

## Poliovirus Excretion among Persons with Primary Immune Deficiency Disorders: Summary of Data from Enhanced Poliovirus Surveillance in Egypt, 2011-2014

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### Abstract

**Background:** If exposed to oral poliovirus vaccine (OPV), persons with primary immune deficiency disorders (PID) are at increased risk of paralytic poliomyelitis; and can chronically excrete poliovirus. However, the risk of excretion of vaccine derived poliovirus among immunodeficient persons (iVDPV) is not well characterized. We present summary of data from poliovirus surveillance project among PID patients collected between 2011 and 2014 from 11 Egyptian Governorates.

**Methods:** Stool was tested for polioviruses in suspected or confirmed PID children regardless of whether Acute Flaccid Paralysis (AFP) was present or not. Those excreting poliovirus were followed until three consecutive negative stool samples were obtained.

**Results:** There were 122 patients with suspected or confirmed PID identified; 13/122 (11%) excreted poliovirus; of these, 6 excreted iVDPVs, the remaining 7 excreted Sabin virus. The duration of iVDPV excretion ranged from 1 to 21 months. AFP was detected in 3/6 (50%) of those excreting iVDPVs. All iVDPV excretors had history of receiving OPV.

**Conclusions:** Chronic poliovirus excretion in PID patients is rare, however, poliovirus eradication requires removal of all polioviruses from circulation; and because PID individuals are not necessarily paralyzed they might be missed by current poliovirus surveillance based on detection of AFP. To achieve poliovirus eradication, surveillance for polioviruses among PID patients should be routinely conducted in all countries, and poliovirus antiviral therapy must be made available for those with chronic excretion.

**Keywords:** Poliovirus excretion; Primary immune deficiency; Vaccine-derived polioviruses; OPV

### Introduction

The global effort to eradicate poliomyelitis owes much of its success to the effective use of the oral poliovirus vaccine (OPV) in routine immunization and supplemental immunization activities (SIAs). OPV, in contrast to the injectable inactivated poliovirus vaccine (IPV), contains live attenuated polioviruses and offers the advantage of easy oral administration appropriate for volunteer use [1]. However, in rare circumstances, OPV polioviruses can cause vaccine-associated paralytic poliomyelitis (VAPP) [2]. In addition, through prolonged replication in a single immunodeficient host or serial transmission in

an under-vaccinated community these attenuated polioviruses can evolve and regain the neurovirulence and transmission characteristics of wild poliovirus [3,4]. These vaccine-derived polioviruses (VDPVs) are capable of circulating and causing paralytic poliomyelitis with an identical clinical presentation as caused by wild poliovirus. VDPVs establishing community circulation are referred to as circulating vaccine-derived polioviruses (cVDPVs) [4].

Primary immunodeficiency disorders (PIDs) represent a large array of immune system disorders jointly characterized by impaired function of the immune system [5,6]. Individuals with PID, especially those disorders affecting the B-cell system, are at highest risk for developing VAPP [7]. In addition, prolonged intestinal poliovirus replication in PID patients may lead to development of VDPVs referred to as

immunodeficiency-related vaccine-derived polioviruses (iVDPVs) [8,9].

Any VDPV is considered a significant threat to polio eradication because it can be reintroduced into the general population and cause outbreaks of paralytic poliomyelitis. Over a dozen such outbreaks have been recorded throughout the world in the last ten years [10]. The largest recorded cVDPV outbreak in Nigeria caused paralysis in 385 predominantly young children between 2005 and 2012 [11-14].

To date, the only documented occurrence of iVDPV community circulation was in the USA, in an Amish community with low immunization coverage in 2005 [15,16]. This iVDPV circulation did not result in any case of paralytic disease.

