

PRELIMINARY STUDY OF SERO-IMMUNITY TO POLIOVIRUSES IN AN URBAN POPULATION IN INDONESIA *)

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Telah dilakukan survey poliomyelitis neutralizing antibody pada anak-anak di bawah umur 5 tahun di Jakarta. Prevalensi type-1 polio virus antibody pada umur di bawah 2 tahun ternyata lebih tinggi di Tanjung Priok, sedangkan type-3 polio virus antibody dari group umur yang sama di Kebayoran Baru lebih tinggi. Proporsi dari triple negatip menurun dengan bertambahnya umur, akan tetapi 78.6-83.7% anak-anak di bawah umur 1 tahun dan 46.7 - 55.6% dari anak-anak yang berumur 1 tahun, kekurangan antibody terhadap setiap type polio virus. Dibandingkan dengan hasil penelitian-penelitian yang pernah dilakukan di Bandung, maka Jakarta kira-kira dua kali lebih tinggi prosentase triple negatipnya daripada Bandung. Hasil dari isolasi virus dari 289 fecal specimen didapatkan 28 (9.7%) Enteroviruses strain. Virus polio type-3, virus Coxsackie B group, Echovirus type-7, adalah prevalent di Tanjung Priok.

Survey ini menunjukkan bahwa di Jakarta, anak-anak di bawah umur 2 tahun sangat baik untuk dipakai sebagai subjek dari pilot polio vaccination trial, untuk mempelajari faktor-faktor yang berpengaruh pada routine vaccinasi.-

KK: vaksin polio, Antibody anak belita

It was the end of the last century when poliomyelitis became an important infectious disease of children in countries of Europe and North America. Since the middle of the present century, epidemic outbreaks of the diseases also began to occur in Asian countries. In 1954, a severe poliomyelitis epidemic with 108 paralytic cases accrued in Yogyakarta and adjacent areas.

Antigenic type of poliovirus which caused this epidemic was obscure because there were neither laboratories nor facilities needed for virological and serological test (Frans E. Perabo, 1956). Severe epidemic of type-1 poliomyelitis occurred in 1958 and 1960 in Singapore and Japan, respectively (I. Tagaya, 1971). Administration of type-2 Sabin vaccine at the

Singapore epidemic and of trivalent Sabin vaccine at the epidemic in Japan has proved great success in controlling epidemic poliomyelitis.

A similar success with the vaccine was achieved in many countries of other regions.

In Indonesia, epidemic outbreaks of poliomyelitis did not occur since after 1954, and therefore, the disease has not become a serious public health problem. However, development of industrialization and social welfare in recent years will improve living conditions of the nation, especially in large cities. This drifts the chance of primary poliovirus infection to older ages, and therefore, form suitable conditions for epidemic occurrence of poliomyelitis. From this respect, the disease will become increasingly important in Indonesia.

A serologic survey of children in urban area for poliovirus infections was carried out assuming the results of the survey would furnish much information for planning of routine vaccination against the disease.

Another task of this survey was to investigate enterovirus prevalent in the area which was also important to make the vaccination effective.

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MATERIAL AND METHOD

Subjects.

Healthy children under 5 years of age in Jakarta were subjects to the present survey. They were selected from the area of the city with different socio-economic status of their family and environmental conditions. One area was Kebayoran Baru which was a residence of people with high socio-economic status and therefore, was good in environmental sanitation.

The other was Tanjung Priok where most residents were low socio-economically and bad in sanitation. The number of subjects in each area and their distribution in age are shown in table 1.

Table 1 Distribution the number of sera examined it's neutralizing antibody to polio virus, from Jakarta (Tanjung Priok and Kebayoran Baru) by age group.

Age group (in years)	Tanjung Priok	Kebayoran Baru	Total
½ - 1 year	49	14	63
1 - 2 years	36	30	66
2 - 3 years	34	40	74
3 - 4 years	44	50	94
4 - 5 years	33	46	79
TOTAL	196	180	376

Collection of specimens.

Blood and fecal specimens were collected in July, 1976. At least 5 ml of whole blood as collected from individual subjects except for young children from whom only 0.5-1 ml of blood could be obtained. Sera separated from the blood clot as stored at -20°C .

About 4 gram of feces collected from the same individuals were made to a 10% suspension with cold sterile saline using a tightly stoppered flask containing glass beads. After vigorous shaking, the suspension was allowed to settle in a cold room, then the supernatant

as centrifuged at 2,500 rpm. for one hour at low temperature. The supernates were mixed with 2-3 ml of ether, shaken and kept at $2-4^{\circ}\text{C}$ for 18 hours. The supernates thus treated were carefully taken out using a pasteur pipette, and warmed at 37°C for one hour to remove the remaining ether. The supernates were stored at -20°C .

