Original article

Status of immunity against poliomyelitis: a study among european and extra-european young immigrants living in Parma

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Abstract. From January 2001 to December 2003 blood specimens obtained from 323 European and extra-European young immigrants were collected for the determination of anti-polio antibody levels. They were sent to the Section of Hygiene, Department of Public Health, by the Local Health Authorities. A neutralization assay was performed to detect the anti-polio antibodies against serotypes 1, 2 and 3, using rhesus monkey kidney cells. The results showed 98,1% prevalence of anti-polio 1 antibodies (titre ≥1:2), 99,1% of anti-polio 2, and 98,8% prevalence of anti-polio 3 antibodies. The seronegativity against only one or two serotypes (antibody titre <1:2) was found in 9 subjects (2,8%) while no subject was found totally seronegative against all 3 serotypes. According to the total amount of the analyzed samples, the estimated Geometric Mean Titre (GMT) resulted from moderate to low (GMT=29 of serotype 3; GMT=48,4 of serotype 2; GMT=56,5 of serotype 1) and it appeared to be similar to the results obtained in the previous years for all 3 serotypes; the GMT of serotype 3 was still the lowest. No difference was observed in the specimens with respect to the country of origin of the examined subjects and the data collected showed a good level of immunity. (www.actabiomedica.it)

Key words: Serological survey, immunity, immigrants, poliomyelitis

Introduction

Poliomyelitis was one of the most feared infectious diseases until the 1950s. In Italy, records showed 4,000 to 8,000 cases/year, but thanks to extensive vaccination campaigns, first with the Salk vaccine (1959) and then with the Sabin vaccine (1964), and to the decision of making polio vaccination mandatory for all newborns (L. No. 51 of February 4,1966), polio became rarer and rarer, until it virtually disappeared in the early 1980s. The last two indigenous paralytic polio cases date back to 1982; in the following ten years only a few cases were recorded among immigrants (three non-vaccinated children from Libia, Iran and India) while others were cases of vaccine-associated paralytic poliomyelitis (VAPP) (1).

During the 1980s the World Health Organization (WHO) reported a mean of 35,000 occurrences per year worldwide (with a real estimate of 350,000 cases), mostly recorded in developing countries (2).

In 1974 the World Health Assembly (WHA) established the EPI (Expanded Program on Immunization), in order to make basic vaccines, oral polio vaccine (OPV) included, available for children worldwide. In 1985 the Pan American Health Organization (PAHO) began a program for the eradication of polio on the American continent by the year 1990; however, it was a 1988 WHA resolution that established the eradication of poliomyelitis worldwide by the year 2000 (3) and that started to produce concrete results (4). Thanks to the consistent implementation of vaccination strategies, the number of endemic countries

decreased from 125 (5) in 1988 to 6 in 2004 with a 60% reduction of paralytic polio cases (6).

These six endemic countries are: Nigeria, India, Pakistan, Egypt, Niger, and Afghanistan (7). Up to October 2004 the number of polio cases in these regions was 851, 75% (637 cases) only in Nigeria (8); the wild virus could also have spread from Nigeria into the neighbouring countries of Chad, Ivory Coast (9), and maybe of Sudan, that had not experienced any polio cases in the previous three years (10).

Twenty-three polio cases among immigrants in countries considered free from polio, such as Burkina Faso, Benin, Cameroon, Chad, Ghana, and Togo were already signalled in 2003 (11) and a genetic analysis of the isolated strains showed that they were related to the same strains circulating in northern Nigeria in 2002-2003 (5).

In the European Region, the last phase of polio eradication started in 1995 and provided for mass immunization campaigns in 18 endemic countries (Operation MECACAR: Eastern Mediterranean, Caucasus region, and Central Asia Republics) (12). In Europe, two important polio outbreaks were reported in the 1990s: one in 1992-1993 in Holland (71 cases) (2), the other in Albania in 1996, during a period of political and social disorder, with 139 confirmed cases (mainly in non- or partially vaccinated children and in young adults). Also, in 1996, sporadic cases were registered in the Federal Republic of Yugoslavia (24 cases), Turkey (19 cases), Greece (5 cases), Russian Federation (3 cases), and Ukraine (1 case) (13).

Since 1998 no other cases have been recorded (12) but in recent times (April 2001) three polio cases have been registered in Bulgaria (Bourgas and Sofia districts) in non-vaccinated gipsy children, and wild type 1 poliovirus antigenically linked to a strain circulating in India in 2000 was isolated from two of them (14).

