The Journal of Infectious Diseases

MAJOR ARTICLE

Isolation of Vaccine-Like Poliovirus Strains in Sewage Samples From the United Kingdom

Manasi Majumdar,[1] Dimitra Klapsa,[1] Thomas Wilton,[1] Joyce Akello,[2] Catherine Anscombe,[2] David Allen,[2] Edward T. Mee,[1] Philip D. Minor,[1] and Javier Martin[1]

[1]Division of Virology, National Institute for Biological Standards and Control, South Mimms, and [2]Enterovirus Unit, Public Health England, London, United Kingdom

Background. Environmental surveillance (ES) is a sensitive method for detecting human enterovirus (HEV) circulation, and it is used worldwide to support global polio eradication. We describe a novel ES approach using next-generation sequencing (NGS) to identify HEVs in sewage samples collected in London, United Kingdom, from June 2016 to May 2017.

Methods. Two different methods were used to process raw sewage specimens: a 2-phase aqueous separation system and size exclusion by filtration and centrifugation. HEVs were isolated using cell cultures and analyzed using NGS.

Results. Type 1 and 3 vaccine-like poliovirus (PV) strains were detected in samples collected from September 2016 through January 2017. NGS analysis allowed us to rapidly obtain whole-genome sequences of PV and non-PV HEV strains. As many as 6 virus strains from different HEV serotypes were identified in a single cell culture flask. PV isolates contained only a small number of mutations from vaccine strains commonly seen in early isolates from vaccinees.

Conclusions. Our ES setup has high sensitivity for polio and non-PV HEV detection, generating nearly whole-genome sequence information. Such ES systems provide critical information to assist the polio eradication endgame and contribute to the improvement of our understanding of HEV circulation patterns in humans.

Keywords. Global Polio Eradication Initiative, environmental surveillance, human enterovirus, vaccine-like poliovirus, intertypic recombination.

The Global Polio Eradication Initiative (GPEI) has been very successful in reducing poliovirus (PV) circulation in humans to the brink of global extinction [1]. However, some areas in Afghanistan and Pakistan remain where PV transmission has never been eliminated and type 1 wild PV (WPV1) and type 2 circulating vaccine-derived PV (cVDPV2) are still being transmitted from person to person. In addition, as of July 2017, PV transmission still occurs in some areas of the Middle East and Africa where there are severe difficulties in accessing children for vaccination. WPV1 was last detected in Nigeria in September 2016, and recent cVDPV2 outbreaks have been reported in Syria and the Democratic Republic of the Congo [2, 3].

As only a small proportion of infections by PV cause paralytic disease, the establishment of efficient and sensitive surveillance systems to guide public health interventions has been essential in ensuring the GPEI's success. The GPEI mostly relies on acute flaccid paralysis surveillance, which is monitored by strict quality performance indicators [4, 5]. A World Health Organization (WHO) global polio laboratory network of >140 laboratories exists where stool samples from acute flaccid paralysis cases are processed and analyzed for the presence of PV. In addition, several countries have successfully conducted environmental surveillance (ES) for PV for many years. Virus genotypes of isolates found in sewage samples from Egypt, Nigeria, India, Afghanistan, and Pakistan closely matched those found in stool samples from acute flaccid paralysis cases, which has helped trace the elimination of WPV in some areas [6–9]. This approach has also been used to monitor the disappearance of vaccine virus after vaccination campaigns in countries such as Cuba, New Zealand, and Japan [10–12]. Furthermore, widespread circulation of WPV1 imported from Pakistan in the absence of reported paralytic cases was identified in Israel in 2013, where the virus was found in sewage samples across the country, clearly suggesting that PV can circulate for long periods without detection in countries using inactivated polio vaccine, even those with high vaccination coverage, such as Israel [13, 14]. Thus, ES is seen as a powerful tool to support the GPEI endgame, helping to identify any remaining PV transmission.

With this in mind, a pilot study was set up to analyze sewage samples from London, United Kingdom, for the presence of PV. The last polio case due to WPV in the United Kingdom was reported in 1982, but the country switched to the exclusive use of inactivated polio vaccine in 2004 and has frequent population exchanges with areas where PV is still circulating and/or oral polio vaccine is being used routinely. We concentrated sewage

Received 1 October 2017; editorial decision 14 December 2017; accepted 20 December 2017; published online December 22, 2017.

Correspondence: J. Martin, PhD Biology, National Institute for Biological Standards and Control, South Mimms, Potters Bar, Herts EN6 3QG, UK (javier.martin@nibsc.org).

The Journal of Infectious Diseases® 2018;217:1222–30

DOI: 10.1093/infdis/jix667

samples by use of 2 different methods and added them to cell cultures susceptible to HEV infection. HEV isolates were then characterized by nucleotide sequencing. Nucleotide sequencing information about HEV clinical and environmental isolates is limited, often to specimens with results (positive or negative) of HEV species–specific real-time polymerase chain reaction (PCR) analysis and/or to short genomic sequences that can help identify the HEV serotype but have limited use for detailed phylogenetic analysis. Next-generation sequencing (NGS) metagenomics and target-specific techniques have recently been described by us and others to obtain nucleotide sequences of HEV strains present in stool, sewage, and cell culture samples [15–21]. A novel approach using NGS analysis is described here that can quickly obtain nearly whole-genome sequences of PV and non-PV HEVs present in cell cultures infected with sewage concentrates. This approach has the power to detect known HEVs, as well as divergent strains and novel serotypes, providing information that should assist with tracing the source and transmission of HEVs, including PV, in human populations.

MATERIALS AND METHODS

Sample Collection

One-liter composite sewage samples were collected during a 24-hour period once monthly at Beckton Sewage Treatment Works in London, which is currently treating the waste of 3.5 million people. Samples were transported to the laboratory on the same day and processed within 1 day of arrival. Twelve samples were collected from June 2016 to May 2017.

