

# Emergence of genetically linked vaccine-originated poliovirus type 2 in the absence of oral polio vaccine, Jerusalem, April to July 2022

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**We report an emergence and increase in poliovirus type 2 detection via routine wastewater surveillance in three non-overlapping regions in the Jerusalem region, Israel, between April and July 2022. Sequencing showed genetic linkage among isolates and accumulation of mutations over time, with two isolates defined as vaccine-derived polioviruses (VDPV). This demonstrates the emergence and potential circulation of type 2 VDPV in a high-income country with high vaccine coverage and underscores the importance of routine wastewater surveillance during the polio eradication.**

Routine acute flaccid paralysis (AFP) surveillance for poliovirus (PV) in Israel has been enhanced by supplementary wastewater environmental surveillance (ES) since 1989 [1]. Sabin-like PV types 1 and 3 are detected in most ES samples because bivalent oral poliovirus vaccine (OPV) is used in Israel routine immunisations. Since the discovery of circulating vaccine-derived poliovirus (cVDPV) type 3 from the end of 2021 throughout 2022 [2], sampling frequency and sites were increased in the Jerusalem region and elsewhere. Here, we describe frequently increasing detections of PV2 in the Jerusalem region in the absence of OPV type 2 immunisations and examine the genetic linkage across these detections.

## Environmental surveillance monitoring sites

The ES monitors 15 sites across the country once a month, with a catchment of ca 50% of the population

in Israel [1]. The Jerusalem region includes four non-overlapping ES sampling sites, three of them located at the inlets of wastewater treatment plants and one in the Kidron River that collects wastewater from central and south-east Jerusalem (Figure 1A).

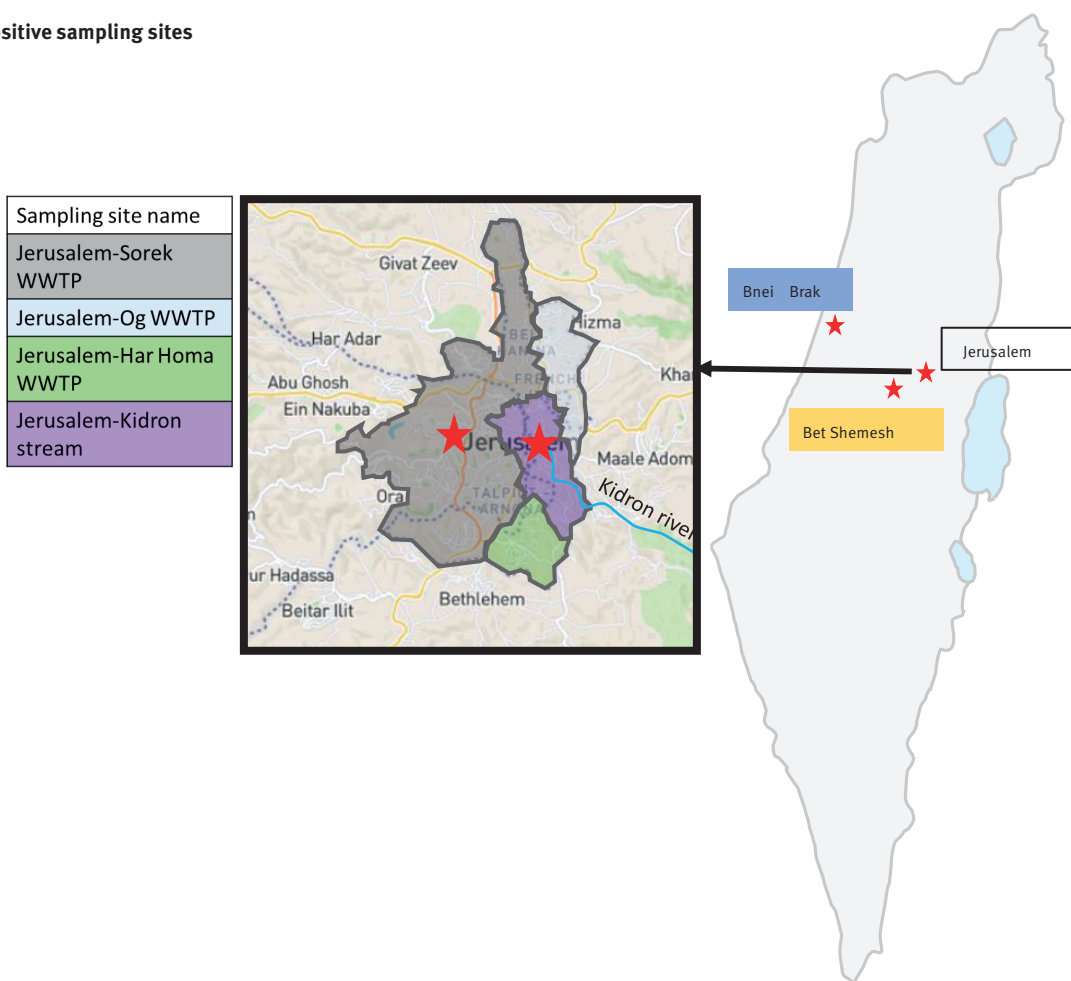
## Detection of poliovirus type 2 vaccine and vaccine-derived virus in wastewater collected from the Jerusalem region

