Genetic Analysis and Characterization of Wild Poliovirus Type 1 During Sustained Transmission in a Population With >95% Vaccine Coverage, Israel 2013

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(See the Editorial Commentary by Delpeyroux and Colbère-Garapin on pages 1065–7.)

Background. Israel has >95% polio vaccine coverage with the last 9 birth cohorts immunized exclusively with inactivated polio vaccine (IPV). Using acute flaccid paralysis and routine, monthly countrywide environmental surveillance, no wild poliovirus circulation was detected between 1989 and February 2013, after which wild type 1 polioviruses South Asia genotype (WPV1-SOAS) have persistently circulated in southern Israel and intermittently in other areas without any paralytic cases as determined by intensified surveillance of environmental and human samples. We aimed to characterize antigenic and neurovirulence properties of WPV1-SOAS silently circulating in a highly vaccinated population.

Methods. WPV1-SOAS capsid genes from environmental and stool surveillance isolates were sequenced, their neurovirulence was determined using transgenic mouse expressing the human poliovirus receptor (Tg21-PVR) mice, and their antigenicity was characterized by in vitro neutralization using human sera, epitope-specific monoclonal murine anti-oral poliovirus vaccine (OPV) antibodies, and sera from IPV-immunized rats and mice.

Results. WPV1 amino acid sequences in neutralizing epitopes varied from Sabin 1 and Mahoney, with little variation among WPV1 isolates. Neutralization by monoclonal antibodies against 3 of 4 OPV epitopes was lost. Three-fold lower geometric mean titers ($Z = -4.018; P < .001$, Wilcoxon signed-rank test) against WPV1 than against Mahoney in human serum correlated with 4- to 6-fold lower neutralization titers in serum from IPV-immunized rats and mice. WPV1-SOAS isolates were neurovirulent (50% intramuscular paralytic dose in Tg21-PVR mice: log107.0). IPV-immunized mice were protected against WPV1-induced paralysis.

Conclusions. Phenotypic and antigenic profile changes of WPV1-SOAS may have contributed to the intense silent transmission, whereas the reduced neurovirulence may have contributed to the absence of paralytic cases in the background of high population immunity.

Keywords. wild type 1 poliovirus; neurovirulence; neutralizing antigenic epitopes; poliovirus receptor; environmental surveillance.

Two trivalent vaccines against poliovirus are used globally: inactivated polio vaccine (IPV; virulent seed strains: Mahoney, MEF-1, and Sukett) [1] and live oral polio vaccine (OPV; attenuated seed strains: Sabin 1, Sabin 2, and Sabin 3) [2]. Israel has been polio-myelitis-free since 1989 as a consequence of high immunization coverage (range since 1990: 92%–95% nationally; 81%–100% within individual health districts).
[3–6]. Differences in the numbers of doses of IPV and/or OPV exist within this population, due to changes in routine childhood polio vaccination schedules plus a large immigrant population [3]. Since 2005, IPV has been used exclusively.

As >90% of poliovirus infections are asymptomatic in naive individuals [1, 2, 7–10], it is important for intervention to identify these silent infections in a population before the first poliomyelitis case appears [7]. Multiple surveillance strategies performed in parallel over extended periods of time increase reliability and strengthen interpretation of data documenting introduction, monitoring of silent circulation, and successful elimination of wild poliovirus in formerly poliovirus-free environments [9]. Israel employs classical acute flaccid paralysis surveillance [11] and routine, country-wide, monthly environmental (sewage) surveillance with catchment areas covering 30%–40% of the entire population [12, 13]. Environmental surveillance identified silent introductions of wild type 1 poliovirus (WPV1) into Ashdod in 1996 [5] and southern and central Israel in 2013 [14, 15]. The 2013 WPV1 was related to the WPV1 South Asia genotype (SOAS) cluster R3A circulating in Pakistan in 2012 and WPV1-SOAS isolated in the absence of cases from sewage collected in Egypt in December 2012 [16]. After detecting WPV1 in sewage collected from the southern city of Rahat on 9 April 2013, sampling frequency was increased, the number of environmental surveillance sites expanded, and a specific semiquantitative real-time reverse transcription polymerase chain reaction (RT-PCR) assay developed that documented, throughout 2013, persistence of WPV1 in sewage at some environmental sites in south and central Israel, intermittence at others, and absence at all sites between 3 April and 28 October 2014 [15–17] (L. M. Shulman, unpublished data). A nationwide vaccine campaign with bivalent OPV (bOPV: Sabin 1 plus Sabin 3) initiated on 8 August 2013 targeting all children <10 years of age who had received ≥1 dose of IPV [14] reintroduced Sabin strains into sewage.

