RESEARCH PAPER

Surveillance of poliomyelitis in Northern Italy: Results of acute flaccid paralysis surveillance and environmental surveillance, 2012–2015

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ABSTRACT

Although in the last years poliovirus (PV) transmission has been reported at the lowest levels ever recorded, the spread of virus from endemic countries endures; the high levels of immigration flows across the Mediterranean Sea jeopardize Italy for PV reintroduction. The World Health Organization (WHO) strategic plan for global poliomyelitis (polio) eradication indicates the nationwide surveillance of Acute Flaccid Paralysis (AFP) as the gold standard for detecting cases of polio. In addition, the Environmental Surveillance (ES), seeking the presence of PV and Non-Polio Enterovirus (NPEV) in sewage, is recognized as a powerful tool to confirm PV circulation in absence of AFP cases, especially in polio-free countries. Here we report the results of AFP surveillance (AFPS) and ES in Lombardy (Northern Italy) from 2012 to 2015.

Forty-eight AFP cases were identified during the study period. No AFP case was caused by PV infection. NPEVs were identified in 6.3% (3/48) of AFP cases. The annual AFP incidence rate was 0.87/100'000 children <15 y in 2012, 1.42/100'000 in 2013, 1.02/100'000 in 2014, and 0.47/100'000 in 2015; according to WHO indicators, the sensitivity of AFPS was adequate in 2013 and 2014. Completeness of case investigation raised progressively during the study period to achieve the WHO standards in 2014 (92.3%) and 2015 (100%). Completeness of follow-up increased from 72.7% in 2012 to 100% in 2014.

In the framework of the ES conducted in Milan, 268 wastewater samples were collected from 2012 to 2015 and no PVs were isolated. In contrast, NPEVs were detected in 65.3% (175/268) of samples. All NPEVs characterized belonged to enterovirus species B: echovirus type 11, 6 and 3 were the most frequently detected viruses, representing 29.1% (41/141), 20.6% (29/141) and 9.2% (13/141) of genotyped NPEVs, respectively.

Keeping strong and encouraging both AFPS and ES is crucial to ensure that PV will not return unnoticed in Italy - as well as in other polio-free countries - and, as a final point, to achieve the global polio eradication goal.

KEYWORDS

acute flaccid paralysis surveillance system; environmental surveillance system; non-polio enterovirus; Polio Eradication Initiative; poliovirus

Introduction

Although in the last years poliovirus (PV) transmission has been reported at the lowest levels ever recorded, the virus is still endemic in 3 countries – Nigeria, Afghanistan and Pakistan.

Until PV transmission is not interrupted, all countries - especially those more vulnerable, with weak public health and immunization services and travel or trade links to endemic countries – remain at risk of virus importation. In May 2014, the World Health Organization (WHO) declared the international spread of wild PV a Public Health Emergency of International Concern (PHEIC) due to PV reintroduction through populations movements in 3 regions: central Asia (from Pakistan to Afghanistan), Middle East (from Syria to Iraq), and central Africa (from Cameroon to Equatorial Guinea).^{1, 2} Since then, a reinforcement of PV surveillance was encouraged here and there in order to detect any virus importation, even though there were no recommendations in polio-free countries.

In Italy, the last case of indigenous polio due to a wild PV infection was reported in 1982, whereas the declaration of

“polio-free country” was obtained in 2002. Since then, polio vaccination schedule consists in 4 doses of inactivated polio vaccine (IPV).³ To date, importation of PV in Italy has never been recorded, albeit the high levels of immigration flows across the Mediterranean Sea jeopardize Italy for PV reintroduction.

The WHO strategic plan for the global polio eradication includes the implementation of infant immunization coverages by routinely vaccination campaign, national immunization days and targeted “mop-up” campaigns, along with the active community-based PV investigation.⁴ In particular, the nationwide surveillance of Acute Flaccid Paralysis (AFP) is recognized by the WHO as the gold standard for detecting cases of polio.⁵ The systematic analysis of stool samples collected from patients identified in the framework of AFP surveillance (AFPS) enables to link PV strains to paralysis cases, allowing a detailed examination of these individuals and their community.

