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Insights from a systematic search for information on designs, costs, and effectiveness of poliovirus environmental surveillance systems

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Abstract

Poliovirus surveillance plays a critical role in achieving and certifying eradication and will play a key role in the polio endgame. Environmental surveillance can provide an opportunity to detect circulating polioviruses prior to the observation of any acute flaccid paralysis (AFP) cases. We completed a systematic review of peer-reviewed publications on environmental surveillance for polio including the search terms “environmental surveillance” or “sewage,” and “polio,” “poliovirus,” or “poliomyelitis,” and compared characteristics of the resulting studies. The review included 146 studies representing 101 environmental surveillance activities from 48 countries published between 1975 and 2016. Studies reported taking samples from sewage treatment facilities, surface waters, and various other environmental sources, although they generally did not present sufficient details to thoroughly evaluate the sewage systems and catchment areas. When reported, catchment areas varied from 50 to over 7.3 million people (median of 500,000 for the 25% of activities that reported catchment areas, notably with 60% of the studies not reporting this information and 16% reporting insufficient information to estimate the catchment area population size). While numerous studies reported the ability of environmental surveillance to detect polioviruses in the absence of clinical cases, the review revealed very limited information about the costs and limited information to support quantitative population effectiveness of conducting environmental surveillance. This review motivates future studies to better characterize poliovirus environmental surveillance systems and the potential value of information that they may provide in the polio endgame.

Keywords

polio; eradication; risk management; environmental surveillance; acute flaccid paralysis

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Introduction

Through intensive vaccination efforts over the past decades, the world continues to make progress towards the 1988 World Health Assembly resolution to eradicate poliomyelitis (polio) (World Health Assembly 1988). Of the three wild poliovirus (WPV) serotypes, endemic circulation of serotype 1 WPV persists in only 3 countries (Afghanistan, Nigeria, and Pakistan), the world certified serotype 2 WPV eradication in 2015 (Global Polio Eradication Initiative 2015a), and serotype 3 WPV has not been detected since late 2012 (Kew et al. 2014). Current vaccination efforts focus most intensively on the interruption of WPV transmission in endemic areas, but all countries must continue sufficient polio vaccination to prevent outbreaks due to imported WPVs or outbreaks of circulating vaccine-derived poliovirus (cVDPV), which can emerge when live, attenuated oral poliovirus vaccine (OPV) viruses find enough susceptible individuals to establish circulation and revert to WPV-like properties (Burns et al. 2014). Besides vaccination, poliovirus surveillance plays a critical role in achieving eradication and will play a key role in the polio endgame, which included the global cessation of serotype 2-containing OPV in late April-early May of 2016 (Hampton et al. 2016).

Currently, most poliovirus surveillance globally involves two different approaches: acute flaccid paralysis (AFP) surveillance and environmental surveillance (ES). AFP surveillance involves the collection of stool samples from clinical AFP cases to determine the presence of poliovirus and provide virological confirmation of polio due to WPV or VDPV. Some high-income countries with good overall disease notification systems and low polio risks do not conduct active AFP surveillance, but instead rely on notifications of any clinical polio cases and/or notification of any poliovirus through enterovirus isolation in laboratories. However, the AFP surveillance system remains the gold standard for polio surveillance (Asghar et al. 2014), because it can investigate a high fraction of all individuals with symptoms that can indicate polio. These approaches make AFP surveillance a comprehensive global system capable of detecting poliovirus circulation in almost all populations (World Health Organization 2016). Thus, AFP surveillance plays a key role in documenting WPV elimination in many countries. Confirmation of WPV or VDPV in an AFP case can also guide outbreak response activities. However, the low paralysis-to-infection ratio (i.e., approximately 1:200 infections in fully susceptible individuals for serotype 1 WPV, with lower ratios for other serotypes (Nathanson and Kew 2010)) means that only a small fraction of first infections result in specific symptoms (i.e., paralysis). In addition, individuals immune to polio paralysis due to prior vaccination or poliovirus infection can still become infected and potentially participate in poliovirus transmission, particularly those vaccinated only with inactivated poliovirus vaccine (IPV) (Hird and Grassly 2012; Duintjer Tebbens et al. 2013). Given that AFP surveillance relies on the reporting of cases with clinical paralytic symptoms, it captures only a small fraction of all poliovirus infections occurring in a population. Thus, as successful polio eradication efforts decrease the number of polio AFP cases, the ability of AFP surveillance to detect any limited remaining WPV circulation decreases (Kalkowska et al. 2015b). For example, in the context of an IPV-vaccinated population that imported a serotype 1 WPV, we estimated that over 7,000 people in Israel became infected with serotype 1 WPV in the absence of a single paralytic case (Kalkowska

et al. 2015a). Based on the same event, a recent study provided a statistical approach to estimate the number of infected people, the ES sensitivity, and the level of confidence about die-out from ES data (Berchenko et al. 2017).

