

# Laboratory Challenges in Response to Silent Introduction and Sustained Transmission of Wild Poliovirus Type 1 in Israel During 2013

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**Wild poliovirus type 1 (WPV1) introduction into southern Israel in early 2013 was detected by routine environmental surveillance. The virus was identified genetically as related to the South Asian (SOAS) R3A lineage endemic to Pakistan in 2012. Intensified, high-throughput environmental surveillance using advanced molecular methods played a critical role in documenting and locating sustained transmission throughout 2013 and early 2014 in the absence of any acute flaccid paralysis. It guided the public health responses, including stool-based surveillance and serosurveys, to determine the point prevalence in silent excretors and measured the effect of vaccination campaigns with inactivated polio vaccine and bivalent oral polio vaccine on stopping transmission.**

**Keywords.** wild poliovirus; silent poliovirus transmission; environmental surveillance; emergency health policy response; epidemiology; vaccine; molecular analysis.

## POLIO HISTORY IN ISRAEL AND ROUTINE VACCINATION SCHEDULE AT TIME OF DETECTION OF WILD POLIOVIRUS (WPV), IN 2013

The road toward polio eradication has been long and by no means smooth [1–3]. This article will describe the latest bump that is currently taking place in Israel

since early 2013, namely, silent sustained transmission e.g., transmission in the absence of any acute flaccid paralysis of WPV type 1 (WPV1) after introduction into a population with very high vaccination coverage (>95%) and 9 birth cohorts vaccinated exclusively with inactivated polio vaccine (IPV). It is written from the perspective of the role of Israel's National Poliovirus Laboratory in providing the evidence necessary for shaping an evidence-based national public health policy in response to this unusual event.

To better understand the context in which this event is occurring, the epidemiological history of poliomyelitis in Israel has been divided into 6 stages on the basis of an epidemiological description of disease within the population [3]. These stages consisted of periods of (1) sporadic cases, first mentioned in reports from the second half of the 19th century; (2) severe annual epidemics between 1949 and 1960, with attack rates of 28.4–128.0 cases per 100 000 population; (3) an annual pattern of endemicity, with periodic small clusters of

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related cases, between 1961 and 1981; (4) sporadic morbidity interspersed between an absence of disease during 1982–1987; (5) a WPV1 outbreak during 1987–1988, with almost all cases manifesting among OPV-recipient Jewish individuals; and (6) absence of cases and sustained transmission between 1989 and the first quarter of 2013. Although there were no cases during the 6th period, environmental surveillance provided evidence for one introduction of WPV1 into Israel, in 1996, and intermittent excretion, presumably by 3 immunodeficient individuals, of neurovirulent, highly diverged, vaccine-derived poliovirus (VDPV) types 1 and 2 between 1998 and 2014 [4–6]. Environmental surveillance also provided evidence for sporadic introductions of WPV1 and WPV3 into nearby Gaza between 1990 and 2002, with presumed local transmission of 1 WPV1 strain [4, 6]. Israel was assessed by the European Regional Certification Commission for Poliovirus Eradication in 2012 as having a low risk for transmission following importation of WPV, based on a low quality of surveillance (measured in terms of acute flaccid paralysis (AFP) reporting rates, without taking into account environmental surveillance), a high population immunity, and an average of other risk factors [7].

Many vaccination formulations and vaccination schedules using live and inactivated vaccines have been used in different countries since 1935 [1, 2, 8, 9]. Changes in schedules and formulations throughout the years resulted in populations with cohorts who received different vaccine formulations and immunization schedules during routine childhood vaccination and vaccination campaigns. Vaccination in Israel prior to 2013 [3] evolved through 5 major phases in response to the changes in the epidemiology of the disease and development of vaccines. The first phase involved exclusive use of IPV (mainly local production) during 1957–1960, with coverage between 75% and 90%. The second phase involved exclusive use of OPV between 1961 and 1981, with an annual coverage of 81%–91%, starting with monovalent type 1 OPV (mOPV1) in 1961; mOPV1, mOPV2, and mOPV3 in 1962; 3 doses of trivalent OPV (tOPV) in 1963; 4 doses of tOPV after 1964; and a supplemental mOPV1 dose in 1979. Phase 3 involved parallel use of the following 3 programs between 1982 and 1987: 4 doses of OPV in 12 of 14 health districts, 4 doses of OPV supplemented with 1 dose of mOPV1 for selected groups at risk, and exclusive use of enhanced IPV (eIPV) in the remaining 2 health districts. Phase 4 involved a combined eIPV/OPV schedule of 3 doses each by 18 months of age, with addition of an IPV booster at age 6–7 years, between 1989 and 2005. Phase 5 involved exclusive use of 4 doses of eIPV (at 2, 4, 6, and 12 months of age), plus an IPV booster at 7 years of age, after 2005, with annual coverage rates of >90%. Further confounding any interpretation of disease in relation to vaccination coverage is the large number of families with children who immigrated to Israel since 1957 whose members were vaccinated according to different vaccination schedules in their countries of origin.

