





RESEARCH PAPER



Is Italian population protected from Poliovirus? Results of a seroprevalence survey in Florence, Italy

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ABSTRACT

Objectives: Periodical assessments of population susceptibility to polioviruses (PV) is essential for evaluating population protection and planning appropriate vaccination strategies. The aim of the current work was to assess serological protective titers against all three polioviruses in the general population of Florence. **Methods:** A convenience sample of 328 sera, collected in 2009 in Florence (Central Italy) was analyzed. Samples were considered protective if neutralizing antibodies were detected at dilutions $\geq 1:8$, according to the WHO protocols. **Results:** The immune coverage was 75.3%, 69.2% and 46% for PV1, PV2 and PV3, respectively. The protective titers of neutralizing antibodies were generally higher in children up to 14 years of age, with 74.4% (PV1), 75.6% (PV2) and 56.7% (PV3) of seroprevalence. From the age of 11 years, most of the study subjects were seronegative for PV3. **Conclusions:** In a polio-free country with strong migration pressures, such as Italy, our results bring clear support to the recent recommendation of Italian health authorities to introduce a fifth dose of IPV vaccine in adolescence all over the country.

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Introduction

Poliomyelitis is an acute communicable disease caused by any of 3 poliovirus serotypes (PV1, PV2 or PV3). Poliomyelitis was until a recent past a devastating disease in terms of morbidity and mortality in Italy, as well as in the rest of the world.¹ Starting from 1988 the World Health Organization (WHO) has engaged an all-out struggle for the eradication of the three polioviruses worldwide through the extensive spread of vaccination. When the eradication campaign started the global incidence of poliomyelitis was >350,000 cases per year, with 125 endemic countries.²

At the end of 2015, only two countries, Afghanistan and Pakistan, were considered endemic for poliomyelitis, but following the report in August 2016 of four cases of paralysis due to WPV1 in Borno state (northern Nigeria) Nigeria lost its “non-endemic” status.³

Overall, in 2017, only 22 cases of WPV1 infection have been reported to WHO (8 in Pakistan and 14 in Afghanistan), compared with 37 cases in 2016 and 74 cases in 2015, but a total of 91 circulating vaccine-derived poliovirus type 2 (cVDPV2) cases were officially reported (17 in the Democratic Republic of the Congo (DRC) and 74 in Syria).² As of 24 January, in 2018 a single case of type 1 wild poliovirus (WPV1) was confirmed in Afghanistan and no cVDPV2 cases were reported.

The type 2 wild poliovirus (WPV2) has been isolated for the last time in 1999 and declared eradicated on September 2015. The wild type poliovirus 3 (WPV3) has not been isolated in polio

cases for more than five years (most recent case in November 2012). These figures raise hopes for a near future without polio.

Salk inactivated vaccine (IPV) is generally adopted in polio-free countries with low risk of importation of wild Polioviruses to rule out the risk of generating live attenuated oral polio vaccine (Sabin vaccine or OPV)-derived virus strains through circulation in human populations with deficient herd immunity. In order to eliminate OPV vaccine-related disease burden, also in countries in which poliomyelitis cases are still notified, the switch from trivalent to bivalent OPV vaccine (containing type 1 and 3 polioviruses only) has been implemented starting from April 2016. However, to maintain immunity level to PV2 the introduction of at least one dose of IPV into routine immunization schedule was planned in 2017 in high risk countries.^{1,4}

In Italy, as in the rest of the world, vaccination strategies have changed over time following vaccine development and epidemiological evolution of the disease. The Salk vaccine has been introduced in Italy in 1957 and it has been replaced by Sabin vaccine in 1964. After the introduction of Sabin vaccine, the number of reported cases quickly dropped from an average of more than 3,000 per year during 1960–64 to 254 in 1965 and 147 in 1966, when polio vaccination became mandatory.⁵ Poliomyelitis incidence further declined rapidly during the ‘70s and the last indigenous case was registered in Italy in 1982. During the ‘80s three imported cases, one each from Iran, India and Libya, have been reported. Thereafter, only a few vaccine-associated poliomyelitis cases were notified.⁶ From 1999 to