These cases were considered to be 'imported' and did not compromise the opportunity for the European Region to be certified as polio-free; the Certification was obtained on June 21st, 2002 during the 14th Regional Commission Certification Meeting (Copenhagen) (1) when Europe was also declared polio-free after the Americas (1994) and after the Western Pacific Region (2000) (2).

In Italy, since it has been free of autoctonous polio cases for more than ten years, and because of certification of this condition issued by the WHO, a modification in the vaccine administration was introduced. The OPV has been gradually substituted with the enhanced potency inactivated polio vaccine (eIPV) (1st and 2nd doses on April 7, 1999; 4 doses on June 18, 2002) in order to avoid the circulation of any attenuated (i.e. weakened) poliovirus in the environment (15).

Keeping the polio-free status until the complete eradication of polio worldwide, demands the continuous monitoring of the population immunization levels and of the circulation of enteric viruses in order to exclude the presence of poliovirus. This is especially important for Italy that has become a destination for many immigrants coming from countries with low sanitation levels. The presence of infected and receptive people could represent a serious danger for the community.

For all these reasons we thought it was important to analyze the immunization levels of immigrants living in Parma, who contacted the Health Authorities to be a part of schools and workplaces.

Methods

This study was carried out between January 2001 and December 2003 and involved 323 immigrants coming from the European Union and from extra-European areas. The age range was 1-30 years old with an average of 12.72±4.641. The obtained serum samples were sent to our Section by the Local Health Authorities.

Table 1 reports age and WHO[1] region of origin of all the examined subjects.

Methods description

The serum samples were sterily collected and kept at -20°C until they were examined. The determi-

[1] AFRO=Africa Region, AMRO=Americas Region, EMRO=Eastern Mediterranean Region, EURO=Europe Region, SEARO=South-East Asia Region, WPRO=Western Pacific Region

Table 1. Sampling

WHO Region	1-5 years		6-10 years		11-15 years		≥ 16 years		Total	
	No. subjects	%	No. subjects	%	No. subjects	%	No. subjects	%	No. subjects	%
AFRO	9	2.8	19	5.9	46	14.2	44	13.6	118	36.5
AMRO	3	0.9	16	5	11	3.4	4	1.2	34	10.5
EMRO	5	1.5	8	2.5	14	4.3	13	4	40	12.4
EURO	7	2.2	13	4	30	9.3	36	11.1	86	26.6
SEARO	4	1.2	4	1.2	6	1.9	5	1.5	19	5.9
WPRO	0	0	3	0.9	19	5.9	4	1.2	26	8
Total	28	8.7	63	19.5	126	39	106	32.8	323	100

nation of the three polioviruses antibody levels was carried out with a long incubation neutralization assay using rhesus monkey kidney cells (RC37) (16).

The sera were kept at 56°C for 30 minutes and then tested into microtiter plates (flat bottom, sterile) simultaneously at dilutions, from 1/2 to 1/512 with polioviruses type 1, type 2 and type 3, respectively. In each dilution series 100 TCID50 of type 1 poliovirus (Mahoney strain), type 2 (Mef1 strain) and type 3 (Saukett strain) were added. The serum/virus mixtures were then incubated at 37°C for 6 hours in an appropriately humidified CO2 incubator and then at 4°C for 18 hours. Aliquots of 0,050 ml of cellular suspension (5-6 x 10^4 RC 37-cells) were added to each well. The microplates were placed at 37°C and microscopically observed for cytopathic effect on the third and fourth days (17).

The antibody titre concerning the three polioviruses was defined as the reciprocal value of the highest dilution of the serum showing a complete neutralization of the cytopathic effect.

Each reaction contained controls for antigens and cells; a serum with known titre towards the three antigens was used as an internal control.

Statistical analysis

We calculated the geometric mean titre (GMT) of all sera with an antibody titre greater or equal to 1:2. In order to compare the frequencies we used the Pearson Chi-squared test with the Fischer correction where necessary; to compare the averages we performed an analysis of the variance (ANOVA) and to verify the link between GMT and age we carried out simple correlation and linear regression tests.

Results

Table 2 shows the results of the antibody titres neutralizing the three polioviruses in relationship with the age range.

If we consider the 1:8 titre to be protective against poliomyelitis according to the WHO guidelines (18-21), 95% of the examined subjects is protected against poliovirus type 1, 96.3% against poliovirus type 2, and 91% against poliovirus type 3. These percentages increase establishing seropositivity starting at the 1:2 titre (98.1% against poliovirus 1, 99.1% against poliovirus 2, and 98.8% against poliovirus 3).