We relate the phenotype of the WPV1-SOAS R3A isolated in Israel to the ability of this strain to circulate in the absence of disease in the highly immunized population. This is the first in-depth phenotypic analysis of an introduced wild poliovirus responsible for sustained circulation in a highly vaccinated population where the youngest birth cohorts were vaccinated exclusively with IPV.

**MATERIALS AND METHODS**

**Isolation of WPV1-SOAS R3A Poliovirus From Sewage Samples and Stool Suspensions**

Twenty-four-hour composite sewage samples were collected from entry points into sewage treatment plants using in-line automatic samplers and from upstream sites using portable automatic composite samplers as described [7, 9, 15]. WPV1-SOAS was isolated from concentrated sewage samples by infecting L20B cell monolayers in tubes (mass viral cultures) or in petri dishes (for plaque-purified isolates) [12, 15, 16] and from stool samples collected during July 2013 from asymptomatic children and adults in WPV1-SOAS-positive catchment areas in southern Israel [16]. Isolate names include a number to indicate the sewage or stool sample followed by the letters “-tt,” “-PL,” or “-s” to indicate isolation from tube culture, plaque isolate, or stool suspension, respectively. Names indicate sewage treatment plants and dates indicate the date of collection. Sabin strains reappeared in sewage after 5 August during the bOPV supplementary immunization activity.

**Sequence Analysis**

Forward primers Y7 or Y7R and reverse primer Q8 were used to amplify and sequence viral capsid protein 1 (VP1) from RNA extracted from WPV1-SOAS isolated before 5 August 2013 [16, 18]. Afterward, WPV1-SOAS VP1 was amplified from mixtures of WPV1 and Sabin poliovirus and sequenced in 2 steps. VP1 cDNA was generated using by RT-PCR using (1) pan-polio primers Y7 and Q8 and/or (2) 2 RT-PCRs, WPV1-SOAS–specific primers SOAS1R (5´-TAAGTATACTCTGACCTTAGAA-3´) and SOAS2F (5´-TATCCAGCACAGGTCACGA-3´) from the semi-quantitative RT-PCR [19] paired with Y7 or Q8, respectively. Amplification products were sequenced with primers SOAS1R and SOAS2F (reaction 1) or SOAS1R, SOAS2F, Y7, and Q8 (reaction 2). Additional primers [18, 20] were used to sequence the remaining capsid proteins and complete genomes.

Sequence accession numbers are listed in Supplementary Table 1. Amino acid differences in neutralizing antigenic (NAg) sites, poliovirus receptor binding amino acid residues, and at sites correlated with attenuation of neurovirulence in Sabin 1 were identified from aligned nucleotide sequences using the Sequencher Program (version 5.2.2, Gene Codes, Ann Arbor, Michigan).

**Neurovirulence in Transgenic Mice Expressing the Human Poliovirus Receptor**

The 50% paralytic dose (PD50) of representative isolates was determined by the probit method as described elsewhere [21] after intramuscular (left hind limb) injection of transgenic mouse expressing the human poliovirus receptor (Tg21-PVR-Bx) transgenic mice with 50 µL of 10-fold serial viral dilutions and determination of the daily clinical score for 14 days.

