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A seroprevalence study of poliovirus antibody against a collection of recombinant and non-recombinant poliovirus vaccine strains in the population of southern Greece

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Abstract

In this study, the serological status of the southern Greek population in the 1–10-year, 11–20-year, 21–30-year and 31–40-year age groups with regard to Sabin vaccine strains and a collection of 15 recombinant and four non-recombinant poliovirus vaccine strains was determined. For all three poliovirus types, the highest neutralization test (NT) titres were observed in the 1–10-year age group, indicating a good response to vaccination. In general, the serological status of the population of southern Greece with regard to poliovirus is better for types 1 and 2 than for type 3. The presence of the lowest NT titre in the 21–30-year age group against poliovirus type 3 suggests the need for a booster dose of monovalent Sabin3 vaccine to ensure personal and herd immunity.

Keywords: Greece, immunity, OPV derivatives, Polioviruses

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Since the 1960s, poliovirus, the causal agent of poliomyelitis, has been effectively controlled by the use of inactivated poliovirus vaccine (IPV) or live attenuated oral poliovirus vaccine (OPV), which is composed of attenuated strains of each of the three serotypes (Sabin1, Sabin2, and Sabin3) [1].

By 1964, OPV was the vaccine that had been adopted throughout most of the world, because of several advantages over IPV, such as: simplicity of administration, induction of mucosal immunity and neutralizing antibodies, and low cost. Since the Poliomyelitis Eradication Initiative was launched in 1988, great progress has been made in stopping the transmission of wild-type poliovirus and in achieving global certification of eradication by 2005 [2]. The number of poliomyelitis cases due to infections with wild-type polioviruses decreased from an estimated 350 000 in over 125 endemic countries in 1988 to just 1310 in four countries in 2007 [3]. Poliomyelitis transmission has been interrupted in the American, European and Western Pacific regions, and by the end of 2002 more than 180 countries and territories had

been declared to be polio-free. At present, the virus remains endemic in four countries: Afghanistan, India, Nigeria, and Pakistan [2].

The eradication strategies recommended by the WHO include: (i) high routine infant immunization coverage with at least three doses of OPV plus a dose at birth in polio-endemic countries; (ii) national immunization days targeting all children aged <5 years; (iii) acute flaccid paralysis surveillance and laboratory investigations; and (iv) mop-up immunization campaigns with OPV to interrupt final chains of transmission [2].

In the late 1990s it was recognized that polio outbreaks can be caused by circulating live-attenuated vaccine viruses (Sabin strain) that have reverted and re-acquired neurovirulence. By late 2005, six outbreaks caused by such circulating vaccine-derived polioviruses (VDPVs), had been documented. Moreover, in rare cases (one case per 750 000 primary vaccinees), OPV strains have been implicated in vaccine-associated paralytic poliomyelitis [4–7].

Emerging concerns, such as the vaccine-associated paralytic poliomyelitis cases and the isolation of VDPV in a number of countries, prompted the WHO, in its 2004–2008 strategic plans, to recommend cessation of OPV administration as soon as possible after interruption of wild-type poliovirus circulation and continuing schedules of immunization, mainly with IPV [8].

OPV was introduced into Greece in 1964, and a standard vaccination schedule was initiated, including four doses at the ages of 2, 4, 6 and 18 months, with a booster dose at 4–6 years. This vaccination schedule has led to the elimination of indigenous cases of poliomyelitis since 1982 [9]. However, since 2005, Greece has switched to the exclusive use of IPV, like most polio-free countries.

In this study, the immunity level of the southern Greek population in the 1–10-year, 11–20-year, 21–30-year and 31–40-year age groups was measured against Sabin vaccine strains and a collection of 15 recombinant and four non-recombinant OPV derivatives. The study was carried out in 2008–2009, and the serum samples were collected from regions of southern Greece. The serotypes of all OPV derivatives were determined by microneutralization assay with type-specific rabbit antisera (RIVM, Bilthoven, The Netherlands) according to the enclosed instructions and using standard procedures [10].