Sample Processing

Raw sewage samples were processed by 2 different methods previously described: a 2-phase (TP) aqueous separation system [22] and concentration by filtration and centrifugation (FC), using Centriprep YM-50 centrifugal concentration devices (Merck) [23].

Cell Lines and Virus Isolation

Virus isolation in cell cultures was performed according to WHO recommendations [24]. Rhabdomyosarcoma (RD) and mouse L20B cells expressing the human PV receptor were used as detailed in the Supplementary Materials.

Intratypic Differentiation of PV Isolates by Real-Time Reverse-Transcription PCR (rRT-PCR) Assays

Conventional rRT-PCR–based intratypic differentiation of PV was performed by means of a PV-diagnostic rRT-PCR kit provided by the Centers for Disease Control and Prevention, using a Rotorgene Q (Qiagen) platform and following the kit instructions [25].

Nucleotide Sequence Analysis of the VP1 Coding Region of PV Isolates

RT-PCR fragments containing the VP1 coding region were generated from purified viral RNAs by 1-step RT-PCR, using a SuperScript III (Invitrogen) system and primers Y7 (5′-GGGTTTGTGTCAGCCTGTAATGA-3′) and Q8 (5′-AAGAGGTCTCTRTTCCACAT-3′) [25]. Amplified products were purified using the QIAquick Gel Extraction Kit (Qiagen) and were sequenced by the Sanger method, using an ABI Prism 3130 genetic analyzer (Applied Biosystems).

Preparation of RT-PCR Templates for NGS Analysis

Whole-genome PV RT-PCR fragments were amplified from purified RNAs from infected cells by a 1-step RT-PCR as described previously [20]. In addition, RT-PCR products were also generated by sequence-independent single-primer amplification of purified RNAs as described elsewhere [18, 19, 26]. Two primer sets were used to generate 2 different double-stranded DNA templates from each sample. Details about the primers and amplification conditions are provided in the Supplementary Materials.

Generation of Sequencing Libraries and Quality Trimming of NGS Reads

Sequencing libraries were prepared using Nextera XT reagents and sequenced on a MiSeq v3 system (Illumina) with a 2 × 301 paired-end flow cell kit according to the manufacturer's protocols. Raw sequence data were imported into Geneious R10 software (Biomatters), and sequence files were processed using a custom work flow. Parameters used for quality trimming of NGS reads are available in the Supplementary Materials. Raw fastq files are available from the National Center for Biotechnology Information's Sequence Read Archive (project code PRJNA417977).

Generation of PV and Non-PV HEV Sequence Contigs From NGS Data by Reference-Guided or De Novo Assembly of Filtered NGS Reads

The filtered reads were mapped to a set of HEV sequences by using a curated HEV sequence database, and contig sequences were generated. Filtered reads were then iteratively reassembled to consensus sequences from the longest contigs, to build whole-genome contig sequences. Final consensus sequences were obtained by assigning the most common nucleotide sequence at each nucleotide position. The filtered reads were also independently assembled de novo, using similar assembly conditions. Whole-genome contig sequences were generated by following the same analysis work flow described above. As a result, we obtained nearly whole-genome nucleotide sequences for various HEV strains in each sample, including various PV strains. Results using the 2 different assembly approaches were almost identical. Manual analyses for visualizing and quantifying assembly results were performed throughout the process. Full details regarding settings used for genome assembly are provided in the Supplementary Materials. Consensus nucleotide sequences are available from DDBJ/EMBL/GenBank (accession numbers MG451802 to MG451811).

Phylogenetic Analysis of Polio and Non-PV HEV Isolates

The closest virus relatives to the London sewage HEV isolates were identified using the RIVM and BLAST online sequence analysis tools [27, 28], and HEV serotypes were assigned on the basis of their VP1 sequence. Whole-genome PV sequences were aligned to type 1 Sabin PV AY184219 or type 3 Sabin PV AY184221 reference genome sequences by using the program ClustalW (within Geneious), to identify mutations and/or recombination events. Any PV3/PV1 recombinant genomic structure was confirmed by independently mapping filtered reads to the PV1 and PV3 Sabin reference sequences mentioned above, with a minimum 50-base overlap, a minimum overlap identity of 95%, a maximum of 5% mismatches per read, and both end pair reads mapping. Percentages of maximum coverage were calculated. Mapped reads against each reference were combined in a graph for each NGS product.

RESULTS

Concentration of Sewage Samples and Virus Isolation in Cell Cultures

Twelve samples from London sewage were analyzed. A total of 500 mL and 120 mL of raw sewage from each sample were used for the TP and FC methods, respectively. Typically, 10 mL and 4 mL were obtained, yielding approximate concentration factors of 50 times for the TP method and 30 times for the FC method, respectively. Aliquot samples of sewage concentrates were used to infect RD and L20B cells. Sewage concentrates from all 12 samples produced a cytopathic effect (CPE) in RD cells after 3–4 days. Only 2 samples, from September 2016 and January 2017, produced a CPE in L20B cells. As shown in Table 1, concentrates from both sewage samples, obtained with both concentration methods, produced a CPE in L20B cells but only in a proportion of flasks (5 of 10 with concentrates from September 2016 and 2 of 12 with concentrates from January 2017).