Cell Cultures.

Primary Cynomolgus monkey kidney cell suspension from Bio Farma Bandung, as routinely used for the study. The cell suspension was diluted to the concentration of 10 X per ml with the growth medium consisting of Eagle's MEM, 1% glutamine, 5% calf sera, 200 I.U. penicillin and 200 ug streptomycin. One ml of the diluted cell suspension was poured into each test tube.

After incubation at 37°C for 5-7 days, the growth medium was replaced by the maintenance medium which was the same in composition to the growth medium except for the absence of calf serum. Cell culture thus prepared as used for isolation and serological test.

Virus Isolation.

About 0.2 ml of fecal extract prepared as mentioned above as inoculated each into 3 tubes with cell cultures. Microscopic examinations of cytopathic effects were made daily to 7th day of incubation at 37°C . Uninoculated cell cultures derived from the same cell suspension were the cell controls. Cell cultures showing cytopathic effects were harvested and stored at -20°C to allow them for further tests. Sample showing no cytopathic effects were cultured serially 3 times to confirm the absence of virus.

Identification of Isolates.

Both the Schmidt's and the Lim and Benyesh-Melnick's antisera pools were used for identification of virus isolates. They contained 20 Units in 0.1 ml of antibody againsts respective viruses. An amount of 100 TCD₅₀ of the isolates as mixed with the same amount of the serum pools. After incubation at 37°C for one hour, 0.2 ml of the serumvirus mixtures were inoculated into 2 tubes with cell

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cultures, and incubated at 37° C. Final reading of the results was made on 7th day. Three serial 10 fold dilutions from 10⁻¹ to 10⁻³ of the virus suspension were also inoculated each into 2 tubes as the virus control.

Examination of Neutralizing Antibody.

Only poliovirus neutralizing antibody was examined for the survey sera. As antigen, Mahoney strain of type-1 poliovirus, MEF-1 strain of type-2 and Saukett strain of type-3 as used.

All sera were diluted serially beginning on 1 : 8. Equal amounts of the diluted serum and 100 TCD₅₀ of virus were mixed and kept at room temperature for one hour, then 0.2 ml of each serumvirus mixture were inoculated into 3 tubes with cell cultures. Final reading was made on 7th day of incubation at 37° C and antibody titer was defined as the reciprocal of highest serum dilution which neutralized 100 TCD₅₀ of virus.

RESULTS

Antibody Prevalence.

The prevalence of neutralizing antibodies to the three type polioviruses among children are shown in table 2. The proportion of positive reactive to every type virus increased with

increasing age in both areas. Ranging from 60.9 to 63.9% of children became positive to every type virus up to the age of 4 years.

It was noted that the prevalence of type-1 poliovirus antibody at age below 2 was distinctly higher in Tanjung Priok, but that of type-3 virus antibody in the same age group was higher in Kebayoran Baru. The proportion of children triple positive and triple negative to the viruses are shown in table 3.

Table 3. Percentage of sera with neutralizing antibody triple negative and triple positive to poliovirus, by age group

Age group (in years)	Tanjung Priok		Kebayoran Baru	
	Triple positive	Triple negative	Triple positive	Triple negative
½ - 1 year	0.0	83.7	0.0	78.6
1 - 2 years	0.0	55.6	0.0	46.7
2 - 3 years	2.9	14.7	0.0	32.5
3 - 4 years	36.4	2.3	36.0	4.0
4 - 5 years	63.6	0.0	60.9	0.0

Table 2 Percentage of sera with neutralizing antibody to each of three type poliovirus, by age, in Tanjung Priok and Kebayoran Baru, Jakarta

Age group (in years)	Tanjung Priok			Kebayoran Baru		
	Polio-1	Polio-2	Polio-3	Polio-1	Polio-2	Polio-3
½ - 1 years	14.2	2.0	0.0	7.1	7.1	14.3
1 - 2 years	22.2	16.7	13.9	13.3	13.3	33.3
2 - 3 years	35.3	38.2	47.1	32.5	20.0	40.0
3 - 4 years	68.2	68.2	75.0	60.0	64.0	80.0
4 - 5 years	81.8	84.8	87.9	78.3	82.6	87.0

Table 4 Enteroviruses Isolation from fecal specimens of healthy children under 5 years old

Location	Number of specimen	Positive isolation and it's identification.									
		P1	P3	CB1	CB2	CB3	E5	E7	E27	UND	Total
Tanjung Priok	146	—	3	1	1	1	2	8	1	3	20
Kebayoran Baru	143	1	1	—	—	—	—	3	—	3	8
TOTAL	289	1	4	1	1	1	2	11	1	6	28

Note : P1, P3 = Poliovirus group CB1, CB2, CB3 = Coxsackie B group
 E5, E7, E27 = Echo virus group UND = Unidentified virus

In Kebayoran Baru, triple negatives occurred a little later in age than in Tanjung Priok. The proportion of triple negatives decreases with increasing age, however, 83.7% of children below 1 year of age and 55.6% of those aged one year were devoid of antibodies to every type poliovirus.