## HISTORY OF AND METHODS FOR ENVIRONMENTAL SURVEILLANCE

The current standard method for poliomyelitis surveillance recommended by the World Health Organization (WHO) [10] is based on the isolation and molecular and serological analysis of all viruses from all cases of AFP to rule in or rule out poliovirus etiology. The rationale for AFP surveillance is based on the observation that AFP from all causes other than poliovirus occurs with an incidence of 1 case per 100 000 in children up to the age of 15 years. A region is considered to be polio free when the number of AFP cases that are investigated falls within this range for nonpoliovirus causes and there is no laboratory evidence for poliovirus in the stool samples. During outbreaks and the current period of silent WPV1 transmission, the number of investigations needed to indicate a negative finding is increased >2-fold to exclude rates of 1 case in >200 000. Most countries use AFP surveillance. However, not all are able to meet the required benchmarks of AFP investigations. Polio-free status requires poliovirus-negative AFP surveillance results at adequate surveillance levels for at least 3 years.

Environmental surveillance, stool surveillance, and enterovirus surveillance are considered supplementary surveillance methods, although in some countries environmental surveillance and/or enteroviral surveillance are used exclusively. Successful environmental surveillance requires selection of the appropriate target population to monitor for evidence for the presence or circulation of poliovirus, a competent and well-equipped laboratory, a plan for routine surveillance and reporting, and cooperation with municipal authorities [11, 12]. Environmental surveillance is resource and labor intensive and may require large-capacity (with the ability to handle 0.5-L bottles), high-speed (10 000 × g) centrifuges that are not commonly present in most national poliovirus laboratories [11]. General principles for selecting surveillance sites have been recommended by the WHO [12]. While environmental surveillance works best with modern centralized sewage systems, each surveillance plan must be carefully tailored to take into account unique aspects of each sewage system and the populations within their catchment areas. The plan must be periodically reevaluated on the basis of long-term routine surveillance results and altered situations. Different methods for analyzing environmental samples are currently used in different laboratories [11, 12], because unlike AFP surveillance [10] there is no single standard method. Sampling include grab sampling during peak sewage use, trapping virus by lowering bags with silicates or gauze into sewage for varying times, and automatic composite sampling of sewage aliquots at given intervals over a 24-hour period. All sample storage and shipment must be at low temperatures (4°C–8°C) to maintain viability, since the WHO-recommended tests for poliovirus in environmental samples require an amplification step in tissue culture [12]. Ranta et al [13] developed a

simulation model for the probability of detecting virus from environmental surveillance samples.

The probability of detecting poliovirus depends on a number of external factors, including the proximity of the excretor to the sample site; the number of excretors, the duration and amount of poliovirus excreted by 1 or more infected individuals, the effect of physical and mechanical factors on the dilution and survival of poliovirus in the sewage system, and the frequency of collection and laboratory processing of the environmental samples [12–15]. Environmental samples frequently contain human enteroviruses other than poliovirus. A tissue culture amplification step that uses transgenic murine cells expressing the human poliovirus receptor (L20B cells) is used to selectively amplify any viable poliovirus in the sewage specimen. In countries using OPV or combined OPV/IPV vaccination schedules, sewage may also contain abundant Sabin virus strains, the quantity of which peaks during campaigns using OPV. However, if OPV is used for routine vaccination or if 1 or only a few individuals become infected with WPV or VDPVs, poliovirus-positive samples normally contain 1 or a few viable poliovirus isolates at best. Since environmental surveillance frequently evaluates samples at this limit of detection, a single negative finding cannot rule out the presence of non-vaccine strains. However, poliovirus-negative findings gain significance when they are part of a long history of negative findings from frequent routine surveillance at a given site [4, 11].

High nucleotide sequence homology of VP1 capsid genes between poliovirus isolates from cases and isolates from environmental samples collected from catchment areas serving the areas in which the cases occurred confirms that population-based environmental surveillance accurately reflects viruses circulating in individuals [15–17]. One of the main advantages of environmental surveillance is its ability to establish the so-called silent presence and/or circulation of WPVs or VDPVs before the appearance of AFP cases [5, 6, 13, 16, 18]. The epidemiological event currently unfolding in Israel [19] emphasizes the need to include some type of supplementary surveillance in national surveillance programs, since AFP surveillance alone was not designed to detect sustained silent transmission in highly immunized populations.

## **ENVIRONMENTAL SURVEILLANCE IN ISRAEL IN 2013: TRENDS, FINDINGS, AND TIME LINE**

Environmental surveillance for poliovirus infections, initiated in northern Israel during the 1987–1988 WPV1 outbreak [6, 17], was expanded in 1989 to include monthly sampling of 11–15 surveillance sites with catchment areas covering 30%–40% of the entire population. These sites have been sampled more or less on a monthly basis since 1989 [5, 6, 15]. Surveillance samples were obtained from inline automatic composite samplers located at the intake of each advanced sewage

