HIT in 275 (C.S.S.R.) and 2750 (G.D.R) sera collected from persons of 7 age categories. The sera

Table 3. Haemagglutination-inhibiting antibodies to C influenza virus in human sera of different age groups

Age group	A*							B**						
	Total number of sera	Negat.		1 : 20		40+		Total number of sera	Negat.		1 : 20		40+	
		no.	%	no.	%	no.	%		no.	%	no.	%	no.	%
0—3	86	59	68.6	20	23.3	7	8.1	486	398	81.9	42	8.6	46	9.5
4—5	40	17	42.5	13	32.5	10	25.0	251	129	51.4	45	17.9	77	30.7
6—14	30	7	23.3	17	56.7	6	20.0	506	169	33.4	147	29.1	190	37.5
15—19	29	0		14	48.3	15	51.7	264	83	31.4	95	36.0	86	32.6
20—29	30	3	10.0	9	30.0	18	60.0	492	149	30.3	154	31.3	189	38.4
30—50	30	0		2	6.7	28	93.3	389	116	29.8	121	31.1	152	39.1
50+	30	0		7	23.3	23	76.7	362	95	26.2	112	31.0	155	42.8
Total number	275							2.750						

** A — sera from C.S.S.R. were tested with C/Taylor/49.
* B — sera from G.D.R. were tested with C/Berlin 2/81.

were collected in both countries during the same period in 1981 and were tested against antigens C/Taylor/49, C/Praha/33/81 and C/Berlin/2/81. The results are presented in Table 3 and Fig. 2. The serum positivity rate rose gradually from the lowest age group of 0—3 years (68—81% of negative sera) to the group of school children of 6—14 years (positive antibody titre of $\geqq 20$ detected in 60—70% fo sera). In the middle and higher age categories (over 20 years), the positivity rate for the C.S.S.R. set of sera was 90—100% and for that of G.D.R. sera only 70—74%. This difference could only in part be due to the use of different antigens. Fig. 2 presents the results of testing the same set of C.S.S.R. sera against antigens C/Taylor/49 and the recent C/Praha/33/81. The results showed that the dynamics of increase of population immunity had the same trend with either strain, but within the age range of 6—50 years the positivity rates with the recent virus isolate were 5—15% lower.

The steep rise in serum positivity in the 4—5 years age group and in 0 to 3-year-old children indicated that contact with type C influenza virus was the most frequent during this age span. Fig. 3 presents some data on young children in greater detail. Tests of 111 sera collected from 1—4 year-old children in C.S.S.R. in 1981 showed a steep decrease in the percentage of negative sera in 2-year-olds and a more gradual increase in positivity rate from the 2nd to 4th year of life. This increase is expressed in Fig. 3 by a change in distribution of antibody titres of 10—40 in the sera of 3 to 4-year-old children. In 3-year-old children the rate for sera lacking antibody to type C virus decreased to 35% and the rate for positive sera with titres of 20 and 40 rose to 52%. The percentage of positive sera was more or less the same in sera of 4-year-old children, but the percentage of sera with higher antibody titres was higher.

Discussion

During the years 1969—1981, 12 epidemics of type A or B influenza occurred in C.S.S.R. and G.D.R., but only 6 and 2 type C influenza strains were isolated in the respective countries. The low frequency of isolation of this virus type, also known from other countries (review by Tůmová, 1972), has been attributed to its difficult cultivation: almost exclusive growth in the epithelium of chick-embryo amniotic sac, poor growth and inability to be passaged in cell culture systems in vitro (Mogabgab, 1962), and the distinctive phenomenon of haemadsorption to chicken or rat erythrocytes instead of the commonly used guinea-pig erythrocytes (Chakraverty, 1974). However, the results of our study indicate that it is not because of the failure of isolation methods but due to the smaller number of manifest illnesses caused by this virus type.

Type C influenza virus has been known as an accompanying agent in type A or B epidemics, being isolated quite accidentally, usually from dual infection (Kilbourne *et al.*, 1951). The seroconversion to type B and C viruses observed by us in the the patient who had yielded the strain C/Praha 1/81 could also be ascribed to dual infection. Outbreaks caused by type C in-

fluenza virus in minor communities, such as described by Styk (1954) in Czechoslovakia and Dykes *et al.* (1980) in U.S.A., are very rare and their detection requires systematic epidemiological surveillance as a prerequistite. Most authors agree that clinically type C influenza is very mild and rather resembles seasonal catarrhs; confirmatory evidence has been furnished by experimental infection of volunteers (Joosting *et al.*, 1968). Thus, it is probable that such illnesses most often escape attention and are not virologically clarified. Hence, discrepancy occurs betwen the small numbers of isolates and the high seropositivity rates in type C influenza.