2002 a sequential schedule with two doses of IPV followed by two doses of OPV was adopted, to be replaced at the achievement of the eradication of poliomyelitis in the European region in 2002 by an IPV-only schedule.⁷ Since 2005 the offer of the fourth dose has been postponed from the 3rd to the 5th year of life.⁸ In Tuscany, the schedule shift was completed in 2007.^{9–11}

According to the current epidemiological data the overall surveillance of polio disease should be based on three cornerstones. The first issue concerns the control of faeces/stool in the environment, through the monitoring of poliovirus presence in untreated municipal sewage, both in endemic areas and in polio-free areas for the possible reappearance of wild clones. This was clearly demonstrated in 2013 in Israel, where IPV-only vaccination had been implemented starting from 2005. The reintroduction and wide circulation in the population of WPV1 was detected by environmental surveillance at sewage treatment plants but no clinical cases were signaled.¹² The second issue is a continuing surveillance of flaccid paralysis in order to identify unexpected diseases due to wild or vaccine-derived polioviruses.

Of course, the third issue relies on the extensive spread of vaccination. Actually, the attainment of the herd immunity and its maintenance have determined the essential requirement for the disappearance of the disease and the eradication of the three viruses. On the other hand, suboptimal vaccination coverage, due to many causes, may be responsible for the reintroduction of wild polioviruses in populations declared for many years polio-free, as occurred in the epidemic of 2013 in Syria due to viruses imported from Pakistan.¹³

The Italian Ministry of Health reported >95% average national immunization coverage rate at 24 months of age against poliovirus until 2013, but unfortunately in the following years values have decreased below 95%. According to the last available data, in 2016 the average national immunization coverage against poliovirus has dropped to 93.3% and this trend leads to some concern.¹⁴

The presence in blood of neutralizing antibodies against polioviruses indicates protective immunity and is an excellent correlate of protection against paralytic disease.¹⁵ Herd immunity is the indirect protection to susceptible subjects conferred by immune individuals in the population. One of the objectives of vaccination programs is to achieve appropriate vaccination coverage to establish the necessary herd immunity, thus preventing outbreaks.¹⁶ Herd immunity is established in a population when the prevalence of protected individuals (vaccinated or naturally infected) is higher than a critical value (herd immunity threshold). The assessment of this threshold depends on various factors: vaccine effectiveness of the immunization program in the community; sensitivity and specificity of serological tests used to assess the prevalence of protected population. It is estimated that the herd immunity threshold for poliovirus is obtained at a minimum level of vaccination coverage equal to 80%–86%. In population groups without herd immunity, additional vaccinations are necessary to increase immunity levels and create herd immunity in the population.¹⁷ Therefore, seroprevalence studies are fundamental, especially if repeated over time, to provide relevant information on the level of immunity induced by the vaccine and to assess the persistence of herd immunity in vaccinated population.

The main aim of the current work was to assess the serological protective titer against all three polioviruses in a sample of sera collected in 2009 from subjects residing in Florence (Central Italy).

Results

As assessed by neutralization tests, the immune coverage calculated on the whole population studied was 75.3%, 69.2% e 46% for PV1, PV2 and PV3, respectively. Protective titers of neutralizing antibodies against PV1 were detected in 74.4% of the pediatric samples (0–14 years of age) and in 78.8% and 80.3% of the people aged 15–43 and 44–65 years, respectively. A reduction to 66.2% was demonstrated in adults over 65 years old. Less than a half (44.4%) of the 9 children under one year of life, including unvaccinated newborns eventually profiting of maternal immunity and infants likely partly immunized (1 or 2 doses), had an immune coverage for PV1. A protective titre for PV1 was found in 85.7% of the sera in the 1–4 years age group but seroprevalence percentages decreased to 58.8% in subjects 5–7 years old, to rise again at 80% or more in those aged 8–14 years (Fig. 1a). Immune protection for PV2 fluctuated from a maximum of 75.6% in children aged 0–14 years to a minimum of 61.8% in the over 65 but was higher (76.1% vs 63.6%) in the 44–65 years age group than in the presumably vaccinated 15–43 years old subjects. Only 22.2% of children under one year of life showed an immune protection for PV2. In the 1–4 years old immune protection from PV2 was found in 90.5% of sera, but lowered to 52.9% in the 5–7 years age group, reached a 100% top in those aged 8–10 years and then decreased to 78.3% in the 11–14 years age group (Fig. 1b). All age-groups analyzed showed a lower prevalence of protective antibodies against PV3 than against PV1 and PV2 (Table 1). The lowest immune coverage for PV3 was found in children under one year of life (22.2%). Immune protection for PV3 reached 76.2% of sera in the 1–4 years group, decreased to 52.9% in the 5–7 years age group, climbed to 75% in those aged 8–10 years and dropped again to 39.1% in the 11–14 years age group. The percentage of PV3 protective sera remained between 38% and 45% in the older age groups (Fig. 1c).