treatment facility; positive specimens were considered indicative of the presence of poliovirus infections within the population served by a sewage treatment plant. Sewage specimens were collected using portable computerized automatic composite samplers (Sigma SD900 portable samplers, HACH, Loveland, CO) at appropriate sites located downstream of specific populations within the catchment area. Specific protocols were designed for concentrating sewage, isolating polioviruses, and characterizing the isolates to monitor the presence and potential circulation of nonvaccine polioviruses in the Israeli population [5, 6]. Most environmental surveillance protocols, including those in Israel, use tissue culture and molecular tests to indicate whether polioviruses are present, and if they are present, molecular tests, serological tests, and sequence analysis are used to determine whether they are vaccine like, vaccine derived, or wild [15]. The Israeli protocols, however, include plaque assays to provide quantitative data on the amount of poliovirus in each sample. The plaque assay is only semiquantitative because of batch-to-batch variations in the percentage recovery of viable polioviruses after processing. Typically, recovery of virus added to the samples before processing ranged between 8.8% and 15% (mean, 12%) [5]. Factors contributing to this variability include co-concentration of varying amounts of substances that specifically (eg, other viruses) or nonspecifically (eg, toxic substances) affect tissue culture, as well as phenotypic characteristics of individual poliovirus isolates that effect the efficiency of plaque formation and plaque size. Furthermore, plaque assay are generally most informative when Sabin strains are absent from the environment, as in countries with vaccination programs exclusively using IPV, since it is expensive and labor intensive and requires additional time to distinguish plaques containing non-vaccine poliovirus strains from plaques of polio vaccine strains. While semiquantitative data on viral loads in sewage significantly increase our understanding of events while they are unfolding, it is still difficult to determine the number of excretors (ie, are  $x$  excretors excreting  $y$  amount of virus or are  $y$  excretors excreting  $x$  amount of virus?). Another shortcoming that is particularly applicable to evaluating the Israeli situation arises when the interval between sampling is shorter than the duration of excretion by an infected individual. This is particularly problematic for determining the end point of transmission, because successive samples will include virus from the same excretor, making it very difficult to distinguish between ebbing yet continued low level person-to-person transmission and cessation of transmission, but continued excretion by infected individuals. The review on environmental surveillance by Hovi et al [11] discusses additional considerations that determine the effectiveness of environmental surveillance.

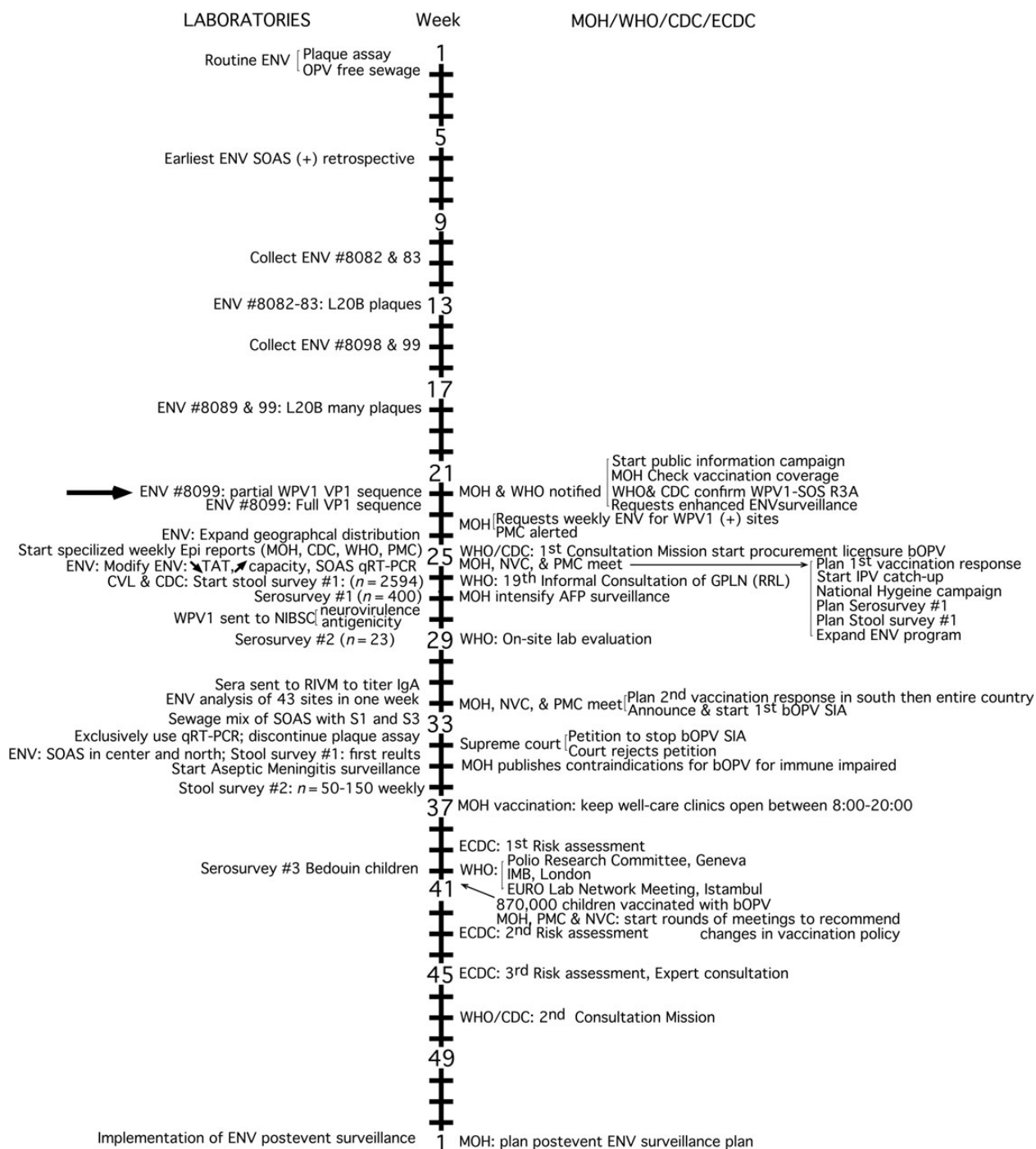
Environmental samples collected at the entrances to the Be'er Sheva and Rahat sewage treatment plants on 9 April 2013 produced plaques on L20B-cell monolayers, and the number of plaques increased in the subsequent samples collected on