Routine systematic Environmental Surveillance (ES) seeks the presence of PVs by the examination of sewage, connects PV strains from uncommon individuals to the population served

by the same Waste-Water Treatment Plant (WWTP) and can allow the identification of PV circulation also in absence of AFP cases.⁵⁻⁸ Moreover, ES can detect the presence of vaccine-related virus like Oral Polio Vaccine (OPV) (Sabin-like PV) and Vaccine-Derived PV (VDPV).⁵ For these reasons, ES is currently recognized as a powerful tool for PV surveillance, especially in polio-free countries.^{7,9}

Since Non-Polio Enteroviruses (NPEVs) are widespread worldwide and - like PVs - can be transmitted by fecal-oral route, NPEVs detection is expected in ES and epidemiological field study on these viruses are encouraged.^{5, 10-14}

In Italy, nationwide AFPS was established in 1997,¹⁵ whereas ES was set up in 2005 only in Milan and other 5 Italian cities (Bolzano, Parma, Sassari, Napoli and Palermo).¹¹

This study described the results of both AFPS and ES in the period spanning from 2012 to 2015 in Lombardy, a region of Northern Italy accounting for nearly 10 million inhabitants (out of 60 million at national level). Critical aspects of the surveillance systems (such as data quality, sensitivity and timeliness) were also discussed. Additionally, the circulation of NPEVs was evaluated in the framework of ES.

Results

Demographical, epidemiological, clinical and virological characteristics of AFP cases

From January 1st, 2012, to December 31st, 2015, 48 AFP cases were reported in Lombardy. The median age of AFP cases was 4.5 y (range: 3.1–5.2 y; inter-quartile range [IQR]: 7.5 y). Thirty-six out of 48 (75%) AFP cases were recorded in children ≤ 5 y old. Half of cases (24/48) were males. The majority of cases came from Milan (14/48; 29.2%) and Bergamo (10/48; 20.8%) districts.

Most (30/48; 62.5%) AFP cases were notified by pediatric units, and the diagnosis at follow-up was Guillain-Barré syndrome (GBS) in 47.9% (23/48) cases, followed by myelitis (7/48; 14.6%). Genetic disorders/malignancies and hypotonia/asthenia were reported at the same extent (6/48; 12.5%); a “not definitive diagnosis” was indicated in 12.5% (6/48) cases.

The AFP cases distribution by month showed 2 peaks: one in July (8/48; 16.7%) and one from October to December (16/48; 33.4%).

As concern virological investigations, no PVs were identified in the AFP cases during the 4-year study period. NPEVs were

detected in 3 (3/48; 6.3%) samples collected from AFP cases; echovirus type 11 was detected in a 5-y-old male with GBS in 2012, echovirus type 6 in an 8-y-old female affected by myelitis in 2013, and echovirus 11 in a 3-y-old female with clinical paralysis of undefined origin in 2014. No NPEVs were identified in 2015.

About 94% (45/48) of AFP cases had at least one serum sample tested and the serological detection of anti-PV antibodies. The proportion of children with protective antibody levels ($\geq 1:8$) against PV1 was 93.3% (42/45) and against PV2 and PV3 was 95.6% (43/45). No seroconversions in paired serum samples of AFP cases (33/45; 73.3%) were reported. The immunization status of AFP cases was available for 85.4% (41/48) of AFP cases: 3 children were aged less than 3 months and thus not vaccinated against PV; no information regarding PV vaccine administration was available for 14.6% (7/48) AFP cases. The remaining children were reported to be vaccinated with IPV. All children with unknown immunization status had protective antibody titers.