The geometric mean titers (GMTs) of neutralizing antibodies for PV1, PV2 and PV3 were also analyzed. As expected, the highest GMTs were observed in the pediatric age groups (0–14 years), 36.2, 72.1 and 63.3, respectively for PV1, PV2 and PV3, with wide variations between age sub-groups. Titers dropped to 28.1, 28.1 and 21.6, respectively, among subjects aged 15–43 years ($p < 0.05$). Subjects aged 44–65 years had antibody titers higher than those found in 16–43 years old ($p < 0.05$). Children under 11 years showed higher GMTs for PV2 and PV3 than for PV1. However, antibody levels against PV1 remained almost constant in all the four age groups analyzed, while immunity against PV2 and PV3 decreased sharply already in the 11–14 age group but was at its lowest also in the 15–43 years old (Table 1).

Serum samples able to neutralize simultaneously all three polioviruses (triple-positive) were found in 37.5% of the whole pediatric population but a progressive reduction from 76.2% in children 1–4 years old to 34.8% in the 11–14 years old ($p < 0.01$) was observed. Conversely, the rate of triple-negative

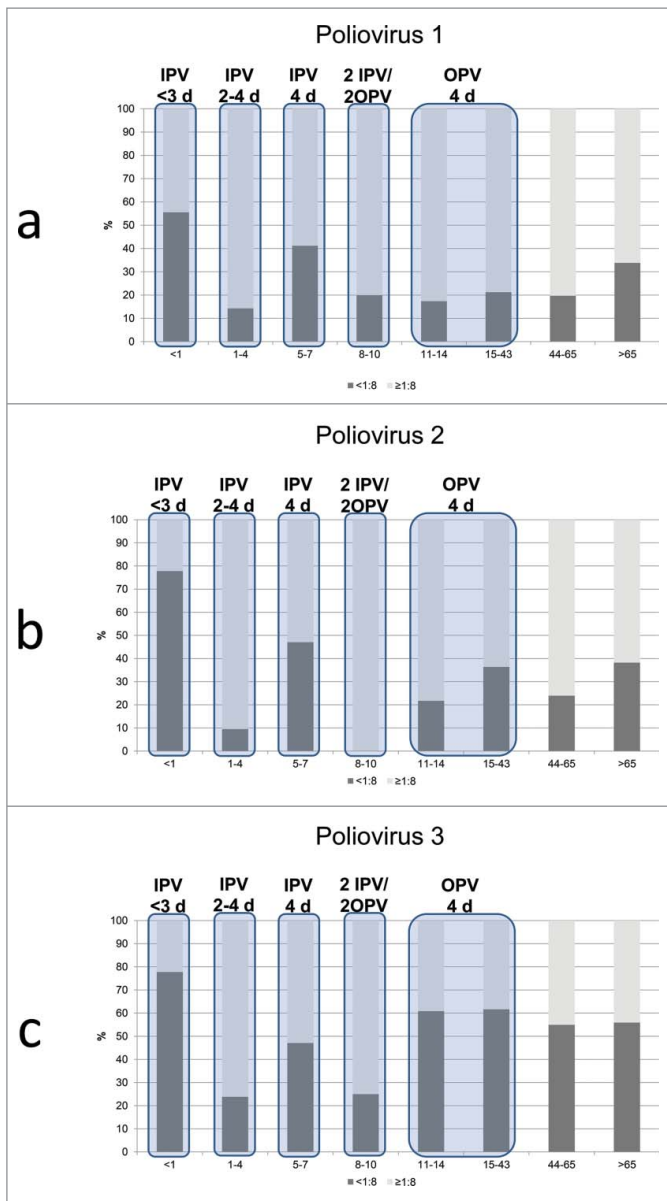


Figure 1. Poliovirus immune coverage rate for PV1 (a), PV2 (b) and PV3 (c) in the whole sample of sera, collected in 2009 in Florence (Tuscany, Central Italy), stratified in sub-groups according to polio vaccination schedule administered. [d: dose; IPV: Inactivated poliovirus vaccine; OPV: Oral poliovirus vaccine].

samples in children was very low (9.5%) in the 1–4 years old, increased to 41.2% in the 5–7 years old and then decreased again to less than 13% in the 11–14 age group (Table 1). In

adults, the prevalence of triple-positive samples decreased from more than 30% in the 15–65 years age groups, to 23.5% in the over 65, while triple-negatives fluctuated in the range 8.5%–14.7% (Table 1).

Discussion

The evaluation of protective immunity against polioviruses, performed on sera collected in 2009 from subjects residing in the Florentine area (Tuscany, Central Italy) demonstrated that, in spite of high national immunization coverage rate against polioviruses for many decades, a low immune protection was found in children and young people in the Florence area, especially for PV3. Apparently, the antibody levels against PV3 rapidly decrease during childhood and most of the presumably vaccinated subjects became seronegative by the age of 15. The results of the analysis of the sera belonging to the pediatric age groups demonstrated that after the completion of the basic four-doses vaccination course a substantial response, although not optimal, is observed against all three polioviruses. However, part of the population quickly lost protective immunity against PV2 and a much higher percentage of sera without evidence of protection was observed for PV3.

