O R I G I N A L   A R T I C L E

Immunity status against poliomyelitis in young migrants: a seroprevalence study

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Summary. *Background and aim of the work:* Recent seroprevalence studies in different population groups have shown low antibody titers against poliomyelitis, especially in young adults. This, together with the reduction of vaccination rates, could favor the reintroduction of poliovirus in long-time polio-free countries. Within the Surveillance system of acute flaccid paralysis, a prevalence study was conducted to estimate the immunological status associated with poliomyelitis in young migrants. *Methods:* Local Health Authority collected serum samples in young migrants, without vaccination documentation. Antibodies levels were assessed with a long incubation neutralization assay. Subjects were stratified by age and by WHO region. Seroprotection was defined by a titer equal or above 1:8 and titers > 1:2 were log-transformed and evaluated as geometric mean titers (GMTs). *Results:* From January 2004 to August 2017, 1138 blood samples were collected. Mean age was 13.3 years with no differences between WHO regions. The percentage of antibody titers below 1:8 was 6.0% versus poliovirus 1 (PV1), 7.7% versus poliovirus 2 (PV2) and 15% versus poliovirus 3 (PV3). The GMTs were 45.5, 29.5 and 20 towards PV1, PV2 and PV3 respectively. In each WHO region, the GMTs towards PV3 were consistently the lowest, and the Europeans showed the lowest GMTs both towards PV2 and PV3 (27.5 and 15.3 respectively). GMTs decreased with age. *Conclusion:* The low GMTs and the clear tendency to decrease with increasing age of the subjects, especially against to PV1, confirm the framework of attention that polio is receiving at national and international level. (www.actabiomedica.it)

Key words: serological survey, seroprevalence, immunity, migrants, poliomyelitis, WHO region

Introduction

Poliomyelitis epidemiology has radically changed since the introduction of intensive vaccination programs against the three polioviruses (PVs) (1,2). The last native case of polio due to wild-type poliovirus (WPV) infection detected in Italy occurred in 1982. At the time, the mandatory vaccination was performed entirely with trivalent oral poliovirus vaccine with Sabin strains (tOPV). In 1999, tOPV was substituted with a sequential schedule: two doses of enhanced inactivated polio vaccine (eIPV) followed by two doses of tOPV. When, in 2002, the European Region was declared "polio-free country" (the last case of indigenous wild poliomyelitis had occurred in Eastern Turkey in 1988) (3), Italy finally decided to adopt the four doses eIPV schedule as well as other high income Countries (4). Several seroprevalence studies, in which the level of neutralizing antibodies against poliovirus 1 (PV1), poliovirus 2 (PV2) and poliovirus 3 (PV3) are considered correlates of protection, conducted in Italy since the Eighties, both in general population and in selected subgroups, showed decreased protective values in terms of geometric mean titers (GMT) and titers considered protective by WHO (equal or higher than 1:8). These studies have also shown, despite good levels of seroprotection in the general population, a reduction in protection among adolescents and subse-

quently among young adults, probably due to the lack of natural boosters 10-15 years after the primary vaccination cycle (5-16). In addition, over the last years, the Italian Ministry of Health observed a lower vaccination coverage nationwide, explained by a loss of trust of the Italian population in these preventive measures. Due to vaccination hesitancy (17,18), anti-polio vaccination coverage dropped from 96.1% in 2013 to 93.4% in 2015, therefore below 95%, which is the requested threshold for polio elimination and to ensure herd immunity (19). For these reason, the 2017-19 National Immunization Prevention Plan confirmed the mandatory vaccination for children, alongside with a fifth booster dose of eIPV for adolescence (20).

Lower immunization rates, in fact, expose the Italian population, at least hypothetically, to a reintroduction of WPV or vaccine-derived polioviruses (cVDPV). Since 2005, when Environmental surveillance (ES, testing sewage for polioviruses) was introduced in Italy, becoming an important tool for early detection of silent reintroduction and circulation of polioviruses, no WPVs were spotted, although there have been several detections of Sabin-like PVs (21-26).

Migration flows towards Europe and Italy have constantly increased since the early Nineties. In many of the cases, migrants come from countries were OPV schedule is still recommended. Unfortunately in some of these areas there is a strong decline of vaccine coverage due to social disruption caused by civil war, Health Services collapse due to major epidemics, or even religious opposition by fundamentalists culminating with acts of violence against polio vaccination workers.