Our studies showed that as many as 60—70% of school children (6—14 years) had unequivocal antibody titres, and the rate was still higher above 20 years of age. The seropositivity dynamics found for children 1 to 4-year-old evidenced that most individuals came in contact with the virus at the age of 2—4 years, with only 35% of children lacking antibody at the age of 4. O'Callaghan *et al.* (1980) have demonstrated significant antibody titres at rates of 36—47% and 96% in the age groups of 1—5 and 20—30 years, respectively. Dykes *et al.* (1980) have found antibodies in 96% of children aged 6—10 years. Glezen (1980) claims that over 60% of children become infected by 5 years of age in small endemic outbreaks which as a rule escape epidemiological attention. This interpretation could explain also our findings. On the basis of immunological analysis in children and adults, Dykes *et al.* (1980) inferred that the circulation of type C influenza virus was continual and reinfections were frequent in the middle age.

Of the type C influenza virus properties that bear on the demonstrability and rapid identifiability of the agent, deserve mentioning selective agglutination of rat erythrocytes and sensitivity to rat serum inhibitor. Inhibiton by normal rat serum demonstrated and later also explained by Styk (1954, 1963) is a characteristic marker of all type C strains and may be used for their rapid differentiation from the other influenza virus types. The results of our antigenic-relationship study are partly in agreement with those of Czelakowski and Prasad (1973) and Chakraverty (1978). Antigenic differences and strain heterogeneity are demonstrable in HIT but depend on the immune sera used and strain avidity. The results of Chakraverty, who included our C/Czechoslovakia/1/69 strain into her work, showed far greater differences from some other strains, probably owing to her usage of postinfection ferret sera.

Despite their differences, the new type C strains cannot, in our opinion, be considered drift variants. On the contrary, the variability of type C influenza virus over the past 30 years can be claimed to have been less than that of type B; this is emhasized by Palese and Young (1982), who explain the phenomenon by a different structure of the genome.

References

Czelakowski, J. W., and Prasad, A. K. (1973): Studies on influenza virus. 1. Antigenic variation in influenza viruses type C. *Arch. ges. Virusforsch.* **42**, 215—277.

Dykes, A. C., Cherry, J. D., and Nolan, C. E. (1980): A clinical, epidemiologic, serologic and virologic study of influenza C virus infection. *Arch. int. Med.* **140** (10), 1295—1299.

El-Rai, F. M., Shaheen, Y. A., El-Diwani, Kh. M., Abdel-Al, M. H. (1977): The incidence of antibodies to influenza C virus in egyptian sera. *J. Egypt. publ. Hlth Ass.* **52/1**, 6—11.
Glezen, N. P. (1980): Influenza C virus infection. *Arch. int. Med.* **140**, 1278.
Chakraverty, P. (1974): The demonstration and multiplication of influenza virus in tissue culture. *J. gen. Virol.* **25**, 421—425.
Chakraverty, P. (1978): Antigenic relationship between influenza C virus. *Arch. Virol.* **58**, 341—348.
Joosting, A. C. C., Head, B., Bynoe, M. L., Tyrrell, D. A. J. (1968): Production of common cold in human volunteers by influenza C virus. *Brit. med. J.* **4**, 153—154.
Kilbourne, E. D., Anderson, H. C., and Horsfall, F. L. Jr. (1951): Concurrent infection with influenza A and B in a single epidemic of influenza. *J. Immunol.* **67**, 547—558.
Mogabdab, W. J. (1962): Influenza C virus in monkey kidney tissue cultures. *J. Bact.* **83**, 209—210.
O'Callaghan, R. J., Gohd, R. S., and Labat, D. D. (1980): Human antibody to influenza C virus: its age-related distribution and distinction from receptor analogs. *Infect. Immun.* **30**, 500—505.
Palese, P., and Young, J. F. (1982): Variation of influenza A, B and C viruses. *Science* **215**, 1468—1474.
Palmer, D. F., Coleman, M. T., Dowdle, W. R., and Schild, G. C. (1975): *Advanced laboratory techniques for influenza diagnosis.* US Dept. HEW, PHS, Atlanta, Georgia, U.S.A.
Strnad, P., Tůmová, B., Syrůček, L., Fedová, D., Brůčková, M., Kunzová, L., Štumpa, A., Střížová, V., Berkovičová, V., Losová, M. (1976): Influenza and other acute respiratory disease in the Czech Socialistic Republic, 1969—1975. *Bull. Wld Hlth Org.* **54**, 657—662.
Styk, B. (1954): Epidemic outbreak of influenza due to type C influenza virus (in Czech). *Čs. Epidem.* **3**, 137—145.
Styk, B. (1963): Effect of some inhibitor-destroying substances on the nonspecific inhibitor of C influenza virus present in normal rat serum. *Acta virol.* **7**, 88—89.
Tůmová, B. (1972): Influenza viruses. pp. 85—130. In M. Majer and S. A. Plotkin (Eds): *Strains of Human Viruses.* Karger, Basel.

Explanation of Micrographs (Plate LIII):

Fig. 1. Relationship among type C influenza strains.
Double diffusion test.
Antigens: 1—C/Taylor/49, 2—C/Paris/67, 3—C/Czechoslovakia/69
Sera: A—C/Praha 33/81, B—C/Berlin/81