The early loss of protective immunity and/or low GMTs in adolescents and young adults, mainly for PV3 but also for PV1, have already been reported in recent years elsewhere in Italy and in some European countries.^{18–25} A previous seroprevalence survey on pediatric sera (0–14 years) collected from the Florentine hinterland in the period 2005–2006 showed a protective coverage for the three polioviruses significantly higher compared to the current survey (93.2%, 94.3% and 90.9%, respectively for PV1, PV2 and PV3). However, also in the 2005–2006 survey the level of immune coverage for PV3 was the lowest and protective antibodies levels against PV3 decreased starting from the twelfth year of age.²⁶ The lack of booster effect due to the eradication of wild type polioviruses and to the implementation of IPV-only vaccination are possibly responsible for the general reduction in protective antibody titers in children and young adults. The 2002 switch to the IPV-only schedule could eventually explain the lower protection levels found in the pediatric population of Florence in 2009 when compared to the previous survey in 2005–2006, when the pediatric population was supposed to have received at least two doses of OPV.²⁶

Table 1. Serum samples tested for each age group and sub-group. Prevalence and Geometric Mean Titers (GMTs) of protective antibodies $\geq 1:8$ against polio 1 (PV1), polio 2 (PV2) and polio 3 (PV3) viruses in sera collected in 2009 in Florence (Tuscany, Central Italy). Prevalence of triple positive and triple negative samples is also shown.

Age group (years)	Age sub-group (years)	No. tested samples	% of protective antibodies ($\geq 1:8$)			GMTs			% Triple positive	% Triple negative
			PV1	PV2	PV3	PV1	PV2	PV3		
0–14	<1	90	74.4	75.6	56.7	36.2	72.1	63.3	52.2	18.9
	1–4	21	44.4	22.2	22.2	29.8	192	256	22.2	55.6
	5–7	17	85.7	90.5	76.2	35.6	81.2	111.5	76.2	9.5
	8–10	20	58.8	52.9	52.9	40.8	189.2	155.3	52.9	41.2
	11–14	20	80	80	100	75	44.3	70.6	34	60
15–43	23	23	82.6	78.3	39.1	32.5	39.7	19.6	34.8	13
44–65	99	99	78.8	63.6	38.4	28.1	28.1	21.6	30.3	14.1
>65	71	71	80.3	76.1	45.1	34.5	45.1	29.3	33.8	8.5
	68	68	66.2	61.8	44.1	47.2	42.7	36.7	23.5	14.7

The lower protection we detected in the 5–7 years old group with respect to the 8–10 group, both theoretically exposed to 4 vaccine doses, could indicate a longer lasting immunity induced by the sequential IPV-OPV schedule (Table 1 and Fig. 1).

