

Surveillance reports

SEROPREVALENCE OF ANTIBODIES TO POLIOVIRUS IN INDIVIDUALS LIVING IN PORTUGAL, 2002

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The last case of poliomyelitis in Portugal caused by indigenous wild poliovirus occurred in 1986 and the country was declared polio-free in 2002. High levels of immunity must be maintained to prevent the importation of wild poliovirus. In this study, we determined the immunity against poliomyelitis of the Portuguese population in order to identify possible immunity gaps. A representative sample of 1,133 individuals older than two years residing in mainland Portugal was studied. Logistical difficulties regarding quick sample transportation precluded the Portuguese islands (Madeira and the Azores) from this study. Sera were collected in 2002 from individuals attending health clinics throughout the 18 districts of Portugal. Levels of neutralizing antibodies against poliovirus types 1, 2 and 3 were determined and a titre of $\geq 1:8$ was defined as indicative of protected immunity. Results were expressed in international units. The antibody prevalence and the geometric mean antibody concentration (GMAC) was 91.6% (GMAC: 2.96 IU/ml), 94.2% (GMAC: 5.03 IU/ml) and 75% (GMAC: 0.53 IU/ml) for poliovirus types 1, 2 and 3, respectively. For poliovirus types 1 and 2, antibody prevalence was close to or above 90% in the majority of age groups. For poliovirus type 3, antibody prevalence was below 80% in teenagers and young adults. Our study shows that the Portuguese are well protected against poliovirus types 1 and 2. For poliovirus type 3, the suboptimal antibody levels observed in teenagers and young adults suggest the need for a booster dose to minimise the risk of wild poliovirus importation.

Introduction

Global immunisation campaigns against poliomyelitis promoted by the World Health Organization (WHO) have resulted in the elimination of this disease from several regions [1]. In Portugal, the last case of poliomyelitis caused by indigenous wild poliovirus occurred in 1986 and the country was certified polio-free in 2002 [2]. Despite imminent eradication, small reservoirs of indigenous transmission persist in Africa and Asia [1]. Thus, there is still a danger of importation of wild poliovirus to polio-free countries, as reported recently [3,4]. Portugal, in particular, could be at risk if protective immunity levels are not sufficiently high, given its close ties with several African countries, including Angola and Cape Verde where outbreaks of poliomyelitis occurred in 2005 and 2000, respectively [4,5].

Mass immunisation against poliomyelitis in Portugal began with a vaccination campaign in 1965, when children aged from three months to nine years were offered two doses of live, attenuated, trivalent oral polio vaccine (tOPV). Subsequently, the national vaccination program has included the administration of three doses of tOPV in the first year of life and since 1990 a tOPV booster at 5-6 years of age. Vaccination coverage has gradually increased since 1965, and since 1991 has reached > 90% of the population at

one year of age [6]. The last reported case of vaccine-associated paralytic poliomyelitis (VAPP) occurred in 1995. To prevent further VAPP cases and the circulation of neurovirulent vaccine-derived polioviruses, tOPV was replaced by inactivated polio vaccine (IPV) in the childhood immunisation schedule in January 2006.

High vaccination coverage and the effective surveillance of acute flaccid paralysis are essential for preventing the re-emergence of wild poliovirus. Additionally, serological surveys are useful for identifying groups with low-immunity that could be at risk of infection. We have determined the immunity of the Portuguese population against poliomyelitis. This study was part of a national serological survey conducted in 2002 aimed at assessing the immunity of the Portuguese against vaccine-preventable diseases [7].

Methods

Study population

The national serologic survey aimed at estimating the percentage of the Portuguese population with antibodies against 15 etiologic agents. The target population, estimated to be 10.3×10^6 by the 2001 census [8], was stratified by eight age groups: 2-4, 5-9, 10-14, 15-19, 20-29, 30-44, 45-64, and >64 years old. Sample sizes aimed at estimating the prevalence of seropositives, p , were computed assuming that p has a normal sampling distribution. If a maximum absolute error of $d=0.05$ is tolerated with 95% probability ($z_{\alpha/2}=1.96$), when estimating the proportion of seropositives at age group i , then an *a priori* conservative estimate of $p=0.5$ leads to a sample size of $n_i = z_{\alpha/2}^2 p(1-p)/d^2 = 384$ [9]. With eight age groups, this prompts a theoretical total sample size of $n=(384 \times 8)=3072$ individuals. For each age group, the theoretical sample of 384 was distributed to the 18 geographic districts of mainland Portugal, in proportion to their population size.

