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Short communication

Field study of fecal excretion as a decision support tool in response to silent reintroduction of wild-type *poliovirus* 1 into Israel

Jacob Moran-Gilad[a,b,*], Ella Mendelson[c,d], Cara C. Burns[e], Ravit Bassal[f], Michael Gdalevich[g], Danit Sofer[c], M. Steven Oberste[e], Lester M. Shulman[c,d], Ehud Kaliner[a], Musa Hindiye[c], Orna Mor[c], Liora Shahar[g], Jane Iber[e], Ruth Yishay[h], Joseph Manor[c], Boaz Lev[i], Ronni Gamzu[d,i], Itamar Grotto[a,b]

[a] *Public Health Services, Ministry of Health, Jerusalem, Israel*
[b] *Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel*
[c] *Central Virology Laboratory, Ministry of Health, Tel-Hashomer, Israel*
[d] *School of Public Health, Tel-Aviv University, Tel-Aviv, Israel*
[e] *Viruses Branch, Centers for Disease Control and Prevention, Atlanta, USA*
[f] *Israel Center for Disease Control, Ministry of Health, Tel-Hashomer, Israel*
[g] *Southern District Health Office, Ministry of Health, Beer-Sheva, Israel*
[h] *Department of Laboratories, Public Health Services, Ministry of Health, Jerusalem, Israel*
[i] *Directorate, Ministry of Health, Jerusalem, Israel*

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ABSTRACT

Background: Israel has used an inactivated polio vaccine (IPV)-only schedule since 2005 (95% coverage). Silent reintroduction of wild type *poliovirus* 1 (WPV1) into Israel in early 2013 was detected in Southern Israel via routine environmental surveillance without clinical cases.
Objectives: To estimate the rate of WPV1 excretion by age and residence and inform decision-making regarding supplemental immunization with OPV.
Study design: A convenience sample of Bedouin and Jewish residential areas in the epicenter of the incident, focusing on under 8 year-olds who not previously given OPV. Fecal samples were directly tested for WPV1 RNA using a novel qRT-PCR assay. Positive samples were confirmed by gold standard cell culture and subject to genotyping.
Results: Overall, 2196 non-duplicate fecal samples were collected and analyzed. WPV1 was detected in 61 samples (2.8%), 55 of which (90.2%) were from Bedouins. WPV1 excretion rates were 5.4% among Bedouins and 0.6% among Jewish individuals. Respective age-specific rates among Bedouin and Jewish children were 4.9% and 0.2% for 0–2 years and 7.2% and 1.7% for 2–8 years. Molecular testing had 89.5% sensitivity (higher than culture) and 100% specificity.
Conclusion: The rapid performance of a field study to evaluate WPV1 excretion unequivocally demonstrated substantial WPV1 infection rates among children under 8 years in Southern Israel, thus informing the decision to vaccinate this age group with bOPV and risk communication to both healthcare personnel and the public. Rapid development and implementation of molecular screening can thus underpin risk assessment and management in complex epidemiological situations.

1. Background

Israel had been free of wild *poliovirus* (WPV) since 1988 based on ongoing acute flaccid paralysis (AFP) and environmental surveillance [1]. Routine immunization in Israel has involved inactivated polio vaccine (IPV) only since 2005. In 2013, WPV1 was discovered into Southern Israel [2] and found in sewage samples obtained from Rahat and Beer-Sheva [3]. Molecular analysis determined the strain to be closely related to the South Asia type 1 lineage (WPV1-SOAS) endemic in Afghanistan and Pakistan [4].

The current World Health Organization (WHO) protocol for WPV detection involves isolation in culture [5]. Virus identification is performed by the intratypic differentiation (ITD) assay, followed by

* Corresponding author at: Public Health Services, Ministry of Health, 39 Yermiyahu St., Jerusalem, Israel. Tel.: +972 506 243900.
E-mail address: giladko@post.bgu.ac.il (J. Moran-Gilad).