**Neutralization Assays**

Neutralizing antibody titers were determined by standard microneutralization assay in 96-well plates [22] and expressed as reciprocals of the highest dilution of serum that protected 50% of the cell cultures (Karber formula [23]). Virus challenge doses were confirmed by back-titration.
Human Serum Samples
Two-fold serial dilutions of convenient human serum samples (23 patients aged 2–72 years; mean = 36 years; median = 16 years; 1 age unknown) chosen from among sera with titers <1:512 (median = 1:64; range = 1:8–1:256) against Mahoney vaccine strain were preincubated overnight at 36°C with one hundred 50% cell culture infectious doses (CCID₅₀) of virus. HEp-2C cells were added to each well, and 5-day survival at 37°C determined by staining with Formalin/Gentian violet solution [22].

Murine Sabin 1–Specific Monoclonal Antibodies
Duplicate, 2-fold serial dilutions of virus containing 10–10,000 CCID₅₀ were preincubated in 96-well plates for ≥2 hours at 36°C with concentrations of monoclonal antibodies (mAbs) to poliovirus antigenic sites 1–4 sufficient to neutralize >100 CCID₅₀ of Mahoney vaccine strain. HEp-2C cells were added per well, and 5-day survival at 37°C was monitored by addition of vital dye (buffered medium containing 0.1% Naphtalene black stain). Tests were considered positive when >100 CCID₅₀ of an isolate was neutralized.

Rat IPV-Specific Antisera Tested In Vitro
Ten female Wistar (ex-Charles River Laboratories) rats were immunized intramuscularly with 0.25 mL per hind leg, for each dilution of a 4-fold serial dilution (2 to 1:256 human vaccine doses/mL) of IPV, international reference Pu91 using a test format used for the batch release of IPV [24]. Rats were bled out 20–22 days postinoculation. Two-fold serial dilutions of rat sera were preincubated in duplicate with 100 CCID₅₀ of virus for at least 2 hours at 36°C before addition of HEp-2C cells. Five-day survival at 37°C was monitored using vital dye (0.1% Naphtalene black stain). Tests were considered positive when >100 CCID₅₀ of an isolate was neutralized.

Immunization/Challenge Experiments in Tg21-PVR Mice
Tg21-PVR mice of both sexes (8 per test group) received 2 intraperitoneal injections of minimum essential medium (controls) or the equivalent of 1 human dose of IPV, international reference Pu91, at an interval of 2 weeks. Twenty-eight days after the last dose, mice were challenged with the equivalent of 25 times the PD₅₀ of live poliovirus and monitored for any sign of paralysis for 14 days [21].

Statistical Analysis
The significance of pairwise differences in NAg titers of human sera against Mahoney and PV1-ISR13 was determined using Wilcoxon signed-rank test (SSPE program, version 17), probit analysis was used to calculate the PD₅₀ with 95% confidence interval (CI) for neurovirulence of type 1 poliovirus strains in transgenic mice, and the relative potency with 95% CIs was based on the number of rats that produced antibodies that neutralized poliovirus in vitro after 1 human dose of IPV.

Ethics Statement
The Sheba Medical Center Ethical Review Board approved this study (SMC-0774-13) and exempted it from requiring informed consent. All links to personal details that could be used to identify individuals were removed, and data were analyzed anonymously.