Typing of PV Isolates by rRT-PCR–Based Intratypic Differentiation and VP1 Sequencing

PV strains present in L20B cell cultures showing a CPE were initially characterized by rRT-PCR–based intratypic differentiation. As shown in Table 1, all PV isolates from September 2016 were identified as PV3 and Sabin (vaccine) like (SL). The sewage sample from January 2017 produced both PV1 and PV3 isolates. The TP-RD isolate from January 2017 was characterized as a PV3-SL strain, while the FC-L20B$_1$ isolate was found to be a PV1-SL strain. The PV serotype and SL classification were confirmed by nucleotide sequencing of the VP1 coding region. PV isolates showed very few VP1 mutations from Sabin references (Table 1).

Genetic Characterization of PV Isolates by NGS

The genomes of the 7 selected PV isolates were further characterized by high resolution sequencing. Whole-genome PV RT-PCR products were obtained and analyzed by NGS. Consensus sequences for each of the PV isolates were generated by de novo assembly. As shown in Table 2, all 5 isolates from September 2016 were confirmed as PV3-SL by NGS analysis, which is very closely related to the Sabin 3 vaccine strain and

Table 1. Isolation and Characterization of Poliovirus (PV) Strains From Infected Cell Cultures

Sample, Method,[a] Cell Line	CPE[b]	Result	Isolate(s)[c]	ITD Result(s)[d]	VP1 Sequence[e]
Sep 2016					
TP					
L20B	1/5	Positive	TP-L20B$_3$	PV3-SL	PV3-SL (1)
RD	1/1	Positive	TP-RD	PV3-SL	PV3-SL (1)
RD to L20B	1/1	Positive	…	…	…
FC					
L20B	2/3	Positive	FC-L20B$_1$ and FC-L20B$_3$	PV3-SL for both	PV3-SL (1)
RD	1/1	Positive	FC-RD	PV3-SL	PV3-SL (3)
RD to L20B	1/1	Positive	…	…	…
Jan 2017					
TP					
L20B	0/5	Negative	…	…	…
RD	1/1	Positive	TP-RD	PV3-SL	PV3-SL (2)
RD to L20B	1/1	Positive	…	…	…
FC					
L20B	1/5	Positive	FC-L20B$_1$	PV1-SL	PV1-SL (0)
RD	1/1	Positive	…	…	…
RD to L20B	0/1	Negative	…	…	…

Abbreviations: RD, rhabdomyosarcoma; SL, Sabin like.

[a]The 2-phase (TP) and filtration-centrifugation concentration (FC) methods are described in Materials Methods.

[b]No. of cell flasks with cytopathic effect/total no. of flasks.

[c]Isolates selected for characterization.

[d]Real-time reverse-transcription polymerase chain reaction–based intratypic differentiation (ITD) results.

[e]The number of nucleotide mutations from the Sabin reference strain is shown in parentheses.

Table 2. Genetic Properties of Poliovirus (PV) Isolates From London Sewage Samples

Sample, Isolate,[a] Nucleotide[b]	Nucleotide Change	Amino Acid[c]	Amino Acid Change
Sep 2016			
TP-L20B$_3$ (PV-3)			
472	U>C	…	…
1729	C>U	…	…
2493	C>U	VP1-6	T>I
TP-RD (PV-3)			
472	U>C	…	…
1912	C>U	…	…
2158	G>A	…	…
2493	U>C	VP1-6	T>I
5832	U>C	3C-135	I>T
FC-L20B$_1$ (PV-3)			
472	U>C	…	…
2493	C>U	VP1-6	T>I
5884	A>G	…	…
FC-L20B$_3$ (PV-3)			
472	U>C	…	…
2493	C>U	VP1-6	T>I
3190	A>G	…	…
4917	C>U	2C-268	T>M
5806	U>C	…	…
6394	A>U	3D-139	K>N
7252	G>A	…	…
FC-RD (PV-3)			
472	U>C	…	…
2493	C>U	VP1-6	T>I
2519	A>C	VP1-15	T>P
2948	A>G	VP1-158	I>V
6364	U>C	…	…
6904	A>G	…	…
Jan 2017			
TP-RD (PV-3/PV-1)			
472	U>C	…	…
1981	C>U	…	…
2493	C>U	VP1-6	T>I
2636	G>A	VP1-54	A>T
5417	A>G	3B-12	N>S
FC-L20B$_1$ (PV-1)			
1219	A>G	…	…

Abbreviation: RD, rhabdomyosarcoma.

[a]The 2-phase (TP) and filtration-centrifugation concentration (FC) methods are described in Materials Methods.

[b]Nucleotide differences from the Sabin vaccine strain.

[c]Amino acid differences from the Sabin vaccine strain.

contains reversions at known Sabin 3 attenuation sites, nucleotide 472 in the 5′ noncoding region, and nucleotide 2493, which codes for capsid amino acid VP1-6. Few additional nucleotide differences from the Sabin 3 sequence were found in the PV isolates, with all showing unique sequences. The TP-RD PV isolate from the 2017 sample was also a PV3-SL strain and also contained reversions at nucleotides 472 and 2493. However, the whole-genome consensus sequence of this 2017 isolate revealed a type 3/type 1 PV recombinant structure with a crossover point between nucleotides 4904 and 4914. As shown in Figure 1, NGS reads from this isolate mapped to both Sabin 1 and Sabin 3 reference sequences in different regions of the genome. This finding was reproducible using the random PCR sequencing approach discussed in next section (Figure 1). Finally, the 2017 L20B isolate from the FC concentrate was confirmed as PV1, which is very closely related to the Sabin 1 vaccine strain, with only 1 nucleotide change from the vaccine strain. The VP1 sequences of all PV isolates determined by NGS analysis were identical to those obtained by the Sanger method

Sequence Analysis of HEV Mixtures Found in RD Cells

RT-PCR products generated using random primers were also analyzed by NGS to sequence any other HEV strain that might be present in the infected RD cell cultures. We first analyzed virus control samples (reference strains enterovirus 20 JV-1, echovirus 7 Wallace, coxsackievirus B5 Faulkner, and PV Sabin 1) to test and optimize our ability to detect and identify HEV mixtures. NGS reads were filtered and analyzed as described in Materials and Methods. Nucleotide sequences obtained from RA01 and M13 random RT-PCR products from these reference strains were almost identical except in the extreme ends, where sequence coverage was low. These extreme regions were discarded from the final consensus sequence assigned to the virus. As shown in Figure 2 and Supplementary Figure 1, there was excellent coverage across most of the genome for all viruses, and nearly whole-genome sequences (≥95% of the genome) were obtained for all 4 reference virus strains in both single and mixed samples. Sequences in these single and mixed samples were identical and highly similar (>99.8%) to the corresponding GenBank Sanger sequences (Table 3).