European countries registered an outbreak of 71 cases (59 paralytic and 2 death) in an unvaccinated religious community in the Netherlands in 1992 (27), whereas other 3 cases were identified among Roma children in Bulgaria in 2001 (28). A large outbreak caused by WPV1 imported from India in late 2009, with 463 laboratory-confirmed and 47 polio-compatible cases, took place in 2010 in Tajikistan and spread to neighbouring countries, Kazakhstan, Russia, Turkmenistan and Uzbekistan (29). Episodes like these ought to remind us that reintroduction of polioviruses cannot be completely ruled out (19).

Migrants who arrive in Italy legally, for work or study reasons, for international adoption or for family reunification and who decide to live permanently in the Italian territory, represent an important population group. Although immunization policies for migrants and refugees vary widely within the WHO European Region (30,31), the Italian Ministry of Health recommends to vaccinate, according to age, all refugee children who have never been vaccinated or who have insufficient documentation regarding prior vaccinations. Additionally, adults with the same characteristics should receive polio vaccination.

The aim of the present study was to estimate the prevalence of antibodies against the three poliomyelitis viruses in subjects of recent immigration who approached the vaccination services for the regularization of their vaccination calendars, to make them coherent with the polio eradication goal.

Methods

Study population

From January 2004 to August 2017, as part of the active surveillance of acute flaccid paralysis (AFP) and of the polio eradication process, all foreign migrants recently arrived in Italy, without or with insufficient vaccination documentation, who have turned to vaccination services of the Local Health Authority of Parma (a city with 190,000 inhabitants, in northern Italy) for the regularization of the vaccination schedule, were subjected to the determination of the antibody titers towards poliomyelitis. The survey was conducted according to the Good Clinical Practice Guidelines: the data collected - age, sex, period elapsed from arrival in Italy and country of origin - were treated anonymously for research purposes. This convenience sample was grouped into the six WHO regions: African Region (AFR), Region of the Americas (AMR), South-East Asia Region (SEAR), European Region (EUR), Eastern Mediterranean Region (EMR), and Western Pacific Region (WPR); by age groups (less than 2 years, 2 to 6 years, 7 to 18 years and equal or more than 19 years). To express graphically the trend of the GMTs in relation to age, instead, the distribution in quintiles of the age, treated as continuous variable, was used.

Serological analysis

Sterile serum samples were collected and kept at -20°C until they were examined. The determination of the three polioviruses antibodies levels was carried out with a long-incubation neutralization assay using 100 $TCID_{50}$, respectively, of poliovirus type 1 (Mahoney), poliovirus type 2 (Mef-1) and poliovirus type 3 (Saukett).

The search for neutralising antibodies (a) and the titration of the viruses (b) were carried out using a laryngeal carcinoma continuous cell line (HEP-2).

(a) The sera, heated to 56°C for 30 minutes, were tested simultaneously in triplicate at dilutions from 1:2 to 1:1024 with polioviruses type 1, type 2 and type 3, respectively. The serum/virus mixtures (0.025 mL each) were then incubated at 37°C for 6 hours in an appropriately humidified CO_2 incubator and then at 4°C for 18 hours.

(b) Aliquots of 0.050 mL of a cellular suspension (5–6 x 104 HEP-2) were added to each well. While being incubated at 37 °C, the microplates were microscopically observed for cytopathic effects (CPEs) on the third and fourth days. The titers of the sera were calculated as the highest dilution capable of neutralising the CPEs. Each reaction included controls of the viral titer, the cells and the sera (32).

Statistical analysis

Seroprotection was defined as a titer equal to or above 1:8. Subjects with antibody titers <1:8 for all the three serotypes were classified as "triple nega-tives". Titers $\geq$ 1:2 were log-transformed and evaluated as GMTs. Continuous variables were summarised as the mean, standard deviation (*SD*) and minimum–maximum values. The Analysis of Variance (Two-Way ANOVA) and Student's t-test were performed when appropriate; to verify the association between GMT and quintile distribution of age, a linear regression test was carried out. A *p*-value of 0.05 was considered significant. All statistical analyses were performed with SPSS 24.0 (IBM SPSS Inc., Chicago, IL).