Table 2. Distribution of neutralizing antibodies against the three polioviruses with relation to age groups

Age groups	No. subjects	Polio 1				Polio 2				Polio 3			
		<1/2	≥1/2	<1/8	≥1/8	<1/2	≥1/2	<1/8	≥1/8	<1/2	≥1/2	<1/8	≥1/8
1-5 years	28	0	28	2	26	0	28	1	27	0	28	1	27
6-10 years	63	1	62	3	60	1	62	2	61	0	63	2	61
11-15 years	126	3	123	6	120	1	125	7	119	3	123	16	110
≥16 years	106	2	104	5	101	1	105	2	104	1	105	10	96
Total	323	6	317	16	307	3	320	12	311	4	319	29	294
%		1.9	98.1	5	95	0.9	99.1	3.7	96.3	1.2	98.8	9	91

Table 3. Seronegative subjects at dilution1/2

Country of origin	WHO region	Age	P1	P2	P3
Cote D'Ivoire	AFRO	16	1:32	1	1
Venezuela	AMRO	8	1	1	1:2
Colombia	AMRO	13	1	1:2	1:4
Morocco	EMRO	13	1:8	1	1
Tunisia	EMRO	15	1	1:2	1
Albania	EURO	16	1	1:16	1:2
Turkey	EURO	12	1:4	1:2	1
China	WPRO	14	1	1:16	1:4
Philippines	WPRO	17	1	1:16	1:64

The absence of antibodies in serum diluted 1/2 was observed 13 times (table 2), but in reality the results refer to 9 individuals: in 5 subjects the absence of antibodies concerned only 1 serotype (4 times for poliovirus 1, and one for poliovirus 3) while in 4 other examined serum samples it was linked to 2 serotypes (poliovirus 1+2, poliovirus 1+3, and poliovirus 2+3) (table 3).

However, in a further examination of the non-diluted serum we found antibodies in all these 9 subjects. Analysis concerning age and origin of these 9 individuals showed an age range of 8-17 years and 9 different birth countries (table 3).

The highest GMT is linked to poliovirus type 1 (GMT=56,5), followed by the type 2 poliovirus (GMT=48,4); poliovirus type 3 showed the lowest and statistically significant titre (GMT= 29) ($p<0.001$) (Figure 1).

Analyzing the GMT in relationship with the age distribution, the highest titres are linked to the 1-5 age range, and are significantly higher if connected with the type 1 poliovirus. The lowest GMT resulted for type 3 poliovirus in the 6-10 age range (Figure 1).

Table 4 shows results with relation to the WHO country of origin. The seropositivity percentages are very similar in subjects coming from different areas; however, some differences emerge in the analysis of the GMT (Figure 2).

The GMT concerning poliovirus 1 is highest in almost all WHO regions, especially in subjects coming from the European Region (GMT= 113.33); on the contrary, in the same subjects it has been observed that the lowest GMT is linked to the type 3 poliovirus (GMT=23.23), with the exception of the WPRO region, where the type 2 poliovirus shows the highest GMT (49.04) followed by poliovirus type 3 that shows the highest values overall (GMT=41.63).