7 May. A preliminary nucleotide sequence analysis of virus plaques isolated from the second Be'er Sheva sample, completed on 29 May, identified the virus as WPV1-SOAS and triggered Israel's poliovirus emergency response plan. Moreover, the finding required that the laboratory rapidly supply epidemiological information required for informed response to the Israeli Ministry of Health. This in turn meant that routine laboratory protocols needed to be revised and refined to decrease turnaround times, increase the number of samples that could be processed in parallel, and develop and validate, within a month's time, specific semiquantitative reverse-transcription polymerase chain reaction (qRT-PCR) assays specific for the circulating WPV1 strain in the absence, and later, following a bOPV vaccination campaign, in the presence of Sabin strains [19, 20]. In addition, the routine environmental monitoring program needed to be enhanced by significantly expanding the geographical distribution and number of sites, especially near areas where samples were found to contain WPV1, and by increasing the frequency of sampling at WPV1-positive sites from once per month to once per week. The time line for major events relating to the discovery of WPV1 is presented in Figure 1. Subsequent environmental surveillance results and results of retrospective analysis of archived aliquots of sewage samples collected before 7 May are presented in Figure 2. For easier comparison, data are presented by epidemiological week in Figures 1 and 2.

A detailed laboratory protocol including the modifications will be provided by the corresponding author (L. M. S.) upon request. In short, staggered work schedules and analysis of half instead of all of each 1-L sample quadrupled daily throughput [20]. The remaining half was stored at 4°C. In addition, we developed, validated, and determined the analytical sensitivity of a qRT-PCR assay for detection of the WPV1 in Israel, using RNA extracted directly from concentrated sewage that might eventually contain homotypic mixtures of Sabin strain of type 1 poliovirus and WPV1. Threshold cycle (Ct) values (ie, the cycle of qRT-PCR at which a specific signal crosses a threshold for detection) were linear over a 7-log range for the semiquantitative WPV1-specific qRT-PCR assay for RNA extracted directly from processed sewage [5, 13]. The limit of detection and quantitation was 200 plaque-forming units (PFU)/mL. The analytical sensitivity was also valid for RNA extracted from serial dilutions of WPV1-SOAS, to which  $\leq 10^7$  PFU of Sabin strain of type 1 and/or type 3 poliovirus had been added. Plaque assays had to be discontinued when Sabin strains began to appear in sewage samples after the start of a supplementary immunization activity (SIA) with bOPV on 5 August (Figures 1–3). The excellent correlation between Ct values and plaque counts enabled us to continue to generate semiquantitative results for WPV1 in samples after 8 August. In addition, 4 replicate L20B-cell tube cultures were challenged and analyzed after 5 days by molecular assay in parallel to the plaque assays. These changes enabled weekly resampling of WPV1-SOAS-positive sites by

reducing turnaround times for preliminary result from approximately 28 days to either 1 week, for molecular results, or 2 weeks, for tissue culture confirmation [21]. It is important to stress that direct molecular tests should be conducted in addition to, not instead of, classic WHO-recommended tissue-culture-based identification protocols for isolation and characterization of polioviruses [10, 12]. Toward the end of 2013, the sensitivity for detection of polioviruses by tissue culture was increased by challenging an additional 4 replicate tube cultures of RD with concentrated sewage, followed by a selective passage in L20B-cell tube cultures for polioviruses, as the number of wild polioviruses in samples approached the limits of detection and the number of WPV1-positive samples began dropping.

A real-time RT-PCR assay for detecting the RNA of Sabin strains of type 1, 2, and 3 poliovirus from tissue cultures that was developed by Kilpatrick et al [22] for use by laboratories in the Polio Laboratory Network was modified for enzymes, buffers, and RT-PCR conditions in our laboratory to also provide a semiquantitative indication of the relative amounts of Sabin strains of 1, 2, and/or 3 poliovirus RNA in the RNA extracted directly from processed environmental samples. The Sabin-strain-specific qRT-PCR was linear over a 6-log interval and had a limit of detection of 400 PFU/mL (M. H., unpublished data). Semiquantitative results for Sabin strains served as a surrogate for monitoring compliance of vaccination with bOPV and the efficacy of its use for intervention. The semiquantitative results provide data within a relevant time frame for determining the response policy, including scheduling for resampling of WPV1-positive sites: results are from direct molecular tests performed 1 week after sample collection and are confirmed by L20B-cell tissue culture the following week and by serial passage in RD and L20B cells within 2 weeks. Cumulative results for 2013–2014 for the catchment area of the Rahat sewage treatment plant (sampled monthly since 1989) are presented in Figure 3 to illustrate how this approach improves the quality of the all-or-none information provided by classic methods for the same site in Figure 2. The Rahat sewage treatment plant serves the Bedouin city of Rahat with a population of 53 600, Giv'ot Bar with a population 400, and kibbutz Mishmar Hanegev with population of 1000. By the end of 2013, WPV1-SOAS RNA levels at most surveillance sites dropped below the limits of detection. In 2–3 sites, qRT-PCR results hovered between the level of quantitation and the level of detection. The algorithm for determining the WPV status of these sites is presented in Table 1. While no paralytic poliomyelitis cases were identified during 2013–2014 in Israel, semiquantitative testing of environmental samples (an example of data from one site is shown in Figure 3) revealed 3 epidemiological patterns [20]: (1) introduction of WPV1 in southern Israeli communities, followed by a rapid increase and persistence of high WPV1-SOAS loads that declined at or before the start of the first round of a bOPV SIA, indicated sustained transmission;



