Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1963, **28**, 217-223

Preliminary Report on Vaccination in Croatia against Poliomyelitis with Type 1 (CHAT) and Type 3 (W-Fox) Attenuated Polioviruses of Koprowski

Laboratory and Epidemiological Data

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The incidence of poliomyelitis in the People's Republic of Croatia has progressively increased since 1945, and in 1953 and 1960 there were serious epidemics. In order to protect the age-groups at greatest risk a mass vaccination campaign was carried out with Koprowski live-virus vaccine in the early spring of 1961, covering the entire population aged 3 months to 20 years. Altogether 1 339 244 persons were given type 1 (CHAT strain), and 1 287 909 received type 3 (W-Fox). Over 100 000 persons were estimated to possess no pre-existing antibody to any of the three types of poliovirus.

Although no unvaccinated control group was set up, it is considered that the epidemiological and serological data indicate the safety and efficacy of the vaccination. The serological conversion rate was 91.5 % for type 1 and 93.5 % for type 3. No post-vaccinal reactions were observed. The usual summer peak in incidence did not occur in 1961, altogether 6 cases being recorded from June (when immunity is considered to have been established) to December. This is the lowest figure since 1945.

The use of live-virus poliomyelitis vaccine in mass vaccination has made possible the eradication of poliomyelitis from entire geographical areas (Courtois et al., 1958; Sabin et al., 1960; Plotkin et al., 1961). This paper records the preliminary results of an effort to this end in the People's Republic of Croatia. It will be seen from Table 1, which gives the history of poliomyelitis in Croatia from 1945 to 1960, that the number of cases of poliomyelitis in 1953 and in 1960 was considerably above the average.

In the period between these two epidemic years, as Salk killed poliomyelitis vaccine was increasingly used all over the world, the possibility of vaccination against poliomyelitis in Croatia came to be seriously considered. In the course of 1959, combined vaccination against poliomyelitis (Salk vaccine), diphtheria, tetanus, and pertussis was undertaken. This combined vaccine, in the composition adopted, caused only mild postvaccinal reactions; there was no interference between the individual components of the vaccine.

Since killed-virus vaccination was used in a comparatively small area only, it could not greatly influence the large poliomyelitis epidemic which spread through Croatia in 1960, when the number of reported poliomyelitis rose to 563, although it was noted that the epidemic was serious only in those areas where no killed vaccine had been administered. In some of these areas, Sabin live-virus poliomyelitis vaccine was then administered.

At the end of 1960 preparations were made for vaccinating the entire population up to 20 years of age with live poliovirus vaccine. The main reason for using live vaccine was that it is cheaper than the inactivated vaccine and much simpler to administer, thus making possible the vaccination of millions of persons within a short time.

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MATERIAL AND METHODS

Vaccination technique

The vaccine was distributed into 5-ml penicillin bottles, sealed with rubber stoppers and metal caps. Each bottle contained 50 0.1-ml doses. Before transportation the filled bottles were stored in the deep-freeze (−20°C).

For application the thawed vaccine was drawn out of the bottle into a 1-ml syringe, on which was then fitted, with a spceial fastening device, a blunt needle, 1 cm long. 0.1 ml of the vaccine was then instilled directly into each child's pharynx. After vaccination, each vaccinee was given milk to drink.

Since the syringe did not enter the mouth of the vaccinee, there was no need to have it changed during vaccination. However, whenever the needle did chance to touch the mouth of the vaccinee, it was immediately replaced by a new one. This simple technique made it possible to vaccinate up to 200 schoolchildren an hour.

Organization of vaccination campaign

The vaccine was transported in special Styropore-coated aluminium boxes containing 48 bottles of frozen vaccine. The vaccine, as a rule, was transported by automobiles, to more distant places by aircraft, and to islands by motorboats.

Fifteen supply centres were set up to cover the entire area to be vaccinated. Each supply centre had a deep-freeze in which the vaccine was stored at −20°C. From there the vaccine was transported directly to the vaccination stations. If a bottle with thawed vaccine was not used on the day of its removal from cold storage, it was discarded.