As part of the national serological survey in 2002, blood samples were collected from individuals attending a network of health-care clinics present throughout the 18 districts of mainland Portugal where routine blood tests are carried out. An extra 10 ml of blood were collected from individuals older than 10 years and 2 ml from children aged two to 10 years. Individuals were randomly sampled as they arrived in order to fulfil the required sample size by age group in their district. Eligible individuals had to be older than 24 months and resident in the district for the past six months. All participants or their guardians (for individuals younger than 18 years) gave written consent allowing extra blood to be taken for this study. Data regarding birth date, sex, nationality, previous known diseases and reason for showing up at the clinic were collected for each donor.

TABLE 1

Number of collected samples for each age group compared to the required sample size (n=384), mainland Portugal, 2002

Age group	2 - 4	5 - 9	10 - 14	15 - 19	20 - 29	30 - 34	45 - 64	> 64	Total
Sample available	327	435	402	340	520	582	541	378	3525
Difference to 384	-57	+51	+18	-44	+136	+198	+157	-6	+453

TABLE 2

Number of samples used for each age group for measuring anti-polio antibodies compared to the required sample size (n=138), mainland Portugal, 2002

Age group	2 - 4	5 - 9	10 - 14	15 - 19	20 - 29	30 - 34	45 - 64	> 64	Total
Sample available	159	135	136	136	152	146	128	141	1133
Difference to 138	+21	-3	-2	-2	+14	+8	-10	+3	+29

A total sample of n=3,525 serum samples was collected, larger than the theoretical n=3,072, but not fulfilling the required sample size for every age group (Table 1). The need to survey 15 etiologic agents from a relatively small blood sample per person, plus the values in deficit shown in Table 1, led us to consider more realistic a priori values for p in the population. In the case of polio, given that mass vaccination is universal since 1965 and that the vaccination coverage is high [6], we set an *a priori* estimate of $p=0.9$. A tolerated error of $d=0.05$ in the estimated proportion of seropositives for polio, at age group i , thus leads to a theoretical sample of $n_i=138$ by age group. A total of 1,133 serum samples (475 from males and 657 from females) were screened for the presence of anti-polio antibodies. The distribution of this sample falls close to the theoretical requirement of $n_i=138$ per age group (Table 2).

Antibody neutralization assays

The titre of neutralizing antibodies against poliovirus types 1, 2 and 3 was determined by microneutralization assay [10]. Sera were diluted two-fold beginning from 1:8 to 1:1024, in duplicate, and each dilution was incubated for three hours at 36°C with 100x50%-cell culture infectious dose of poliovirus strains Sabin 1, 2 or 3 (NIBSC, UK). The virus-serum mixtures were added to Hep-2 cells and, after a five-day incubation at 36°C, the cytopathic effect was assessed by phase contrast microscopy. Titres were calculated as the reciprocal of the highest dilution that protected 50% of the cultures against challenge virus and a titre $\geq 1:8$ was defined

as indicative of protective immunity. Titres were converted to IU/ml by comparison with the titre of an in-house reference serum (IHS) of known potency. The potency of the IHS was determined by comparison with the titre of an International Standard Serum (NIBSC, UK) as described previously [10]. Titres of test serum were converted to IU/ml by dividing the serum titre by the geometric mean titre (GMT) of the IHS and multiplying by the potency of the IHS.

Data analysis

Data were inserted into an Access database. Analysis of the results consisted of the determination of relative frequencies of protective immunity, geometric mean titres and respective 95% confidence intervals using SPSS 11.01 software.

Results

Our data indicated that a titre of 1:8 corresponded to 0.331 IU/ml, 0.667 IU/ml and 0.151 IU/ml for poliovirus types 1, 2 and 3, respectively, and the geometric mean of antibody concentration for test sera were 2.96 IU/ml (95% confidence interval (CI): 2.73-3.20) for poliovirus type 1, 5.03 IU/ml (95% CI: 4.68-5.41) for poliovirus type 2 and 0.53 IU/ml (95% CI: 0.50-0.57) for poliovirus type 3.