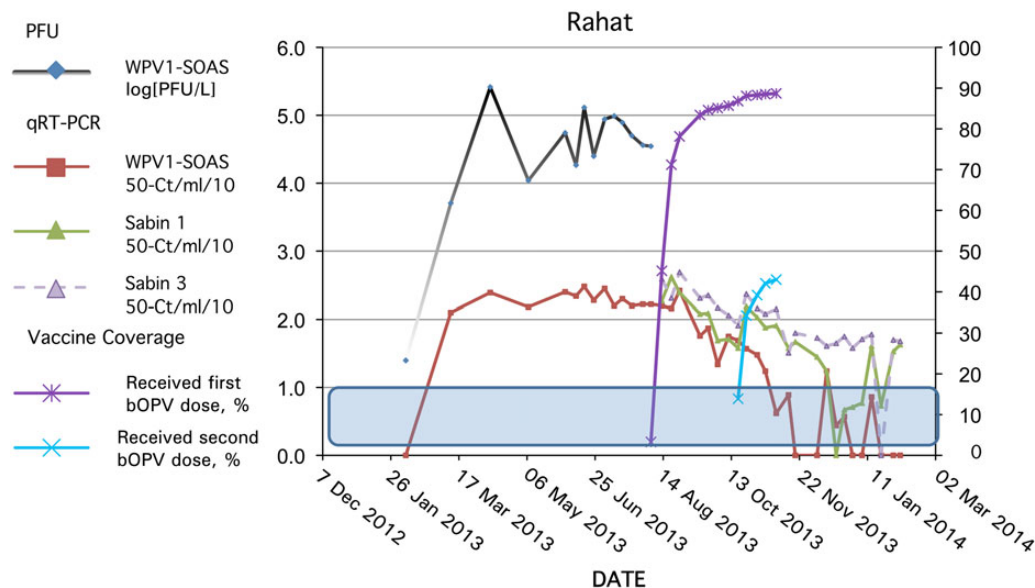
**Figure 1.** Time line of public health policy and laboratory actions after introduction and sustained silent transmission of wild poliovirus type 1 (WPV1-SOAS) in Israel in 2013, by epidemiological week. The numbers in the time line running through the center of the figure indicate the epidemiological week in 2013. Laboratory actions appear to the left of the time line. Public health actions appear to the right of the time line. The black arrow indicates the first laboratory confirmation for isolation of WPV1-SOAS from concentrated sewage. Abbreviations: bOPV, bivalent oral polio vaccine; CDC, Centers for Disease Control and Prevention; CVL, Israel Central Virology Laboratory; ECDC, European Centers for Disease Control; ENV, environmental surveillance; GPLN, Global Polio Laboratory Network; IgA, immunoglobulin A; IMB, World Health Organization Independent Monitoring Board of the Global Polio Eradication Initiative; MOH, Israel Ministry of Health; NIBSC, United Kingdom National Institute for Biological Controls and Standards; NVC, Israel National Committee for Vaccinations; PMC, Israel National Polio Monitoring Committee; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; RIVM, Netherlands National Institute for Public Health and Environment; RRL, WHO regional reference laboratory for polio; SIA, supplemental immunization activity; SOAS, South Asian lineage of wild poliovirus type 1; VP1, viral capsid protein 1; WHO, World Health Organization European Region; WPV1, wild poliovirus type 1.



**Figure 2.** Environmental surveillance results for polioviruses, by epidemiological week and sample site. Results for isolation of polioviruses from environmental surveillance sites in Israel are presented by epidemiological week. The color key appears at the bottom of the figure. The names of the sewage treatment plants (not cities) or trunk line are on the left column, and their location within Israel is on the right. The last column on the right indicates whether the sample was taken at the entry point into the sewage treatment facility (STF) or from a trunk line (TL) located upstream. The results were determined according to the algorithm presented in Table 1. The information was made available on a weekly basis to the Israel Ministry of Health and the World Health Organization Regional Office for Europe, Regional Polio Laboratory Network (Copenhagen, Denmark).

