Immunity Status to Poliovirus in Veneto Region (North-East Italy)
A Seroepidemiological Survey*†

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ABSTRACT

The immunity state to poliovirus types 1, 2, and 3 of a population aged 2 to 75 years was determined by examining 274 sera collected in the Venice mainland (North-East Italy).

Altogether, the neutralizing antibody prevalences (at a titre ≥1:2) for poliovirus 1, 2, and 3 were 99.0 percent (geometrical mean titres [GMT]: 72.1), 99.6 percent (GMT: 95.9) and 98.2 percent (GMT: 17.3), respectively, and all the age groups also showed very good levels of humoral immunity.

High antibody titres (≥1:256) to one or more types of poliovirus were demonstrated in older age groups also, possibly indicating exposure to natural polioviruses or contact with vaccine strains.

Although probably all the older subjects had practically acquired their antibodies as a result of natural infection and those under 30 through vaccination, these results indicate that the humoral immunity against poliomyelitis in our population is satisfactory, and the maintenance of such good protection level depends on an effective immunization program.

Introduction

In most of the countries in North America and Europe, as well as some other countries such as Australia and Japan, the
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introduction of mass immunization pro­grams against poliomyelitis since the 1950s, whether using live attenuated or inacti­vated poliovirus vaccines, have succeeded in dramatically reducing to near zero the incidence of paralytic poliomyelitis.

However, the wild polioviruses can still circulate in regions with an overall high rate of immunization coverage, and limited outbreaks of natural poliomyelitis were reported in developed countries, even in recent years. These epidemic episodes are generally related to the introduction of wild strains in unimmunized or inadequately immu­nized population groups.

In Italy, mass vaccination against poliomyelitis by Sabin live attenuated virus (OPV) started in 1964 and became compulsory in 1966. The annual inci­dence of poliomyelitis quickly declined. In the 1980s, when only five autochtho­ nous cases (four in unvaccined children and one vaccine-associated) were con­firmed, the virtual disappearance of the paralytic disease was reached.

Even if the risk of acquiring polio­myelitis is minimal in our country, as the immunization program seems very effec­tive, the presence of possible residual circulation of indigenous or imported neuropathogenic polioviruses stresses the essential importance of accurate sur­veillance systems. Such systems are cru­cial (1) to define the extent of the polio­virus circulation (asymptomatic infection is much more frequent than paralytic dis­ease) and to identify which types of poliovirus are circulating in a given area; (2) to determine the prevalence and the persistence over the years of the vaccine­induced humoral immunity; and (3) to guide possible control efforts (e.g., vacci­nation campaigns that prevent spreading of disease).

The aim of this study was to assess the age-specific prevalence of poliovirus neutralizing antibody in our population about 30 years after the introduction of compulsory antipoliomyelitis vaccination in Italy and to assess whether or not humoral immunity is lacking in an area where high coverage for polio immuni­zation, good sanitation, and hygienic prac­tices might have interrupted endemic cir­culation of indigenous wild poliovirus, diminishing the contribution of natural infection to overall immunity levels in the general population.

Materials and Methods

Population

The population studied included 274 subjects of both sexes, 2 to 75 years old, all resident in the Venice mainland (North-East Italy).

Blood specimens were drawn consecu­tively from March 1993 to May 1993 in the course of routine checks for minor ail­ments and health check-ups. Subjects with abnormal biochemistry, with sus­pected or acclaimed infectious or neo­plastic pathologies, and with immunolog­ical deficiencies were excluded from the study.

Sera were stored at −20°C until tested.

Neutralization Test

Sera were inactivated at 56°C for 30 minutes before testing. Two-fold dilu­tions of serum samples (from 1:2 to 1:512) were tested against 100 TCID_{50} of attenu­ated Sabin polioviruses (L Sc2ab for type 1, P 712 Ch 2ab for type 2, and Leon 12ab for type 3). After overnight incuba­tion at 4°C, the serum/virus mixtures were spread in microwells with a Vero cell suspension (10,000 cells/ml), and the plates were incubated at 36°C in carbon dioxide atmosphere for 6 days. Serum and virus dilutions were made in Eagle’s minimum essential medium (MEM).