Before the vaccination began, two conferences were held, at which regional leaders were briefed regarding the organization and the objectives of the campaign. The regional leaders then held meetings with physicians taking part in the vaccination campaign in their areas. After vaccination with type 1 and type 3 was completed, another conference was held at which the regional leaders commented on the campaign.

TABLE 1
MONTHLY POLIOMYELITIS INCIDENCE IN CROATIA, 1945-61

Year	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total	Total June-Sept.
1945	1	1	1	1	1	3	1	8	2	3	0	0	22	14
1946	1	3	1	2	5	4	12	13	6	6	4	0	57	35
1947	0	2	2	6	1	1	3	5	1	0	0	0	21	10
1948	1	1	1	1	4	4	3	5	4	3	1	0	28	16
1949	2	4	0	2	4	5	8	11	7	5	5	1	54	31
1950	4	4	0	0	3	10	2	3	7	5	3	1	42	22
1951	2	2	2	4	1	15	17	15	12	6	4	2	82	59
1952	2	3	0	1	0	3	3	4	4	7	2	1	30	14
1953	4	6	7	7	7	23	79	140	51	29	23	12	388	293
1954	18	7	4	9	7	4	12	15	13	7	9	14	119	44
1955	3	8	4	9	6	6	14	6	6	5	7	4	78	32
1956	4	2	0	1	3	5	16	37	32	23	12	5	140	90
1957	8	5	7	5	7	13	27	41	23	6	3	1	146	104
1958	5	4	8	4	4	2	13	26	25	15	7	7	120	66
1959	8	3	1	4	11	15	28	23	5	5	7	3	123	81
1960	9	5	5	7	10	35	73	213	115	55	22	14	563	436
1961	10	11	3	3	1	2	1	1	0	1	1	0	34	4

Before the campaign began, an intensive publicity campaign was organized, inviting all the population to take part (although vaccination had been made compulsory). This campaign was conducted through television, the radio, the press and the cinema and by means of lectures, posters, etc.

Vaccine and vaccination

Live-virus vaccine made available by Dr H. Koprowski was used. The concentration of type 1 (CHAT) of the attenuated virus amounted to 230 000 $TCID_{50}$ and that of type 3 (W-Fox) of attenuated virus to 80 000 $TCID_{50}$. This difference in concentration proved to be justified, as antibody titres in the sera of persons vaccinated with type 3 attenuated virus were very high.

The vaccination campaign was designed to include the whole population from 3 months to 20 years of age—a total of 1 566 008 persons.

Vaccination with type 1 was carried out from 18 February to 4 March 1961, and with type 3 from 25 March to 7 April. The entire population to be vaccinated was divided into two groups, one of which started vaccination seven days earlier than the other. Vaccination in the various areas lasted about seven days, although some areas with highly developed health services succeeded in completing the work within 3-4 days, using Sunday for the vaccination of pre-school children and weekdays for the vaccination of schoolchildren and the remainder of the population up to 20 years of age.

In order to achieve satisfactory results from a large-scale vaccination campaign with live poliomyelitis vaccine and in order to be able to draw valid conclusions as to the safety and efficacy of the vaccine, it is necessary not only to collect epidemiological data but also to determine: (1) the immune status of the population before vaccination; (2) the circulation of enteroviruses before vaccination, and (3) the immune status of the population after vaccination.

With this purpose in mind, 3704 persons were chosen by random sampling from the list of those to be vaccinated, and from them were drawn 3465 blood samples for testing the prevaccinal immune status. From the same persons 2118 stool specimens and 285 rectal swabs were taken in order to test the circulation of wild polioviruses and other enteroviruses at the time of vaccination. So far, 2746 sera have been tested. The results are presented in Table 2.

Five weeks after vaccination with type 3, blood samples were collected from persons who were triple- or type-negative before vaccination in order to test the rise in antibody titres to type 1 and type 3 poliovirus.