The overall antibody prevalence was 91.6%, 94.2% and 75.1% for poliovirus types 1, 2 and 3, respectively (Table 2). For poliovirus types 1 and 2 the antibody prevalence was highest in children

TABLE 3

Age-specific antibody prevalence for poliovirus types 1, 2, and 3 in individuals residing in mainland Portugal, 2002 (n=1,133)

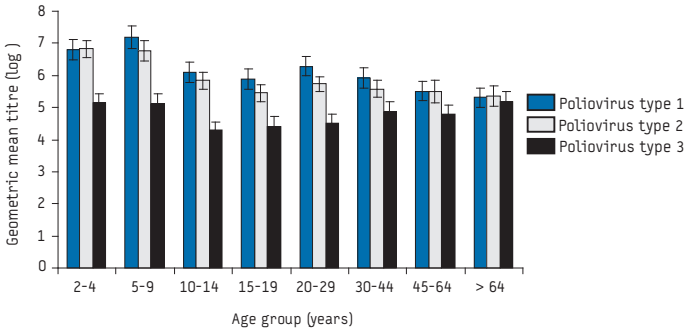
Age group	n tested	Poliovirus type 1		Poliovirus type 2		Poliovirus type 3	
		%	95% CI*	%	95% CI*	%	95% CI*
2 - 4	159	93.1	88.0 - 96.5	98.7	95.5 - 99.8	84.9	78.4 - 90.1
5 - 9	135	99.3	95.9 - 100.0	100.0	97.3 - 100.0	83.7	76.4 - 89.5
10 - 14	136	93.4	87.8 - 96.9	95.6	90.6 - 98.4	68.4	59.9 - 76.1
15 - 19	136	91.2	85.1 - 95.4	95.6	90.6 - 98.4	56.6	47.9 - 65.1
20 - 29	152	93.4	88.2 - 96.8	98.7	95.3 - 99.8	73.0	65.2 - 79.9
30 - 44	146	87.7	81.2 - 92.5	95.2	90.4 - 98.1	72.6	64.6 - 79.7
45 - 64	128	88.3	81.4 - 93.3	82.0	74.3 - 88.3	80.5	72.5 - 86.9
> 64	141	86.5	79.8 - 91.7	85.8	78.9 - 91.1	80.1	72.6 - 86.4
Total	1133	91.6	89.8 - 93.2	94.2	92.6 - 95.5	75.1	72.5 - 77.6

* CI= confidence interval

aged 5-9 years and was close to or above 90% in the majority of age groups (Table 3). For these two serotypes, antibody titres were highest in children (5-9 years), then decreased in teenagers, but were relatively stable thereafter (Figure). For poliovirus type 3 we observed lower antibody prevalence in all age groups and this was mirrored by lower antibody titres against this serotype (Table 3 and Figure). The antibody prevalence was close to 85% in children younger than 10 years and then decreased to levels below 70% in teenagers (10-19 years) or and to 70%-80% in young adults (20-44 years) (Table 3). Antibody titres were lowest in persons aged 10-29 years and reached highest levels in children up to nine years and persons older than 30 years (Figure).

FIGURE

Figure. Age-specific geometric mean titres, mainland Portugal, 2002. Whisks represent 95% confidence intervals



We considered three birth cohorts: persons born before 1956 who were not eligible for childhood vaccination; persons born between 1956 and 1964 who represent the first vaccinated cohorts; and persons born after 1964 who would have followed the complete vaccination schedule since birth (Table 4). For poliovirus types 1 and 2 antibody prevalence was highest in persons born after 1964, whereas for poliovirus type 3, the percentage of seropositives was highest in persons born before 1956 (Table 4).

Although there were no overall differences between seroprevalence of male and female individuals, we found that women older than 30 years had better protection than males against all polioviruses (data not shown).

Discussion

Our results show that the Portuguese are well protected against poliovirus types 1 and 2 in most age groups. Additionally, children

had very high antibody prevalence and presented the highest antibody titres, consistent with a good response to immunisation and high anti-polio vaccination coverage. The decrease in titre observed in teenagers is most likely due to waning immunity, which is faster in the initial years following vaccination [11]. However we can not exclude the possibility that these lower titres are due to a failure in receiving a booster dose at 5-6 years of age.

