Poliovirus Neutralizing Antibody Persistence after Vaccination 
with the Sabin Vaccine: 
A Follow-Up Study*†

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ABSTRACT

In 1976, a cohort survey on the persistence of neutralizing antibodies in 
children regularly immunized 1 to 4 years earlier with the Sabin attenuated 
vaccine (OPV) was undertaken in the Venice mainland. Subsequent sero­
logical evaluations in the same cohort were carried out in 1983 and 1993. 
A macroneutralization test using a 1:4 initial serum dilution was utilized 
in the 1976 and 1983 survey years. In the 1993 survey a microneutralization 
test using a 1:2 initial dilution was utilized. In this survey, however, sera 
were tested using both the latter microneutralization test and the for­
ter test.

Using the former method, the results indicate that the OPV-induced 
humoral immunity to poliovirus 1 and 2 remain fairly stable after the initial 
decrease, whereas antibodies to poliovirus 3 are further declining.

Using the latter more sensitive method the seropositivity rates were 
found to be equal or close to 100 percent. The results of our follow-up 
survey thus indicate that the OPV-induced humoral immunity is long­
lasting when tested with a highly sensitive and reproducible method. The 
clinical protection that ensues after OPV-immunization is probably lifelong 
similar to that which follows the natural infection.

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Introduction

Poliomyelitis has been successfully controlled and virtually eliminated in most developed countries by the systematic use of vaccines, the most widely used during the past 30 years being the Sabin live attenuated poliovirus vaccine (OPV). In Italy, mass vaccination by OPV started in 1964 and became compulsory in 1966. The routine immunization program soon resulted in a marked downward trend in morbidity from poliomyelitis (figure 1). During the 1980 to 1991 years, only nine cases of polioparalysis were confirmed in our country, six of which were autochthonous (4 in unvaccinated children, one vaccine-associated, and one probably vaccine-associated) whereas three were imported from Libya, Iran, and India (with repeat isolation of wild poliovirus type 3). The trend, therefore, clearly indicates the progressive establishment of a very high vaccine-induced level of protection in our population. It appears quite realistic that Italy could achieve global eradication of poliomyelitis by the year 2000, as recommended by the World Health Organization.

However, wild poliomyelitis is still endemic in many parts of the world. Sporadic outbreaks related to importation of wild-type poliovirus continue to be a problem in developed countries, pointing out the importance of a careful appli-

![Figure 1](image-url)  
TABLE I
Seropositivity Rates and Geometrical Mean Titres to Polioviruses in Cohort Study by Control Year

<table>
<thead>
<tr>
<th>Control Year</th>
<th>Type 1 %</th>
<th>GMT</th>
<th>Type 2 %</th>
<th>GMT</th>
<th>Type 3 %</th>
<th>GMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1976 a</td>
<td>95.2</td>
<td>(20.3)</td>
<td>96.6</td>
<td>(32.5)</td>
<td>83.4</td>
<td>(10.2)</td>
</tr>
<tr>
<td>1983 a</td>
<td>93.1</td>
<td>(14.1)</td>
<td>95.9</td>
<td>(13.4)</td>
<td>76.6</td>
<td>(7.1)</td>
</tr>
<tr>
<td>1993 a</td>
<td>93.8</td>
<td>(11.4)</td>
<td>93.8</td>
<td>(12.1)</td>
<td>77.2</td>
<td>(6.7)</td>
</tr>
<tr>
<td>1993 b</td>
<td>100</td>
<td>(32.3)</td>
<td>100</td>
<td>(48.9)</td>
<td>98.6</td>
<td>(14.8)</td>
</tr>
</tbody>
</table>

GMT = geometrical mean titre.

a Macroneutralization test; serum initial dilution 1:4.
b Microneutralization test; serum initial dilution 1:2.

cation of appropriate immunization programs and periodic control on the immunological condition of the population.

