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Seroepidemiology of Polioviruses among University Students in Northern Italy

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The widespread use of poliovirus vaccination schemes has led to a marked decline in the incidence of paralytic poliomyelitis worldwide, but wild poliovirus is still endemic in some developing countries, and in 2009 a total of 23 countries reported at least 1 case of poliomyelitis caused by wild-strain polio viruses. A serological survey was thus conducted on the immunological status against polioviruses of 318 young adults, classified by their country of origin. Immunity to poliomyelitis was assessed by neutralizing antibody titration in tissues cultured on microplates. The rate of seronegativity ($\leq 1:8$) in the study population was 26.7% for poliovirus type 1, 7.2% for type 2, and 22.6% for type 3. In our sample of 318 individuals, 219 (68.9%) were Italian and 99 (31.1%) were from outside the European Union (EU). The proportion of cases found seropositive to polioviruses 1 and 3 decreased significantly with older age; this age-related decrease was more evident in the Italian group than among the non-EU subjects. Any risk of the wild virus recurring and causing paralytic poliomyelitis must be prevented, keeping Europe polio free by means of appropriate immunological protection, until polio has been conclusively eradicated all over the world. Judging from our findings, it may be worth considering administering a fifth dose of polio vaccine to adolescents.

Poliovirus, the etiologic agent of paralytic poliomyelitis, once crippled hundreds of thousands of people in the world (13,000 to 20,000 people every year in the United States and 4,000 to 8,000 per year in Italy). As a result of the Global Polio Eradication Initiative, poliomyelitis has been successfully brought under control and eradicated in most developed countries by the systematic use of vaccines. The most widely used vaccine in the last 50 years has been the Sabin live attenuated oral poliovirus vaccine (OPV). The oral polio vaccine provides good individual protection and better community protection, which is particularly important when the natural poliovirus is circulating. Worldwide, the number of children paralyzed by polio has fallen from over 350,000 in 1988 to as few as 2,000 in 2009, and the number of countries where the disease is endemic has dropped from 125 to 4 during the same period. Since 1994, three of the six WHO regions have been certified free of wild-strain polioviruses (WPVs) and no WPV type 2 (WPV2) has been detected anywhere in the world since 1999 (11). Despite such progress toward the eradication of polio, a number of countries—23 in all—reported at least 1 case of polio occurring due to WPVs in 2009 (20).

On 21 June 2002, the European Regional Commission for the Certification of the Eradication of Poliomyelitis certified that the European Region was free of indigenous WPVs. After wild poliovirus type 1 (WPV1) was imported in 2010, Europe's polio-free status was recently confirmed again (23).

In recent years, Europe has had to face mass immigration and the risks of importing neuropathogenic polioviruses from areas of endemicity, or of poliovirus infections developing in nonimmunized immigrants, making it important to effectively monitor the immunity status of the European population. The last indigenous case of poliomyelitis was diagnosed in Italy in 1982. The last two imported wild viruses were detected in nonvaccinated children coming from Iran (1984) and India (1988). Since 2000, there have not been found any cases of vaccine-associated paralytic poliomyelitis.

The aim of this study is to assess the level of immunological

protection against polioviruses among young adults in a polio-free environment that—in principle—is at risk of reacquiring neuropathogenic polioviruses originating from abroad.

MATERIALS AND METHODS

Setting. OPV immunization began in Italy in 1964 and became compulsory in 1966. The vaccination schedule provided for the administration of three doses of OPV at 3, 5, and 11 months of age, plus a booster dose at 3 years old. From 1999 onwards, a sequential schedule was adopted, consisting of two doses of inactivated poliovirus (IPV) vaccine at 3 and 5 months old and two doses of oral polio vaccine at 11 months and 3 years of age. In July 2002, this was replaced by a schedule consisting of four doses of IPV vaccine. In 2005, the Italian National Vaccine Plan for 2005 to 2007 introduced a schedule consisting of four doses of IPV vaccine at 3, 5, and 11 months and at 4 or 5 years of age. Since 2001, a combined DTPa-HBV-IPV/Hib vaccine has been commercially available in Italy, containing components for diphtheria (D), tetanus (T), and acellular pertussis (Pa); hepatitis B virus (HBV); and inactivated poliovirus (IPV) types 1, 2, and 3, mixed with a conjugated *Haemophilus influenzae* type b (Hib) vaccine, administered, from 2002 onward, according to a 3-, 5-, and 11- to 13-month vaccination schedule (10).