The same analytical process was followed for TP-RD isolates from the 2 London sewage samples. Six and 4 different HEV strains were identified in the September 2016 and January 2017 samples, respectively, including PV3 strains found in both samples. Results are shown in Figure 2, Supplementary Figure 1, and Table 3. Again, nearly whole-genome sequences (>90% of the genome) were obtained for all virus strains identified in both samples, and nucleotide sequences determined by either RA01 or M13 primers were almost identical. The relative proportions of VP1 sequence reads mapping to each of the different HEV strains identified in each sample are shown in Figure 3 and Supplementary Figure 2. There were some differences in the results for the September 2016 sample in that echovirus 3 sequence reads were the most prevalent in sequences from the M13 RT-PCR, while coxsackievirus B3 reads showed the highest proportion when the RA01 RT-PCR product was analyzed. These differences likely reflect some degree of bias in the amplification of viral genomes from some strains, using different primer sets. Echovirus 7 was the most prevalent strain in the 2017 RD culture, with >92% of reads mapping to this strain. The proportion of sequence reads mapping to PV sequences was relative low, with only 6.28% and 0.76% of the total number of reads mapping

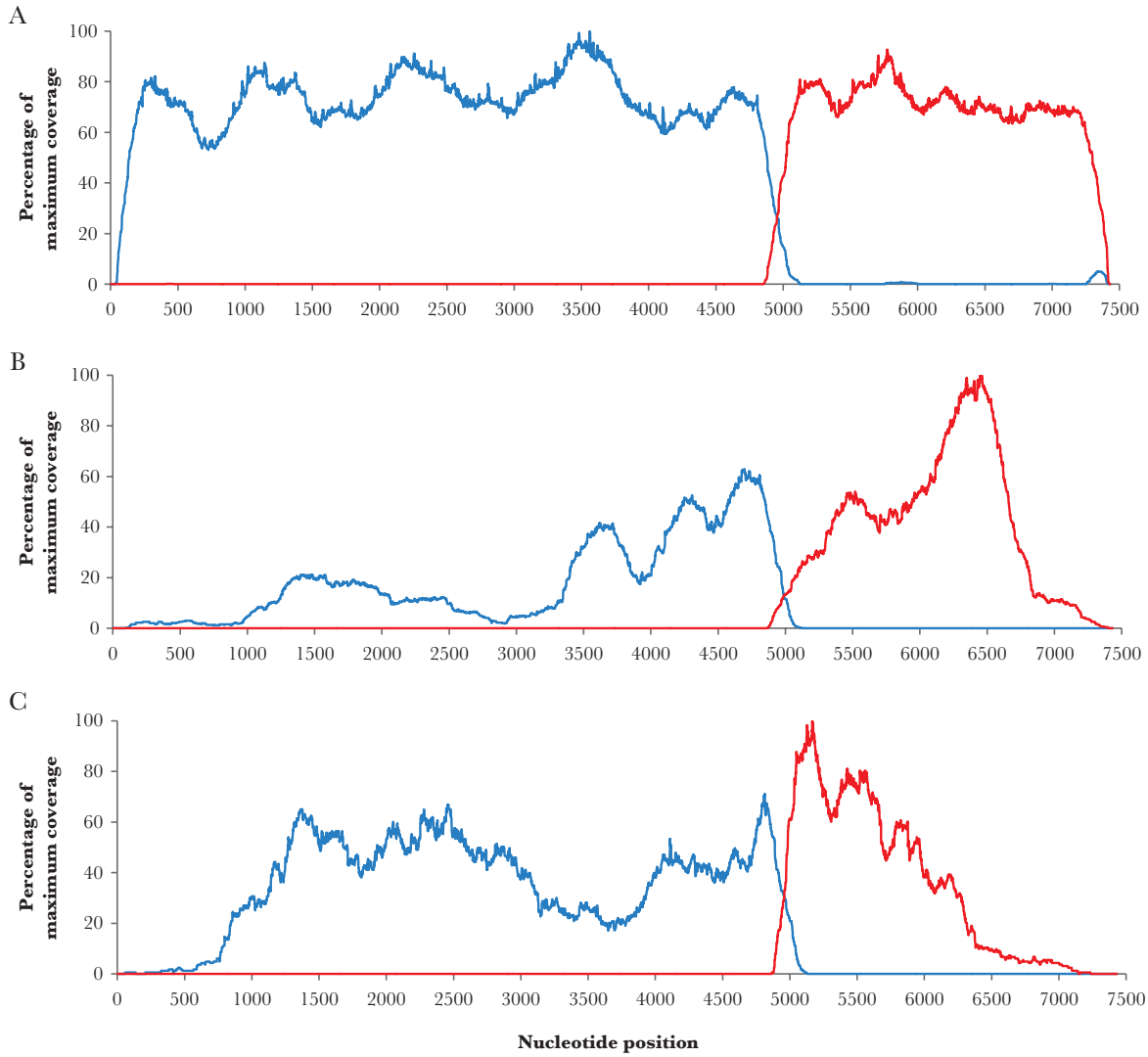

Figure 1. Coverage of Sabin PV 1 and 3 genomes following reverse-transcription polymerase chain reaction (RT-PCR) analysis and next-generation sequencing (NGS) of the TP-RD poliovirus (PV) isolate from the January 2017 sewage sample. Filtered reads from PV-specific (*A*), M13-random (*B*), or RA01-random (*C*) RT-PCR products obtained from RNA purified from infected cells were independently mapped against type 1 Sabin PV AY184219 (red line) or type 3 Sabin PV AY184221 (blue line) reference genome sequences. Mapped reads against each reference were combined in the graph for each NGS product. Percentages of maximum coverage are reported. The results were identical for all RT-PCR products and identified a type 3/type 1 PV recombinant with a crossover point between nucleotides 4904 and 4914.