In 1976, a prospective study on the persistence of poliovirus neutralizing antibodies was undertaken in the Venice mainland on children regularly immunized 1 to 4 years earlier with OPV. A second serological evaluation in the same cohort was carried out in 1983. On the whole, the results showed appreciable seronegativity rates to all three poliovirus types (table I). The difference among the serotypes was mainly due to type 3, to which the percentages of subjects with detectable neutralizing antibodies (titre >1:4) were 83.4 percent in 1976 and 76.6 percent in 1983, while over 93 percent of children had detectable antibodies to poliovirus types 1 and 2.4,5

However, in a subsequent survey carried out in 1988 in subjects vaccinated 16 to 23 years earlier with OPV, it was observed that the humoral immunity persisted at acceptable levels (97.6 percent, 99.3 percent, and 97.3 percent for poliovirus types 1, 2 and 3, respectively) for more than 20 years after immunization.6

In this study, however, the method used for the titration of neutralizing antibodies was significantly modified in order to assure a better sensibility and reproducibility of the test according to Albrecht et al.,7 and utilizing a 1:2 initial dilution of sera which was considered more adequate in detecting low levels of neutralizing antibody.8*

This paper reports the results of the third serological survey on the subjects enrolled in the 1976 cohort study.

Materials and Methods

Study Population

The study population was described in a previous paper.4 Briefly, a prospective study on poliovirus neutralizing antibody persistence was started in 1976 on a cohort of 281 children attending a public nursery in a town on the Venice mainland (North-East Italy). All the subjects had regularly completed immunization schedules with the OPV 1 to 4 years before testing; in all cases, parents were given complete information about the study, and their written consent was obtained. The serosurvey was repeated on the same group in 1983; 197 youngsters (follow-up rate 70.1 percent) were traced and participated in the second serosurvey.

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* Sabin, personal communication.
In the 1993 serological survey, only 145 subjects (follow-up rate 51.6 percent) who were present in the previous two serosurveys were included. The blood samples were drawn in May, 1993, 18 to 22 years after primary immunization, and the sera stored at −20°C until tested in batches.

Neutralization Test

A microneutralization test according to Albrecht et al\textsuperscript{7} was used with some minor modifications. The test was performed in 96-well plastic microtitration plates.\textsuperscript{*} The serum samples were complement-inactivated at 56°C for 30 minutes and diluted from 1:2 to 1:512. They were then placed in contact with 100 TCID\textsubscript{50} of the three types of Sabin attenuated polioviruses (type 1: L Sc 2ab strain; type 2: P 712, Ch 2ab strain; type 3: Leon 12, a1b strain). Safety considerations indicated the substitution of attenuated strains for wild strains as no difference was observed when either poliovirus strain was used.\textsuperscript{7,9} After an overnight incubation at 4°C, a suspension of freshly trypsinized Vero cells (approximately 10\textsuperscript{5}/ml) was added to each well containing the serum/virus mixture and the solution was incubated at 36°C in a 5 percent CO\textsubscript{2} incubator. The sera and polioviruses dilutions were performed in Eagle's minimum essential medium. For the Vero cell cultures, the medium was added with fetal calf serum. Virus, serum, and cell controls were included in each microplate. The final test reading was made after 6 days. The value for the 50 percent end point was used as the value for the serum titre, and geometrical mean titres (GMTs) were computed by log\textsubscript{10} of reciprocal antibody titres \(\geq 1:2\).

The 1993 sera were tested using both this microneutralization test, including a 1:2 initial dilution, and the former macro-neutralization test, described by Hoskins,\textsuperscript{10} at a beginning serum dilution of 1:4 for a better comparison with the 1976 and 1983 serological results.

Results

The neutralizing antibody positivity rates and GMTs in the cohort study population are reported in table I in relation to the survey years (1976, 1983, and 1993) and to the type of neutralization procedures for 1993.

Data analyses were performed only on the 145 subjects who were present in all the three serosurvey years.

At enrollment in 1976, 1 to 4 years after completion of a full course of OPV, the percentage of children with detectable neutralizing antibodies (titres \(\geq 1:4\)) was similar for poliovirus types 1 and 2 (95.2 percent and 96.6 percent, respectively), but consistently lower for type 3 (83.4 percent).

In the 1983 serosurvey, 8 to 11 years after vaccination, the seropositivity rates (titres \(\geq 1:4\)) showed a slight decline for poliovirus types 1 and 2 (93.1 percent and 95.9 percent, respectively) and a substantial drop for type 3 (from 83.4 percent to 76.6 percent).

