ORIGINAL ARTICLE

# Immunity to poliovirus among children and the elderly in north-east Italy

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Key words

Immunity • Poliovirus • Italy

Summary

***Introduction.*** *Italy and Europe were declared polio-free in June 2002, but increasing migration, even from undeveloped countries where polio still exists, may lead to a come-back of circulating poliovirus (PV) and infection in previously polio-free areas. It is consequently advisable to continue to monitor the immunity of the population in polio-free areas. The aim of this study was to assess the current prevalence of neutralizing antibodies in recently-immunized children and in elderly people who were never vaccinated.*

***Methods.*** *Sera from 511 healthy subjects resident in the Veneto Region were examined to assay their antibody titer for PV 1, 2 and 3, using the microneutralization test. Data were analyzed by chi-squared test, Student's t-test and linear regression analysis, using EPI-Info 2000 supplied by the Centers for Disease Control and Prevention (Atlanta, GA, USA).*

***Results.*** *Neutralizing antibodies in group A (231 subjects aged 1-17 years) showed significantly higher geometric mean titers (GMTs) than in group B (280 subjects aged 65-100 years) for all three PV serotypes ($P < 0.001$). Nobody simultaneously lacked neutralizing antibodies for all three serotypes. There were no difference between the two groups in terms of male/female GMTs for the three PV. Antibodies decreased with time since vaccination, but the difference was only significant for PV 3. GMTs were lower in the elderly, with no significant difference among the three PV.*

***Discussion.*** *The population examined showed a good level of protection against the three PV strains: both groups A (vaccinated) and B (naturally immunized) revealed a valid immunity to poliovirus.*

***Conclusion.*** *Immunization programs and immunity status population screening are still advisable until polio has been worldwide eradicated.*

## Introduction

As a result of the Global Polio Eradication Initiative, poliomyelitis has been successfully controlled and eliminated in most developed countries by the systematic use of vaccines. The most widely used in the last 50 years has been the Sabin live attenuated oral poliovirus vaccine (OPV). The number of children paralyzed by the disease has fallen from over 350,000 in 1988 to as few as 1,900 in 2003; the number of countries where the disease is endemic has dropped from 127 or more to 7 respectively between 1988 and the end of 2001 [1].

On 21 June 2002, the European Regional Commission for the Certification of the Eradication of Poliomyelitis certified that the European Region is free of indigenous wild polioviruses [2].

In Italy, mass vaccination with OPV began in 1964 and became compulsory in 1966; it soon led to a marked downward trend in poliomyelitis morbidity; the last cases of paralytic poliomyelitis in the early Eighties were only vaccine-associated or imported from endemic areas [3, 4].

Italy became polio-free from around the Eighties as a result of high immunization coverage with routine services and good-quality surveillance [5].

Wild poliomyelitis is still endemic in some undeveloped areas of the world, however, especially where it is more difficult to reach people for religious, political and geographical reasons. There is increasing migration from developed countries, with a consequent risk of wild poliovirus strains being imported and circulated [6-10].

Although the risk of acquiring poliomyelitis is minimal in Italy, it may be important to know the prevalence of polio-neutralizing antibodies in our geographical area today, given the immigration problems and with a view to achieving the goal of suspending vaccination now that polio has been declared eradicated.

The aim of this study was to assess the current prevalence of neutralizing antibodies in two population groups, i.e. in recently-immunized children and in elderly people who had never been vaccinated.

## Methods

During the study period, 511 healthy subjects were examined. They were all resident in the Veneto Region and were consecutively enrolled at a laboratory for clinical analyses in one of the Region's public hospi-

tals. Blood specimens were drawn consecutively during routine tests for health check-ups.
The subjects were grouped according to age: Group A: subjects aged 1-17 years; Group B: subjects aged 65-100 years.
All subjects, or the parents of children, were informed about the study and consent was obtained from each participant.
Venous blood samples were drawn from each subject at the time of enrolment and the sera obtained were stored at - 20 °C until they were tested in batches.
A microneutralization test was used to evaluate the serum titer of poliovirus 1, 2 and 3 antibodies. 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 TCID50 of the three types of Sabin attenuated polioviruses (type 1: L Sc2ab strain-, type 2: P712ch2ab, type 3: Leon 12alb). After overnight incubation at 4 °C, a suspension of freshly trypsinized Vero cells (approximately 105/ml) was added to each well containing the serum/virus mixture, and the solution was incubated at 36 °C in a 5% $CO_2$ incubator. The final test reading was carried out after 6 days. The 50% endpoint value was used as the serum titer, and geometrical mean titers (GMTs) were computed by $\log_{10}$ of reciprocal antibody titers ≥ 1:2. Titers ≥ 1:2 were considered positive.
Data were analyzed using the chi-squared test, Student's t-test for unpaired data and linear regression analysis, as appropriate. Analyses were performed using EPI-Info 2000 supplied by the Centers for Disease Control and Prevention (Atlanta, GA, USA).