The level of immunity of the human population against poliovirus types 1, 2 and 3 was determined with a microneutralization assay, according to the WHO guidelines. Pooled sera (40 mixed serum samples) were used from each age group for the neutralization test (NT) [11]. Serum pools were diluted 1 : 10 in MEM, heat-inactivated for 50 min at 56°C, diluted two-fold from 1 : 10 to 1 : 1280, and incubated in duplicate for 1 h at 37°C with 100 50% tissue culture infective doses with each one of the 19 OPV derivatives as well as with the Sabin vaccine strains (Sabin1, Sabin2, and Sabin3). Finally, a cell suspension containing 10^4 Hep2 cells/0.1 mL was added. Cell and virus controls were included in each batch. The plates were examined daily (3–5 days) for the development of cytopathic effect. When the virus controls showed complete cytopathic effect, the final results were recorded 24 h later. The highest dilution of serum pool that protected the cultures was recorded. Results were expressed as $\log_{10}$ reciprocal titres ($\log_{10}$ titre 1 : 10 = 1). Student's *t*-test (paired samples test) was used to compare the mean NT titres of four age groups (1–10, 11–20, 21–30 and 31–40 years) against each Sabin vaccine strain (Sabin1, Sabin2, or Sabin3) with those against same serotype OPV derivatives. A one-way ANOVA test (Duncan's multiple range test) was used to compare the mean NT titres against the Sabin vaccine strain and OPV derivatives of the same serotype between the 1–10-year, 11–20-year, 21–30-year and 31–40-year age groups.

Table 1 shows the serotype and the recombination site of each of the 19 OPV derivatives identified in previous studies [12–17]. The serotype of OPV derivatives was identified as P1 for five, as P2 for six, and as P3 for eight. The majority of OPV derivatives were characterized as OPV-related polioviruses displaying <1% divergence from the VP1 region of the reference Sabin vaccine strain. However, an OPV derivative of serotype 1 was characterized as VDPV in a previous study [12]. Specifically, it revealed 1.87% divergence from the VP1 region of reference strain Sabin1 and a recombination event between the Sabin1 vaccine strain and a member of enterovirus group C in the 2A genomic region.

Table 2 shows the statistical analysis of the $\log_{10}$ reciprocal NT titres against Sabin vaccine strains and OPV derivatives. The population of the 1–40-year age group shows significantly lower NT titres against two Sabin1 derivatives (742 and 522) in comparison with the Sabin1 vaccine strain. No significant differences in NT titres were observed for Sabin2 and Sabin3 derivatives in comparison with the Sabin2 and Sabin3 vaccine strains, respectively.

A sequential decrease in NT titre was observed from the 1–10-year age group to the 11–20-year and 21–30-year age groups in all three poliovirus types (P1, P2, and P3). Specifically, a significant decrease was observed from the 1–10-year to the 11–20-year age group for poliovirus type 1 and from the 1–10-year age group to the 11–20-year and 21–30-year age groups for poliovirus types 2 and 3. An increase in NT titre was observed from the 21–30-year to

TABLE 1. The serotype, the recombination type and the recombination sites of all oral poliovirus vaccine (OPV) derivatives

Serotype 1 (P1)	Serotype 2 (P2)	Serotype 3 (P3)
742	I34	EPC
S1/S3/S2[a] (2A/2C)[b]	S2/S1/S2/S1 (2C/3D/3D)	S3/S2/S3 (2C/3D)
This study	Karakasiliotis *et al.* (2005) [13]	Paximadi *et al.* (2007) [16]
7/b/97	EP9	EPB
S1/EVC[c] (2A)	S2/S1 (3A)	S3/S2/S3 (2C/3D)
Dedepsidis *et al.* (2007) [12]	Paximadi *et al.* (2006) [14]	Paximadi *et al.* (2007) [16]
522	EP12	738
S1 (non-recombinant)	S2/S1 (3D)	S3/S2/S1 (3C/3D)
This study	Paximadi *et al.* (2006) [14]	This study
II	ID	584
S1 (non-recombinant)	S2/S1 (3C)	S3/S2/S1 (2C/3D)
This study	Karakasiliotis *et al.* (2004) [15]	This study
152	IF	EPA
S1 (non-recombinant)	S2/S1 (3D)	S3/S2/S3 (2C/3D)
This study	Karakasiliotis *et al.* (2004) [15]	Paximadi *et al.* (2007) [16]
	8001	K/2002
	S2 (non-recombinant)	S3/S2 (VP1)
	This study	Dedepsidis *et al.* (2008) [17]
		EP16
		S3/S2 (2C)
		Paximadi *et al.* (2006) [14]
		EP23
		S3/S1 (2C)
		Paximadi *et al.* (2006) [14]

All isolates were characterized as OPV-related polioviruses except for one, which was characterized as a vaccine-derived poliovirus strain (VDPV). The recombination sites of some OPV derivatives were identified previously by our group (references are indicated).
[a]The recombinations of OPV-related polioviruses are among Sabin vaccine strains (S1, Sabin1; S2, Sabin2; S3, Sabin3).
[b]Recombination sites are located in the 2A, 2C, 3A, 3C, 3D or VP1 genomic regions.
[c]Isolate 7/b/97 showed a recombination event between Sabin1 vaccine strain and a member of enterovirus group C in the 2A genomic region. Moreover, it revealed 1.87% divergence from the VP1 region of reference strain Sabin1 and was characterized as VDPV.