Recognizing the risk that iVDPVs pose to global poliovirus eradication, the World Health Organization (WHO) maintains a registry of known iVDPV excretors and is promoting global surveillance for iVDPVs. Between 1962 and 2015, 107 individuals with long-term iVDPV excretion have been included in this registry [WHO data as of February 1, 2016]. The vast majority of these cases were identified from upper or middle-income countries and have either spontaneously stopped shedding poliovirus or died. In seven cases, iVDPV excretion was reported to last for over five years. In one reported case, an individual has been excreting iVDPV for over 28 years [17]. All of the chronic excretors included in the registry suffered from common variable immune deficiency (CVID) and therefore CVID patients represent the most important reservoir of potential long-term poliovirus excretors. To protect PID patients with prolonged poliovirus excretion against development of paralysis and to prevent community spread, polio antiviral products are being developed, with a goal for drug approval in 2017 [18,19]. However, surveillance to detect PID patients excreting iVDPVs remains limited, particularly for those PID patients without paralytic manifestation.

Acute flaccid paralysis (AFP) surveillance is a cornerstone of surveillance for polioviruses worldwide and is well established in most countries. AFP surveillance relies on timely identification of paralyzed patients and stool testing for polioviruses [20]. Some reports of iVDPV excretion in paralyzed PID patients diagnosed through AFP surveillance are available; however, little data exist on those PID patients who had not been paralyzed and therefore had not been captured through AFP surveillance [21]. These individuals are the potentially missed cases of poliovirus excretion that, if not identified and treated, may pose risk to poliovirus eradication.

Because limited data exist on the prevalence and outcome of prolonged or chronic poliovirus excretors in middle and lower income countries, WHO initiated a study series with the objective to better describe epidemiology of iVDPV and to estimate the risk of iVDPV excretion among PID patients [22]. In this report, we summarized data collected between January 2011 and December 2014 from the iVDPV excretor surveillance project in Egypt, one of the countries that participated in the study series. The objectives of this surveillance project was to estimate the prevalence of iVDPV excretion among persons diagnosed with PID; to establish a pilot national iVDPV surveillance project, integrated with AFP surveillance, that could serve as a model for other countries to implement as a means of minimizing the global risk of iVDPVs in the post-OPV cessation era; and to build capacity to monitor and respond to iVDPVs. During the period covered by this report, Egypt has used only OPV in its routine immunization program, with a plan to add IPV in 2016.

## Methods

The surveillance for iVDPV was established in participating health facilities, initially in 3 governorates of Egypt: Cairo, Giza and Alexandria. In 2013 it expanded to involve 8 additional governorates (Gharbiya, Kafr El Sheikh, Dakahliya, Qalioubiya, Sharkiya, Port Saeid, Ismailiya, Beheira), which comprise approximately 54/87 million (62%) of the total population of Egypt. As part of this project, 960 physicians and other AFP surveillance personnel were trained on how to recognize PID patients and implement the surveillance for iVDPV.

Persons below 18 years of age who were diagnosed with either suspected or confirmed PIDs were identified as potential iVDPV excretors; and their stools were tested for the presence of poliovirus using standard poliovirus detection methodology used in the Global Polio Laboratory Network [23,24]. Two stool samples were collected from each patient; the collection of the second sample was performed 24 hours after the collection of the first sample. In addition, one blood sample was collected for complete blood counts (CBC) and quantitative measurement of immune globulins (QIGs).

A broad definition was used for suspected PID patients based on increased susceptibility to infections. Patients who presented at participated health facilities with at least one of the 10 warning signs for PID, which were developed by the Jeffrey Modell Foundation, were classified as suspected PIDs [25]. A confirmed PID diagnosis, for the purpose of this project, was based on CBC, QIG values, and clinical assessment. Definitive diagnosis, treatment and follow-up of patients with PID was carried out in the three main focal pediatric immunology centers (at Ain Shams University, Cairo University and Alexandria University) and required further investigations such as CD markers, phagocytic functions, imaging studies, and molecular diagnosis, as necessary.

If stool sample was positive for VDPV or Sabin-like virus (SL), it was repeated monthly until either the sample was no longer positive on three consecutive occasions, or the subject was lost to follow-up or passed away.

Partial genome sequences were determined for all detected polioviruses. VDPV was defined as poliovirus isolates with at least 10 nucleotide differences in the VP1 region from Sabin vaccine strain for serotypes 1 and 3, or 6 nucleotide differences for serotype 2 [4]. SL virus was defined as virus related to Sabin vaccine virus with 0-9 nucleotide substitutions (0-5 for type 2).