(2) intermittent detection in communities such as Kiryat Gat, suggesting that transmission was either at a lower rate or that there was occasional importation by visitors or daily workers; and (3) sites that remained free from WPV1. At the peak of surveillance, during the week of 4 August, 43 sites were sampled in parallel within 1 week (Figure 2). All sites were sampled at

frequencies determined by previous positive results and proximity to positive sites. The pattern of positive environmental results (Figure 3) was consistent with demographic data. This observation was further supported by the pattern of negative and positive results of surveillance involving samples collected at the same time at the mouths of different upstream trunk lines



**Figure 3.** Results of environmental surveillance for polioviruses in Rahat. Environmental surveillance results are presented for composite environmental samples collected at the inlet into the sewage treatment plant for the Bedouin city of Rahat as an example of the semiquantitative cumulative data presented on a weekly basis to the Israel Ministry of Health. Results are presented for the number of plaques per environmental sample and the threshold cycle of the quantitative reverse-transcription polymerase chain reaction (qRT-PCR) assay for RNA extracted directly from concentrated sewage for wild poliovirus type 1 with a South Asian lineage (WPV1-SOAS), the Sabin strain of type 1 poliovirus, and the Sabin strain of type 3 poliovirus. Superimposed on the figure is the percentage of vaccine coverage for the first and second doses of bivalent oral polio vaccine (bOPV). The following calculations were made to place the results on the same graph: (1) the number of plaques/L is presented as the log of the number of plaques/L (left scale); (2) the qRT-PCR results for WpV1-SOAS, the Sabin strain of type 1 poliovirus, and the Sabin strain of type 3 poliovirus are presented as 50-Ct/ml/10; e.g. cycle 50 minus the cycle at which the reaction results first appeared above the threshold of detection (Ct) divided by 10 (left scale); and (3) the vaccine coverage is presented as the percentage of children aged <10 years of age in Rahat who received 1 dose of bOPV or the percentage of bOPV-recipient children who received a second dose of bOPV (right scale). Values that fall within the shaded box at the bottom of the graph were between the limits of quantitation and detection. Abbreviation: PFU, plaque-forming units.

serving different subpopulations within the catchment areas served by 4 sewage treatment plants: Arad, Ayalon, Beer Sheva, and Tnuvot (Figure 3).

Sequence and phylogenetic analyses are essential epidemiological tools for tracing the origin and routes of transmission of polioviruses from poliovirus-endemic regions to poliovirus-free regions, to characterize the evolution of poliovirus during person-to-person transmission in outbreaks, and for risk assessment of the virus for causing disease [1, 17, 23, 24]. The WPV1 isolated from all surveillance sites were most closely related to WPV1-SOAS R3A endemic to Pakistan and to WPV1 isolated from sewage samples obtained during December 2012 in Egypt [25]. Bayesian phylogenetic time clock analysis using complete capsid coding sequences (2643 nt) indicated that the Israeli and EGY WPV1-SOAS lineages diverged in September 2012 and that the Israeli isolates formed 2 subbranches after January 2013, shortly before the earliest WPV1-positive sample was identified by retrospective analysis. Sequence data were essential for designing primers and probes for the WPV1 qRT-PCR reaction described above. The WPV1 were reported to have evolved at rates expected for WPVs [25].

A stool survey was commissioned by the Ministry of Health to determine the point prevalence of asymptomatic excretors in the south of Israel and to correlate this with the viral loads in sewage estimated from semiquantitative qRT-PCR after environmental surveillance indicated high amounts of virus in successive weekly samples collected from a number of surveillance sites. WPV1-positive samples were identified by 2 methods, based on parallel testing: one method was performed at the Centers for Disease Control and Prevention (Atlanta, GA), using tissue-culture-based protocols as described by the WHO [10], and the other was performed at the Central Virology Laboratory (Israel), using qRT-PCR screening followed by virus isolation from WPV1-positive suspensions. The highest prevalence of silent excretors was among children in Bedouin communities where rates of positive results of semiquantitative molecular assays performed during sewage surveillance were persistently high [20]. The Bedouins in the Negev region are living in 2 forms of settlements. About 60% live in 7 Bedouin towns, which are relatively crowded and where most residents are connected to central sewage systems. The remaining 40% are scattered throughout the Negev desert. They have access

**Table 1. Algorithm for Interpretation of Results for Environmental Samples**

Sample	qRT-PCR Finding	Reaction(s)	MS2 $\Delta$ Ct	Preliminary Report	Action	TC Result <sup>a</sup>	Final Interpretation
1	$\leq 37$	$\geq 1$	$\leq 3$	Positive	None	Positive	Positive
2	$\leq 37$	$\geq 1$	$\leq 3$	Positive	Redo TC	Negative	Positive
3	$>37$ and $\leq 45$	2	$\leq 3$	Weakly positive	None	Positive	Positive
4	$>37$ and $\leq 45$	2	$\leq 3$	Weakly positive	None	Negative	Weakly positive
5	$>37$ and $\leq 45$	1, 1 negative	$\leq 3$	Pending	Repeat qRT-PCR in triplicate <sup>a</sup>	Positive	Positive
6	$>37$ and $\leq 45$	1, 1 negative	$\leq 3$	Pending	Repeat qRT-PCR in triplicate <sup>a</sup>	Negative	Positive or weakly positive or inconclusive <sup>b</sup>
7	$>45$	2	$\leq 3$	Pending	None	Positive	Positive
8	$>45$	2	$< 3$	Pending	None	Negative	Negative
9	$>45$	2	$>6$	Pending	Dilute RNA 1:10 and/or add 0.5% BSA; repeat qRT-PCR <sup>a</sup> in triplicate	Positive	Positive
10	$>45$	2	$>6$	Pending	Dilute RNA 1:10 and/or add 0.5% BSA; repeat qRT-PCR <sup>a</sup> in triplicate	Negative	Indeterminate (inhibition not resolved) or result of qPCR <sup>c</sup>

Abbreviations: BSA, bovine serum albumin; Ct, threshold cycle; L20B, mouse L cells transfected with the gene for the human cellular receptor for poliovirus; MS2, RNA from coliphage MS2; qRT-PCR, quantitative reverse-transcription polymerase chain reaction. RD, a continuous cell line derived from a human rhabdomyosarcoma; TC, RNA extracted from tissue cultures.