Performance indicators of AFPS

According to the size of Lombardy population aged less than 15 y, 13 AFP cases per year were expected. The annual AFP incidence rate was 0.87/100'000 children <15 y in 2012, 1.42/100'000 in 2013, 1.02/100'000 in 2014, and 0.47/100'000 in 2015 (Table 1). According to WHO indicators, the sensitivity of AFPS was adequate ($\geq 1.00/100'000$) in 2013 and 2014. Completeness of case investigation ($\geq 80\%$) was achieved in 2014 (12/13; 92.3%) and 2015 (6/6; 100%), whereas in 2012 and 2013 paired stool samples were collected in 63.6% (7/11) and 77.8% (14/18) of AFP cases, respectively (Table 1). Completeness of follow-up increased from 72.7% (8/11) in 2012 to 100% (13/13) in 2014. In 2015, follow-up examinations were available for about one-third of AFP cases (Table 1).

ES

During the 4-year study, 268 wastewater samples were collected from 3 WWTP. Viral isolation in L20b cell culture showed no virus growth, thus excluding PV presence in tested samples. NPEVs were detected in 65.3% (175/268) of samples. The proportion of NPEV-positive samples by year of study was 70%

Table 1. Performance of AFPS carried out in Lombardy (Northern Italy) from January 1st, 2012, to December 31st, 2015. Indicators (incidence index, completeness of case investigation and completeness of follow-up) are reported by year of study. Results that fulfil the WHO target are highlighted in bold.

	Expected AFP cases*	Reported AFP cases**	Incidence index† (per 100'000 children <15 y)	Completeness of case investigation†† (%)	Completeness of follow-up# (%)
2012	13	11	0.87	63.6%	72.7%
2013	13	18	1.42	77.8%	83.3%
2014	13	13	1.02	92.3%	100.0%
2015	13	6	0.47	100.0%	33.3%
WHO target			≥ 1	$\geq 80\%$	$\geq 80\%$

*Number of AFP cases expected according with the number of inhabitants <15 y in Lombardy for the relevant year.

**Number of AFP cases reported in children <15 y in Lombardy.

†Reported AFP cases / Expected AFP cases.

††AFP cases with 2 stool samples collected 24–48 hours apart and within 14 d from paralysis onset.

#Clinical follow-up completed within 60–90 d from paralysis onset.

Figure 1. Frequency of NPEV genotypes identified in Lombardy (Northern Italy) from January 1st, 2012, to December 31st, 2015 in the framework of ES. Genotype results were available for 141 out of 175 (80.6%) NPEVs. (CVB: coxsackievirus B; E: echovirus).

(42/60) in 2012, 56.9% (41/72) in 2013, 66.7% (48/72) in 2014, and 68.8% (44/64) in 2015.

Overall, 80.6% (141/175) of NPEV-positive wastewater samples were molecularly characterized. All characterized NPEVs belonged to species B, and according to sequencing results 16 different NPEV genotypes were identified: 4 were group B coxsackievirus and 12 echovirus (Fig. 1). Echovirus type 11 was the genotype detected most frequently (41/141; 29.1%) followed by echovirus type 6 (29/141; 20.6%), and echovirus type 3 (13/141; 9.2%) (Fig. 1), accounting for 58.9% (83/141) of all characterized NPEVs.

As shown in Fig. 2, a distinctive temporal distribution of echovirus type 6, 11 and 3 was observed. The analysis of circulating NPEV genotypes per year showed that echovirus type 11 was detected in 78.6% (33/42) of wastewater samples collected in 2012, 9.8% (4/41) in 2013, 2.1% (1/48) in 2014, and 6.8% (3/44) in 2015. Echovirus type 3 and type 6 started to increase from 2012 reaching a detection rate of 20.8% (10/48) and 27.1% (13/48), respectively, in 2014 (Fig. 2).