The WHO polio reference laboratory at VACSERA, Cairo, carried out virus isolation from the stool samples and the Polio and Picornavirus Laboratory, Centers for Disease Control and Prevention, Atlanta, USA sequenced the polioviruses found in the stool samples.

## Results

Between January 2011 and December 2014, 122 patients were identified as having suspected (18/122) or confirmed (104/122) PID. They were predominantly male (81%) and less than 5 years of age (67%). In those with confirmed PID diagnoses (104/122, 85%), combined immune deficiency (CID) was the most predominant (29/104, 28%); CVID was diagnosed in 8/104 (8%) (Table 1).

Among the PID patients, 7/122 (6%) excreted SL poliovirus and 6/122 (5%) excreted iVDPVs (one excreted iVDVP1, four excreted iVDPV2, and one excreted iVDPV3).

Characteristic		n/N (%) N=122
<b>Gender</b>	Male	81/122 (64%)
<b>Age*</b>	0-1 year	32/97 (33%)
	1-5 years	33/97 (34%)
	5-15 years	30/97 (31%)
	15-18 years	2/97 (2%)
	Confirmed	104/122 (85%)
<b>PID Diagnosis</b>	CID (including SCID)	29/104 (28%)
	CVID/ Hypogammaglobulinemia	22/104 (21%)
	Agammaglobulinemia	18/104 (17%)
	Other PID	35/104 (34%)
	Suspected PID	18/122 (15%)

Received IVIG*	Yes	51/92 (55%)
*Patients whose data were available PID: primary immune deficiency; CID: combined immune deficiency syndrome; CVID: common variable immune deficiency; IVIG: intravenous immunoglobulin; SCID: severe combined immune deficiency syndrome		

**Table 1:** Demographic characteristics of detected PID patients and their diagnosis.

The patient who excreted iVDPV 3 had had SL3 detected in the first collected stool sample; however, the SL3 evolved into iVDPV3 in the sample taken one year later and remained detectable in another three monthly samples; the three subsequent samples were negative. The patient underwent stem cell transplantation and, in the end of 2015, was surviving with mild residual paralysis (Table 2, patient 5). This patient has a twin sibling, also diagnosed with PID, who was found to excrete iVDPV2 (Table 2, patient 2). In addition to poliovirus excretors, we found 20/122 (16%) patients excreting non-polio enteroviruses (NPEV). The NPEVs were not characterized further.

	Case #	Initial Sample Type	Initial Sample (Date)	Nucleotide changes from Sabin poliovirus of initial sample	Final positive Sample Type	Nucleotide changes from Sabin poliovirus of final positive sample	First negative Sample (Date)	Number of Samples Obtained	Duration of shedding (weeks)	Last OPV Dose (Date)	PID Type	Age at PID Diagnosis	AFP (yes/no)	
								(two stool samples obtained each time)						
iVDPV Excretors	1	iVDPV1	05-03-2011	19	iVDPV1	19	06-08-2012	6	7	Mar-11	Agamma globulinea	21 mo	yes	
	2	iVDPV2	06-03-2011	13	-	-	-	1	13	Feb-11	SCID	9 mo	no	
	3	iVDPV2	07-03-2012	11	-	-	-	1	8	May-12	SCID	5 mo	no	
	4	iVDPV2	12-04-2012	10	-	-	-	1	10	Sep-12	CID	6 mo	yes	
	5	SL3	06-08-2011	-	-	iVDPV3	48	12-08-2012	8	82	Feb-11	SCID	9 mo	yes
	6	iVDPV2	12/21/2013	11	-	iVDPV2	11	--	2	12	Nov-13	SCID	6 mo	no
Sabin Virus Excretors	7	SL1	3/15/2013	-	-	-	8/20/2013	2	-	Jan-13	CVID	67 mo	no	
	8	SL1	01-06-2014	-	-	-	7/22/2014	2	-	Apr-14	CID	6 mo	no	
	9	SL1+S L3	10-11-2011	-	-	-	7/20/2012	4	-	Sep-11	Hypogamma globulinea	12 mo	no	
	10	SL2	11/29/2013	-	-	-	12/29/2013	2	-	Sep-13	CID	5 mo	no	
	11	SL2	11-04-2014	-	-	-	-	1	-	NA	Suspected PID	-	no	