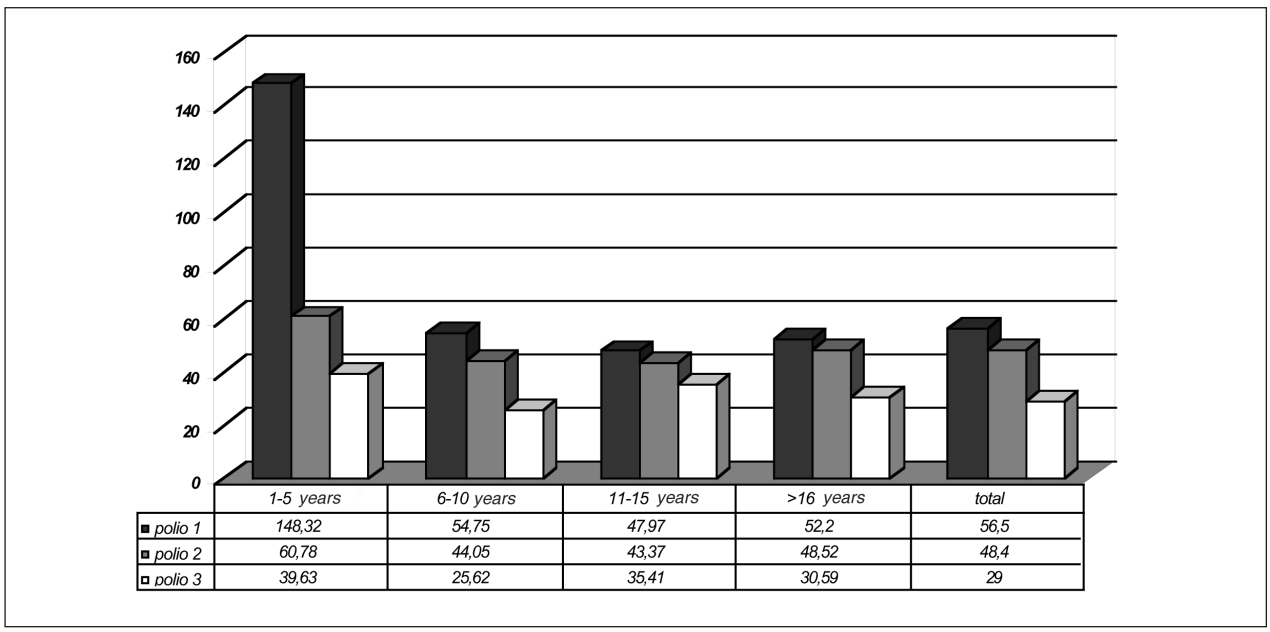

Figure 1. Geometric Mean Titre related to age groups

Table 4. Distribution of antibodies that neutralize the three polioviruses with relation to the country of origin

WHO Region	No. subjects	Polio 1			Polio 2			Polio 3		
		<1/2	≥1/2	≥1/8	<1/2	≥1/2	≥1/8	<1/2	≥1/2	≥1/8
AFRO	116	-	116	110	1	115	113	1	115	108
AMRO	34	2	32	30	1	33	32	-	34	32
EMRO	42	1	41	41	1	41	40	2	40	37
EURO	86	1	85	83	-	86	82	1	85	77
SEARO	19	-	19	19	-	19	18	-	19	18
WPRO	26	2	24	24	-	26	26	-	26	22
Total	323	6	317	307	3	320	311	4	319	294
%		1.86	98.14	95.05	0.93	99.07	96.28	1.24	98.76	91.02

Discussion and conclusions

The control of polio disease depends on an appropriate immunization coverage and an efficient monitoring system.

Altough governments and humanitarian organizations have made numerous efforts in organizational and monetary terms, in many African countries the vaccination coverage against poliomyelitis has not reached optimum levels. Wars and especially religious beliefs, have represented obstacles for a thorough diffusion of polio vaccination and therefore have put the population at risk of a possible reintroduction of the polio virus, even in countries that had been polio-free for years (22, 23). The existence of susceptible groups living in degraded economic and hygienic conditions, could originate new epidemics potentially hard to circumscribe.

The local and international monitoring systems play an essential role, thanks to the diagnostic systems that can identify the genetic origins of epidemic clusters, providing in this way an immediate awareness of the epidemiologic changes in the community.

In Italy, the polio-free certification obtained in 2002 is the result of the high vaccination coverage rates achieved in these years (third dose of vaccine, 96%) and of the efficient surveillance system of Acute Flaccid Paralysis (AFP), which is coordinated by