Results

From January 2004 to August 2017, 2,138 samples were analyzed to determine immunization levels in migrants recently moved to Italy. Such group was mostly composed of male subjects (59.07%), average age was 13.3 years old (sd 6.1), range, 1– 55 yrs, median 13.6 yrs, with no statistically significant differences regarding the WHO region of origin. The most represented age group was the one in school age (Table 1). Median time interval between arrival in Italy and sampling date was 3 months (range = 15 days-5 yrs), resulting higher in population arriving from the European Region (median, 7 months).

The African Region was the most represented with an elevated number of subjects coming from Senegal, Ivory Coast, Ghana and Nigeria (which is still an endemic country), followed by the EMR which includes two still endemic countries (Pakistan and Afghanistan). SEAR was extensively represented by the Indian sub-continent. Over time, the relative

Table 1. Characteristics of the study sample

WHO Region*		Age				Age group (%)			
	Subjects (No.)	Mean (sd)	Median	Min	Max	< 2 years	2 - 6 years	7 - 18 years	=>19 years
AMR	195	13.00 (5.38)	13.00	1	51	0.5%	9.9%	83.9%	5.7%
AFR	1,038	13.52 (6.01)	14.00	1	55	1.3%	10.8%	80.7%	7.2%
SEAR	223	12.61 (5.63)	12.2	2	44	0.0%	12.7%	80.1%	7.2%
EUR	240	13.56 (7.70)	14.00	1	49	1.3%	16.4%	72.3%	10.1%
EMR	271	13.18 (6.48)	13.40	1	40	0.7%	17.2%	73.9%	8.2%
WPR	171	12.38 (4.76)	13.00	0	31	2.4%	11.2%	82.2%	4.1%
Overall	2,138	13.25 (6.11)	13.63	0	55	1.1%	12.4%	79.2%	7.3%

* See abbreviations in the text

percentage of subjects from the African continent has increased, while the number of subjects coming from AMR has decreased (Figure 1).

The percentage of antibody titers below 1:8 was 6.0% versus poliovirus 1, 7.7% versus poliovirus 2 and 15% versus poliovirus 3. Twenty-seven subjects resulted triple negatives (antibody titers <1:8 for all the three serotypes).

Stratifying population by WHO region of ori-

gin, the WPR had the highest percent of non-sero-protected subjects against poliovirus 1 (8.8%), while the European Region had the highest percent of non-seroprotected against polio 2 and 3 (respectively 11.7% and 24.6%). Overall, the European subjects showed the highest percentages of seronegativity towards one or more serotypes, in fact only 70% of them, at the same time, showed protective antibodies to the three polio viruses. (Table 2).
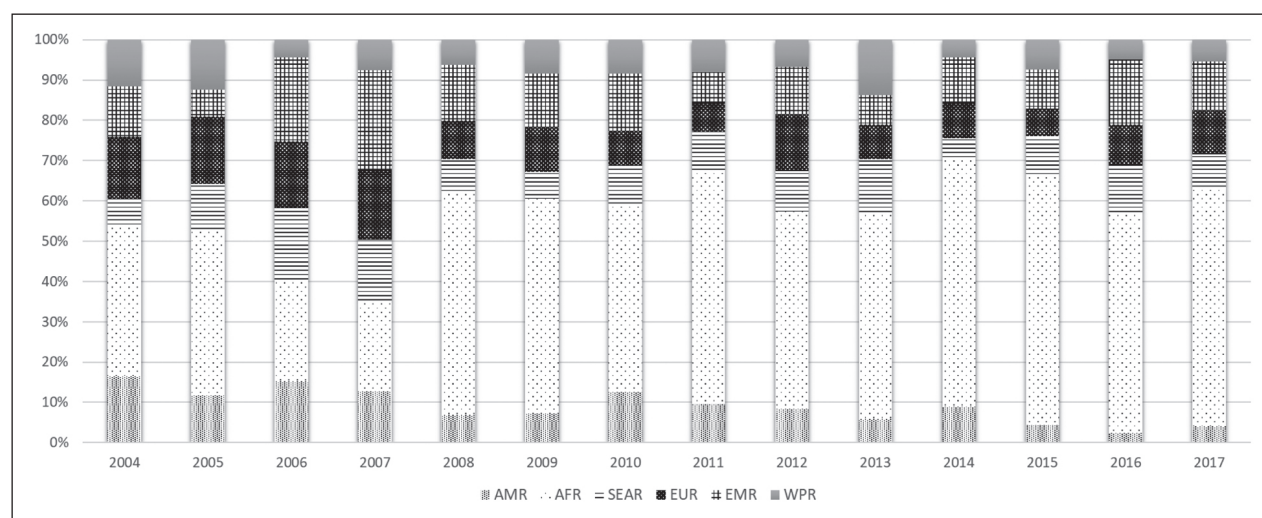