<sup>a</sup> Because TC results are available 1 (for L20B tissue cultures) or 2 (for serial transfer in RD and then L20B cell cultures) weeks after qRT-PCR, samples are analyzed again before TC results are known.

<sup>b</sup> TC Negative: The sample is considered positive, if qRT-PCR findings for at least one of the triplicate repeats for RNA extracted from tissue cultures are  $\leq 37$ ; weakly positive, if findings for at least one of the triplicates are  $>37$  to  $\leq 45$ ; and inconclusive, if findings for 0 triplicate repeats are  $\leq 45$  and the TC is negative.

<sup>c</sup> If the difference between the MS2 Ct results for the sample and the MS2 control are  $>6$ , the qRT-PCR is considered to have been completely inhibited. qRT-PCR is then repeated using conditions for reducing inhibition. TC Negative: If qRT-PCR findings for the repeat triplicates for RNA extracted from tissue cultures are  $\leq 45$ , results are interpreted as positive, weakly positive, or inconclusive according to qRT-PCR criteria for negative TC results. If the results are  $>45$  and the sample is still inhibited, then the result is considered indeterminate.

to health and education systems, but most of them use local sewage treatment devices, such as septic tanks. Sequences of the stool isolates closely matched sequences of isolates in environmental samples collected from catchment areas that included the excretors [25]. The epicenter of the virus circulation was in the Bedouin communities in southern Israel [20], and the main reservoir of infected individuals in this community was the cohort of IPV recipients aged  $<9$  years (unpublished data). A subsequent weekly stool survey was performed to measure the effectiveness of intervention with bOPV. Phenotype analyses of the isolates (neurovirulence and reactivity of antibodies raised against serotype 1 OPV and IPV strains to neutralizing epitopes) were also conducted during the outbreak. The phenotype of WPV1-SOAS was not exceptional (L. S., unpublished data), although in addition to high population immunity, antigenic profile changes may have contributed to the intense “silent” transmission, and a slightly reduced neurovirulence may have contributed to the absence of paralytic cases. Thus, studies did not indicate any unusual properties of the WPV1 strain that would enable it to circulate in the highly immune population in Israel.

Host factors that might have contributed to the ability of the virus to circulate were also investigated. A serosurvey ( $n = 400$ ) was launched on week 27 (Figure 1) to determine whether

epidemiological records indicating vaccination coverage of  $>90\%$  corresponded to a similar percentage of sera with neutralizing antibody titers. The results confirmed the presence of neutralizing titers for the type 1 IPV Mahoney vaccine strain in  $>98\%$  of the population [26]. A second survey comparing titers against the Mahoney strain and WPV1 in 23 sera with low titers to the Mahoney strain indicated a 3-fold lower titer against WPV1, as expected for heterotypic WPVs, and revealed that some individuals with titers greater than 1:8 against the Mahoney strain had titers of less than 1:8 for WPV1 (D. S., unpublished data). Two other serosurveys are ongoing, one comparing sera from Bedouin children before (in 2012) and after (in 2013) introduction of WPV1 into southern Israel and the other measuring immunoglobulin A antibody levels during the initial stages of the silent transmission.

### **PUBLIC HEALTH RESPONSE: VACCINATION CAMPAIGNS, TARGET POPULATION, CHOICE OF VACCINE, AND COVERAGE LEVELS**

Despite a risk assessment presented in 2012 by the European Regional Certification Commission for Poliovirus Eradication, in which Israel was described as a country with a low risk



for transmission following importation [7], Israel has witnessed prolonged circulation after importation of an endemic strain of WPV1 from Pakistan in 2013. This strain lacked any unusual genetic [25] or phenotypic (L. S., unpublished data) characteristics that could alone account for circulation in a population with laboratory-confirmed vaccine coverage of >98%, although antigenic profile changes may have contributed to the intense silent transmission and a slightly reduced neurovirulence to the absence of paralytic cases, in addition to the high population immunity. There were immediate goals: to prevent clinical poliomyelitis and to halt sustained transmission. Overcoming these challenges was dependent on making informed decisions, risk communication, and operational considerations.