In Tuscany, a study conducted in 1963,²⁷ before mass vaccination was implemented, reported the simultaneous presence of antibodies against PV1, PV2 and PV3 in 50% of healthy children aged 1–5 years and in 76% of the 21–22 years old. Antibodies against PV2 appeared earlier in children and they were more frequently detected also reaching the highest titers in the whole population. Similar findings were reported in the same years in Southern Italy.²⁸ The wide circulation of wild polioviruses could explain the high prevalence of protective anti-polio antibodies we found in our study in subjects born before the introduction of compulsory vaccination in 1966. Consistently, the detection of higher antibody levels for all three polio types in subjects aged 44–65 years than in younger people was probably due to several expositions to wild type and/or vaccine polioviruses circulating in the population and acting as boosters for immunity. The reduction of antibodies titers in the over 65 years old that were exposed to the same boosters opportunities of the preceding age-group could merely correlate to their immune senescence.

Although there is no evidence that loss of detectable antibodies puts at risk of disease immunocompetent vaccinees,¹ in the absence of natural boosters, IPV recipients will remain susceptible to poliovirus infection. IPV vaccination alone is insufficient to induce a mucosal IgA response against poliovirus. In mucosally (OPV-) primed individuals, however, booster vaccination with IPV leads to a strong mucosal IgA response.²⁹ Declining protective immunity in the Italian population might favor silent transmission of neurovirulent polioviruses to unvaccinated subjects. Although there is no indication that vaccination with IPV only confers lower levels of immune protection than provided by the sequential IPV-OPV or by the OPV-only schedule,¹ IPV-only schedules permit WPV transmission, even at high IPV vaccination coverage, as demonstrated by the persistent circulation of WPV in Israel in 2013.³⁰

In the years of birth of the pediatric cohort analyzed in the present study the poliovirus vaccination coverage in the Florentine pediatric population was inferred from the Regional coverage level in Tuscany that remained stable at around 96%.³¹

This study has some limitations. First, the serum samples analyzed belonged to a collection stored in 2009, thus the prevalence of protection reflected the serological profile of the population at that time. Second, according to the Italian Data Protection Act, only limited data elements were collected and it was not possible to determine vaccination status. Third, our results were representative of a restricted geographical area. Larger seroprevalence surveys, involving a nationally representative sample population, including recent immigrants, are needed to update the Italian sero-epidemiological situation.

The introduction of a fifth IPV dose in adolescence is intended to raise protective antibody titers and to extend protective immunity.³² Moreover, a single IPV dose has been demonstrated to boost and maintain at high levels mucosal IgA response in previously OPV-vaccinated persons.²⁹ Some European Countries, such as United Kingdom, France and Germany have already introduced the fifth polio dose into their

Table 2. Type of vaccine and vaccine doses supposedly administered to the pediatric cohort of subjects (up to 14 years) included in the seroprevalence survey of sera collected in Florence (Tuscany, Central Italy), 2009, according to the vaccination offer recommended.

Year of birth	Age (years)	Doses of Polio vaccines received at the time of 2009 ^a	Type of Polio vaccine administered	Age at 4th dose (years)
1995	14	4	OPV	3
1996	13	4	OPV	3
1997	12	4	OPV	3
1998	11	4	OPV	3
1999	10	4	2IPV-2OPV	3
2000	9	4	2IPV-2OPV	3
2001	8	4	2IPV-2OPV	3
2002	7	4	IPV	3
2003	6	4	IPV	3
2004	5	4	IPV	3
2005	4	4	IPV	3
2006	3	3 or 4	IPV	3
2007	2	3	IPV	5
2008	1	2 or 3	IPV	5
2009	<1	None or <3	IPV	5

Note. ^aExpected according to the vaccination schedules recommended in Tuscany over time; OPV: oral polio vaccine; IPV: inactivated polio vaccine.