to PV3 sequences for the September 2016 and January 2017 samples, respectively. The closest virus relatives to the London sewage non-PV HEV isolates were identified by BLAST analysis of VP1 sequences. VP1 genetic similarities to related non-PV HEV strains ranged between 87.4% and 96.9 % for nucleotides and between 97.9% and 100% for amino acid sequences, confirming the HEV serotype assignment (Table 3). Two different echovirus 7 strains were found in the September 2016 sewage sample, with 83.4% and 98.2% nucleotide and amino acid sequence identity, respectively, between them across the whole genome.

DISCUSSION

All 12 sewage samples from London, tested as part of a pilot ES study, were positive for HEVs, and 2 contained PV. Type 1 and 3 vaccine-like PV isolates were found in samples collected in September 2016 and January 2017. Both concentration methods resulted in PV isolation, and we found the FC method to be simpler, quicker, less technically demanding, and free from bacterial contamination as compared to the TP separation system used in most WHO laboratories. Finding PV in these samples was rather unexpected because the United Kingdom has exclusively used inactivated polio vaccine for polio immunization since 2004 [29]. However, vaccine-like PVs have occasionally been found in environmental samples collected in countries using only inactivated polio vaccine, presumably imported by people coming from countries where oral polio vaccine is still used [30]. The London sewage sampling site serves a population that includes large migrant groups from countries where there is still oral polio vaccine use.

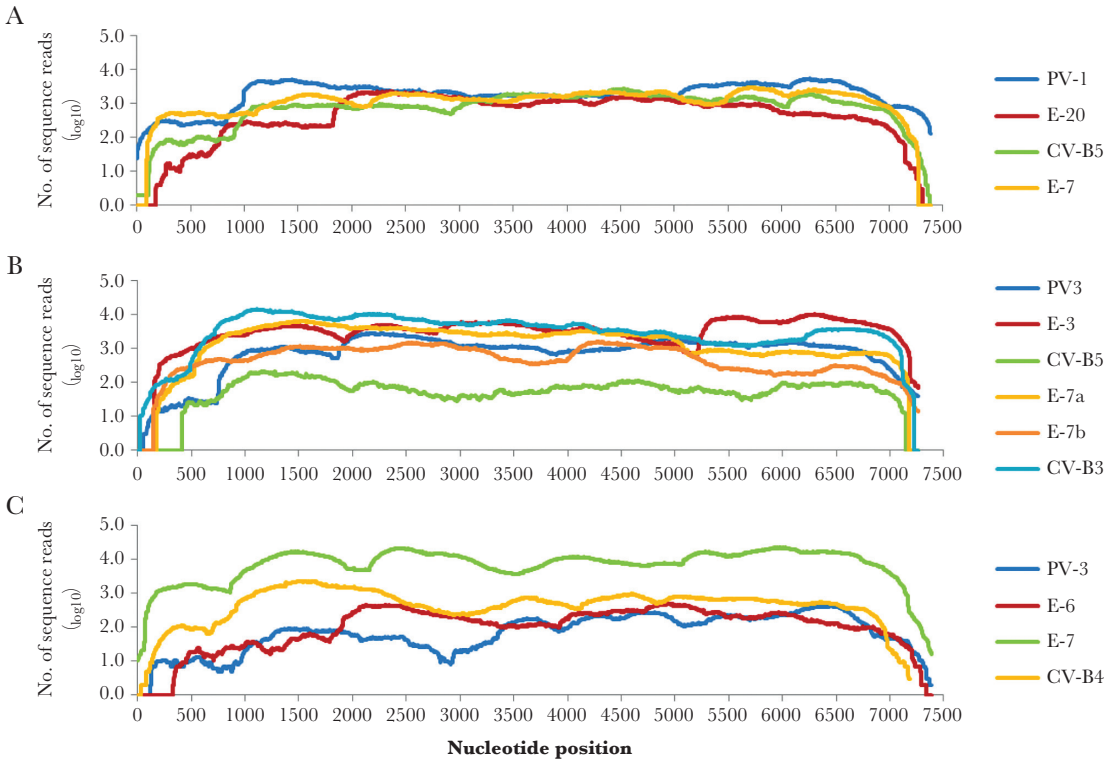

Figure 2. Genome coverage of human enterovirus (HEV) genomes following reverse-transcription polymerase chain reaction (RT-PCR) analysis and next-generation sequencing (NGS) of TP-RD isolates from sewage samples. Filtered reads from the virus control mixture (*A*), a sewage sample from September 2016 (*B*), and a sewage sample from January 2017 (*C*) were mapped to the respective final HEV consensus sequences identified by de novo assembly. Results obtained with NGS reads generated with RA01-random RT-PCR products are shown. The number of sequence reads at each nucleotide position is shown for each HEV strain. The results show excellent coverage across most of the genome for all viruses. Similar results were obtained when using M13-random RT-PCR products (Supplementary Figure 1).