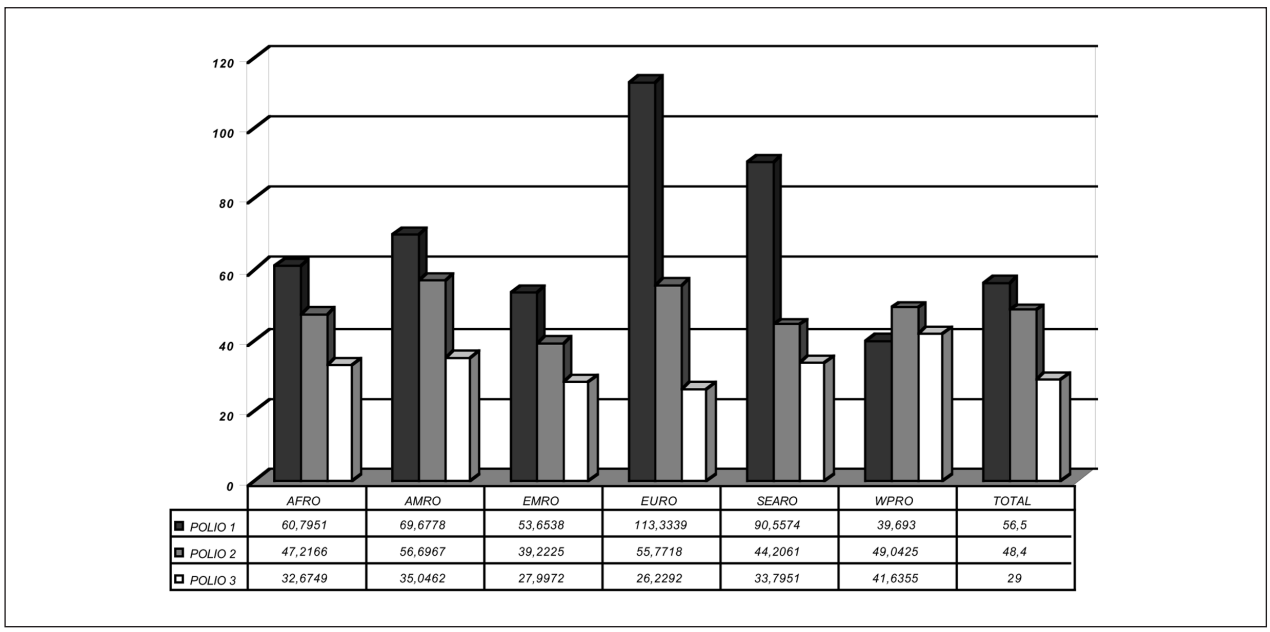

Figure 2. Geometric Mean Titre related to WHO Regions of origin

Table 5. GMT and seronegativity data with relation to the three polioviruses in young immigrants compared to previous research data

	Years 89-91		Years 92-93		Years 94-00		Years 01-03	
	GMT	N.<2	GMT	N.<2	GMT	N.<2	GMT	N.<2
Polio 1	19.43	4	25.26	5	43.22	8	56.5	4
Polio 2	34.06	-	49.67	-	41.94	4	48.4	3
Polio 3	19.34	5	24.67	-	33.56	7	29	3
Seronegativity	(9/111) 9,1%		(5/93) 5,3%		(15/506) 2,9%		(10/323) 3,1%	

the National Health Institute since 1997. In order to preserve the polio-free status, a continuous monitoring of the environment and population is important, since polioviruses (wild viruses or retro-mutants viruses) could be introduced in Italy by immigrants coming from endemic areas. This risk should not be underestimated since national data shows a constant increase in foreign residents: between 2000 and 2004 the percentage raised from 1.8 % to 5.7 % (24).

In the province of Parma data regarding foreign residents shows a presence of 3.1% in 1999 and of 5.8% in 2004 (24). In particular, 17% of the subjects (56) examined in our study come from areas that have been recently struck with polio outbreaks.

Although the group analyzed in this survey is not completely representative of the immigrant population, it still gives a good idea of the immunization status of those who wish to be integrated into the Italian communities. This group is made up of young adults living in Italy for work or educational reasons and of children joining their families in Italy. They are probably health conscious and aware that it is important to be part of the community they live in.

With respect to the results obtained in our previous surveys, data collected in this study revealed a better immunization status of the examined subjects, with higher antibody titres and a lower seronegativity frequency (Table 5).

Antibody titres ≥1:2 have been found in 98-99% of the collected serum samples. But all of the 323 examined subjects had antibodies, because also the 9 subjects with antibody titres <1:2 on one or two serotypes showed some level of immunity according to the analysis of the non-diluted serum carried out using a very sensible and specific method (25). This result is particularly important considering that even low levels of neutralizing antibodies create the condition for an immunological memory that can promptly respond to antigen stimuli and prevent potential viremias (26).

The antibody titres are constantly higher in connection with type 1 and 2 poliovirus (GMT=56.5 and 48.4 respectively) and progressively lower for poliovirus 3, considering both the sample size and the country of origin. This is in agreement with the data obtained in previous studies showing constantly lower immunization levels for poliovirus type 3 (1, 27, 28). With regards to the country of origin we noted an exception in the WPRO region where the GMTs are higher for the type 2 poliovirus and absolutely lowest for the type 1 poliovirus (GMT=49.06 and 39.69 respectively).

Analyzing the GMT obtained in relationship with the age of the examined subjects, we found significantly higher GMT values in the youngest age range (Univariate Anova $p=0.037$, Test post hoc LSD $p<0.001$).

The results of this serological survey offer a rather optimistic view regarding the status of poliomyelitis among immigrants. However these findings cannot be applied to the total immigrant population because of the small sample size and because of illegal immigrants. Illegal immigrants are an issue for our country, because it is difficult to determine how many they are, their origin, and their sanitary conditions. For these reasons it would be wrong to apply also to them the data we collected from those who contacted the Public Hygiene Services and are willing to be part of the Italian society.

Therefore, we recommend a continuous and better offer of serological check-ups for all immigrants and in particular for those who come from endemic areas and are not aware of their immunization history.

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