ES offers additional information by testing composite human fecal samples from the environment for the presence of poliovirus (i.e., WPV, VDPVs, and/or OPV-related viruses) (Asghar et al. 2014). Researchers succeeded in detecting poliovirus in sewage as early as the 1930s, which contributed to early efforts to understand poliovirus epidemiology (Paul et al. 1939; Paul and Trask 1941; Melnick 1947). Typically, ES involves the collection of water containing human sewage, concentration of matter in the collected sample likely to contain viruses into a small volume, and isolation of poliovirus through inoculation and observation for cytopathic effect in cell culture and/or direct poliovirus detection through polymerase chain reaction (PCR) techniques (Zaidi et al. 2016a). Poliovirus ES uses a variety of methods globally and no standards for specimen processing exist, which complicates characterization of ES system sensitivity and specificity. The World Health Organization (WHO) issued draft guidelines that describe a general approach to site selection and sample collection and recommended laboratory methods, which most of the recently implemented ES activities follow (Global Polio Eradication Initiative 2015b). Most of these ES activities involve monthly collection of grab samples and transport to a Global Polio Laboratory Network (GPLN) laboratory for processing using standardized virus isolation methods (World Health Organization 2004). No established global criteria for optimization or accreditation of poliovirus ES exist. While ES does not directly identify infected individuals, it can provide valuable information regarding the presence of virus in the catchment area. In populations characterized by high risk of transmission and/or low AFP surveillance rates, ES can potentially identify areas with clinically undetected circulation for further intervention and it may lead to more efficient use of resources by targeting specific locations for which stool samples will more likely yield potentially positive results for poliovirus. With accumulating experience in many countries, ES increasingly supplements AFP surveillance and plays a role in documenting WPV elimination, detecting re-introductions into polio-free areas, and detecting VDPVs. In addition, past ES activities documented the disappearance of OPV-related virus following the cessation of OPV vaccination in several countries (Mas Lago et al. 2003; Huang et al. 2005; Zurbriggen et al. 2008; Mueller et al. 2009; Wahjuhono et al. 2014; Nakamura et al. 2015). The expanding global ES system currently monitors the decline of serotype 2 OPV viruses following the globally coordinated switch from trivalent OPV (which contains attenuated strains of all 3 serotypes) to bivalent OPV (which contains attenuated strains of serotypes 1 and 3 only). This ES monitoring effort already detected persistent transmission of a serotype 2 VDPV at the time of the switch in Nigeria (Etsano et al. 2016), detected several instances of likely inadvertent use of trivalent OPV (Bahl et al. 2017), and monitored the prevalence of serotype 2 OPV-related viruses following the use of serotype 2 monovalent OPV to respond to serotype 2 cVDPV outbreaks after the switch.

Although prior reviews provide some relevant insights (Hovi 2006; Asghar et al. 2014; Snider et al. 2016; Zaidi et al. 2016b), no prior evaluation systematically categorized poliovirus ES systems or quantified the costs or effectiveness of ES in various settings. A few studies exist that quantify the cost-effectiveness of an ES system aimed at rapid detection (Kman and Bachmann 2012) or that provide analytical approaches to optimally

design an ES system to monitor chemical pollutants (Wang et al. 2015). Neither of these provide data that can inform global poliovirus ES cost and design choices or support estimates of the value of information provided by global poliovirus ES. This review aims to evaluate historical and current use of ES for polioviruses and to synthesize the available information on potential ES designs, costs, and effectiveness. We sought to synthesize the available evidence to support future evaluation of the potential feasibility of targeted implementation of ES in many settings globally and future valuation of the costs and benefits of the information provided by poliovirus ES. Despite the WHO guidelines for poliovirus ES (World Health Organization 2003), no existing analyses provide insights about the variability in approaches that ES systems worldwide use.

Methods

We searched PubMed and Web of Science for any publications in English including the terms “environmental surveillance” or “sewage,” and “polio,” “poliovirus,” or “poliomyelitis” from 1975 through 2016. We screened each study for relevance and identified studies that described a poliovirus ES system or reported the results of attempts to detect polioviruses in ES samples. We excluded reviews, outbreak reports that mention ES without any details about the activities, commentaries, summary papers, and any studies that discussed lab or extraction methods or presented modeling results without reporting sample collection results. We also reviewed the bibliographies of reviews and the relevant papers to identify potential studies missed by the database search, which we subsequently assessed for eligibility. We grouped studies that reported different results related to the same ES activity or event (e.g., outbreak) during the data extraction process. We assigned ES activities as either activities conducted by the GPLN or not based on the laboratory that performed the primary sample processing.

To fully explore available information on costs of an ES system and review any specific costing results we conducted several searches using terms related to any enteroviruses and costs of any ES system or design of sewage systems. We reviewed any studies that mentioned or evaluated costs regardless of meeting other inclusion criteria.