In April 2022, PV2 was detected in three sampling sites – two in the Jerusalem region (Kidron, Bet Shemesh) and one in Bnei Brak in central Israel (Figure 1A). In June, PV2 was detected in three sampling sites in the Jerusalem region (Kidron, Bet Shemesh and Sorek, Figure 1A). In July, we observed a substantial increase in the number of PV2 detections in the Jerusalem region (n=10, Figure 1B), although the final number of PV2-positive samples in July may have been higher as some isolations were still under processing at the time of publication of this article. The PV2 isolates detected during 2022 by RT-PCR were partially sequenced using the Sanger method (VP1 gene, 903 nt) [3] and compared with the Sabin 2 vaccine strain reference sequence (AY.184220) along with VP1 sequences from all PV2 previously detected in ES during 2020 and 2021 in Israel (n=11, Figure 2). We observed an accumulation in nucleotide changes in sequences from June and July 2022, classifying two isolates as VDPV type 2 (defined as  $\geq 6$  nt changes from Sabin 2 [4]). Five additional isolates with  $\geq 6$  nt changes including mixed bases were not classified as VDPV type 2. Most

**FIGURE 1**

Environmental detections of poliovirus type 2, Israel, 2022

**A. Poliovirus type 2 - positive sampling sites**



**B. Frequency of wastewater-based surveillance PV2 detections**

Places name	Sampling site	Covered region in Israel	Estimation of population	2022						
				1	2	3	4	5	6	7
Sorek	Basin and WWTP	North and west Jerusalem	680,000	0/7	0/9	0/9	0/8	0/6	2/4	3/5 <sup>a</sup>
Jerusalem-Og	WWTP	North-east Jerusalem	220,000	0/4	0/2	0/3	0/2	0/2	0/2	0/1
Jerusalem-Har Homa	WWTP	South-west Jerusalem	38,000	0/1	0/1	0/2	0/2	0/2	0/2	0/1
Jerusalem-Kidron river	River	South-east and central Jerusalem	300,000	0/4	0/4	0/5	2/5	0/9	1/7	2/6 <sup>a</sup>
Bet Shemesh area	City manhole and WWTP	Bet Shemesh, Har Hadar, Abu Gosh, Mate Yehuda	225,000	NT	0/1	0/4	1/9	0/9	1/9	5/10 <sup>a</sup>
Bnei Brak area	City manholes	Bnei Brak City	220,000	NT	NT	0/1	1/15	0/5	0/16	0/10 <sup>a</sup>
Total PV2-positive				0	0	0	4	0	4	10

NT: not tested; PV2: poliovirus type 2; WWTP: wastewater treatment plant.

<sup>a</sup> Samples still under processing.

Panel A: Red stars indicate the geographical locations of PV2-positive detections in Israel. The Jerusalem region is magnified to show the specific regions being sampled in environmental surveillance and where PV2 was detected.

Panel B: Sampling sites with PV2-positive detections only are indicated, including catchment area, description of region coverage, frequency of detection in each site (nominator is the number of PV2-positive samples; denominator is the total number of samples examined) and the total number of PV2-positive samples identified overall in each month, January–July 2022.