The systematic immunization of infants practically eradicated poliovirus and soon led to a marked downward trend in the morbidity of poliomyelitis. The last cases of paralytic poliomyelitis in Italy, which occurred in the early 1980s, were either vaccine associated or imported from areas of endemicity (6).

Subjects. The study was conducted on a sample of healthy students attending Padua University. Subjects were consecutively enrolled at Padua University's Preventive Medicine Service in 2010. Although blood

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TABLE 1 Distribution of neutralizing antibodies to polioviruses in 318 students attending Padua University, by country of origin and serum titer

Titer	Poliovirus type 1				Poliovirus type 2				Poliovirus type 3			
	Italian		Non-EU		Italian		Non-EU		Italian		Non-EU	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
<8	67	30.6	18	18.2	19	8.7	5	5.1	47	21.5	25	25.3
8	26	11.9	10	10.1	24	11.0	9	9.1	37	16.9	11	11.1
16	44	20.1	24	24.2	41	18.7	11	11.1	47	21.5	23	23.2
32	37	16.9	13	13.1	49	22.4	20	20.2	38	17.4	19	19.2
64	28	12.8	14	14.1	33	15.1	24	24.2	26	11.9	9	9.1
128	14	6.4	9	9.1	37	16.9	11	11.1	14	6.4	8	8.1
256	3	1.4	5	5.1	14	6.4	19	19.2	6	2.7	2	2.0
512	0	0.0	6	6.1	2	0.9	0	0.0	4	1.8	2	2.0
Total	219	100.0	99	100.0	219	100.0	99	100.0	219	100.0	99	100.0

samples were collected during routine general health and immunological checkups, as stated in the University's Health Surveillance Program (7), subjects were enrolled for the study only after their written informed consent was obtained. An anonymous questionnaire identified by a code was completed for each individual.

Procedures. Venous blood samples were drawn from each subject at the time of enrollment, and the sera obtained were stored at -20°C until they were tested in batches. A microneutralization test was used to assess the serum titer of antibodies to poliovirus types 1, 2, and 3. Serum samples were complement inactivated at 56°C for 30 min and diluted from 1:2 to 1:512. They were then placed in contact with 100 50% tissue culture infective doses (TCID₅₀) of the three types of Sabin attenuated poliovirus (type 1, L Sc2ab strain; type 2, P712ch2ab; type 3, Leon 12alb). After overnight incubation at 4°C , freshly trypsinized Vero cells in suspension (approximately 1×10^5 to 2×10^5 per ml) were added to each well containing the serum-virus mixture and the solution was incubated at 36°C in a 5% CO₂ incubator. The final test reading was obtained after 6 days. The 50% endpoint value was used as the serum titer, and geometric mean titers (GMTs) were computed by \log_{10} of reciprocal antibody titers. Titers of $\geq 1:8$ were considered to be protective (18).

Statistical analyses. Data were analyzed using the χ^2 test, Student's *t* test for unpaired data, and linear regression analysis, as appropriate. Analyses were performed using the EPI-Info 2000 supplied by the Centers for Disease Control and Prevention (Atlanta, GA).

RESULTS

The study population included 318 subjects (mean age \pm standard deviation [SD], 26.3 ± 4.3 years), classified by country of origin, i.e., 219 (68.9%) were Italian and 99 (31.1%) came from outside the European Union.

The subjects in the Italian group were a mean 27.1 ± 4.3 years of age; 77 (35.2%) were male, and 142 (64.8%) were female; they had all been vaccinated with oral poliovirus vaccines (OPVs) according to the Italian vaccination schedule in use when they were children. The non-EU subjects were a mean 25.6 ± 4.2 years old; 43 (43.4%) were male, and 56 (56.6%) were female; 59.6% of them had no reliable documentation of prior vaccination. Among the 99 non-EU students considered, 55 (55.5%) were from Eastern Europe (63.6% of them from Albania and the former Yugoslav Republic), 27 (27.3%) were from sub-Saharan Africa (70.4% from Cameroon), and 17 (17.2%) were from other countries.