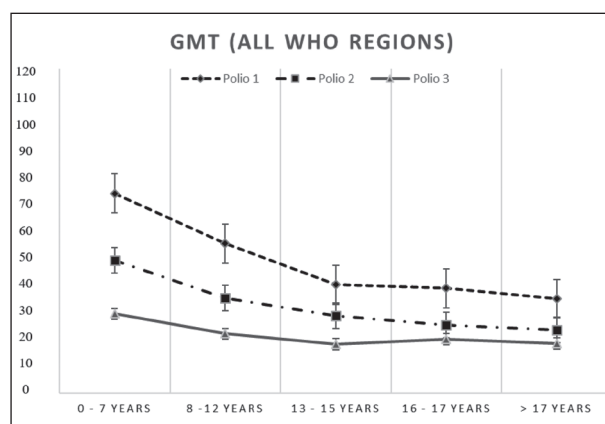**Figure 1.** WHO regions of origin: distribution of subjects per year of study

Table 2. Numbers and percentages of subjects with protective (≥ 1: 8) and non-protective (<1: 8) antibodies and numbers and percentages of subjects without antibodies to one or more of the polioviruses, by WHO regions

WHO regions			Poliovirus 1		Poliovirus 2		Poliovirus 3		Triple	1/3	2/3	Triple
			≥ 1:8	< 1:8	≥ 1:8	< 1:8	≥ 1:8	< 1:8	positives	negatives	negatives	negatives
AMR	No.	195	185	10	180	15	159	36	147	37	9	2
	%		94.9%	5.1%	92.3%	7.7%	81.5%	18.5%	75.4%	19.0%	4.6%	1.0%
AFR	No.	1038	974	64	953	85	896	142	826	141	63	8
	%		93.8%	6.2%	91.8%	8.2%	86.3%	13.7%	79.6%	13.6%	6.1%	0.8%
SEAR	No.	223	212	11	213	10	206	17	195	19	8	1
	%		95.1%	4.9%	95.5%	4.5%	92.4%	7.6%	87.4%	8.5%	3.6%	0.4%
EUR	No.	240	226	14	212	28	181	59	168	48	19	5
	%		94.2%	5.8%	88.3%	11.7%	75.4%	24.6%	70.0%	20.0%	7.9%	2.1%
EMR	No.	271	257	14	254	17	230	41	221	33	12	5
	%		94.8%	5.2%	93.7%	6.3%	84.9%	15.1%	81.5%	12.2%	4.4%	1.8%
WPR	No.	171	156	15	161	10	145	26	137	23	5	6
	%		91.2%	8.8%	94.2%	5.8%	84.8%	15.2%	80.1%	13.5%	2.9%	3.5%
Overall	No.	2138	2010	128	1973	165	1817	321	1694	301	116	27
	%		94.0%	6.0%	92.3%	7.7%	85.0%	15.0%	79.2%	14.1%	5.4%	1.3%

Table 3. GMTs toward PV1, PV2, PV3, by WHO region

WHO Region	Subjects (No.)	GMT (Poliovirus 1)	GMT (Poliovirus 2)	GMT (Poliovirus 3)
AMR	195	44.8	31.1	17.2
AFR	1038	43.0	28.6	21.2
SEAR	223	50.9	35.0	22.8
EUR	240	56.0	27.5	15.3
EMR	271	50.7	29.3	20.6
WPR	171	35.1	30.2	19.2
Overall	2138	45.5	29.5	20.0

Figure 2. GMTs calculated by quintile of age group

The GMTs towards the 3 polioviruses were 45.5 for PV1, 29.5 for PV2 and 20 for PV3 respectively (Table 3). In each WHO region, the GMTs for PV3 were consistently the lowest, and even in this case the EUR prevailing subjects showed the lowest GMTs for both PV2 and PV3 (respectively 27.5 and 15.3). The GMTs referring to each of the 14 years of study have experienced strong fluctuations (from 21.3 to 89.3 for the PV1, from 16.8 to 55.6 for the PV2, from 12.4 to 36.6 for the PV3). The analysis conducted on the distribution in quintiles of the ages, confirmed the reduction of GMTs that show a decrease in relation to age classes especially those towards polio 1 and polio 2. (Figure 2). The age group below 2 years of age showed the greatest prevalence of non-seroprotected subjects towards the 3 polioviruses; 34.8% of subjects had no protection against at least one of the 3 serotypes. Even the very large group of children and adolescents showed a high percentage of subjects lacking protective antibodies, in particular towards poliovirus 3 (15.4%) (Table 4).