Discussion

Since the launch of the Global Polio Eradication Initiative in 1998, the incidence of wild PV cases has decreased by >99%;

nonetheless, international spread of wild PV from endemic countries remains possible, as recognized in 2014 by the WHO with the PHEIC declaration.^{1, 2}

Italy is a country of huge immigration impact and this may facilitate the re-introduction of wild PV. In this setting, it is mandatory to strengthen the AFPS at regional and national level in order to detect rapidly any virus importation or emergence, and enable a prompt public health response. Although AFPS remains the gold standard for PV control, systematic ES has been recognized as a powerful tool to detect PV in the absence of AFP cases, especially in polio-free countries.^{5, 7-9}

According to Centralized Information System for Infectious Diseases (CISID), Italy - as several European countries - achieves barely the WHO target of AFPS performance indicators,¹⁶ probably because of the low priority given to AFPS by national public health authorities and because Italy was declared “polio-free” in 2002 and since then PV has not been considered a health hazard anymore.

In our 4-year study, AFPS met the WHO criteria for sensitivity from 2013 onwards and the level of completeness of case investigation improved significantly from 2014 after the PHEIC declaration.² Physicians and healthcare workers involved in surveillance activities have proved to give special attention to

Figure 2. Temporal distribution of echovirus type 11, echovirus type 6 and echovirus type 3 detected in Lombardy (Northern Italy), from January 1st, 2012, to December 31st, 2015 in the framework of ES. (E: echovirus).

AFPS thanks to several initiatives proposed by SNRL and implemented since 2013, such as the sharing of surveillance reports, epidemiological alerts and updates on polio eradication proceedings. The utmost attention was paid in 2014 after the PHEIC declaration when all AFPS performance indicators of sensitivity were achieved.

Overall, results of AFPS in the period spanning from 2012 to 2015 were adequate in terms of data quality, sensitivity, and timeliness, thus underlining an improvement of AFPS compared with the results obtained from 1997 to 2011¹⁵ in the same surveyed area and those reached in other Italian regions.¹⁷⁻²⁰

Epidemiological characteristics of AFP cases were similar to those reported in the current scientific literature; no differences of the AFP frequency between males and females were observed. 75% of AFP cases were recorded in children aged below 5 y, as also reported by others.^{21, 22}

The temporal distribution of AFP was characteristic with notifications peaks in July (16.7%) and in the period spanning from October to December (33.5%), differently to the results reported in Lombardy in the past¹⁵ and in another region of Central Italy.²¹

A number of causes can bring about AFP, and GBS is the most frequently recognized clinical manifestation of AFP onset, particularly after the interruption of wild PV transmission.²³ Here, GBS was confirmed as the most common conclusive diagnosis linked to AFP; this is consistent with other findings reported in Italy^{15,21,24} and in other countries such as South Africa and Hong Kong.^{25, 26}

No wild PV were detected during this 4-year study supporting the epidemiological data deriving from AFPS at national level. Three children (6.3% of AFP cases) resulted positive to NPEV; a 5-y-old male presented GBS was echovirus type 11-positive, an 8-y-old female with myelitis was echovirus type 6-positive, and a 3-y-old female with paralysis of undefined origin was echovirus 11-positive. The rate of NPEVs detected in our AFP series is lower than that noticed in India (21.4%)²⁷ but it is in line with findings of WHO European region (*unpublished data*). It remains challenging to associate the detection of NPEV in clinical samples to the onset of AFP, even if several NPEVs, such as Enterovirus (EV) 71 and D68, have been recognized to cause paralysis.^{28, 29}

As long as in the current polio endgame PV outbreaks reflect serious gaps in immunity to PV due to the weakness of routine immunization coverage in otherwise polio-free countries, all nations should maintain uniformly high routine immunization coverage at the district level to minimize the consequences of any new virus introduction. In Italy, a satisfactory vaccination coverage with 3 doses of vaccine in children less than 2-y-old is currently reached.³⁰ Our data confirm adequate levels of immune protection in the study population since more than 93% of children showed a protective antibody titer against PV. In Italy, PV vaccination is realized by the administration of IPV only; since IPV does not elicit a consistent mucosal immunity,³¹ silent transmission of PV can happen through IPV-immunized individuals, favoring possible infection of unvaccinated subjects or children receiving delayed IPV vaccination.