## Results

The population studied included 511 people grouped by age:

- Group A: 231 subjects aged 1-17 years (mean age 8.0 ± 4.1 years), 120 (51.9%) males and 111 (48.1%) females, who had been vaccinated with oral poliovirus vaccines (OPV) according to the Italian compulsory vaccination schedule;
- Group B: 280 subjects aged 65-100 years (mean age 83.4 ± 9.1 years), 68 (24.3%) males and 212 (75.7%) females, none of whom had received any doses of polio vaccine during their life.

Table I shows the prevalence of neutralizing antibodies according to serum titer, group and poliovirus type. The GMTs were significantly higher in the younger group than in the elderly for all serotypes ($p < 0.001$). No subjects simultaneously lacked neutralizing antibodies for all three polioviruses. Only poliovirus type 1 revealed any seronegativity: in a 5 year-old female in Group A and in a 65-year-old male and an 85-year-old female in group B.
The GMTs for polioviruses 1, 2 and 3 did not differ significantly between males and females in either group (Tab. II).
The GMTs of neutralizing antibodies in group A showed a constant decline after vaccination. This decline was smaller for poliovirus 1 and greater for polioviruses 2 and 3. Linear regression analysis showed a significant decrease only for serotype 3 ($r = 0.958$; $p = 0.042$).
Group B had lower GMTs and the declining trend of the three serotypes showed no significant differences, while it revealed a slight increase beyond 89 years old (Fig. 1).

## Discussion

This study was conducted to check on the humoral immunity status in two opposite age brackets, children/adolescents and the elderly, a few years since

**Tab. I.** Neutralizing antibody prevalence according to serum titer, group and type of poliovirus.

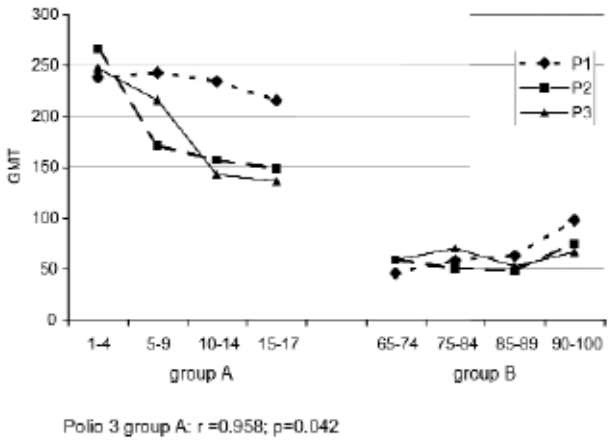
| serum titer | group A (children) | | | | | | group B (elderly) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | poliovirus | | | | | | poliovirus | | | | | |
| | type 1 | | type 2 | | type 3 | | type 1 | | type 2 | | type 3 | |
| | n | (%) | n | (%) | n | (%) | n | (%) | n | (%) | n | (%) |
| negative | 1 | (0.4) | 0 | (0.0) | 0 | (0.0) | 2 | (0.7) | 0 | (0.0) | 0 | (0.0) |
| 2 | 1 | (0.4) | 1 | (0.4) | 1 | (0.4) | 2 | (0.7) | 4 | (1.4) | 3 | (1.1) |
| 4 | 3 | (1.3) | 2 | (0.9) | 0 | (0.0) | 19 | (6.8) | 19 | (6.8) | 17 | (6.1) |
| 8 | 5 | (2.2) | 4 | (1.7) | 4 | (1.7) | 26 | (9.3) | 28 | (10.0) | 37 | (13.2) |
| 16 | 8 | (3.5) | 3 | (1.3) | 3 | (1.3) | 32 | (11.4) | 37 | (13.2) | 29 | (10.4) |
| 32 | 5 | (2.2) | 13 | (5.6) | 8 | (3.5) | 26 | (9.3) | 35 | (12.5) | 24 | (8.6) |
| 64 | 6 | (2.6) | 18 | (7.8) | 25 | (10.8) | 33 | (11.8) | 31 | (11.1) | 36 | (12.9) |
| 128 | 23 | (10.0) | 50 | (21.6) | 51 | (22.1) | 38 | (13.6) | 50 | (17.9) | 47 | (16.8) |
| 256 | 44 | (19.0) | 71 | (30.7) | 73 | (31.6) | 67 | (23.9) | 40 | (14.3) | 52 | (18.6) |
| 512 | 135 | (58.4) | 69 | (29.9) | 66 | (28.6) | 35 | (12.5) | 36 | (12.9) | 35 | (12.5) |
| GMTs* | 252.2 | | 185.7 | | 190.8 | | 65.8 | | 56.7 | | 60.9 | |