Table 3. Genetic Identity of Human Enterovirus Isolates Found in Cultures of Rhabdomyosarcoma Cells Infected With London Sewage Samples

		Closest Relative From NCBI Sequence Database				
Sample, Virus	Coverage, %	NCBI Accession No.	Nucleotide Identity, %	Amino Acid Identity, %	Country	Year
Control						
PV-1 Sabin 1	96.26	AY184219	100	100	US	1956
E-20 JV-1	94.94	AY302546	99.9	100	US	1956
E-7 Wallace	97.02	AY302559	99.8	99.7	US	1953
CV-B5 Faulkner	97.62	AF114383	99.8	100	US	1952
Sep 2016						
PV-3	99.31	AY184221	99.9	99.7	US	1956
E-7a	96.88	KY433706	96.1	100	Niger	2013
E-7b	97.92	KY433706	87.4	97.9	Niger	2013
E-3[a]	98.33	GU359052	92.0	98.6	Pakistan	2009
CV-B5	91.22	HF948225	95.4	99.3	Germany	2010
CV-B3	98.73	LC012523	95.4	99.3	Japan	2013
Jan 2017						
PV-3	90.08	AY184221	99.8	99.3	US	1956
E-7	97.13	KY433706	95.2	100	Niger	2013
E-6	85.86	HM852754	91.3	99.3	Greece	2006
CV-B4	92.15	KU841464	96.9	99.3	Russia	2013

Abbreviation: NCBI, National Center for Biotechnology Information.

[a]Partial 414-nucleotide VP1 sequence.

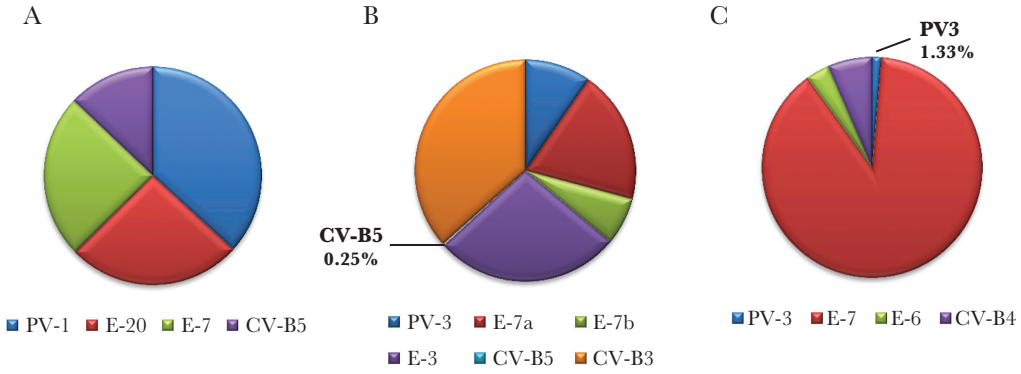

Figure 3. Percentage of filtered reads mapping to VP1 sequences. Filtered reads from the virus control mixture (*A*), a sewage sample from September 2016 (*B*), and a sewage sample from January 2017 (*C*) were mapped to VP1 consensus sequences of each of the human enterovirus (HEV) strains identified by de novo assembly. Results for RA01-random reverse-transcription polymerase chain reaction (RT-PCR) products are shown. Percentages of total reads mapping to each of the HEV VP1 sequences are indicated. No HEV isolates were identified in any of the negative controls analyzed. The results show that the proportion of poliovirus sequences found in infected rhabdomyosarcoma cells was low, particularly in the sample from January 2017. Similar results were obtained when using M13-random RT-PCR products (Supplementary Figure 2).

Nearly whole-genome viral sequences were rapidly generated by NGS analysis of RT-PCR products obtained using random or PV-specific primers. Five different vaccine-like PV3 strains were identified in the 2016 sewage sample, all containing few mutations from the PV3 Sabin vaccine strain. These included reversions at attenuation mutations at nucleotide 472 in domain V of the 5′ noncoding region, with a role in protein translation, and at nucleotide 2493, which codes for amino acid VP1-6 and is possibly involved in virus particle stability [31, 32]. A different PV3 strain was found in the 2017 sewage sample, which also contained few mutations from Sabin 3 and the 2 reversion mutations mentioned above. This 2017 PV3 isolate had a PV3/PV1 recombinant genomic structure with a crossover point located in the region coding for nonstructural protein 2C. In addition, a PV1 vaccine-like isolate with a single mutation from the Sabin 1 vaccine strain was also found in the sewage sample from 2017. The fact that all PV isolates from sewage specimens contained a very low number of mutations from the Sabin vaccine strains indicates a very short period of replication/transmission in humans, from several days to few weeks after vaccination, with these PV strains possibly having been excreted by just 1or few recent vaccinees and/or their immediate contacts.

It is striking that significant sequence differences were found between virus isolates found in the same sewage sample, including the presence of unique PV3 genetic variants in the sample from September 2016 and a PV1 strain together with a PV3/PV1 recombinant virus in the sample from January 2017. This is likely due to PV being in very low concentration in sewage, leading to a strong sampling effect that is reflected when using different aliquots of concentrate to infect different cell culture flasks. This is in agreement with the fact that only a proportion of L20B cell culture flasks incubated with sewage concentrates showed a CPE. Indeed, complex virus mixtures are commonly found in sewage samples, with parallel cell culture flasks infected with aliquots of the same sewage concentrate producing very different results. This might include PV in different homotypic and/or heterotypic mixtures, often in combination with non-PV HEVs [6]. Furthermore, it is not at all unexpected that vaccinees excrete virus mixtures containing mixed serotype and recombinant variants [20, 33]. PV3/PV1 recombinant strains similar to the 2017 isolate found in this study are commonly found in stool samples from vaccinees collected soon after vaccination in combination with other nonrecombinant and recombinant variants from all serotypes present in the oral polio vaccine [33, 34].