Reciprocal antibody titre was expressed as the highest serum dilution
yielding 50 percent inhibition of cytopathogenic effects (CPE) of the cell cultures against 100 TCID<sub>50</sub> of virus.

Sera with antibody titres less than 1:2 were considered negative. Geometrical mean titres (GMT) were computed by log<sub>10</sub> of reciprocal antibody titres only for positive sera (log 10).

Each assay was accompanied by virus, cell culture and sera controls.

**Results**

The prevalence of serum neutralizing antibodies at a titre ≥1:2 is shown in table I. Seropositivity was present in 99 percent of the 274 subjects for poliovirus type 1 (GMT 72.1), in 99.6 percent for type 2 (GMT 95.9), and in 98.2 percent for type 3 (GMT 17.3).

Relatively higher prevalence rates (>10 percent) were included between 1:32 and 1:256 for poliovirus 1 (peak of 28.8 percent at dilution 1:64), between 1:32 and 1:512 for poliovirus 2 (peak of 23.0 percent at dilution 1:128), and between 1:8 and 1:64 for poliovirus 3 (peak of 32.9 percent at dilution 1:16).

The data analysis after disaggregation of the study population in 10-year age groups are reported in figure 1. Seronegativity appeared to concern mainly the age group 41 to 50 years, in which subjects without detectable antibody (titre <1:2) against all the three poliovirus were present (5.2 percent, 2.6 percent, and 5.2 percent for types 1, 2, and 3, respectively). In the 21 to 30 age group, subjects without neutralizing antibodies were present only for poliovirus 1 (1.7 percent); in the 51 to 60 age group, only for poliovirus 3 (5.3 percent). In all the other groups, humoral immunity for all the three types of poliovirus was present in 100 percent of the subjects.

In figure 1, it is also shown that the neutralizing antibodies GMT were particularly high in the first age group for polioviruses 1 and 2 (128.0 and 165.2, respectively). Nevertheless, the antibody levels showed a consistent decline in the second age group (76.8 and 85.7, respectively), then remaining relatively stable in all the subsequent age groups. While the patterns for polioviruses 1 and 2 closely resembled each other, that for poliovirus 3 was different. In all the age groups, the GMT were remarkably

![Figure 1. Poliovirus neutralizing antibodies: prevalence and geometrical mean titres in 274 subjects, according to age groups.](image-url)
lower than for the two other types, and no appreciable decline was noted with aging.

High antibody titres (≥1:256) to one or more poliovirus types were demonstrated in older age groups also, possibly indicating contact with vaccine strains or exposure to natural polioviruses.

Discussion

Former surveys on the humoral immunity against poliomyelitis in the population of the Veneto region³,⁴,⁹,¹⁰,¹¹,¹²,¹³ allowed us to observe in vaccinated subjects that the prevalence of seropositivity and levels of neutralizing antibody, according to other published data,¹⁴,¹⁵,¹⁶,¹⁷,¹⁸,¹⁹ showed a progressive and at times significant decline over the years. The unvaccinated adult population, on the contrary, generally presented much more satisfactory seroimmunity levels.

However, these surveys considered a titre of 1:4 as the lowest positive value and were carried out using serological tests which were considered inadequate in detecting low levels of neutralizing antibody ²⁰ (Sabin, personal communication).

Recently, to improve the sensitivity and the reproducibility of the neutralization test, some modifications have been proposed ²¹. The use of this kind of serological test and the evaluation even of a 1:2 dilution, already considered possibly aspecific by some authors,¹⁹ presently enable us to point out results indicating, on the whole, a very satisfactory protection level against poliomyelitis in both vaccinated and unvaccinated population of the Veneto region.