(circulating cVDPV) or following extended replication

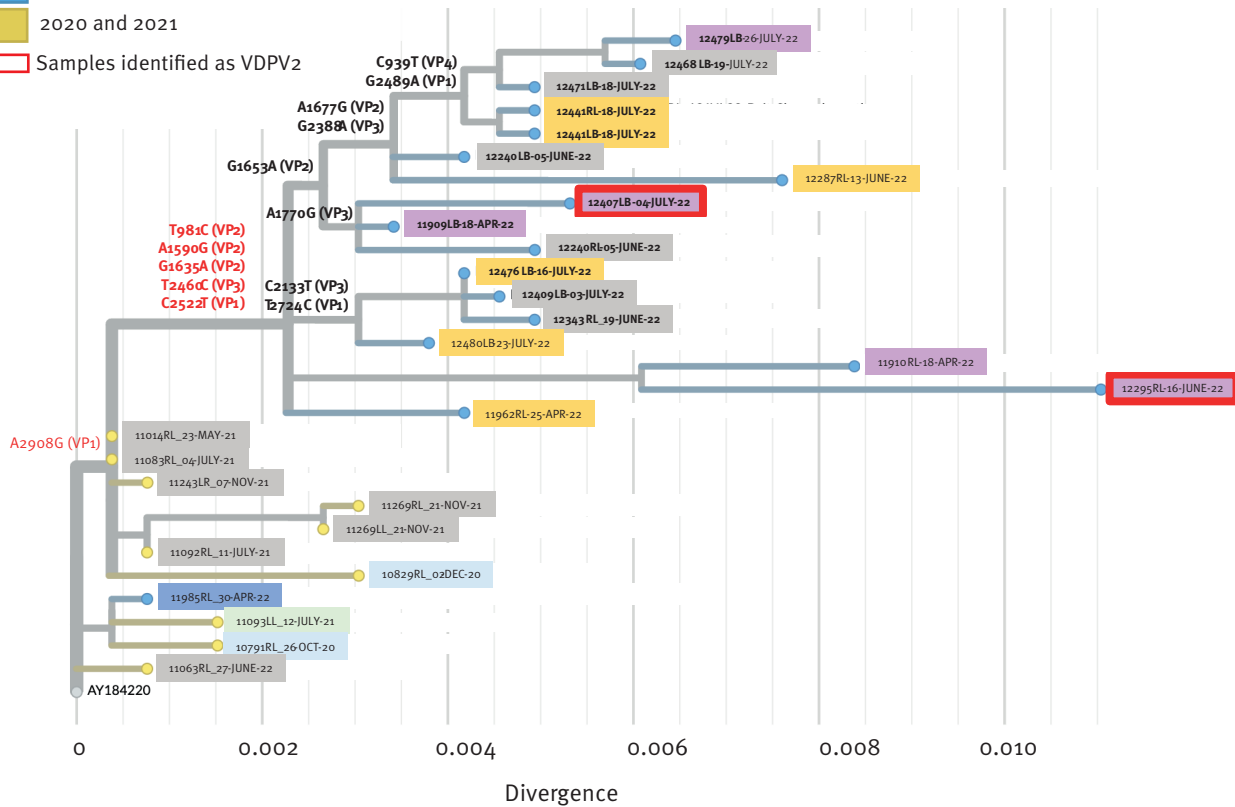
Here we report for the first time an increase in the fre-

**FIGURE 3**

Phylogenetic analysis of the P1 region of polioviruses wastewater isolates, Israel, 2020–2022 (n = 28)

**Year of detection**

- 2022
- 2020 and 2021
- Samples identified as VDPV2



VDPV: vaccine-derived poliovirus.

High resolution phylogenetic tree of the P1 region (includes the VP1, 2, 3 and 4 genes) representing genomic differences across the samples. Shared mutation locations relative to the whole genome are indicated in the tree for splits representing three or more samples.

The expanded signature mutations shared across all the 2022 strain are coloured in red.

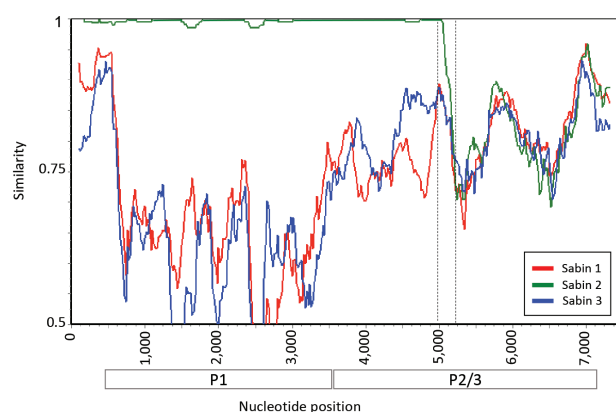
in the intestine and/or oropharyngeal mucosa of an immunodeficient vaccinated individual (immunodeficient iVDPV) [4].