RESULTS

Response of the population to vaccination

As noted above, 1 566 008 persons ranging in age from 3 months to 20 years were to be vaccinated. Of these, 1 339 244 persons (85.5%) were vaccinated with type 1 and 1 287 909 persons (82.2%) with type 3 poliovirus. Of the total number of persons given type 1 vaccine, 96.2% also received type 3.

This is the biggest vaccination campaign organized by the Croatian Health Service since the last war. The extremely satisfactory response of the population and the fact that the vaccination was carried out within a very short period, in some areas within no more than a few days, show that preparations were adequate and that the health service is today capable of performing extensive work in this field.

Serological and other laboratory examinations

The testing of the immune status of the population before vaccination covered not only poliomyelitis but also diphtheria, tetanus and pertussis. In addition, testing was also done to determine the influence of the vaccination with live poliomyelitis vaccine on the response to immunization against diphtheria, tetanus, and pertussis, and *vice versa*, when applied simultaneously. Data on this aspect will be published in the final report.

Table 2 presents the percentage of type- or triple-negative persons by age-group. "Type-negative" refers to persons whose titre before inoculation was below 1 : 4. Out of a sample of 2746 sera examined, the percentage of type- and triple-negatives was highest in the pre-school age, especially in the first year of life. Above the age of 7 years the immune status was relatively constant.

The number of type- and triple-negative persons before vaccination, as projected from the data in Table 2, is shown in Table 3 by age-group. Among the 1 566 008 persons who were to be vaccinated, there were probably 404 772 (25.8%) type 1 negative, 516 138 (33.0%) type 2 negative, 502 595 (32.1%) type 3 negative, and 110 953 (7.1%) triple-negative. Accordingly among the 1 339 244 persons actually vaccinated with type 1, there were probably 345 525 type 1 negatives and 95 086 triple-negatives. Among

TABLE 2

TYPE-NEGATIVE AND TRIPLE-NEGATIVE PERSONS IN RANDOM SAMPLE BEFORE VACCINATION

Age (years)	No. in sample	Negative for type 1		Negative for type 2		Negative for type 3		Negative for types 1, 2 & 3	
		No.	%	No.	%	No.	%	No.	%
< 1	171	112	65.5	91	53.2	96	56.1	61	35.7
1- 2	222	121	54.5	108	48.6	102	45.9	45	20.3
2- 3	271	116	42.8	146	53.9	147	54.2	39	14.4
3- 4	239	99	41.4	109	45.6	126	52.7	30	12.6
4- 5	145	38	26.2	65	44.8	66	45.5	12	8.3
5- 6	111	29	26.1	44	39.6	50	45.0	6	5.4
6- 7	113	22	19.5	32	28.3	31	27.4	4	3.5
7- 8	103	20	19.4	32	31.1	33	32.0	2	1.9
8- 9	130	26	20.0	31	23.8	31	23.8	0	—
9-10	114	24	21.1	25	21.9	31	27.2	0	—
10-11	135	24	17.8	40	29.6	30	22.2	1	0.7
11-12	114	27	23.7	30	26.3	25	21.9	4	3.5
12-13	126	20	15.9	40	31.7	33	26.2	0	—
13-14	150	18	12.0	30	20.0	20	13.3	0	—
14-15	160	26	16.2	39	24.4	35	21.9	0	—
15-16	78	8	10.3	27	34.6	21	26.9	2	2.6
16-17	79	7	8.9	15	19.0	14	17.7	1	1.3
17-18	96	16	16.7	27	28.1	22	22.9	3	3.1
18-19	86	12	14.0	15	17.4	15	17.4	1	1.2
19-20	103	11	10.7	17	16.5	19	18.4	2	1.9

the 1 287 909 persons vaccinated with type 3, there were probably 413 419 type 3 negatives and 91 442 triple-negatives.