* $p < 0.001$ group A vs. group B for all serotypes; group A: 231 children; group B: 280 elderly people.

**Tab. II.** Neutralizing antibody GMTs according to gender, group and type of poliovirus.

| gender | group A (n = 231) poliovirus | | | group B (n = 280) poliovirus | | |
|---|---|---|---|---|---|---|
| | type 1 | type 2 | type 3 | type 1 | type 2 | Type 3 |
| males | 274.2 | 188.9 | 195.6 | 54.9 | 70.2 | 80.1 |
| females | 229.9 | 182.2 | 185.6 | 69.7 | 52.9 | 55.8 |
| total | 252.2 | 185.7 | 190.8 | 65.8 | 56.7 | 60.9 |

**Fig. 1** - Poliovirus neutralizing antibodies: GMTs according to age group and type of poliovirus




Europe was declared a polio-free region [2] in view of the eradication of poliomyelitis.

The Veneto Region population enrolled in the study globally revealed a good level of immunity against poliomyelitis. The younger group (A) had significantly higher GMTs than the elderly people (group B), judging from the time-related decline in antibody titer since exposure to wild or vaccine strains of poliovirus [11, 12].

A good natural immunity to the three poliovirus types was revealed among the elderly, even over 89 years old: the majority of the subjects had high GMTs, probably due to their repeated exposure to poliovirus throughout their lives, initially to the wild type and subsequently to vaccine-derived polio virus (VDPV).

As expected, immune levels to poliovirus 1 and 2 induced by OPV remain fairly stable after an initial decrease during the early years after vaccination, whereas the drop in antibody to poliovirus 3 is more marked [13].

Massive vaccination with OPV has been effective in protecting the newborn and also a natural booster reaching people who had been immunized a long time before, or had never been immunized with vaccine strains. Today the indigenous population reveals an excellent protection against poliovirus infection. IPV immunization is currently used in our polio-free country to avoid even the remote possibility of circulating VDPV and retromutated PV.

Specific immunological surveillance and/or far-reaching vaccination campaigns, as the case may be, should be implemented or maintained in immigrants, foreign workers and adopted children coming from developing countries in order to prevent any risk of the wild virus reappearing and inducing polio paralysis, to keep Italy polio-free until polio eradication has been unequivocally achieved worldwide.

The world now has a good chance of putting a stop to the transmission of wild poliovirus, because wild poliovirus transmission is confined to a limited number of polio 'hot-spots' in very few countries.

There are still poliomyelitis outbreaks [9] in some countries, however, indicating that wild strains of PV may circulate. The outbreak in the Netherlands in the winter of 1992-93 [7] goes to show that wild strains may be brought into polio-free areas and can lead to symptomatic infection in communities with no herd immunity.

Up until such a time when polio as been eradicated successfully, a non-immune population cluster may represent a reservoir in which poliovirus can multiply, possibly infecting non-immune subjects outside the cluster.

As long as endemic areas continue to exist, it will be necessary (even in areas declared polio-free) to continue to routinely vaccinate against polio and screen the population to check the immunity state of both indigenous and immigrant populations [6, 14, 15].

## Conclusions

Antibody levels indicate a good protection against poliomyelitis in the population considered, but until polio has been eradicated, high levels of immunity against the three polio strains must be maintained because of the risk of PV being imported from non polio-free areas (i.e. due to people travelling in endemic areas, immigration, international adoptions, etc.).

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