The seropositivity rates for the three polioviruses in our population sample are in fact close to 100 percent both in the younger age groups immunized through vaccination and in the older subjects born during the prevaccine era who had probably acquired antibodies as a result of natural infection with wild poliovirus or possibly, after the mass vaccination started, with vaccine strains excreted by immunized children.

The antibody levels also appear very good for poliovirus types 1 and 2. This phenomenon is particularly evident for the latter and may be due to the higher immunogenicity of this poliovirus type ²² and/or to more frequent reinfection by the vaccine-derived type 2 strains circulating in the environment. The antibody levels for poliovirus 3 are always significantly lower, probably owing to a lower antigenicity of this poliovirus type.

Overall, the results indicate that the humoral immunity against the classical wild polioviruses is very satisfactory in our population. However, after the mass immunization programs started, several important problems have come up. First, the attenuated poliovirus used in the oral vaccine may cause paralytic poliomyelitis in vaccine recipients and their household or community contacts. In fact, the live attenuated polioviruses excreted by

| TABLE I |
| Neutralizing Antibody Prevalence to Polioviruses by Serum Titre* |
| Serum Titre | Type 1 | Type 2 | Type 3 |
| < 2 | 1.0 | 0.4 | 1.8 |
| 2 | 0.7 | 1.0 | 2.2 |
| 4 | 0.4 | 0.7 | 7.7 |
| 8 | 4.0 | 2.2 | 18.2 |
| 16 | 9.6 | 6.9 | 32.9 |
| 32 | 16.9 | 13.1 | 23.3 |
| 64 | 28.8 | 21.9 | 10.2 |
| 128 | 19.3 | 23.0 | 2.9 |
| 256 | 13.5 | 16.9 | 0.4 |
| 512 | 5.8 | 13.9 | 0.4 |
| GMT | 72.1 | 95.9 | 17.3 |

*274 subjects aged 2 to 75 years.
Vaccines are known to mutate, particularly type 3, and they could revert towards neurovirulence and cause poliomyelitis. The true risk of inducing paralytic poliomyelitis by OPV is not precisely defined; nevertheless, the rarity of the risk may be indicated by the estimated overall frequency of one case per 2.6 million doses distributed and one case per 500,000 first doses given to infants in the USA from 1973 through 1984. Sabin, however, does not accept such figures, pointing out the difference between temporal and causal associations, and reminding that there are other causes of paralytic poliomyelitis (e.g., enteroviruses). There is general agreement that this rate of risk is acceptable, taking into consideration the enormous benefits provided by oral vaccine; possibly, a combined vaccine approach, in which inactivated poliovirus vaccine (IPV) is used for all first doses and OPV is given later, should help to reduce the number of vaccine-associated cases in vaccine recipients. Secondly, the possible antigenic evolution of wild polioviruses, as shown by the recent outbreak of poliomyelitis in Finland, where the wild type 3 poliovirus, responsible for the paralytic and nonparalytic cases in subjects immunized with IPV, was found to have drifted significantly antigenically and to be poorly neutralized by antibodies to the classical poliovirus type 3 vaccine strain. However, as reported by Böthig et al., the immunity induced by OPV is protective also for poliomyelitis caused by modified wild strains. Finally, it is also important to point out the risk related to immigration from and travelling to developing countries, where wild polioviruses are still widespread. In Italy, with its many outside contacts, it is unlikely that these strains would not be imported, and, hence, it is essential to maintain the present high immunization levels, to carry out periodic surveillance to monitor the pattern of the immunity of the population to the three polioviruses, and to ensure that poliomyelitis vaccination is available for all new foreign arrivals. When new generations of nonrevertant vaccines produced by recombinant DNA technology will become available for global use, they should be able not only to avoid the small number of vaccine-associated poliomyelitis cases and to block infections with wild polioviruses but also to replace them in nature and possibly to reach the global eradication of paralytic poliomyelitis.

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