Conclusions

Sub-optimal vaccination coverage, often the result of the disintegration of social and health systems due to ongoing conflicts, may be responsible for the circulation or reintroduction of wild polioviruses in polio-free populations as evidenced by recent episodes in Tajikistan (2010) or in the Arab Republic of Syria (2013-2014) (29,33).

In this survey, 79.2% of subjects showed protective antibodies to the three polioviruses. As in investi-

Table 4. Numbers and percentages of subjects with protective (> 1: 8) and non-protective (<1: 8) antibodies, percentages of subjects without antibodies to one or more of the polioviruses and GMTs by age class

Age	Subjects (No.)	Poliovirus 1			Poliovirus 2			Poliovirus 3			All strains	
		titres ≥1:8	titres <1:8	GMT	titres ≥1:8	titres <1:8	GMT	titres ≥1:8	titres <1:8	GMT	triple positives	triple negatives
< 2 years	23	82.6%	17.4%	70.1	87.0%	13.0%	40.7	65.2%	34.8%	14.6	65.2%	13.0%
2 - 6 years	263	96.6%	3.4%	79.6	95.1%	4.9%	47.8	87.8%	12.2%	28.0	86.3%	1.9%
7 - 18 years	1,679	94.0%	6.0%	42.5	92.3%	7.7%	27.8	84.6%	15.4%	18.9	78.5%	0.9%
=>19 years	155	90.3%	9.7%	34.5	73.1%	6.1%	25.1	87.1%	12.9%	22.2	76.1%	2.6%
Overall	2,120	94.0%	6.0%	45.5	88.4%	11.6%	29.6	85.0%	15.0%	20.0	79.2%	1.3%

gations of the past and in recent seroprevalence studies on the Italian population, PV1 antigen was the most immunogenic with GMTs constantly higher than PV2 and PV3 during the 14 years of the survey and considering the WHO regions of origin. Fifteen percent of the subjects, on the other hand, were found not to have protective antibodies against PV3. In particular, subjects from the European region showed high percentages of low protection both towards PV2 (11.7%) and PV3 (24.6%). Children under the age of 2 were poorly represented (23 overall): they showed elevated GMTs, but a high percentage of unprotected subjects towards at least one of the 3 poliovirus.

GMTs tend to decrease significantly with age, especially PV1 and PV2 and, as in the case of the Italian population, low titers could depend on the absence of natural boosters.

The sample considered, coming from the 6 WHO regions and from 78 different countries, showed a low prevalence of subjects without antibodies; in 14 years of investigation only 27 subjects (1.3%) were triple-negatives.

However, a substantial percentage of sample showed not optimal antibodies levels as considered by the WHO, in a scenario of possible circulation of wild polioviruses. The low GMTs and the clear tendency to decrease with the increasing age of the subjects, especially against PV1, confirm the framework of attention that polio is receiving at national and international level.

The main limitation of this study is the convenience sample represented by the most stable foreign population that, for study and work reasons, turns to the Local Health Services to regularize its vaccination situation. Furthermore, due to the absence of vaccination documentation it was not possible to trace the type of vaccine used, however most of the subjects (> 95%) came from Countries where OPV Sabin is still used and, in this case, all subjects were hypothetically vaccinated with the trivalent vaccine (tOPV) before the switch to bivalent OPV (bOPV), which occurred between April and May 2016, due to the disappearance of PV 2 worldwide and the consequent removal of the type 2 component (OPV2) from immunization programmes. (34).

The population residing in Italy, vaccinated with eIPV, no longer exposed to vaccine polioviruses since 2002, if not those eventually imported by subjects with recent vaccination, could have GMTs and seroprotection levels lower than those found in our study (15,16).

The addition of a 5th dose of eIPV to the adolescent vaccination calendar, could be evaluated on serum epidemiological data collected in controlled investigations on representative population samples identified on the basis of age, origin and vaccination status.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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