Considering that oral polio vaccine is not used in the United Kingdom and that this sewage site covers a population of >3.5 million people, we conclude that our ES set up is sensitive enough to detect low concentrations of PV. In this context, it is expected that, should transmission of WPV1 or cVDPV2 occur in the United Kingdom following importation, it would be readily detected using our system. Importantly, no such PV isolates were detected in the London samples. It is also reassuring that no PV2 vaccine sequences were detected in any of the PV isolates identified since the type 2 component was removed from oral polio vaccine in August 2016. The results shown here are compatible with the viruses found being derived from vaccinees who received type 1 and 3 bivalent oral polio vaccine.

It is also expected that several non-PV HEV strains would be present in sewage concentrates and, hence, in RD cultures from them, reflecting their circulation in human populations. Using NGS analysis, we found 5 and 3 non-PV HEV strains of different serotypes, in addition to PV, in the London sewage samples from September 2016 and January 2017, respectively. A recent report, also presenting NGS findings, described

the presence of coxsackievirus B1, B3, and B5 strains in single RD cultures from sewage samples in Pakistan [17]. All non-PV HEV strains found in the 2016 and 2017 London samples correspond to species B HEV serotypes, a common finding that, rather than reflecting the actual prevalence of HEV serotypes in human populations, might be a consequence of the high sensitivity of RD cells for infection with species B HEVs [35]. Virus strains from all 4 HEV species can infect RD cells, as shown by the analysis of stool extracts from acute flaccid paralysis cases [36]. However, the complex HEV composition in sewage means that species B HEVs would likely outcompete viruses from other species when growing on RD cells. Indeed, some studies have shown that HEV strains from all 4 species (ie, A, B, C, and D) are frequently found in stool and sewage samples [7, 30, 37, 38]. Identifying them has required the use of several cell culture systems and/or sequencing RT-PCR products from multiple PCR reactions or from a large number of complementary DNA clones. Our NGS approach can reveal the presence of several non-PV HEV strains in a single cell culture system, providing nearly whole-genome nucleotide sequence information about each of them.

PV strains are known to replicate efficiently on RD cells, but cultures of RD-infected cells from the 2 London samples were found to contain only a low proportion of PV relative to other species B non-PV HEV strains. This observation highlights the relevance of using L20B cells to increase the sensitivity for PV detection in clinical and environmental samples. Several studies have described the microbiome in stool and sewage samples, but information on HEV content is very limited. Our results show the great value of using NGS technology for HEV surveillance, particularly for PV, as it can detect low concentrations of PV possibly excreted by 1 or few individuals and can quickly provide whole-genome genetic information, including evidence for recombination events. Identifying genetic features that link PV isolates is essential to establish temporal and geographical relationships between them that help trace virus transmission. Previous work in our laboratory, using NGS for the analysis of both vaccine products and isolates from vaccinees, has shown that these methods can also accurately identify PV strains in homotypic and heterotypic mixtures [18, 20], a critical feature that will help identify WPV and cVDPV strains in a background of oral polio vaccine use. The use of NGS methods for HEV identification and characterization represents a major step forward in HEV molecular diagnosis and will greatly contribute to improving our knowledge about HEV circulation patterns in human populations and their association with human disease.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We thank Jim McLauchlin and Chris Roberts, for organizing the regular composite sampling of sewage from Becton between June 2016 and May 2017 that enabled this project to take place; and the NGS team at the National Institute for Biological Standards and Control, for their technical support.

Financial support. This work was supported by the Department of Health Policy Research Programme (National Institute for Biological Standards and Control Regulatory Science Research Unit, 044/0069).

Potential conflicts of interest. All authors: No reported conflicts of interest. 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

1. World Health Organization. Poliomyelitis fact sheet. Updated 2017. http://www.who.int/mediacentre/factsheets/fs114/en/. Accessed 28 November 2017.
2. World Health Organization. Wild polio and vaccine derived polio in Nigeria. 6 October 2016. http://www.who.int/csr/don/06-october-2016-polio-nigeria/en/. Accessed 28 November 2017.
3. World Health Organization. Circulating vaccine-derived poliovirus type 2—Syrian Arab Republic. 13 June 2017. http://www.who.int/csr/don/13-June-2017-polio-syrian-arab-republic/en/. Accessed 28 November 2017.
4. World Health Organization. WHO-recommended surveillance standard of poliomyelitis. http://www.who.int/immunization/monitoring_surveillance/burden/vpd/surveillance_type/active/poliomyelitis_standards/en/. Accessed 28 November 2017.
5. Diop OM, Kew OM, de Gourville EM, Pallansch MA. The Global Polio Laboratory Network as a Platform for the Viral Vaccine-Preventable and Emerging Diseases Laboratory Networks. J Infect Dis **2017**; 216:299–307.
6. Hovi T, Blomqvist S, Nasr E, et al. Environmental surveillance of wild poliovirus circulation in Egypt–balancing between detection sensitivity and workload. J Virol Methods **2005**; 126:127–34.
7. Ndiaye AK, Diop PA, Diop OM. Environmental surveillance of poliovirus and non-polio enterovirus in urban sewage in Dakar, Senegal (2007–2013). Pan Afr Med J **2014**; 19:243.
8. Johnson Muluh T, Hamisu AW, Craig K, et al. Contribution of environmental surveillance toward interruption of poliovirus transmission in Nigeria, 2012–2015. J Infect Dis **2016**; 213:S131–5.