RESULTS
Molecular Characterization of Environmental and Stool Survey Isolates
Variations in amino acids among Israeli WPV1-SOAS isolates from 95 sewage or stool suspensions between 6 February and 7 August 2013 occurred at 27 sites for VPI (per isolate: 2–6, median = 2; for 3–12 nucleotide differences). Within NAg site 1 in VPI (P1; 669–681), site 2a in VPI (P1; 800–805), site 2b in VP2 (P1; 233–242), site 3b in VP3 (P1; 399–401), and site 4 in VP3 (P1; 77–80, and 141), there were 3–4, 2, 2, 1, and 0 differences from Sabin 1; and 2–3, 2, 2, 0, and 0 from Mahoney, respectively. Israeli sequences were also highly conserved with wild polioviruses circulating from the 1980s in South America to recent 2013 strains in Nigeria and Pakistan for antigenic site 1 and with most wild isolates at site 3. Antigenic site 2 contained the highest variations among wild polioviruses isolated at different time periods and/or geographical areas.

Neurovirulence
The neurovirulence of WPV1-SOAS compared with other wild isolates was measured using a transgenic mouse challenge model to see whether a relative decrease might have contributed together with high vaccine coverage [25] to absence of paralytic cases despite continued circulation of WPV1-SOAS in Israel between February 2013 and April 2014 [14, 16]. The results (Table 1) show that although 3 Israeli isolates were neurovirent, they were less so than a type 1 vaccine-derived poliovirus and a WPV1-SOAS isolate from Pakistan.

Neutralization Assays
Neutralization experiments with antibodies raised against type 1 OPV (Sabin 1), as expected from amino acid differences (Figure 1), revealed that only mAbs specific for antigenic site 4

Table 1. Reactivity of Type 1 Poliovirus Strains With Anti–Sabin 1 Monoclonal Antibodies

<table>
<thead>
<tr>
<th>Neutralizing Antigenic Site</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal antibody</td>
<td>955</td>
<td>234</td>
<td>423</td>
<td>234</td>
<td>1585</td>
</tr>
<tr>
<td>Sabin 1</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>Mahoney</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>PV1-8062-PL1_ISR13</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>PV1_8149-PL2_ISR13</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>PV1_8150-PL1_ISR13</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
</tr>
</tbody>
</table>

Abbreviations: Neg, negative; Pos, positive.
Amino acid substitutions in neutralizing antigenic (NAg) sites and poliovirus receptor binding residues during sustained transmission of wild poliovirus type 1 South Asia genotype (WPV1-SOAS). Complete P1 capsid protein RNA sequences of 8 Israeli WPV1 isolates were computer translated and the resultant amino acid sequences compared with the equivalent sequence from the earliest WPV1-SOAS environmental isolate, PV1_8062-PL1_ISR13 that is used here as the reference strain. 

**Figure 1.** Amino acid substitutions in neutralizing antigenic (NAg) sites and poliovirus receptor binding residues during sustained transmission of wild poliovirus type 1 South Asia genotype (WPV1-SOAS). Complete P1 capsid protein RNA sequences of 8 Israeli WPV1 isolates were computer translated and the resultant amino acid sequences compared with the equivalent sequence from the earliest WPV1-SOAS environmental isolate, PV1_8062-PL1_ISR13 that is used here as the reference strain. A, The rectangle represents the 4 capsid proteins (VP1, VP2, VP3, and VP4) within the P1 capsid protein encoding region of the type 1 poliovirus genome. Blue and red vertical bars indicate the location of amino acid residues in NAg epitopes and receptor binding sites, respectively. B, The table indicates variations in the amino acid residues within these NAg and receptor binding sites among WPV1-SOAS isolates relative to reference strain PV1_8062-PL1_ISR13. The starting and ending residue of each site are indicated relative to the P1 of the reference strain and the amino acid residues of the reference strain appear in the line below using a single letter amino acid code. When NAg epitopes and receptor binding residues overlap, the combined amino acid sequence containing both is shown for each with asterisks (*) for amino acids that are not part of the peptide encoding the NAg epitopes or the receptor binding site, respectively. For clarity, lines below each bar in (A) connect to columns in (B) that contain the reference amino acids and variations among 8 other WPV1-SOAS isolates, Sabin 1 and Mahoney vaccine seed strains. A dashed line indicates identity with the amino acid in the reference strain. Days below each isolate are indicated as the number of days post-symptomatic onset. Differences in amino acids in outbreak strains in amino acid residues associated with neurovirulence in Sabin 1 are shown in the Supplementary Data.
neutralized 3 WPV1-SOAS strains from Israel (Table 2). Variations in receptor binding residues were also determined (Figure 1) as these residues frequently overlap with NAg residues and changes in one may result in altering properties of the other relevant for silent circulation.