As cVDPV type 2 caused most VDPV outbreaks, OPV type 2 was removed globally in April 2016 from the routine trivalent OPV which now contains only types 1 and 3 (bivalent OPV) [9]. Since then, monovalent OPV2 has been given only as a response to cVDPV2 outbreaks [10]. Nevertheless, an increase in the number of cVDPV type 2 outbreaks has been reported worldwide in recent years [11]. During 2022, a new challenge in the World Health Organization (WHO) poliovirus eradication programme includes controlling PV2 events not only in low-income countries [12] but also in countries with high vaccination coverage such as the US (New York), the United Kingdom and Israel [13].

quency of PV2 isolates detected in the Jerusalem region in Israel via wastewater surveillance. Sequencing of PV2 showed genetic linkage across the isolates and accumulation of mutations over time, with two isolates defined as VDPV. These PV2 isolates were detected in three different non-overlapping wastewater catchment regions in the Jerusalem region over a period of 100 days, which makes it unlikely that these isolates originated from one individual. We therefore assume that this VP2 strain might be circulating in this region, although not currently defined as such by the WHO. Genetically linked PV2 isolates were also identified in New York and London [14]. As monovalent OPV2 is not given in routine immunisations in these countries, these isolates may have originated from countries holding OPV2 campaigns due to recent VDPV2 outbreaks. Interestingly, in 2020 and 2021, importations of PV2 strains to Israel did not result in prolonged, evolving circulation, and those strains disappeared.

## FIGURE 4

Recombination event in poliovirus 2 sequences from wastewater isolates, Israel, 2022 (n =28)



Simplot [8] of the Sabin 1, 2 and 3 strains compared with 2022 poliovirus 2 isolates demonstrates highest similarity to Sabin 2 (AY.184220) in the P1 region. Similarity to Sabin 2 declines in the P2/3 region at a position around 5,100 (dashed vertical lines), with low similarity of the 2022 PV2 isolates to all three Sabin strains, suggesting recombination with a non-polio enterovirus.

The establishment of this PV2 strain from 2022 in Israel started during a cVDPV3 outbreak in the same region. The subsequent emergence of two such rare events is unexpected, especially in a high-income setting with high vaccination coverage. Israel responded to the first event (cVDPV3) by administering an inactivated poliovirus (IPV) including inactivated PV2 and a bivalent OPV that does not include Sabin 2. Thus, the vaccination programme cannot prevent transmission of PV2 but can prevent the disease in vaccinated individuals. As per July 2022, transmission of this PV2 strain in Israel has remained local and has not been detected outside the Jerusalem region, nor have any AFP cases been caused by PV2. Despite the high vaccination coverage in Jerusalem, there are still sub-populations with lower coverage in this region. As a response to the recent PV2 findings, the Israeli Ministry of Health initiates specialised programmes to vaccinate every child with IPV to prevent the disease, specifically targeting susceptible under-vaccinated communities in Jerusalem that were identified during the earlier emergence of cVDPV3. The early detection and identification of the genetic linkage across the isolates of this strain were based on WGS and extensive wastewater surveillance with advanced technologies. Indeed, with only two mutations in the routinely Sanger-sequenced VP1 gene linking the isolates (one of which was also observed in PV2 sequences from 2020 and 2021), WGS strengthened the genetic linkage by identifying additional mutations and a unique recombination shared across the 2022 isolates. Taking this case as an example, characterisation methods and working protocols may require updates in order to improve resolution of identification and response time to future outbreaks.

## Conclusion

This event emphasises the importance of routine environmental/AFP surveillance as a means for outbreak control and rapid immunisation campaigns, not only in low-income countries, but also in high-income countries with high vaccination coverage.

## Ethical statement

Not relevant. Samples used in this study are non-clinical environmental samples.

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## Conflict of interest

None declared.

## Authors' contributions

NSZ, MW, IBO conceived the manuscript. NSZ, MW, IBO, EB, NL, LW, IA, ZC, KK, RV, ME, ISF, MG, MWa generated the laboratory data. NSZ, MW, IBO, HM performed the phylogenetic and data analysis. NSZ, MW, IBO, LMS, DS, OE, RY, LHS, SAP and EM and prepared a draft of the manuscript. NSZ, MW and EM critically revised the manuscript and all authors reviewed it.