We recorded attributes of each study to characterize the location, timing and nature of the activity, sampling characteristics, laboratory methods, and any information about polio cases reported from the population. We use letter codes to categorize different attributes for the extracted data. For the nature of the activity, we distinguished between time-limited research studies (R, e.g., pilot studies to set up ES, ad-hoc monitoring of water quality), continuous surveillance or monitoring (S), or activities triggered by an event (T, e.g., by an outbreak, transmission event, isolating of a highly-diverged virus related to OPV, or a vaccine policy change). For sampling characteristics, we reported the sampling interval (i.e., daily (D), weekly (W), monthly (M), yearly (Y), or variable (V) intervals or at a single point in time (S)), the collection method (i.e., grab (G), composite grab (CG), or trap (T)), the collected volume, the type of ES sites sampled (i.e., wastewater treatment plants (WP), flowing surface waters (FW) such as open drains, canals, rivers, sea, and creeks, standing surface waters (SW), pumping stations (PS), other sewage access points (AP), or other sources (O)) and the whether the sample contained treated (T), partially treated (PT), or untreated (U)

sewage, and the estimated catchment area based on the information provided by the authors. For laboratory methods, we reported the concentration method (i.e., filter adsorption/elution or ultrafiltration methods (FAE), inorganic flocculation (IF) or organic flocculation (OF), the pellet method (PM); precipitation using polyethylene glycol (PP); the two-phase separation method (TP), and other methods (O)), the volume of concentrate inoculated into cell cultures, the effective volume of the environmental sample tested for the presence of poliovirus (i.e., volume initially processed for concentration multiplied by fraction of final concentrate volume inoculated into cell culture(s)), the virus isolation methods (i.e., cell line abbreviation, and/or polymerase chain reaction (PCR) based methods), and whether the study reports results from spiking studies that seed ES samples with poliovirus prior to concentration.

We recognize the importance of evaluating the effectiveness of an ES system, which requires information about the absence or presence of poliovirus from an alternative surveillance system and/or known presence of the virus due to intentional use (i.e., a supplemental immunization activity) or release (i.e., flushing studies). We recorded whether each study reported any information about known symptomatic polio cases in the catchment area and at the time of the ES activity, which could facilitate some comparison between ES and AFP.

Results

We identified 569 studies published between 1975 and 2016 that met the search criteria (Figure 1). Of 432 studies that did not meet the inclusion criteria, 146 studies examined the fate of polioviruses in different environmental media or different approaches to inactivate or purify them. Another 100 studies examined methods for ES surveillance without reporting any poliovirus ES results. We also excluded reviews, summaries, and reports (n=91), studies that did not perform any ES sample analysis (n=49) or did not report any polio ES results (n=40), and modeling studies (n=6) (Figure 1). Given the wide availability of OPV and its relative safety, many studies used OPV strains as a model for the survival of enteric viruses in environmental media or to assess concentration methods, cell lines, or viral transport through soil columns.

While we did not review excluded studies in detail, comprehensive reviews of environmental fate studies (Dowdle and Birmingham 1997; Fine and Carneiro 1998; Dowdle et al. 2006) suggests that polioviruses can survive for days to months in different environmental media, depending on temperature, organic content, moisture, and acidity of the medium. After adding 6 studies identified through the references, we identified 146 studies from 48 countries that met all inclusion criteria. Table 1 groups these into 101 separate activities listed chronologically and alphabetically (rows) and shows the data we extracted from each study.

As shown in Table 1, we found 13 studies that describe ES results from Israel and the Palestinian Authority, which began routine ES in 1989 and repeatedly detected evidence of WPV transmission (Manor et al. 2007; Manor et al. 2014; Shulman et al. 2014a; Shulman et al. 2014b; Shulman et al. 2015) and the existence of VDPV excretors (Shulman et al. 2000b; Shulman et al. 2006a; Shulman et al. 2006b; Shulman et al. 2011) in the absence of reported

clinical polio cases. We identified 12 ES studies conducted in Italy, and 10 in Japan, 9 in the US (including one by US authors that does not report the location (Sheladia et al. 1982), 8 in Canada, 7 in Nigeria, between 2 and 5 in 19 countries, and a single study in 23 countries each (Table 1). Figure 2 maps the number of ES activities (i.e., rows in Table 1) by country using different symbols to indicate GPLN or non-GPLN activities, with 10 countries for which we found both GPLN and non-GPLN activities (i.e., Brazil, China, Cyprus, Finland, Israel, Japan, Mexico, the Netherlands, Poland, and the Slovak Republic). We emphasize the map captures all ES activities published between 1975 and 2016, including many one-off studies, and thus does not reflect the current extent of poliovirus ES. Moreover, we recognize that some countries with extensive ES systems serve programmatic functions and may not publish the results of routine surveillance in peer-reviewed manuscripts. Consequently, the activities included in Figure 2 and Table 1 probably reflect some bias toward one-off ES studies rather than continuous ES activities with the most frequent and robust sampling.