12	SL3	10/21/2012		SL3		-	2	-	Jul-12	CID	4 mo	no
13	SL3	11-03-2014	-	-	-	-	1	-	Oct-14	Other PID	8 mo	no

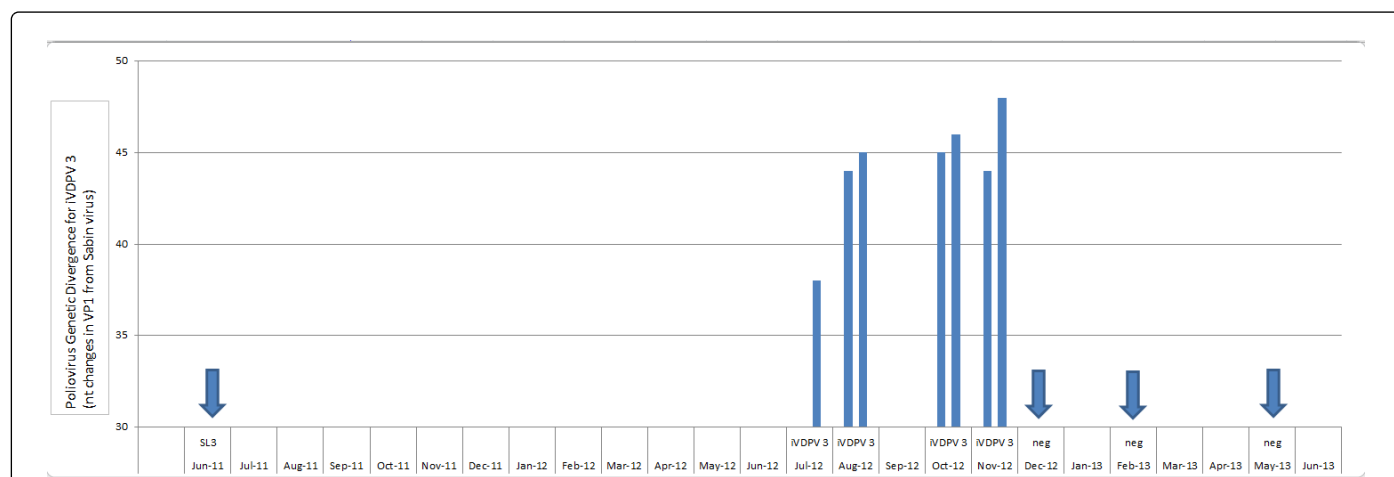
**Table 2:** Line list of poliovirus excretors among the primary immunodeficient children.

Among the patients with confirmed PID diagnoses who excreted iVDPVs, 5/6 (83%) were diagnosed with severe combined immunodeficiency (SCID) of whom 4 passed away; 1/6 (17%) was diagnosed with agammaglobulinemia (surviving); both of the surviving children were maintained on intravenous immunoglobulins (IVIG). One iVDPV excretor with SCID had normal QIG values at diagnosis yet his B cell count was repeatedly zero.

The duration of iVDPV excretion ranged from 2 months to 20 months but only one person excreted iVDPV for more than 6 months; since December 2014, there have been no PID patients who excreted iVDPVs. Further, no secondary transmission of iVDPVs was reported.

Acute flaccid paralysis (AFP) was detected in 3/6 (50%) of those excreting iVDPVs. All iVDPV excretors had history of receiving multiple doses of OPV. AFP was detected within a median of one month following last OPV dose.

Sequencing of iVDPVs detected divergence from Sabin polioviruses to be between 10 and 48 nucleotide substitutions in the VP1 region. Multiple samples were collected from several patients. There was one patient (patient 5 on Table 2) who had evolution of VDPV in multiple stool samples, and Figure 1 demonstrates the evolution of the poliovirus mutations over the 8 collections of stool samples (each stool collection is comprised of 2 samples). The screening of the first polio isolates indicated that the virus isolates were Sabin-Like (SL). Following the WHO algorithm, these isolates were not referred for sequencing. The second collection of stools more than one year later yielded virus isolate containing 38 nucleotide substitutions in VP1. From July 2012 to November 2012, the number of nucleotide substitutions increased to a maximum of 48.