## References

1. Shulman LM, Manor Y, Hindiyeh M, Sofer D, Mendelson E. Molecular characterization of polio from environmental samples: ISSP, the Israeli sewage surveillance protocol. In: Martín, J. (eds). Poliovirus. Methods in molecular biology, vol 1387. New York: Humana Press; 2016. pp. 55-107. [https://doi.org/10.1007/978-1-4939-3292-4\\_5](https://doi.org/10.1007/978-1-4939-3292-4_5).
2. World Health organization (WHO). Circulating vaccine-derived poliovirus type 3 – Israel. Geneva: WHO; 2022. Available from: <https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON366>
3. Burns CC, Kilpatrick DR, Iber JC, Chen Q, Kew OM. Molecular properties of poliovirus isolates: nucleotide sequence analysis, typing by PCR and real-time RT-PCR. In: Martín, J. (eds). Poliovirus. Methods in molecular biology, vol 1387. New York: Humana Press; 2016. pp. 177-212. doi: [https://doi.org/10.1007/978-1-4939-3292-4\\_9](https://doi.org/10.1007/978-1-4939-3292-4_9)
4. Burns CC, Diop OM, Sutter RW, Kew OM. Vaccine-derived polioviruses. J Infect Dis. 2014;210(Suppl 1):S283-93.
5. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012;19(5):455-77. <https://doi.org/10.1089/cmb.2012.0021> PMID: 22506599
6. Nikolaidis M, Mimouli K, Kyriakopoulou Z, Tsimpidis M, Tsakogiannis D, Markoulatos P, et al. Large-scale genomic analysis reveals recurrent patterns of intertypic recombination in human enteroviruses. Virology. 2019;526:72-80. <https://doi.org/10.1016/j.virol.2018.10.006> PMID: 30366300
7. Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, et al. Nextstrain: real-time tracking of pathogen evolution. Bioinformatics. 2018;34(23):4121-3. <https://doi.org/10.1093/bioinformatics/bty407> PMID: 29790939



8. Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, Novak NG, et al. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. *J Virol.* 1999;73(1):152-60. <https://doi.org/10.1128/JVI.73.1.152-160.1999> PMID: 9847317
9. Hampton LM, Farrell M, Ramirez-Gonzalez A, Menning L, Shendale S, Lewis I, et al. cessation of trivalent oral poliovirus vaccine and introduction of inactivated poliovirus vaccine - worldwide, 2016. *MMWR Morb Mortal Wkly Rep.* 2016;65(35):934-8. <https://doi.org/10.15585/mmwr.mm6535a3> PMID: 27606675
10. World Health Organization (WHO). Standard operating procedures: responding to a poliovirus event or outbreak. Geneva: WHO; 2020. ISBN 978-92-4-000299-9. Available from: <https://www.who.int/publications/i/item/9789240002999>
11. Cooper LV, Bandyopadhyay AS, Gumedé N, Mach O, Mkanda P, Ndoutabé M, et al. Risk factors for the spread of vaccine-derived type 2 polioviruses after global withdrawal of trivalent oral poliovirus vaccine and the effects of outbreak responses with monovalent vaccine: a retrospective analysis of surveillance data for 51 countries in Africa. *Lancet Infect Dis.* 2022;22(2):284-94. [https://doi.org/10.1016/S1473-3099\(21\)00453-9](https://doi.org/10.1016/S1473-3099(21)00453-9) PMID: 34648733
12. Rachlin A, Patel JC, Burns CC, Jorba J, Tallis G, O'Leary A, et al. Progress toward polio eradication - worldwide, January 2020-April 2022. *MMWR Morb Mortal Wkly Rep.* 2022;71(19):650-5. <https://doi.org/10.15585/mmwr.mm7119a2> PMID: 35552352
13. Hill M, Bandyopadhyay AS, Pollard AJ. Emergence of vaccine-derived poliovirus in high-income settings in the absence of oral polio vaccine use. *Lancet.* 2022;400(10354):713-5. [https://doi.org/10.1016/S0140-6736\(22\)01582-3](https://doi.org/10.1016/S0140-6736(22)01582-3) PMID: 35988575
14. Global Polio Eradication Initiative. Updated statement on report of polio detection in United States. Geneva: World Health Organization; 2022. Available from: <https://polioeradication.org/news-post/report-of-polio-detection-in-united-states>

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