Early studies typically reflected research activities, including many that report on microbial testing of different water sources or evaluate the ability to recover viruses before and after sewage treatment. Other studies involved opportunistic or one-off ES sampling in populations with suspected poliovirus transmission following the detection of an outbreak (i.e., after the area reported one or more clinical polio cases). Starting in the mid-2000s, larger scale ES systems and programs began, leading to larger and more systematic surveillance and studies, and some reviews. In recent years, studies increasingly reported ES results from developing countries as the GPEI began efforts to globally expand poliovirus ES.

Designs

The design of ES sampling significantly impacts the quality of ES. Table 1 reveals significant variability with respect to sampling interval, collection method, ES sample sources, laboratory methods, and catchment areas. The variability in sampling designs reflects different study aims, ranging from assessment of water quality or sewage treatment efficiency, which may involve infrequent and irregular sample collection from sources with minimal potential sewage contamination (e.g., bathing or drinking waters, treated wastewater), to studies specifically targeting poliovirus detection, which may involve frequent sample collection at regular intervals from sources with high fecal contamination. As noted in Table 1, 54 of the 101 ES activities (53%) sampled from wastewater treatment plants (coded as WP), 38 (38%) from flowing surface waters (FW) such as open drains, canals, rivers, sea, and creeks, 8 (8%) from standing surface waters (SW) such as ponds, lakes, and lagoons, 4 (4%) from pumping stations (PP), 7 (7%) from other sewage access points (AP), and the remaining 20 (20%) activities reported sampling from other sources (O, e.g., drinking or domestic use water, locally collected household or institutional sewage, central sewage collection points, mussels, soil or marine sediments, airplane lavatory waste tanks, aerosol samples above sewage effluent-irrigated fields, a sponge tissue found in flowing water, swimming pool water, or bore water). Studies that compared isolation rates before and after sewage treatment did not always find lower isolation rates after treatment, consistently reported poliovirus in treated sewage sources, and frequently highlighted that

sewage treatment does not effectively inactivate polioviruses (Sekla et al. 1980; Payment 1981; Sheladia et al. 1982; van Olphen et al. 1984; Towianska and Potajallo 1990; Ansari et al. 1992; Patti et al. 2003; Sano et al. 2004; Kim et al. 2006; Belguith et al. 2007).

The collection method depends on the available technology and the nature of the sampling sites. Trap (coded as T in Table 1) sampling can draw from flowing surface waters and typically results in a small sample representative of matter passing through over a period of time. Grab (G) samples typically collect larger volumes, including some very large volumes between 10–520 L reported by some studies that took river or treated water samples that they passed through filters as an initial concentration step (van Olphen et al. 1984; Kueh and Grohmann 1989; Akiyama et al. 1999; Sano et al. 2004). Like trap sampling, composite grab (CG) sampling aims to catch viruses passing through at different hours of the day while avoiding laboratory analysis of a large number of samples. However, a study that directly introduced poliovirus at a single point in time in the sewage system recovered the virus on each of four subsequent days at a downstream location, suggesting that a single grab sample during a longer period of time may suffice to detect a naturally occurring virus (Hovi et al. 2001). Moreover, excreted viruses typically enter the sewage system over a period of time rather than a single point in time, thus increasing the period of time during which the virus exists at downstream locations. The WHO guidelines recommend grab sampling because it allows better “quantitative estimation of the detection sensitivity of the system and long-term experience suggests that programmes exploiting concentrated grab samples detect polioviruses and non-polio enteroviruses more often than those using trap sampling” (World Health Organization 2003, p. 5). In the event of practical infeasibility of grab sampling, the guidelines permit trap sampling preferably using “macroporous glass in permeable bags” (World Health Organization 2003, p. 5) and not gauze pads. However, almost all studies in Table 1 that reported the use of trap sampling used gauze or cotton pads and none reported using macroporous glass or glass powder (although some studies used glass powder as part of their concentration process, e.g., Grabow et al. 1999). More recently, the GPEI piloted the use of a bag-mediated filtration system, which effectively increases the sample volume and thus detection sensitivity by allowing on-site filtration of the sample (Fagnant et al. 2014). Given that bag-mediated filtration requires more time on-site to collect a sample, the local circumstances will determine whether this disadvantage negates the advantage of higher volume collection.

Given the large variation in sampling facilities and locations, studies presented a wide range of catchment area populations. We found only 25 activities (25%) that provided sufficient information to estimate the catchment area population size (Table 1), with a median of 500,000, mean of 1.1 million, and range from 50 in an experimental study that locally monitored sewage from a crèche in conjunction with an OPV campaign in the Prague (Zdrazilek et al. 1977) to 7.3 million involving 8 cities across Italy (Cesari et al. 2010; Pellegrinelli et al. 2013; Battistone et al. 2014a; Battistone et al. 2014b). We found 10 activities (10%) that provided an upper bound (i.e., studies that reported the total number of people living in the area, without specifying the population coverage of the sewage system), 3 (3%) that provided a lower bound (i.e., studies that reported catchment populations from some but not all sites), 3 (3%) that provided only a population coverage estimate for the sewage system without specifying the number of people living in the covered area, and 1

(%) that specified only the child population in the catchment area. If we hypothetically assume 100 active ES sites currently exist that cover populations around the median, then this implies approximately 50 million of the 7.1 billion people in the world living in the catchment area of an actively monitored ES site (i.e., less than 1%). Table 1 includes 60 (60%) activities that did not provide any information about catchment population size. The limited information about catchment populations of sewage systems compromises our current understanding of ES and therefore estimation of required sampling frequencies and volumes for timely and sensitive detection.