TABLE 2. Statistical analysis of the $\log_{10}$ reciprocal neutralization test (NT) titres against Sabin vaccine strains and oral poliovirus vaccine derivatives

Serotype	Virus strain	Mean values of $\log_{10}$ reciprocal NT titres of four age groups (1–10, 11–20, 21–30 and 31–40 years) against each poliovirus strain[a]	Mean values of $\log_{10}$ reciprocal NT titres of each age group (1–10,11–20,21–30 or 31–40 years) against polioviruses of the same serotype[b]			
			1–10	11–20	21–30	31–40
1	Sabin1	2.80	3.00 (p 1.0)	*2.45* (p 0.087)	*2.10* (p 0.087)	*2.40* (p 0.087)
	742	*2.35* (p 0.014)				
	7/b/97	2.80 (p 1.0)				
	522	*2.35* (p 0.014)				
	II	2.42 (p 0.080)				
	152	2.20 (p 0.066)				
2	Sabin2	2.35	3.02 (p 1.0)	2.54 (p 1.0)	*2.16* (p 1.0)	*2.16* (p 1.0)
	134	2.35 (p 1.0)				
	EP9	2.42 (p 0.761)				
	EP12	2.58 (p 0.215)				
	ID	2.50 (p 0.182)				
	IF	2.50 (p 0.182)				
	8001	2.58 (p 0.058)				
3	Sabin3	2.27	2.99 (p 1.0)	2.24 (p 1.0)	1.63 (p 1.0)	1.86 (p 1.0)
	EPC	2.42 (p 0.495)				
	EPB	2.12 (p 0.182)				
	738	1.97 (p 0.092)				
	584	2.27 (p 1.0)				
	EPA	2.12 (p 0.182)				
	K/2002	1.97 (p 0.092)				
	EP16	2.27 (p 1.0)				
	EP23	2.12 (p 0.182)				

[a]Means which are in italics have significant differences according to Student's *t*-test.
[b]Means which are in italics in each poliovirus serotype have no significant differences according to ANOVA test.

the 31–40-year age group for poliovirus types 1 and 3, but not for poliovirus type 2. However, this increase was significant only for poliovirus type 3. For all three types, the highest NT titres were observed in the 1–10-year age group, indicating a good response to vaccination. The lowest NT titre was observed in the 21–30-year age group

against poliovirus type 3. This indicates an unsatisfactory level of immunity against poliovirus type 3 in young adults. These results are consistent with those of some previous studies [18–20].

In general, the serological status of the population of southern Greece with regard to poliovirus is better for types 1 and 2 than for type 3. The presence of the lowest NT titre in the 21–30-year age group against poliovirus type 3 suggests the need for a booster dose of monovalent Sabin3 vaccine to ensure personal and herd immunity. Moreover, the higher NT titres of the 31–40-year age group against polioviruses 1 and 3 than those of the 21–30-year age group could be attributed to the acquisition of antibodies following natural infection with circulating poliovirus strains of types 1 and 3 during the first 10 years of their life (time period: 1967–1977). Moreover, a booster effect from babies to their parents could contribute to the rise in NT titres observed in the 31–40-year age group. In contrast, the 21–30-year and 31–40-year age groups displayed the same NT titres against poliovirus strains of type 2, and this could be explained by the earlier eradication of poliovirus type 2 than of types 1 and 3 from Greece and worldwide, as their worldwide transmission was successfully interrupted after 1999 [21].

The existence of circulating VDPVs, their ability to produce outbreaks and the fact that they exhibit pathogenicity similar to that of wild-type strains has significantly changed the risk–benefit analysis associated with the final stages of the polio eradication campaign. It has become obvious that the emergence of populations of unvaccinated individuals following OPV cessation could risk restarting a polio pandemic caused by either circulating VDPV or wild-type polioviruses. Today, following the replacement of OPV by IPV in most developed countries, well-maintained herd immunity is a priority. It is tempting to assume that the changes in antigenic properties frequently observed in OPV derivatives represent a selection of viral variants that are less prone to be neutralized by human antibodies. Taking into consideration that IPV does not induce the same immunity as OPV, the need for immunological studies in all age groups is urgent in order to avoid epidemics due to the circulation of highly evolved OPV derivatives and the importation of wild-type polioviruses from endemic countries.

Transparency Declaration

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