In this setting, ES is able to detect the silent introduction and circulation of wild PV⁸ and VDPV.³² In Italy, ES was set up in 2005, since then no wild PV were detected whereas Sabin-like PV were identified here and there,^{10,11,33,34} even if in Italy OPV

administration was stopped in 2002. These findings underline the ability of ES to detect any PV strains and the need to implement this surveillance to monitor the effectiveness of OPV containment after the interruption of OPV administration in these latest eradication stages.

In the framework of ES, no PV reintroduction was detected from 2012 to 2015 in Lombardy. In contrast, NPEVs were detected in 65.3% of tested samples, in accord to our previous data¹¹ and to results obtained by ES conducted in Tehran (60.2%) in 2007¹⁴ and in Poland (77%)¹² in 2011. In our study, all characterized NPEVs belonged to enterovirus species B and echovirus type 11, 6 and 3 were the predominant genotypes. Overall, echovirus type 11 was the genotype most frequently identified in this study; similarly, echovirus type 11 was detected in 31.5% of wastewater samples in Tehran and echovirus type 11, 3 and 6 represented 2-third of NPEVs detected in ES in Poland.¹²

In Guangdong province of China, the role of echovirus type 30 in an outbreak of aseptic meningitis has been assessed through continuing ES³⁵; in Wisconsin (US) an association between NPEVs detected in environmental samples and those affecting the population has been documented.³⁶ These pieces of evidence emphasize that ES is able to provide comprehensive epidemiological information about EVs circulation and phylogenetic study can be used to assess links of NPEV dynamics and transmission route in a population.

To date, no systematic NPEV hospital- and community-based surveillance systems are ongoing in Italy, contrary to other countries (i.e. France, Spain, and Australia); information on NPEV circulation in Italy derived exclusively from occasional studies in particular population, especially in children affected by severe pathological conditions.³⁷⁻⁴²

In conclusion, AFPS proved to be successful especially after the PHEIC declaration and ES revealed a wide NPEVs circulation, mainly supported by echovirus type 11, 6 and 3.

Keeping strong and encouraging these surveillance activities all over the world is crucial to ensure the PV will not return unnoticed in polio-free countries and, as a final point, to achieve the global polio eradication goal.

Materials and methods

This study was conducted from January, 1st 2012, to December, 31st 2015, in Lombardy region (Northern Italy) by the Department of Biomedical Sciences for Health, University of Milan, which is one of the 6 Italian Sub-National Reference Laboratories (SNRLs) performing virological and serological investigations on samples collected in the operational framework of AFPS and ES.²⁴ SNRLs strictly complies with WHO guidelines, methods and recommendations for conducting AFPS and ES activities.^{43,44} SNRLs are regularly evaluated by the National Polio Reference Laboratory (NPRL) for proficiency in isolation and typing of EVs.²⁴

SNRLs share the results of AFPS and ES activities obtained at regional level with the NPRL and the Italian Ministry of Health.

Surveillance systems

AFPS – According to WHO case definition of AFP, all children <15 y with a clinically evident AFP of one or more limbs with

deceased or absent deep tendon reflex with sudden onset or with a bulbar paralysis were enrolled by collaborating hospitals of the surveillance network. AFP cases were notified by sending an 'AFP case report' to the reference SNRL twice a month, according to the "zero reporting" methodology. Prompt collection of i) 2 stool specimens taken 24–48 hours apart within 14 d since the onset of paralysis, and ii) paired serum samples, one acute and one convalescent, from each AFP case was requested for virological and serological tests, respectively. Epidemiological (i.e., type of PV vaccine received and date of administration), demographical (i.e., age, gender, country of origin), and clinical (i.e., symptoms and date of onset) data were collected by means of an 'ad hoc' questionnaire by physicians or health-care workers.⁴³ After case notification, another 'ad hoc' questionnaire with more comprehensive medical information about the case should be filled to disclose the final outcome of AFP case (i.e., residual paralysis or death 60–90 d after the onset of paralysis) and to clarify the conclusive diagnosis.⁴³ Patients' privacy and confidentiality issues were managed in full agreement with national legislations.