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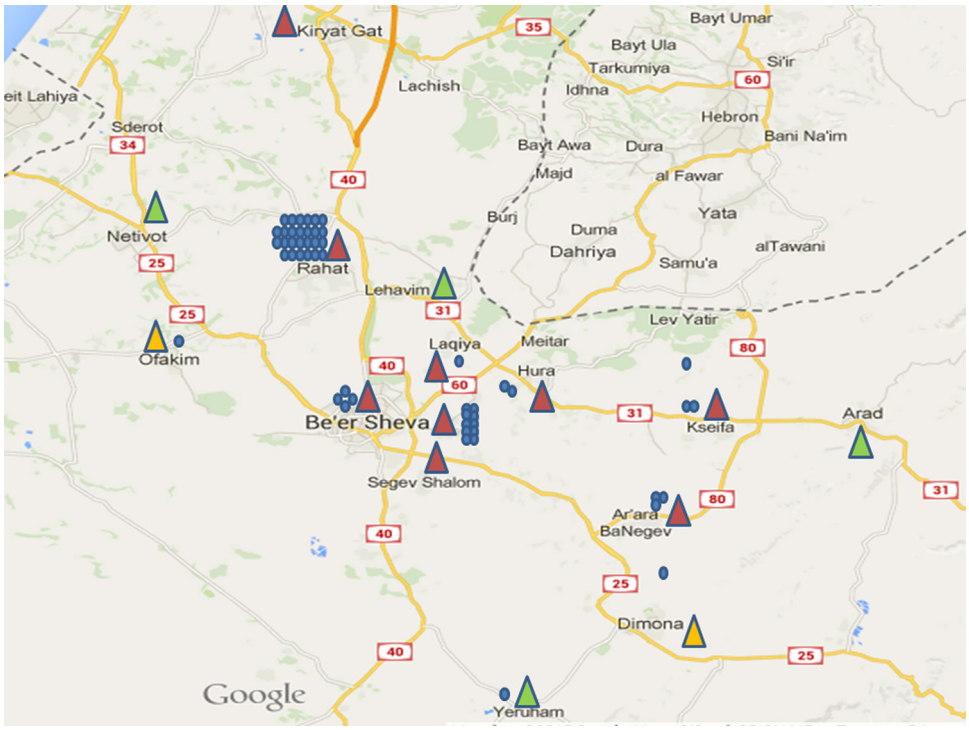

Fig. 1. Geographic distribution of WPV1 positive individuals in Southern Israel. The map shows the Northern and Central parts of the Negev region, Southern Israel where stool samples were obtained. Environmental sampling sites are marked by triangles (Green – continuously negative; Amber – intermittently positive; Red – continuously positive). Individual cases of WPV1 excreters are marked by blue dots and represent actual residential locations (either permanent or semi-nomadic). (For interpretation of the reference to color in this figure legend, the reader is referred to the web version of this article).

complete VP1 sequencing [6]. These protocols are labor intensive and therefore a specific qRT-PCR assay was developed and deployed in Israel early in the course of the investigation [7].

2. Objectives

In the framework of Israel's response to this unprecedented silent reintroduction (WPV1 transmission lacking clinical cases in a population immunized with IPV only), data regarding the force of infection in the community were needed in order to determine the most appropriate supplementary immunization activity (SIA). An urgent field survey of WPV1 excretion was thus carried out.

3. Study design

The study involved a convenience sample of the population of Southern Israel in July 2013. The primary goal was to estimate the rate of WPV1 excretion and secondary goals were to estimate excretion rates by age group and residence. Our assumption was that children <9 years old, representing birth cohorts not given oral polio vaccine (OPV) were the main source of transmission. Since prevalence was unknown, sampling efforts were concentrated in the Bedouin and Jewish populations at the epicenter of circulation with sample size set at 2000.

Fresh stools were collected in multiple locations including day-care centers, "drop of milk" stations, primary clinics, and clinical laboratories routinely processing stools, transported within 24 h to the Central Virology Laboratory (CVL) and aliquoted into two subsets. The first subset was analyzed by direct qRT-PCR with internal controls [7] after nucleic acid extraction using Thermo Scientific™ KingFisher System (Waltham, MA, USA). When PCR inhibition was suspected, extraction was attempted using the NucliSENS® easyMAG™ system (bioMérieux, Marcy l'Etoile, France). A positive was defined as cycle threshold (C_T) < 37 and a negative at C_T > 45. Samples with C_T value >37 and <45 were re-tested in triplicate and reported as weak positive if ≥2 were positive. Results were considered inconclusive if inhibition was not resolved. qRT-PCR-positive samples were inoculated into L20B cultures for virus isolation with blind passage after seven days to propagate virus.

The second subset was shipped on dry ice to CDC and analyzed according to reference culture-based methods [5]. RD and L20B cell cultures were inoculated in parallel with supernatants from cultures. Those showing cytopathic effects were subjected to molecular analysis [6]. Testing at both sites was performed independently and blindly. Phylogenetic characterization based on complete VP1 sequence for isolates from representative stool and environmental samples was performed and deposited as previously described [8,9].

Demographic data were extracted from Ministry of Internal Affairs database. Data regarding vaccination were extracted from immunization registries. Data were analyzed anonymously following de-duplication. Statistical analysis was performed using SPSS v.21. Rates were compared using chi-square test. The study was approved by the Institutional Review Board of Sheba Medical Center (SMC-0774-13) and the CDC.

4. Results

Of 2395 collected samples, 2196 non-duplicate samples were initially analyzed (49% from Bedouin, 51% from Jewish residents). Of 2065 samples with age data, 1576 (76.3%) were obtained from <8 year-olds (OPV-naive), 40 (1.94%) from 8–10 year-olds (transition between vaccine schedules), 122 (5.91%) from 10–22 year-olds (cohorts given OPV and IPV) and 327 (15.84%) from >22 year-olds (cohorts given OPV only).

Sixty-one subjects excreted WPV1-SOAS (2.77%), of whom 55 were Bedouin (90.16%). Among 59 positives with age data, 31 (52.5%) were <2 years, 22 (37.3%) were 2–8 years, 4 (6.8%) were