To cover a large catchment population, the collection site must represent a converging sewage system and flowing water. In developed countries, collection often occurs from the inlet of untreated water of a sewage treatment plant, which represents a relatively large population covered by the catchment area. While some studies not specific to poliovirus ES explore the technical specifications of sewage systems (Astaraie-Imani et al. 2012; Mara 2013; Qu et al. 2013), to our knowledge no study systematically categorized the types of sewage system used by each country in the world. The less sophisticated sewage systems in developing countries identified in our review included a variety of surface water systems (e.g., rivers, trenches, canals), which may vary in catchment area representation and flow rates.

Surface water sources can change seasonally or even daily due to variable volume per catchment area and flow rates, and the contents can change hourly, which implies significant potential variability in these sites. Many studies report generic sewage collection sites for sampling without providing details about the nature of the site, which we cannot use to support the determinations of ES quality or best practices. Time and transportation choices also represent important determinants of ES system performance. None of the studies reported information about the time from sample collection to the time of reporting results or the distance from sample site to lab. However, one study detailed the lab processing times in the context of adjustments to the process to accommodate a large number of samples during an episode of WPV transmission in Israel (Manor et al. 2014). The authors reported reducing the turnaround time from arrival of the sample in the lab to sequencing results from 16–23 days during routine surveillance to 10–16 during the event by collecting lower volumes and changing the algorithm of testing from a serial to a more parallel approach (Manor et al. 2014).

Cost

We found only one paper when we added “cost” to the literature search terms, but this study did not estimate ES costs (Manor et al. 1999a), which left us with very limited information about costs. One review estimated ‘start-up’ costs of equipment of approximately US $50,000 for an existing poliovirus laboratory and a cost of approximately $33,000 for supplies used to support the processing of 100 specimens, based on experience of facilitating polio ES within GPLN (Hovi et al. 2012). The same review reported that “processing and analysis of 100 sewage samples requires trained staff at about the same level as for processing and analysis of stools from 200 AFP cases with two specimens from each case” (Hovi et al. 2012, p. 3) based on nearly a decade of experience with ES in Egypt. Another

review explored filter costs in the evaluation of virus concentration techniques for water samples (Cashdollar and Wymer 2013). Although studies suggest low costs associated with routine sample collection, training and staff salaries require additional support. A few studies discuss lab techniques or processing methods that may reduce costs and/or technician hands-on time for sample processing without explicit presentation of costs (Singh et al. 1983; Reynolds et al. 1995; Manor et al. 1999a; Tansuphasiri et al. 2000; Donaldson et al. 2002; Li et al. 2002; Fuhrman et al. 2005; Cashdollar and Dahling 2006; Karim et al. 2009). The technical skill, effort, time, and cost needed to develop and maintain the laboratory techniques for ES appear similar to existing techniques used to detect poliovirus in clinical samples (Vinjé et al. 2004). We did not find any other relevant costing information by exploring alternative searches.

Effectiveness

We break down the overall effectiveness of an ES system to detect poliovirus circulation in a population into three components (i.e., sample-specific detection probability, site-specific detection probability, and population-specific detection probability). We discuss the information we identified in the literature for each.

The first component reflects the sample-specific probability (i.e. the probability of detecting a virus given the presence of virus in the sample). This depends on the concentration of virus in the ES sample and the laboratory methods used. Concentration methods and virus detection methods vary, with 36 (36%) activities using filters to adsorb and elute the virus for concentration (coded as FAE in Table 1), 22 activities (22%) using the two-phase separation (TP) methods, 20 (20%) using polyethylene glycol precipitation (PP), 13 (13%) using organic or inorganic flocculation (OF or IF, respectively), and 2 (2%) using the pellet method (PM). Three (3%) activities did not report a concentration method and 20 (20%) reported various other (O) methods, e.g., extraction/separation through chloroform treatment, centrifugation, freezing, and thawing (Zdrazilek et al. 1977; Böttiger 1978; Böttiger et al. 1979; Kasova et al. 1981; Matyasova and Koza 1982; Zdrazilek et al. 1982; Matyasova and Koza 1985; Bottiger and Herrstrom 1992); protein precipitation, ultrasonic water bath, and centrifugation (Sellwood et al. 1981); oyster homogenate adsorption with two elution and concentration steps (Sheladia et al. 1982); beef extract elution only or with ultracentrifugation (Hamparian et al. 1985; Manor et al. 1999b; Shulman et al. 2000a); aluminum sulfate coagulation (Towianska and Potajallo 1990); virus adsorption-enumeration; hollow fiber concentration/dialysis (Murphy et al. 1983); cellulose method (Sano et al. 2004); precipitation using SiO_2 (Zurbriggen et al. 2008; Wieczorek et al. 2013; Kuryk et al. 2014; Wieczorek et al. 2015); silica adsorption (de Oliveira Pereira et al. 2016). Fifteen activities (15%) reported either using a combination of the above methods or using different methods for different samples. The WHO guidelines for the isolation of polioviruses in ES (World Health Organization 2003) recommend use of the two-phase method due to its relative simplicity and universal applicability. Consequently, a higher fraction of activities since 2003 report using the two-phase method (i.e., 50%). The guidelines further recommended validating the concentration method by spiking experiments with poliovirus and they characterize a good method as capable of detecting 10–20 tissue-culture infectious doses ($TCID_{50}$) per 500 ml sample (World Health