To evaluate AFPS performance, a number of indicators were assessed.⁴³ In particular: i) the incidence index (i.e., at least one AFP case detected per 100'000 children <15 y annually); ii) completeness of case investigation (i.e., virological analysis of 2 stool samples collected 24–48 hours apart, within 14 d after the onset of paralysis, in at least 80% of AFP cases); and iii) completeness of follow-up (i.e., clinical follow-up 60–90 d after the onset of paralysis completed in at least 80% of AFP cases).

ES – The sample size representative for study population is defined according to the WHO guidelines.⁴⁴ Wastewater samples (1000 ml) were collected twice a month at the inlet of 3 WWTPs, placed in high-density urban setting in Milan, serving a population of about 1 million inhabitants. Wastewater samples were collected during 24h-automated flow at the WWTPs and shipped frozen to the reference SNRL for PV and NPEVs identification and molecular characterization. Half volume (500 ml) of wastewater sample was used for virological investigation; the left volume was kept frozen (–80 °C) until virological assessment.⁴⁴

PV and NPEVs identification and molecular characterization

For viral detection, 10% of stool sample collected from each AFP case was prepared in phosphate-buffered saline and clarified by micro-tube centrifugation (13,000 rpm for 15 min). Wastewater samples were treated using the 2-phase (poly-ethylene-glycol and dextran) separation method, as previously described, in order to obtain a 50-fold volume concentration.⁴⁴

Once processed, stool and wastewater samples were analyzed to detect EVs by virus isolation in RD (human rhabdomyosarcoma cells, permissive to EVs) and L20B (murine transgenic L cells expressing the gene for the human cellular receptor for PV, sensitive to PV and few other NPEVs) cell cultures,⁴⁴ according to WHO algorithm.⁵ For rapid virus identification, the lysate of cell cultures that showed cytopathic effect were tested by RT-nested-PCR specific for the 5' noncoding region (5'NCR) (nucleotide [nt.] 179 to 575) common to all EVs.⁴⁵ The molecular characterization of EVs was performed by sequence analysis

of the VP1 gene (nt. 2'602–2'977).⁴⁶ The nucleotide sequences were obtained by automated DNA sequencing (Macrogen Inc., Korea) and nucleotide sequence alignments were obtained using BioEdit software.⁴⁷

Detection of anti-PV antibodies in serum samples

The presence of anti-PV antibodies was assessed in paired serum samples collected from each AFP case by microneutralization assay.⁴³ According to WHO guidelines, an antibody titer $\geq 1:8$ was considered protective; seroconversion was defined as the 4-fold or greater increase in antibody titer between the paired serum samples.⁴⁸

Abbreviations

AFP	Acute Flaccid Paralysis
AFPS	AFP Surveillance
CISID	Centralized Information System for Infectious Diseases
ES	Environmental Surveillance
EV	Enterovirus
GBS	Guillain-Barré Syndrome
IPV	Inactivated Polio Vaccine
NPEV	Non-Polio Enterovirus
NPRL	National Polio Reference Laboratory
OPV	Oral Polio Vaccine
PHEIC	Public Health Emergency of International Concern
Polio	Poliomyelitis
PV	Poliovirus
SNRL	Sub-National Reference Laboratory
VDPV	Vaccine Derived Poliovirus
WHO	World Health Organization
WWTP	Waste-Water Treatment Plant

Disclosure of potential conflicts of interest

The authors have no competing interest with respect to this manuscript.

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