vaccination schedule.³³ Its geographical position puts Italy at high risk for polioviruses importation. Therefore, the Italian Ministry of Health recently officially recommended the administration of a fifth IPV dose in adolescents and the new schedule has already been implemented by some regional health authorities.³⁴

In order to counter the gradual decline in vaccinations observed in Italy since 2013, the Italian Ministry of Health extended to ten (including IPV) the panel of mandatory vaccinations for preschool and school-age children and for adolescents up to 16 years, starting from September 2017.³⁵ Other European countries have already approved mandatory vaccinations.³⁶ A new policy in France requires all children born January 2018 or later to receive 11 mandatory vaccines.³⁷

Conclusions

Further surveys, would be necessary to confirm our observations and to better explain the apparent loss of vaccine-induced immunity observed in the Florentine population in recent years. However, our results encourage to introduce into the national vaccination schedule a fifth IPV dose in adolescence, in order to obtain a longer-lasting immunity in the population. Seroprevalence studies provide valuable information to assess the levels of protection against diseases in a population and suggest to maintain a state of attention against this specific disease also in countries, like Italy, where polio cases have not been notified since many decades. Italy and the other European countries are fully committed to prepare the global objective of a Polio-Free World with all available measures.

Materials and methods

Study population

The evaluation of immunity against PV1, 2 and 3 was carried out on a population of 328 immunocompetent subjects, 161 females and 167 males aged 0 to 88 years (mean age 38 years,

median age 35 years). Serum samples were collected in 2009 from the University Hospital Careggi and the Meyer Children's Hospital of Florence during routine general health and immunological checkups. According to the Italian Data Protection Act, only limited data elements (including initials, gender and year of birth) were collected; therefore, it was not possible to assess vaccination status. All samples were stored at -20°C in the serum bank of the serology laboratory of the Department of Health Sciences, University of Florence. In Table 2 is reported the expected number of polio vaccine doses administered to the subjects included in the study, according to the evolution of the Tuscan vaccination schedule in recent years, for the pediatric birth cohort (0–14 years). The study population was divided into four different age groups and five sub-groups for the pediatric subjects, according to the vaccination offer recommended: the first age group included children, from 0 to 14 years of age, vaccinated with OPV and/or IPV (subjects up to 7 years received only IPV vaccines, from none to four doses, on the basis of their age at the moment of the sera sampling; subjects belonging to the 8–10 years age group received a sequential schedule of two IPV doses and two OPV doses and subjects between 11 and 14 years of age received a whole OPV schedule); subjects aged 15–43 years, born after the mandatory OPV vaccination was introduced, were included in the second group; the last two groups included probably unvaccinated adults, aged from 44 to 65 years and over 65 years, respectively. The distribution of sera samples into age groups is reported in Table 1.

Serology

Neutralizing antibodies titers were determined at the Department of Health Promotion Sciences and Mother and Child Care "Giuseppe D'Alessandro", University of Palermo, according to the WHO protocols.³⁸ Briefly, serum samples were complement inactivated at 56°C for 30 minutes and two fold diluted to 1:1024. Dilutions from 1:8 to 1:1024 were placed in contact (1 hour at 37°C) with 100 TCID₅₀ (50% tissue culture infective doses) of the Sabin attenuated type 1, 2 and 3 strains received from the Italian National Institute of Health, Rome. Then, freshly trypsinized Vero cells were added. After 3–4 days of incubation at 37°C , the highest serum dilution that protected 50% of the cultures was recorded and samples were considered protective if neutralizing antibodies were detected at dilutions $\geq 1:8$. Complying with the biocontainment requirements, connected to the global withdrawal of the type 2 component of OPV, all neutralization tests involving PV2 have been carried out before April 2016.

Statistical analysis

The chi-square test was used to compare antibodies prevalence and Student's *t*-test to analyze differences in the geometric mean titers (GMTs) calculated for each age group.

Compliance with Ethical Standards

All procedures performed in the current study involving human participants were in accordance with the ethical

standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of retrospective study formal consent is not required.

Disclosure of potential conflicts of interest

No potential conflicts of interest to declare.

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