**Figure 1:** Divergence in VP1 sequence of poliovirus in Patient 5 (y axis: number of nucleotide changes in the VP1 sequence, arrows show dates of stool collection with SL3 or negative result), iVDPV: vaccine derived poliovirus in immunodeficient individual; SL: Sabin-like poliovirus.

## Discussion

This is the first population-based surveillance study of PIDs with or without paralytic manifestations. Our study covered a population of >50 million, and focused on stimulation of identification (based on the ten Jeffrey Modell Foundation warning signs), reporting, and follow-up investigations in tertiary referral centers.

Our study in Egypt demonstrates that it is possible to establish a system to detect PID patients and test their stools for polioviruses in a low-middle income country. Prevalence of iVDPV excretion was relatively low and similar to previous observations [22,26,27]. The duration of excretion was generally short, with only with one patient excreting for more than 6 months. The short duration of excretion was influenced by early death due to severe infections unrelated to poliovirus in 4/6 excretors. We observed clearance of iVDPV excretion

in the 2 surviving children who were maintained on replacement therapy (IVIG). Current evidence regarding the effect of IVIG therapy on clearing persistent poliovirus infection is inconclusive [8,28].

Patients' referral to pediatric immunology units and their follow-up was a consistent challenge, due to logistical and cultural barriers. Accordingly, the number of recruited cases might not represent the true frequency of PID in the general population and hence it may become necessary to establish a national registry to generate a more accurate estimate of PID prevalence for the purpose of identifying poliovirus excretors.

A study limitation was that surveillance was conducted on the pediatric age group only; hence, all detected PID patients were below 18 years of age with the more severe forms of PID. PIDs are usually recognized during infancy and early childhood, but some types of PID,

such as CVID may be diagnosed later in life, commonly during the second decade of life or even later [29,30]. An additional limitation of this study was that there is incomplete data from several of the PID patients, due to loss to follow-up.

It is estimated that approximately 12 nucleotide mutations are expected to occur in one year in the VP1 genome of poliovirus [31]. Our finding demonstrated that iVDPV excretion was likely undetected for a prolonged period of time because we found patients excreting highly divergent iVDPVs. One complication in detecting and sequencing VDPVs from immunodeficient individuals is the presence of multiple virus lineages, which are closely genetically linked and are evolving over time. The mixed virus lineages can result in sequences with mixed base positions, which can be hard to resolve into distinct sequences [9]. This might have contributed to the classification of the first virus isolates as Sabin-Like, with no need for sequencing. Mixed virus lineages can also complicate the assignment of numbers of nucleotide substitutions, since more than one nucleotide is present and can lead to different consensus base calls. Limited amino acid substitutions in the neutralizing antigenic sites were observed (data not shown). The last dose of OPV for this patient (Table 2) in February 2011 is not likely to be the dose that initiated the emergence of VDPV because of the extensive number of nucleotide substitutions; 38 substitutions is about 3 fold more than would be predicted for 13 months using the poliovirus molecular clock [32].

Despite the low probability of iVDPV being transmitted to the general community and causing cVDPV outbreaks, it is imperative to continue with surveillance efforts to identify all VDPV excretors: the existence of VDPVs potentially circulating in communities may threaten poliovirus eradication [8,33]. OPV will be globally withdrawn from use; in April 2016, the trivalent OPV was replaced by bivalent OPV containing Sabin poliovirus serotypes 1 and 3.

Wild poliovirus serotype 2 has been eradicated, with the last case reported in 1999; after the switch from trivalent OPV to bivalent OPV, the iVDPV chronic excretors will remain the only potential source of poliovirus serotype 2. Our ability to detect these individuals, and potentially treat them with antivirals, will be of high importance in the immediate post-eradication era.

Efforts to integrate AFP and iVDPV surveillance in non-paralyzed PID patients should continue in Egypt, as well as in other countries using OPV. Expansion of population-based surveillance efforts are being actively discussed, and should be considered by the Global Polio Eradication Initiative to identify PID patients with poliovirus infection, make therapeutic interventions available to clear the infection, and thus, to remove the threat of paralysis caused by poliovirus from these patients, but also to ultimately secure complete poliovirus eradication.

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