Organization 2003). Eighteen (18%) activities reported results of poliovirus spiking experiments, including three studies from the same activity that performed spiking experiments for the two-phase separation method and achieved the recommended target (Pellegrinelli et al. 2013; Battistone et al. 2014a; Battistone et al. 2014b). An experimental study in 1975 that sampled sewage at a crèche following an immunization campaign also reported a detection limit of approximately 20 $TCID_{50}$ using a trap sampling approach and a concentration method involving chloroform treatment, centrifugation, freezing, and thawing (Zdrazilek et al. 1977). Consistent with the GPLN manual (World Health Organization 2004), the ES guidelines recommend the use of human rhabdomyosarcoma (RD) and poliovirus receptor-expressing mouse (L20B) cell lines. However, given that the studies in Table 1 cover a broad time period and studies that may not specifically target polio, we found 18 additional cell lines used for virus isolation. In studies that compared multiple cell lines, L20B cells generally provided the highest sensitivity to poliovirus detection. Some studies reported discordant results between cell lines, which could reflect variable sensitivity and specificity and/or the variability of viruses present in the samples. Given that ES samples may contain fecal matter from a large number of different people, the sample-specific probability of detecting poliovirus involves inherent uncertainty about the content of individual samples. PCR-based methods may allow direct detection without the need to inoculate cells, which could save time and costs, but measuring the concordance between cell culture methods and direct detection methods for ES samples that may contain a mixture of viruses remains challenging. Some studies provide promising results with the use of (RT-)PCR at different stages of laboratory processing (Grabow et al. 1999; Tansuphasiri et al. 2000; Donaldson et al. 2002; Zhang et al. 2010; Troy et al. 2011; Troy et al. 2012), but not all studies consistently report greater sensitivity (Schlindwein et al. 2009; Wieczorek et al. 2015). The GPLN continues to consider direct detection methods for both AFP surveillance and ES, but in the absence of clear data demonstrating comparability of direct detection, virus isolation remains the gold standard for virus detection in the GPLN. The sample-specific sensitivity also depends on the collected volume and the proportion of the harvested sample (i.e., concentrate) ultimately tested for polioviruses. Table 1 reveals that many activities (45%) do not report the volume tested for poliovirus by inoculation into cell cultures. Among 56 activities that report the tested volume, volumes range from 0.1 and 25 ml, with 71% of reported volumes of between 0.1 and 1 ml per cell culture. For direct detection using PCR methods, the volumes typically remained much smaller and not directly comparable to cell cultures. Computing the effective volume tested requires information about both the volume after concentration and the number of cell cultures (i.e., flasks, tubes, monolayers, wells, plaques, depending on procedures) for each cell line. Given that few studies provide this information, we could only determine the effective volume tested for approximately 25% of ES activities. The effective volumes vary greatly from a few milliliters to almost 100 liters, reflecting variation in the source of the samples (typically much greater volumes for flowing water than sewage with high solid content), concentration methods, and cell culture procedures. However, for most of the more recent sewage sampling studies, Table 1 shows relatively consistent effective volumes of approximately 0.2–0.9 liters. .