After vaccination the antibody titres of 612 persons who had been type 1 negative before vaccination were tested along with the titres of 753 persons who had been type 3 negative before vaccination. In each of these groups, 148 had been triple-negative before vaccination. It will be seen from Table 4 that, after vaccination, antibodies against type 1 appeared in 91.5% of the type 1 negative persons, and against type 3 in 93.5% of type 3 negatives.

Safety of the attenuated strains CHAT and W-Fox (Koprowski types 1 and 3)

One of the factors to be investigated in the vaccination campaign was the safety of Koprowski strains of types 1 and 3 poliovirus. We had at our disposal data on the previous incidence of poliomyelitis and data on the immunological status of the population to be vaccinated. The vaccination itself was carried out within a very short period of time, when the prevalence of "wild" virus was comparatively low.

The number of poliomyelitis cases before vaccination amounted to 10 in January and to 11 in February. By the end of February and the beginning of March, 1 339 244 persons were vaccinated with type 1 (CHAT). Of this number, 345 525 probably did not have antibodies against type 1, and were therefore sensitive to infection with that type. If the administration of live poliomyelitis vaccine were dangerous, vaccination of such a large number of type-negative persons in so short a period could be expected to increase the number of poliomyelitis cases beyond that to be expected from an extrapola-

TABLE 3

ESTIMATED NUMBER OF TYPE-NEGATIVE AND TRIPLE-NEGATIVE PERSONS BEFORE VACCINATION IN THE POPULATION GROUP TO BE VACCINATED

Age (years)	No. of persons in age-group	Negative for type 1	Negative for type 2	Negative for type 3	Negative for types 1, 2 & 3
< 1	129 665	84 931	68 982	72 742	46 290
1- 2	82 842	45 149	40 261	38 024	16 817
2- 3	88 636	37 936	47 775	48 041	12 764
3- 4	79 553	32 935	36 276	41 924	10 024
4- 5	78 770	20 638	35 289	35 840	6 538
5- 6	78 770	20 559	31 193	35 447	4 254
6- 7	54 340	10 596	15 378	14 889	1 902
7- 8	56 846	11 028	17 679	18 191	1 080
8- 9	63 423	12 685	15 095	15 095	0
9-10	70 470	14 869	15 433	19 168	0
10-11	71 723	12 767	21 230	15 923	502
11-12	72 506	17 184	19 069	15 879	2 538
12-13	75 482	12 002	23 928	19 776	0
13-14	75 012	9 001	15 002	9 977	0
14-15	79 240	12 837	19 335	17 354	0
15-16	80 493	8 291	27 851	21 653	2 093
16-17	79 241	7 052	15 056	14 026	1 030
17-18	81 589	13 625	22 927	18 684	2 529
18-19	84 095	11 773	14 633	14 633	1 009
19-20	83 312	8 914	13 746	15 329	1 583
Total	1 566 008	404 772 (25.8 %)	516 138 (33.0 %)	502 595 (32.1 %)	110 953 (7.1 %)

TABLE 4

ANTIBODY CONVERSION RATES AFTER VACCINATION WITH TYPE 1 AND 3 POLIOVIRUS

Type	Negative before vaccination	Positive after vaccination	
		No.	%
1	612 [a]	560	91.5
2	588 [b]	307	52.2
3	753 [c]	704	93.5

[a] Of 612 type 1 negative sera, 148, or 24.2 %, were triple-negative.

[b] Of 588 type 2 negative sera, 148, or 25.2 %, were triple-negative.

[c] Of 753 type 3 negative sera, 148, or 19.7 %, were triple-negative.