In the 1993 serosurvey, using the former neutralization procedures, similar seropositivity prevalences and GMTs were observed as compared to 1983 for all the three types of poliovirus. However, some subjects demonstrated a rise in antibody titre to one or more serotypes, possibly indicating exposure to wild poliovirus or subsequent contact with vaccine strains. With the latter more sensitive method and utilizing a 1:2 initial serum dilution, the results were markedly different: the seropositivity rates were equal or close to 100 percent (100 percent had antibody for types 1 and

\* Microtest III plate, Falcon, Becton Dickinson, NJ, USA.
2 and 98.6 percent for type 3) and the GMTs were two- to four-fold higher than the values observed using the former method.

Discussion

The dramatic decline of poliomyelitis, up to its virtual disappearance in recent years clearly indicates a progressive establishment of a very good level of protection in our country related to vaccination practice.

The safety and efficacy of OPV are well documented, but certain important issues still remain unresolved. One of the main problems is the long-term persistence of neutralizing antibody following oral immunization. Pertaining to this, it has been shown that OPV-induced humoral immunity declines over the years, but reported data have been contradictory. However, these surveys were carried out using neutralization tests which differ from one another either in the incubation times of cell-virus mixtures or in the initial serum dilution as well as in the macro- or microneutralization method. The reported results, therefore, are difficult to compare, and it is still debatable whether such differences are real or mostly depend on the lack of standardization in the performing the neutralization tests.

Until the mid-1980s, our seroepidemiological studies on OPV-induced immunity, carried out using a macroneutralization method which was considered inadequate for detecting low antibody levels, showed that the humoral immunity declines over the years. Since the mid-'80s, a more sensitive microneutralization method has been adopted in our laboratory. A subsequent cross-sectional survey carried out in subjects 16 to 23 years after OPV immunization showed a good persistence of immunity status.

In the present cohort study, both methods mentioned previously were used. The former method was used to compare antibody levels among the three different survey years, while the latter method was used only in the 1993 survey to compare the level of sensitivity between both methods.

With the former method, high seropositivity rates were observed for polioviruses type 1 and 2 (95.2 percent and 96.6 percent, respectively) and a relatively lower rate for poliovirus type 3 (83.4 percent) 1 to 4 years after vaccination. Seven to 11 years after vaccination, in 1983, the seropositivity rates showed a further slight decrease for poliovirus types 1 and 2; the decrease was more marked for poliovirus type 3 (table I). In the 1993 serosurvey, the seroprevalence rates were similar to those observed in 1983. The GMTs showed an analogous drop from 1976 to 1983 with values leveling off in 1993. Overall, these results indicate the OPV-induced immune levels to poliovirus 1 and 2 remain fairly stable after the initial decrease during the first post-vaccination years, whereas antibody to poliovirus 3 are further declining.

Using the latter method, a higher seropositivity rate, though often with low antibody titres, was observed for all poliovirus types with total coverage for virus types 1 and 2; the GMTs were two- to four-fold higher for all three types of poliovirus. The results of our follow-up survey therefore indicate that the OPV-induced humoral immunity is long-lasting when tested with a highly sensitive and reproducible method. Similar results have been reported by other authors. However, serum antibody is only one manifestation of immunity. OPV, like the natural poliovirus infection, provides both serum humoral immunity and intestinal immunity. It is likely that OPV-vaccinees may be resistant to reinfection even in the absence of detectable serum
neutralizing antibodies if the level of secretory antibody is sufficiently high.31 Furthermore, even vaccinated subjects with undetectable antibody levels can develop secondary humoral response when challenged antigenically.9 These observations suggest that subjects correctly vaccinated maintain an immunological memory. The clinical protection that ensues after OPV-immunization appears to be lifelong similar to that which follows the natural infection.

Given the recent trend in the incidence of poliomyelitis, one may conclude that Italy may soon be free of indigenously transmitted paralytic disease. However, it is the authors' opinion that the immunization program should be strictly maintained in our country. High-coverage vaccination campaigns should be implemented in immigrants, foreign workers, and adopted children originating from developing countries to prevent the risk of reappearance of wild-virus-induced poliomyelitis, until global eradication of poliomyelitis is unequivocally achieved.

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References