Lower prevalence and antibody titres were observed for poliovirus 3. These results are similar to those of other European countries such as Greece [12], Germany [13], the Netherlands [14] and Italy [15] and with the lower seroconversion rates observed for poliovirus type 3 following vaccination with OPV [16]. These observations may be explained by a lower potency of poliovirus type 3 antigens in the vaccine. For this serotype, suboptimal levels of protection were observed, particularly in teenagers and young adults. This has been reported in other countries in Europe [12, 15]. Nevertheless, seroprevalence in children was high as expected under high vaccination coverage. Furthermore, we retested all negative sera at a single dilution of 1:4 and found that for poliovirus type 3 the seroprevalence increased significantly (88.9% seropositives) and was closer to or above 90% in most age groups (Table 5). These results suggest that despite the lower antibody levels against poliovirus type 3 a large proportion of individuals had been primed. The suboptimal antibody prevalence observed in teenagers and young adults is therefore most likely due to waning immunity. It is generally accepted that the presence of antibodies at a dilution of 1:8 confers immunity against polio. Individuals with lower or undetectable antibody levels may be protected from disease by memory immunity that provides a rapid immune response to infection. However, they may be susceptible to re-infection [17]. Examples of importations in Albania and Namibia stress the risk of an age-dependent immunity gap [18,19]. Thus to improve immunity to poliovirus type 3 and minimize the risk of wild poliovirus importation a booster dose in teenagers may be required.

For poliovirus types 1 and 2, antibody prevalence was highest in individuals who most likely acquired immunity through vaccination (persons under 37 years in our study), rather than contact with wild poliovirus, which reinforces the success of anti-polio vaccination in Portugal. Still, a large proportion of persons who would have acquired immunity naturally were seropositive, suggesting that naturally-induced immunity is long-lasting, as described previously [14,20]. Interestingly, for poliovirus type 3, the antibody prevalence was higher in groups born before the vaccination era and the elderly (>64 years) had antibody levels similar to recently vaccinated children. A possible explanation for this result is that immunity

TABLE 4

Antibody prevalence for poliovirus types 1, 2, and 3, according to birth cohort, mainland Portugal, 2002 (n=1,133)

	Poliovirus type 1		Poliovirus type 2		Poliovirus type 3	
	%	95% CI*	%	95% CI *	%	95% CI*
cohort						
Born 1955 (n=261)	87.7	83.1, 91.5	83.9	78.9, 88.1	79.7	74.3, 84.4
Born between 1956-64 (n=90)	87.8	79.2, 93.7	93.3	86.1, 97.5	74.4	64.2, 83.1
Born 1965 (n=782)	93.4	91.4, 95.0	97.7	96.4, 98.6	73.7	70.4, 76.7

• CI= confidence interval

TABLE 5

Analysis of age-specific seroprevalence using a 1:4 titre^a, mainland Portugal, 2002 (n=1,133)

Age group	n	Poliovirus type 1		Poliovirus type 2		Poliovirus type 3	
		%	95% CI*	%	95% CI*	%	95% CI*
2 - 4	159	95.0	90.3 - 97.8	98.7	95.5 - 99.8	92.5	87.2 - 96.0
5 - 9	135	100.0	97.3 - 100.0	100.0	97.3 - 100.0	93.3	87.7 - 96.9
10 -14	136	94.9	89.7 - 97.9	97.1	92.6 - 99.2	86.0	79.0 - 91.4
15 - 19	136	92.6	86.9 - 96.4	96.3	91.7 - 98.4	80.9	73.5 - 86.6
20 - 29	152	95.4	90.8 - 97.8	98.7	95.3 - 99.6	94.1	89.1 - 96.9
30 - 44	146	93.2	87.9 - 96.2	97.9	94.1 - 99.3	86.3	79.8 - 91.0
45 - 64	128	93.8	88.2 - 96.8	91.4	85.3 - 95.1	90.6	84.3 - 94.6
> 64	141	97.2	92.9 - 99.2	95.7	91.0 - 98.4	86.5	79.8 - 91.7
Total	1133	95.2	93.8 - 96.4	97.1	95.9 - 98.0	88.9	86.9 - 90.7

^aResults combine previously seropositive results at 1:8

*CI= confidence interval

induced by exposure to wild poliovirus type 3 antigens, circulating before mass vaccination, is stronger than vaccine-induced immunity.

This study allows one to draw conclusions on the seroprevalence of the whole Portuguese population. However, important subpopulations, such as immigrant communities, were not specifically examined. We can not exclude the existence of low-immunity pockets in the population that were not detected in our study. Surveys aimed at determining anti-polio immunity in subpopulations as well as in the general population, to evaluate the impact of introducing IPV, should be carried out. We have expressed results as titres and in international units, to facilitate comparison of our data with that of future studies.

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