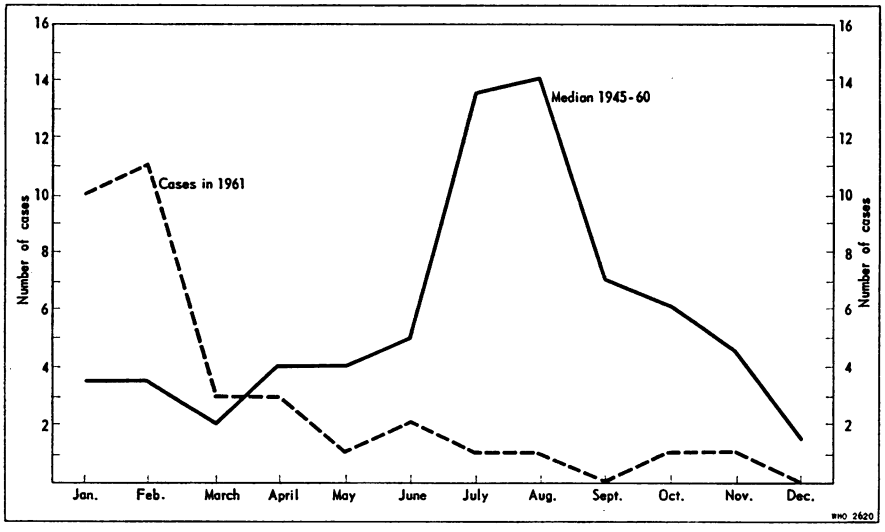
tion of previous incidence figures. In fact, the number of poliomyelitis cases fell to three in March; and in April, after mass vaccination with type 3, there were again only three cases. This is less than would have been expected on the basis of epidemiological estimates.

Two cases of poliomyelitis occurred within 1-3 weeks (more precisely, 7 and 11 days) after vaccination. These were in the district of Zadar where a small outbreak of poliomyelitis had occurred in January and February (with eight cases recorded).

Epidemiological data

Epidemiological data on the incidence of poliomyelitis in 1961 have been shown in Table 1. From

MONTHLY INCIDENCE OF POLIOMYELITIS IN CROATIA, 1961 [a]



[a] Vaccination with type 1 poliovirus was started on 18 February 1961 and with type 3 on 25 March.

that table and the accompanying graph it appears that the majority of poliomyelitis cases usually occurs in August, the incidence generally rising in June, increasing rapidly in July and reaching its peak in August. It decreases in September, and thereafter declines steadily in October, November and December.

The poliomyelitis curve for 1961 is unusual, in comparison with that for the years 1945-60, in that the greatest number of cases occurred in January and February. Then, after vaccination, the curve fell, and in June, July, August and September together only four cases of poliomyelitis were recorded. Two more cases occurred during the remainder of the year. The usual July-August peak was completely cut off in 1961.

DISCUSSION

The administration of poliomyelitis vaccine during an epidemic may raise doubts as to the safety of the vaccine if cases of poliomyelitis occur shortly after vaccination as a result of infection with " wild " polioviruses that are circulating at the same time. According to suggestions made to the WHO Study Group on Requirements for Poliomyelitis Vaccine (Oral) [1, 2] and according to the *Public Health Service Regulations* of the US Department of Health, Education, and Welfare (1961; 73.110), an attenuated poliovirus can be considered safe and suitable for large-scale administration if it can be shown to produce no harmful effects in 100 000 persons without antibodies to the strain fed; if possible, this number should include about 10 000 triple-negative persons. As to its antigenic and immunogenic properties, an attenuated virus may be considered to give satisfactory results if it gives rise to antibody formation in about 90% of susceptible persons (WHO Expert Committee on Poliomyelitis, 1960; Public Health Service, 1961, 73.117).

The production of a lower degree of immunity than might have been expected in a vaccinated community is very often attributable, especially in summer, to interference by other enteroviruses.

From the careful collection of data on, and the thorough observation of, over 300 000 type-negative

[1] Nagler, F. P. (1960) *Proposed regulations for live oral poliovirus vaccine* (unpublished working document WHO/BS/IR/80).

[2] Koprowski, H. & Plotkin, S. A. (1960) *Notes on acceptance criteria and requirements for live poliovirus vaccines* (unpublished working document WHO/BS/IR/85).

and over 100 000 triple-negative persons we may confidently assert that the CHAT and W-Fox attenuated type 1 and type 3 (Koprowski) polioviruses are quite suitable for mass administration, not only in view of their safety, but also in view of the fact that a high percentage of persons who had been negative before vaccination became serologically positive.