The relative in vitro potency of antisera to Mahoney vaccine virus from rats immunized with 1 human dose of IPV to neutralize outbreak strains was less than against OPV Sabin 1 and IPV Mahoney strains (Table 3). However, reverse cumulative distribution curves of virus neutralizing titers indicated that antibodies raised against the single dose of IPV were still capable of neutralizing the WPV1-SOAS strains, albeit at titers 4- and 6-fold lower than against Mahoney and 2- to 4-fold lower than against Sabin 1, respectively (Figure 2A). IPV also induced good protection in vivo over 14 days in PVR transgenic mice (Table 2). IPV also induced neurovirulence in the mouse Tg21-PVR model, and although they appeared to be somewhat less neurovirulent than the other WPV1 or VDPV strains tested, the difference between ISV-8149 and Mahoney was not significant, as the 95% CIs between PD50 values partially overlapped. The low incidence of paralytic disease in naive individuals is significantly reduced among vaccinated individuals [8, 10]. Thus, even a slight reduction in neurovirulence might have contributed to the absence of paralytic poliomyelitis in the highly vaccinated population of Israel by

<table>
<thead>
<tr>
<th>Virus Strain</th>
<th>PD50 (95% CI)a</th>
</tr>
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<tbody>
<tr>
<td>Sabin 1</td>
<td>~8.3</td>
</tr>
<tr>
<td>Mahoney</td>
<td>5.9 (5.4–6.6)</td>
</tr>
<tr>
<td>02v9529</td>
<td>5.7 (5.4–6.1)</td>
</tr>
<tr>
<td>PAK-5388</td>
<td>6.0 (5.3–6.8)</td>
</tr>
<tr>
<td>VP1_8062-PL1_ISR13</td>
<td>7.2 (6.9–7.6)</td>
</tr>
<tr>
<td>VP1_8149-PL2_ISR13</td>
<td>6.7 (6.3–7.0)</td>
</tr>
<tr>
<td>VP1_8150-PL1_ISR13</td>
<td>6.8 (6.4–7.1)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; PD50, 50% paralytic dose.

a Estimates of the PD50 with 95% CIs calculated by the probit method.

Table 2. Neurovirulence of Type 1 Poliovirus Strains in Transgenic Mice

Table 3. In Vitro Neutralization Potency of Antisera From Rats Immunized With 1 Human Dose of Inactivated Polio Vaccine

The geometric mean titer (GMT) of antibodies neutralizing Mahoney virus in 400 serum samples collected in 2013 from cohorts of Israelis aged 1–7 (n = 100), 8–17 (n = 100), 18–24 (n = 100), 25–44 (n = 46), 45–64 (n = 35), and ≥65 (n = 19) years of age was reported to range between 124.2 ± 4.8 and 537.5 ± 3, while 95.6%–100% of the individuals in each cohort had titers ≥1:8, consistent with the very high vaccine coverage in the general population [25].

Levels of WPV1-SOAS neutralizing antibodies in individuals with low titers (1:8–1:256) against Mahoney were determined (Table 4) as continuous exposure to high infectious doses occurs during outbreaks [26]. As expected, the GMT of neutralizing titers against the heterotypic strain was significantly lower (Z = −4.018; P < .001 for paired results from the same run for each of the 23 individuals using the Wilcoxon signed-rank test). Moreover, WPV1-SOAS titers were ≤1:8 in 4 individuals (22%; 3 aged 31–41 years, and 1 aged 10 years).