The second component reflects the site specific sensitivity (i.e., the probability of capturing a poliovirus given the presence of virus in the catchment area). Detection of virus excreted by one or more people in the catchment areas depends on a sufficient amount of virus ending up in the sample at a level above the detection limit. The poliovirus concentration of an ES sample collected at an ES sample collection site depends on the number of people living in the catchment area of the site, the mass of stool produced per person, the amount of poliovirus excreted by any infected people in the catchment area, and the sewage flow rate at the sampling site. Furthermore, higher sampling frequencies and greater sample volumes will increase the probability of detecting the virus in a site at the cost of increasing the laboratory workload, which may impact workflow. Although sample volumes historically varied significantly, more recent studies typically collect 1 L per sample, with half of it processed for concentration and virus isolation, consistent with the guidelines (World Health Organization 2003; Global Polio Eradication Initiative 2015b). Our review suggests a very high ability of continued monitoring by ES sites to detect poliovirus transmission, although the studies in Table 1 likely reflect a publication bias towards positive findings. For example, studies in the current Czech Republic (Zdrazilek et al. 1977; Matyasova and Koza 1982; Zdrazilek et al. 1982), Canada (Sekla et al. 1980), Spain (Lucena et al. 1986), Japan (Akiyama et al. 1999; Matsuura et al. 2000), South Africa (Grabow et al. 1999), Cuba (Mas Lago et al. 2003), and Mexico (Troy et al. 2011; Troy et al. 2012; Esteves-Jaramillo et al. 2014) readily detected OPV-related virus in areas with known immunization campaigns. More impressively, several studies detected imported OPV-related viruses in populations that do not use OPV (van Olphen et al. 1984; Huang et al. 2005; Battistone et al. 2014b) or detected WPV in the absence of any reported WPV cases (Bottiger and Herrstrom 1992; Manor et al. 1999b; Shulman et al. 2014a). Other ES studies likely detected individual excretors of immunodeficiency-associated VDPV from within total ES system catchment areas of up to over 2 million people (Blomqvist et al. 2004; Cherkasova et al. 2005; Shulman et al. 2006a; Shulman et al. 2006b; Al-Hello et al. 2013; Klement et al. 2013; Cassemiro et al. 2016).

Despite this anecdotal evidence of the high potential site-specific sensitivity of ES, we found very little information to quantify the site-specific detection probability. Two controlled experiments deliberately introduced different amounts of OPV to the sewage and attempted to isolate the virus at a downstream location. The first, conducted in Sweden in 1970s, detected as little as 8×10^6 $TCID_{50}$ (the lowest amount tested) introduced 4.5 km from the sampled sewage treatment inlet with an unspecified catchment area population size (Böttiger 1978). The second introduced $3–6 \times 10^{10}$ cell culture-infective doses ($CCID_{50}$) into the Helsinki sewage system with a catchment area population of 740,000 people and successfully recovered the virus on all four consecutive sampling days in a location 20 km downstream from the virus introduction site (Hovi et al. 2001). Based on this experiment, the authors estimated a widely-cited minimum prevalence of 1 excreting individual per 10,000 people to detect the poliovirus in a single sewage sample from this system (Hovi et al. 2001). These findings reflect the specific setting of a developed country with a converging sewage network. We did not find any comparable controlled study from a developing country, so we do not know if this sensitivity would apply in developing country settings. However, studies from developed (Zdrazilek et al. 1977; Huang et al. 2005; Lodder

et al. 2012) and developing (Mas Lago et al. 2003; Troy et al. 2011; Esteves-Jaramillo et al. 2014) countries exist that simultaneously collected stool and ES samples in the context of known exposure to OPV. These studies found a close match between stool and ES isolation patterns and generally suggested that ES surveillance appears at least as sensitive as random stool collection in the detection of OPV-related viruses. One sewage sampling study conducted in the context of a limited OPV challenge study reported the ability to detect poliovirus if at least 100 people excrete poliovirus out of a population of tens of thousands of uninfected people (Lodder et al. 2012), which remains consistent with an earlier small-scale study that reported a minimal infection prevalence of 1% to detect poliovirus in local sewage (Zdrazilek et al. 1977). Another study estimated a 75% probability of detecting poliovirus using ES in a population shedding at a rate of 31% (Mas Lago et al. 2003).

The final component of effectiveness reflects population wide sensitivity (i.e., the probability of capturing a poliovirus in the catchment area given poliovirus circulation in the wider population). This probability will depend on the ratio of the catchment population to the total population and thus on the number and location of ES sites in a population. An analysis of the ES system in New Zealand suggested that it could represent 28% of its population (Huang et al. 2005) and studies from the Israeli ES system report 30–40% coverage of the entire country (Shulman et al. 2014a; Shulman et al. 2014b). With the current expansion of ES globally, the global population sensitivity of ES continues to increase. However, it remains very challenging to estimate the actual ratio because of the challenges associated with estimating catchment area population sizes (Table 1), and the possibility of overlapping catchment areas from different ES sites.

Ideally, we could measure the overall effectiveness of detecting a virus using an ES system (i.e., the compound probability that combines each of the three component probabilities) by comparing detection rates to the true prevalence of virus in a population. Unfortunately, given the inability to directly observe transmission due to low paralysis-to-infection ratios, we can only estimate but not observe true prevalence of infection. Estimation of the effectiveness of ES for detecting poliovirus may also involve comparison of ES to a different surveillance system, such as AFP surveillance. However, given that historically, most countries established ES only in the context of relatively inactive transmission, many studies did not occur in the context of reported polio cases. We identified 14 (14%) ES activities that reported at least one polio case at the time of sampling and from the same population, although uncertainty remains about whether these all actually excreted into the monitored ES system. Two studies reported several transmission pathways in which ES detected WPV well before AFP surveillance identified a polio case (van der Avoort et al. 1995; Alam et al. 2016). The lack of deliberate development of large-scale ES by the GPEI until relatively recently most likely limited the design and conduct of systematic studies to compare the information simultaneously collected for ES and AFP.