### **Risk Communication**

As reviewed elsewhere [27], special task forces were set up to inform professionals, to reach a consensus within the medical community, to convincingly and transparently convey decisions to a substantially anxious public through traditional (television, radio, and newspapers) and new (the Internet and Facebook) routes, and to record, analyze, and report any adverse effects, since vaccination in Israel is voluntary and must be performed by licensed health professionals. Public compliance with recommendations/decisions required active countering of arguments by vocal antivaccination groups and conspiracy theories, successful arguments against an appeal to the Israeli Supreme Court to block the emergency vaccination program, and responses from health professionals to questions presented during public forums. Safety was considered paramount and contributed to the choice of bOPV over tOPV; the presentation of a comprehensive list of contraindications associated with use of live-virus vaccines that affect 4.3% of children (personal communication); and the presentation of information conveying the necessity of administering of a live vaccine (through a routine program and the catch-up SIA with IPV) that had not been used since 2005 to children who were fully protected by IPV. Risk analysis included consideration of the potential impact on implementation and the public confidence in other routine vaccination programs.

Laboratory operations and priorities evolved in response to the needs and directives of the minister of health, the director general of the Ministry of Health, and the head of the public health services. Regularly advising them was an emergency response group consisting of local experts that included members of the Polio Monitoring Committee and the National Vaccination Committee, the Central Virology Laboratory, experts in infectious diseases and risk communication, and health department administrators. In addition, 2 WHO/CDC consultation missions visited Israel on weeks 25 and 47 of 2013, there was a WHO on-site laboratory visit on week 29, and Israeli experts participated in additional international meetings and consultations.

### **Operational Considerations**

IPV and bOPV vaccine stocks were procured under emergency health regulations. A catch-up IPV campaign was conducted among children aged 2 months to 7 years in Rahat and the surrounding areas. This was followed by a bOPV (Sabin strain of type 1 poliovirus plus the Sabin strain of type 3 poliovirus) campaign initiated in the southern health district on 5 August 2013 and extended to the rest of the country starting on 18 August 2013 (Figures 1 and 2). Together, 76% of all Israeli children (902 192 of 1 181 604 eligible children <10 years of age who had received at least 1 dose of IPV) received 1 dose of bOPV administered largely through the Healthy Baby Clinics (Drop of Milk Clinics). While the southern health district had an overall bOPV coverage rate of 85% during the SIA in 2013, the bOPV1 coverage rate reached 90% (calculated as  $[50\,876 \text{ children aged } <10 \text{ years who were vaccinated}] / [56\,695 \text{ children aged } <10 \text{ years in the population}] \times 100$ ) in the epicenter where the WPV transmission was sustained (eg, the area of Bedouin settlements), according to environmental surveillance findings. Moreover, a second dose of bOPV was administered to 51% of the vaccinated children in this southern health district. Finances were procured for expanding activities in the laboratory and by health professionals, for risk communication, and for enhancing surveillance during silent transmission. Finally, the same sensitive laboratory methods and an extensive period of enhanced surveillance will be needed to provide convincing evidence that transmission has ceased. As outlined above, the laboratory, municipal sanitation engineers, and district health offices worked together to pinpoint the location of the populations where silent transmission was highest, through enhanced environmental surveillance supplemented with stool surveys of asymptomatic individuals residing in the southern part of Israel. In addition, molecular characterization of the WPV1 [25] and serosurveys of the population at risk were conducted with international cooperation to determine the virological and host factors that contributed to the ability of this lineage to circulate and to assess the risk for reoccurrence of a WPV1 penetration and subsequent silent circulation in highly vaccinated populations in Israel and elsewhere.

### **CHANGES IN POLICY AS A RESULT OF SILENT WPV1 TRANSMISSION IN ISRAEL**

The first major change in policy was to reintroduce the use of live vaccine after 9 years of exclusive use of IPV. The Polio Monitoring Committee and the National Vaccination Committee held a series of meetings in the first months of 2014 to review the administrative, operational, and laboratory responses to the introduction and subsequent circulation of WPV1 in Israel, with the goal of modifying the emergency response plan in light of the knowledge and experience gained during 2013. One of the aims of these meetings was to recommend short-term postevent changes in vaccination policy to guide the

switch from vaccination campaigns to age-based administration of live vaccine to new birth cohorts. Another was to strike a balance between the previous routine surveillance program and the program at the height of transmission, since elements of the current expanded sensitive environmental surveillance will need to remain in place to provide convincing documentation for the disappearance of WPV1-SOAS R3A from Israel.

## CONCLUSION

The sustained silent transmission of WPV1 in Israel was considered to be very unlikely. The risk for similar occurrence in other countries using IPV exclusively may need to be reassessed to ensure a successful endgame for poliomyelitis and poliovirus infections. The responses outlined in this article, especially when the public is well informed, will serve as precedent for handling such events. Our experience emphasizes the importance of maintaining a scalable response capacity in national polio laboratories, to allow rapid risk assessment and risk management in emergency situations. Specifically, the capability and capacity to develop, validate, and implement novel laboratory assays within short times is crucial, to facilitate an informed evidence-based public health decision-making process. It also points out the need for establishing long-term surveillance as the primary method to detect silent transmission events that may occur in IPV-only developed countries with high vaccine coverage, since the likelihood of cases is significantly reduced in such settings. The relative inferiority of IPV over OPV in preventing subsequent poliovirus infections and transmission [8, 9], as well as crowded living conditions with continued exposure to high amounts of virus, may have contributed the most toward enabling sustained transmission. Finally, endemic transmission of poliovirus must be interrupted at its source to prevent future exportations to polio-free countries.

## Notes

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