The study population's seronegativity rates ($\leq 1:8$) were 26.7% for poliovirus type 1, 7.2% for poliovirus type 2, and 22.6% for poliovirus type 3. Table 1 shows the prevalence of neutralizing antibodies by serum titer, nationality, and type of poliovirus.

None of the subjects simultaneously lacked neutralizing antibodies for all three polioviruses.

The data were analyzed after separating the study sample by age group (Fig. 1). The proportion of cases seropositive ($>1:8$) for poliovirus types 1 and 3 dropped significantly ($P < 0.01$) with older age. In the Italian group, but not in the non-EU group, the age-related drop was greater for the poliovirus types 1 and 3, i.e., which fell by 21.5% and 23.4%, respectively ($P < 0.01$).

Overall, the GMTs of neutralizing antibodies were 18.3, 36.1, and 18.6 for poliovirus types 1, 2 and 3, respectively. These GMTs were significantly higher ($P < 0.05$) for poliovirus type 2 than for the other two types of poliovirus, 1 and 3 (Fig. 1). In the Italian sample, the neutralizing antibody GMTs dropped from 15.4 in the group of 19- to 24-year-olds to 13.7 among the students over 28 years old for poliovirus type 1 and dropped from 23.6 to 17.3 for poliovirus type 3.

DISCUSSION

It is important to monitor vaccination protection to check on the population's immunity to polioviruses. Our study on the humoral immunity status of a sample of university students in Padua identified seronegativity rates ($\leq 1:8$) of 26.7% for poliovirus type 1, 7.2% for type 2, and 22.6% for type 3. These data are consistent with a national serum surveillance scheme conducted in 2006 to 2007, which revealed a significant, age-related decline in the study population's level of seroprotection. In our sample of Italian students, the GMTs were low mainly for poliovirus types 1 and 3, and the persistence of the vaccine-induced antibodies fell off with time, as reported in other studies (8, 12). This means that immunological protection against the risk of new cases cannot be guaranteed. The world now has a good chance of stopping the transmission of wild poliovirus for good, but although there has been a marked decrease in the number of cases of polio, the goal of global eradication has been proving difficult to reach.

Wild poliovirus type 2 was not detected after 1999, while types 1 and 3 are both circulating and are endemic in Pakistan, Afghanistan, and Nigeria—all countries where vaccination rates are low, due mainly to inadequate vaccination strategies or for religious, political, and geographical reasons (4). Some countries still experience outbreaks of poliomyelitis, indicating that WPVs may be circulating. Infections imported from countries where polio is still endemic are continuously reported; however, the general situation has recently improved. In March 2012, India was removed