The usual seasonal peak of poliomyelitis incidence was not seen after vaccination. It is perhaps too early to attribute the change in the curve to vaccination alone, because 1960 was the year in which the severest post-war epidemic occurred and it is likely that the number of cases would have fallen in 1961 even without vaccination. The serological conversion rates of over 90% suggest, however, that vaccination was a factor in the marked reduction in incidence.

In the 1961 vaccination campaign in Croatia there were no control groups against which to assess the efficacy of this vaccine, but we are nevertheless under the impression that the vaccination was effective. It can at any rate be said that there are no data pointing to its failure. This may be seen from the last column in Table 1, which shows the number of cases in June, July, August and September from 1945 to 1961. The number of cases in 1961 is the lowest in the last 17 years.

RÉSUMÉ

Une campagne de vaccination massive de la population avec des vaccins antipoliomyélitiques vivants de type 1 et de type 3 a été effectuée en Croatie. Le vaccin a été fourni par le Dr H. Koprowski. La concentration en virus atténué du vaccin de type 1 (Chat) était de 230 000 $DICT_{50}$ et celle du vaccin de type 3 (Fox) de 80 000 $DICT_{50}$. Chaque type de vaccin a été administré séparément, sous forme d'instillation dans le pharynx de chaque enfant de 0,1 ml de vaccin contenu dans une seringue pourvue d'une aiguille épointée.

La vaccination s'est adressée à toute la population âgée de 3 mois à 20 ans. C'est ainsi que 1 566 008 personnes devaient s'y soumettre. Du 18 février au 4 mars 1 339 244 sujets (soit 85,5%) ont reçu du vaccin du type 1; du 25 mars au 7 avril 1 287 909 sujets (soit 82,2% du nombre total des personnes devant être vaccinées) ont reçu du vaccin du type 3.

Afin de se rendre compte de l'état d'immunité prévaccinal, 3465 échantillons de sang ont été prélevés au hasard parmi ces sujets. En outre 2118 selles et 285 frottis rectaux ont été examinés pour déterminer l'éventuelle circulation de poliovirus sauvages ou d'autres entérovirus à l'époque de la vaccination.

Ces examens ont montré que 25,8% des sujets étaient type 1 négatifs, 33% type 2 négatifs et 32,1% type 3 négatifs, tandis que 7,1% étaient triplement négatifs.

Trois semaines après la vaccination par le type 3, du sang a été prélevé chez ceux qui étaient triplement négatifs et chez ceux qui étaient négatifs aux divers types avant la vaccination, en vue de suivre la montée du titre des anticorps contre les poliovirus type 1 et 3. Après la vaccination le taux des anticorps contre le virus type 1 était de 91,50% chez les sujets type 1 négatifs; celui des anticorps contre le type 3 était de 93,5% chez les sujets précédemment type 3 négatifs.

D'après les données de l'épidémiologie, le maximum saisonnier de la poliomyélite en Croatie s'est situé, pendant les 16 ans précédant cette vaccination massive, en juillet et août. En 1961 la plus grand nombre de cas a été observé en janvier et février. Après la vaccination le nombre de cas est tombé si bas que pendant les mois de juin, juillet, août et septembre l'on n'a enregistré que 4 cas. Deux autres cas ont été observés pendant le reste de l'année. Le chiffre des cas déclarés de juin à septembre 1961 est le plus faible de ceux obtenus en 17 ans.

Il est peut-être prématuré d'attribuer ces résultats à la seule vaccination, car 1960 a été l'année de la plus grande épidémie jamais observée dans ce pays et il est probable que de toute façon les chiffres de 1961 fussent tombés. Cependant l'augmentation brusque de 90% du taux des anticorps indique que cette vaccination a joué un rôle dans la réduction du nombre des cas de poliomyélite.

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