9. Deshpande JM, Shetty SJ, Siddiqui ZA. Environmental surveillance system to track wild poliovirus transmission. Appl Environ Microbiol **2003**; 69:2919–27.
10. Más Lago P, Cáceres VM, Galindo MA, et al. Persistence of vaccine-derived poliovirus following a mass vaccination campaign in Cuba: implications for stopping polio vaccination after global eradication. Int J Epidemiol **2001**; 30:1029–34.
11. Huang QS, Greening G, Baker MG, et al. Persistence of oral polio vaccine virus after its removal from the immunisation schedule in New Zealand. Lancet **2005**; 366:394–6.
12. Nakamura T, Hamasaki M, Yoshitomi H, et al. Environmental surveillance of poliovirus in sewage water around the introduction period for inactivated polio vaccine in Japan. Appl Environ Microbiol **2015**; 81:1859–64.
13. Shulman LM, Gavrilin E, Jorba J, et al. Molecular epidemiology of silent introduction and sustained transmission of wild poliovirus type 1, Israel, 2013. Euro Surveill **2014**; 19:20709.
14. Shulman LM, Martin J, Sofer D, et al. Genetic analysis and characterization of wild poliovirus type 1 during sustained transmission in a population with >95% vaccine coverage, Israel 2013. Clin Infect Dis **2015**; 60:1057–64.
15. Victoria JG, Kapoor A, Li L, et al. Metagenomic analyses of viruses in stool samples from children with acute flaccid paralysis. J Virol **2009**; 83:4642–51.
16. Bessaud M, Sadeuh-Mba SA, Joffret ML, et al. Whole Genome Sequencing of Enterovirus species C Isolates by High-Throughput Sequencing: Development of Generic Primers. Front Microbiol **2016**; 7:1294.
17. Furtak V, Roivainen M, Mirochnichenko O, et al. Environmental surveillance of viruses by tangential flow filtration and metagenomic reconstruction. Euro Surveill **2016**; 21.
18. Mee ET, Minor PD, Martin J. High resolution identity testing of inactivated poliovirus vaccines. Vaccine **2015**; 33:3533–41.
19. Montmayeur AM, Ng TF, Schmidt A, et al. High-Throughput Next-Generation Sequencing of Polioviruses. J Clin Microbiol **2017**; 55:606–15.
20. Stern A, Yeh MT, Zinger T, et al. The Evolutionary Pathway to Virulence of an RNA Virus. Cell **2017**; 169:35–46 e19.
21. Ng TF, Marine R, Wang C, et al. High variety of known and new RNA and DNA viruses of diverse origins in untreated sewage. J Virol **2012**; 86:12161–75.
22. Pöyry T, Stenvik M, Hovi T. Viruses in sewage waters during and after a poliomyelitis outbreak and subsequent nationwide oral poliovirus vaccination campaign in Finland. Appl Environ Microbiol **1988**; 54:371–4.
23. Harvala H, Calvert J, Van Nguyen D, et al. Comparison of diagnostic clinical samples and environmental sampling for enterovirus and parechovirus surveillance in Scotland, 2010 to 2012. Euro Surveill **2014**; 19.
24. Guidelines for environmental surveillance of poliovirus circulation. http://archives.who.int/vaccines-documents/DocsPDF03/www737.pdf. Accessed 25 September 2017.
25. 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. Methods Mol Biol **2016**; 1387:177–212.
26. Victoria JG, Kapoor A, Dupuis K, Schnurr DP, Delwart EL. Rapid identification of known and new RNA viruses from animal tissues. PLoS Pathog **2008**; 4:e1000163.
27. Madden T. The BLAST sequence analysis tool. In: McEntyre J OJ, ed. The NCBI handbook. Bethesda, MD: National Center for Biotechnology Information, **2002**.
28. Kroneman A, Vennema H, Deforche K, et al. An automated genotyping tool for enteroviruses and noroviruses. J Clin Virol **2011**; 51:121–5.
29. Minor PD. The polio-eradication programme and issues of the end game. J Gen Virol **2012**; 93:457–74.
30. Pellegrinelli L, Binda S, Chiaramonte I, et al. Detection and distribution of culturable Human Enteroviruses through environmental surveillance in Milan, Italy. J Appl Microbiol **2013**; 115:1231–9.
31. Minor PD. Poliovirus biology. Structure **1996**; 4:775–8.
32. Weeks-Levy C, Tatem JM, DiMichele SJ, Waterfield W, Georgiu AF, Mento SJ. Identification and characterization of a new base substitution in the vaccine strain of Sabin 3 poliovirus. Virology **1991**; 185:934–7.
33. Minor PD. The molecular biology of poliovaccines. J Gen Virol **1992**; 73 (Pt 12):3065–77.
34. Cuervo NS, Guillot S, Romanenkova N, et al. Genomic features of intertypic recombinant sabin poliovirus strains excreted by primary vaccinees. J Virol **2001**; 75:5740–51.
35. Faleye TO, Adeniji JA. Enterovirus Species B Bias of RD Cell Line and Its Influence on Enterovirus Diversity Landscape. Food Environ Virol **2015**; 7:390–402.
36. Fernandez-Garcia MD, Kebe O, Fall AD, Ndiaye K. Identification and molecular characterization of non-polio enteroviruses from children with acute flaccid paralysis in West Africa, 2013–2014. Sci Rep **2017**; 7:3808.
37. Apostol LN, Imagawa T, Suzuki A, et al. Genetic diversity and molecular characterization of enteroviruses from sewage-polluted urban and rural rivers in the Philippines. Virus Genes **2012**; 45:207–17.
38. Wang H, Tao Z, Li Y, et al. Environmental surveillance of human enteroviruses in Shandong Province, China, 2008 to 2012: serotypes, temporal fluctuation, and molecular epidemiology. Appl Environ Microbiol **2014**; 80:4683–91.