DISCUSSION

Routine monthly environmental surveillance coupled with molecular [19] and sequence analyses [16] detected introduction of WPV1-SOAS clade R3A into Israel in February 2013 and subsequent continued silent circulation throughout the rest of 2013 [14, 15]. Wild poliovirus spread within a population initially with >95% IPV vaccine coverage, extended to 99.5% through a catch-up IPV campaign in multiple birth cohorts vaccinated exclusively with IPV [14]. Most of this cohort (nationally 76%, 85% in southern heath districts, and 90% in the epicenter, unpublished data) received at least 1 dose of bOPV (Sabin 1 and Sabin 3), starting on 5 August 2013, to boost mucosal immunity in OPV-naive children. The first detailed genotypic characterization of a wild poliovirus associated with sustained silent transmission in a highly vaccinated population [16] indicated that the virus was very similar to the WPV1-SOAS R3A that still circulates in Pakistan and that was recovered from environmental samples in Egypt in December of 2012.

The WPV1- SOAS R3A polioviruses circulating in Israel were phenotyped to identify any unusual antigenic characteristics or any alteration in neurovirulence that may have contributed to its ability to spread in the highly vaccinated population without causing paralytic disease. They were neurovirulent in the mouse Tg21-PVR mouse neurovirulence model, and although they appeared to be somewhat less neurovirulent than the other WPV1 or VDPV strains tested, the difference between ISV-8149 and Mahoney was not significant, as the 95% CIs between PD50 values partially overlapped. The low incidence of paralytic disease in naive individuals is significantly reduced among vaccinated individuals [8, 10]. Thus, even a slight reduction in neurovirulence might have contributed to the absence of paralytic poliomyelitis in the highly vaccinated population of Israel by

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relative Potency</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP1_8062-PL1_ISR13</td>
<td>0.50</td>
<td>0.25–0.97</td>
</tr>
<tr>
<td>VP1_8149-PL2_ISR13</td>
<td>0.46</td>
<td>0.23–0.89</td>
</tr>
<tr>
<td>Sabin 1</td>
<td>0.84</td>
<td>0.32–1.27</td>
</tr>
<tr>
<td>Mahoney</td>
<td>SD = 1</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; NA, not applicable; SD, standard.

a Significant; the upper 95% CI limit is <1.0.
reducing the proportion of viral infections that could have resulted in paralysis. Acute flaccid paralysis cases reported from closely related viruses circulating in an undervaccinated population in Syria in 2013 [27] suggest that immunization was more important in the lack of disease in Israel than decreased neurovirulence.

WPV1 SOAS R3A viruses isolated in Israel in 2013 showed little amino acid sequence variation within antigenic epitopes and receptor binding epitopes (Figure 1), suggesting that most shared similar antigenic and receptor binding properties with analyzed isolates. Amino acid differences from Sabin 1 in 3 of 4 major antigenic epitopes were consistent with loss of reactivity to murine mAbs to these epitopes. In vitro and in vivo neutralization assays with murine mAbs and rat and murine antisera to OPV and or IPV demonstrated that the tested WPV1-SOAS R3A isolates shared 1 or more cross-reacting neutralizing epitopes with both OPV and IPV vaccine strains. Both vaccines contributed to the prevention of paralytic disease from WPV1-SOAS R3A circulating in Israel. Despite a reduced reactivity to antibodies raised against the IPV vaccine strain, IPV-immunized mice did not show any signs of paralytic disease in vivo when challenged with paralytic doses of WPV1-SOAS R3A strains. However, the finding of significantly lower antibody levels against WPV1-SOAS than vaccine strains in human sera and the loss of cross-reactivity with 3 of the 4 epitopes against Sabin 1 murine mAbs leaves some room for concern. Loss of neutralization by mAbs indicates changed antigenicity but does not necessarily predict abrogation of cross-reactivity with human polyclonal antisera. When impaired herd immunity to epidemic strains occurs, as in Finland in 1984 [28], poliomyelitis cases may appear. Immune profiles of WPV1 from a poliomyelitis epidemic in the Republic of Congo in 2010 were similar to those found in the Israeli strains—that is, differences in antigenic sites 1, 2, and 3 with respect to both Mahoney and Sabin 1 vaccine strains [29]. The unusually high 47% case-fatality rate (5%–10% is typically seen in WPV1 outbreaks) was attributed to inefficient immune protection due to gaps in immunization in particular age groups [30], whereas changes found in antigenic site 2 of virus isolates from fatal cases might have contributed to the severity of the outbreak in immunized individuals [29]. Despite this, the outbreak was readily interrupted with OPV, similar to many other outbreaks caused by different wild-type poliovirus strains, and this lineage is no longer in circulation [31]. Information on relative neurovirulence, as far as we are aware, was not available.