Enteroviruses Isolated from Feces.

Of 289 fecal samples tested, virus was isolated from 28 (9.7%) specimens. The results of identification of the isolates are shown in table 4. Of the 28 isolates, 20 were obtained in Tanjung Priok. It is clear that viruses of type-3 polio, Coxsackie B group and Echo-7 were prevalent in the area. There were 6 isolates for which identification test failed.

DISCUSSION

The present survey disclosed poliovirus types prevalent in two areas of Jakarta. About 80% of preschool children in both areas were found have become infected with every type poliovirus by the age 5 years.

However, half of children aged one year were devoid of antibodies to three types of the virus and percentage of triple negatives rose

to about 80 in those below 1 year of age. It was unexpected that the percentage of triple negatives was very high in young children of Tanjung Priok which was chosen as an area of bad environmental sanitation. Lie King Ting (1960) reported that 17% of healthy preschool children in Bandung were triple negative to poliovirus. In the same city, Soenarko and Lo Siau Goen (1966) found triple negatives at a rate of 14% in a child population of which 86% were those of ages from 6 months to 2 years and the remaining was from 3 to 6 years old. Comparing the results with those from Bandung, Jakarta is about 2 fold higher in the percentage of negatives than Bandung, suggesting that Jakarta would be better in personal hygiene and sanitary conditions. A distinct difference was observed in the prevalence of type-1 and type-3 poliovirus antibodies between Kebayoran Baru and Tanjung Priok. Namely if only children below 2 years of age are taken into observation, the percentage of children having type-1 poliovirus antibody was about 2 fold higher in Tanjung Priok, but reverse was the case in type-3 virus antibody. This would mean that poliovirus type prevalence in the same city differ with its areas.

It seemed curious that epidemic poliomyelitis did not occur in Jakarta in the past several years despite large proportion of children were devoid of antibodies to every type virus. This could be explained in part by the result of virus isolation from fecal specimens. Of 28 virus isolates, 11 (39%) were Echo-7 virus which was proved to interfere with poliovirus (Kono et al.). Coxsackie B group viruses, which are also known to inhibit poliovirus transmission in human populations, were isolated in Tanjung Priok although in small number. It is very likely therefore that these enteroviruses were highly prevalent and inhibited poliovirus transmission among young children.

Melnick et al. (1959) pointed out that children lacking maternal immunity are highly susceptible to poliovirus like those not exposed to natural infection. The lesser the chance of mothers to contact with the virus, the greater is the number of offsprings without maternal immunity. Therefore, preventive measures against paralytic poliomyelitis must be taken in action earlier after birth with the improvement of environmental sanitation. (Soeprapti, 1971). Much technical cautions are needed to make routine live poliovirus vaccination effective, and it is wise to carry out a pilot vaccination trial on a small scale to study factors affecting the routine vaccination. The present survey indicate that, in Jakarta, children below 2 years of age are the most appropriate subjects to the trial.

SUMMARY

A survey for poliomyelitis neutralizing antibody in children under 5 years of age was conducted in Jakarta. Prevalence of type-1 poliovirus antibody at ages below 2 years was distinctly higher in Tanjung Priok, but that of type-3 virus antibody in the same age group was higher in Kebayoran Baru. The proportion of triple negatives decreases with increasing age; however, about 80% of children below 1 year of age and about 50% of those age 1 year were devoid of antibodies to every type poliovirus. Comparing the result with those from previous investigations in Bandung,

Jakarta is about 2-fold higher in the percentage of triple negatives than in Bandung. Of 289 fecal specimens tested, virus was isolated from 28 (9.7%) specimens. Of the 28 isolates, viruses of type-3 polio, Coxsackie B group and Echo-7 were prevalent in Tanjung Priok. The present survey indicate that, in Jakarta, children below 2 years of age are the most appropriate subjects to the pilot vaccination trial on a small scale to study factors affecting the routine vaccination.

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