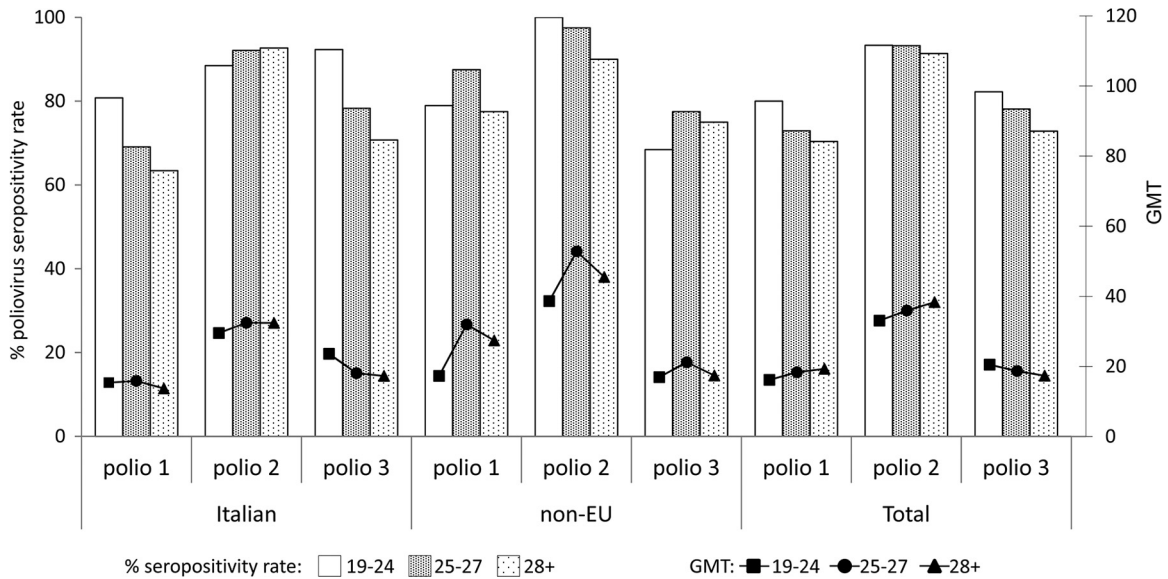


FIG 1 Poliovirus-neutralizing antibodies: prevalence of seropositivity and geometric mean titers in 318 subjects, by age group. This graph shows a significant drop ($P < 0.01$) for poliovirus types 1 and 3 in the Italian group with increasing age. The graph also shows the GMTs of neutralizing antibodies, which are significantly higher ($P < 0.05$) for poliovirus type 2 than for the other polioviruses in both study groups (Italian and non-EU).

from the list of countries in which polio is endemic, and the total WPV cases in countries in which polio is not endemic were 309 in 2011, as opposed to 1,120 in 2010 (22).

For the European region, the recent outbreak of WPV1 in Tajikistan should serve as a reminder that polio-free regions may import polioviruses at any time, as long as polioviruses continue to circulate somewhere in the world. Many European countries may have pockets of the population with poor vaccination coverage, in which case the arrival of polioviruses from elsewhere can restore the circulation of the virus. As the outbreak in the Netherlands in the winter of 1992-1993 showed, wild strains may be brought into polio-free areas and lead to symptomatic infections in communities with generally very high vaccination coverage (16). Evidence was also found for circulating vaccine-derived polioviruses (cVDPVs). In Romania in 2002, a type 1 VDPV was isolated from one acute flaccid paralysis (AFP) case (an unimmunized 5-month-old child) and seven healthy unimmunized children within a Roma community with low rates of OPV coverage (19). However, episodes of cVDPVs are rare and occur if the population is seriously underimmunized. In contrast, if a population is entirely immunized against polio, it will be protected against the spread of both wild and vaccine strains of poliovirus (3). More serum surveillance is needed in order to confirm the potential risk of disease spread due to low protection against poliomyelitis, and seroprevalence surveys are necessary to identify population groups susceptible to polio for targeting specific vaccination activities.

The decrease of seroprevalence was observed for type 3 virus in other serological studies performed in the European region (1, 2, 13, 14), and adolescents and young adults seem to be at major risk (2, 9, 17). The low percentages of protective antibodies to poliovirus type 1 described for the Italian students in the present study require further investigations to consolidate such results. Data concerning the non-European students suggest a change in immunity level to poliovirus in immigrants if compared to a

previous study which found that immigrants in the Veneto region were seropositive to WPV types 1, 2, and 3 at rates of 93.3%, 99.6%, and 95.9%, respectively (15). The aim of OPV immunization is to block poliovirus circulation. From 1988, when the WHO resolved to eradicate polio worldwide, the total number of polio cases showed a reduction greater than 99% (21). The reduction of poliovirus circulation has contributed to the reduction of the percentages of protective antibodies in young people. This phenomenon is described in developed countries with high rates of OPV coverage, but even developing countries will be interested in consideration of increasing population immunity rates above the thresholds required to block poliovirus transmission. If contact with poliovirus becomes sporadic, the booster effect caused by continued exposure to wild virus decreases. In addition, recent data suggest that even children are to be considered at risk of low levels of immunity to poliovirus type 1 and type 3. This phenomenon may be linked to the implementation of IPV vaccination (14). If so, in areas where only IPV is used, in the future both adolescents and young adults may have a greater reduction in protective antibody titers.

Considering all the above data and the epidemiological pattern identified in our study, it would make sense to administer a fifth dose of polio vaccine to adolescents in Italy, as is already done in other European countries (12). Administering a fifth dose of IPV vaccine to adolescents previously vaccinated with OPV would not only contribute to reinforcing the bonds of circulating antibodies but would also induce an IgA response at the mucosal level, as described in studies in which the mucosal response to IPV was found to depend on prior contact with poliovirus (5). Any risk of poliovirus reappearing and causing paralytic poliomyelitis must be prevented, keeping Europe polio free by ensuring adequate immunological protection until polio has been unequivocally eradicated worldwide.

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