Response during sustained silent transmission in highly immunized populations must differentiate between risk of disease and transmission. High antibody titers protect against disease but do not induce “sterilizing immunity” (eg, prevent infection), but rather reduce the numbers of excretors, the amounts excreted, and/or duration of excretion ([32–36] and references therein). Individuals vaccinated with IPV are more likely to become infected and transmit poliovirus than those vaccinated with OPV as IPV does not induce a significant mucosal immune response.

Table 4. Levels of Wild Poliovirus Type 1 South Asia Genotype Neutralizing Antibodies in Individuals With Low Titersa Against Mahoney

<table>
<thead>
<tr>
<th>Strain</th>
<th>GMT</th>
<th>Significanceb</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV1_8099-PL9_ISR13</td>
<td>14.2</td>
<td>P &lt; .001; Z = 4.018</td>
</tr>
<tr>
<td>Mahoney</td>
<td>46</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: GMT, geometric mean titer.

a 1:8–1:256.
b Wilcoxon signed-rank test.
Thus, while high population immunity enhanced by supplementary immunization [14] prevented paralytic cases in Israel in 2013, all individuals, in particular the 9 birth cohorts of children immunized exclusively with IPV since 2005, potentially contributed to sustained WPV1 circulation and WPV1 recovered from the environment [15].

In conclusion, phenotypic analysis of WPV1-SOAS R3A virus isolates from Israel did not show any major characteristic that might easily explain its widespread and sustained circulation after penetration in a population that was protected against poliomyelitis by >90% IPV coverage, but less well protected against transmission through lack of exposure to live poliovirus; nonetheless, wild poliovirus gradually disappeared from the epicenter sites such that after 3 April 2014, none has been detected to date (28 October 2014) [17] (L. M. Shulman, unpublished data). Epidemiological factors such as large families exposed over extended periods of time to high viral loads, rather than the virus, may have contributed to sustained transmission ([14, 15, 16]. As long as there are countries where endemic transmission of wild poliovirus has never been interrupted, there remains the potential for wild poliovirus to spread to polio virus–free regions, including those with very high vaccine coverage. Antibodies to IPV neutralized WPV1-SOAS. In vitro neutralization experiments with mAbs indicated that OPV would still be an effective tool to interrupt transmission of WPV1-SOAS in individuals previously vaccinated exclusively with IPV. However, the loss of reactivity against 3 of the 4 major NAg epitopes by mAbs raised against Sabin 1 emphasizes the need to rapidly interrupt the chain of transmission of the WPV1-SOAS currently circulating in the Middle East before amino acid substitutions in the remaining NAg site reduce effectiveness of bOPV. Finally, the importation and subsequent silent circulation of WPV1-SOAS in the highly immunized population in Israel, reported here and elsewhere [14, 15], illustrates the importance of environmental surveillance to achieve eradication [7], as this major event would not have been recognized without environmental surveillance.

**Supplementary Data**

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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**Potential conflicts of interest.** All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**