Aside from the probability of detection, the timing of detection represents another important component of ES system effectiveness. The time until an ES system detects a poliovirus depends on the concentration of virus, the number of sampling sites and concentration labs sending specimens to a poliovirus lab, the distances and travel times between each of these locations, the frequency of sampling, and the time required to concentrate and process a

sample in the lab. Even in the absence of prevalence data, efforts to measure effectiveness of ES would benefit from comparison to the time it takes to detect a virus using ES relative to that for AFP surveillance in the area (i.e., if ES does not provide a faster result than AFP surveillance, then its value as a tool for detection could be limited). However, in most studies that reported ES results and polio cases, the ES began after the detection of a polio case by AFP surveillance.

Given the numerous uncertainties related to measuring real world effectiveness of ES, modeling may provide some useful insights. Our review identified one study that modeled the effects of changing the quality of laboratory techniques and loss percentages (i.e., due to households not connected to sewage system, disposable diapers, etc.) and produced detection probability curves based on these inputs (Ranta et al. 2001). The study concluded that ES would perform better than AFP surveillance except in the context of high case-to-infection ratios or for the worst scenarios for ES sampling quality.

Discussion

This review finds that the published literature provides very limited information about the costs of poliovirus ES and some limited and variable information about the design and effectiveness of ES systems. No information exists in the literature to support rigorous costs estimation and no systematic categorizations allow determination of the optimal ES system designs or assessments of the population effectiveness of ES relative to AFP surveillance. Further studies will need to quantify the complete costs of ES. Additional controlled studies of ES effectiveness in developing countries represent an important research opportunity to support more rigorous estimation of the benefits of ES.

This review underscores the very low fraction of the global population potentially covered by ES at any given point of time in the past (less than 1%). Although ES may represent an opportunity to see circulating polioviruses early, its value will depend on the area covered and any promises about the benefits of ES should take this into consideration.

The successful design and implementation of ES in any area requires consideration of key attributes of the geographical area, catchment population (size and location), type and characteristics of sewage and treatment system, and laboratory resources available. The variability in these factors limits both the categorization of ES and the ability to determine, optimize, and standardize the quality of its information. Assuming ES aims to detect poliovirus more quickly than AFP surveillance, measuring the quality of ES and ensuring rapid sample transport and processing emerge as critical hurdles. For GPLN laboratories, increasing the laboratory workload may interfere with the primary duties of these laboratories to support AFP surveillance, suggesting the need to expand laboratory capacity to accommodate the ES workload (Figure 2).

The limitations of our findings underscore the need for further efforts to characterize the benefits and potential expanded role of poliovirus ES and to obtain better information about costs and effectiveness of existing systems. We (RDT, MAP, KMT) conducted a prior survey of the GPLN to characterize the costs and value of the GPLN (de Gourville et al. 2006),

which we hoped would motivate further reporting of costs and effectiveness information in poliovirus surveillance studies. This review demonstrates the need for further efforts to encourage the inclusion of economic information in future studies, and we anticipate the need to conduct a repeat survey to collect data from GPLN laboratories. We recommend that future poliovirus ES studies should explicitly state key characteristics of the ES system (sampling site locations and characteristics, size of catchment areas for sampling sites, nature of the sewage system, sampling frequency, laboratory and sampling methods) and provide data about costs and effectiveness of the systems, even if reported in supplementary materials.

The insights in this analysis remain limited by the available literature, which does not include unpublished reports. For example, the review did not include one study submitted after the literature search that examined the benefits of an ongoing ES system to detect polio sooner than AFP surveillance in 2009–2012 in 6 provinces of Pakistan (Cowger et al. 2017). The study reported on the results of monthly samples from sewage systems examined using WHO recommended methods and found that ES detected poliovirus circulation before AFP surveillance for 60% of polio cases, on average nearly 4 months earlier (Cowger et al. 2017).

ES may play an important role in reaching and maintaining the goal of global polio eradication. In combination with AFP surveillance, ES may help to identify final reservoirs of WPV and high-risk areas for VDPVs. As the GPEI plans for ES expansion, addressing the issues of prioritizing populations, site selection, and sampling schemes will become increasingly important for efficient use of surveillance resources. Therefore, estimating the effectiveness and costs of a global ES system remains critical to supporting optimal global investments. Unfortunately, significant data gaps exist in this area. Optimization will likely involve some form of targeting high-risk locations, which may change over time as the sources of risk evolve (Duintjer Tebbens et al. 2015). This review highlights some insights as well as the significant limitations in the published literature that can help inform programmatic choices.

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List of abbreviations

AFP	acute flaccid paralysis
cVDPV	circulating VDPV
ES	environmental surveillance
GPEI	Global Polio Eradication Initiative
IPV	inactivated poliovirus vaccine
OPV	oral poliovirus vaccine

PV	poliovirus
VDPV	vaccine-derived poliovirus
WPV	wild poliovirus

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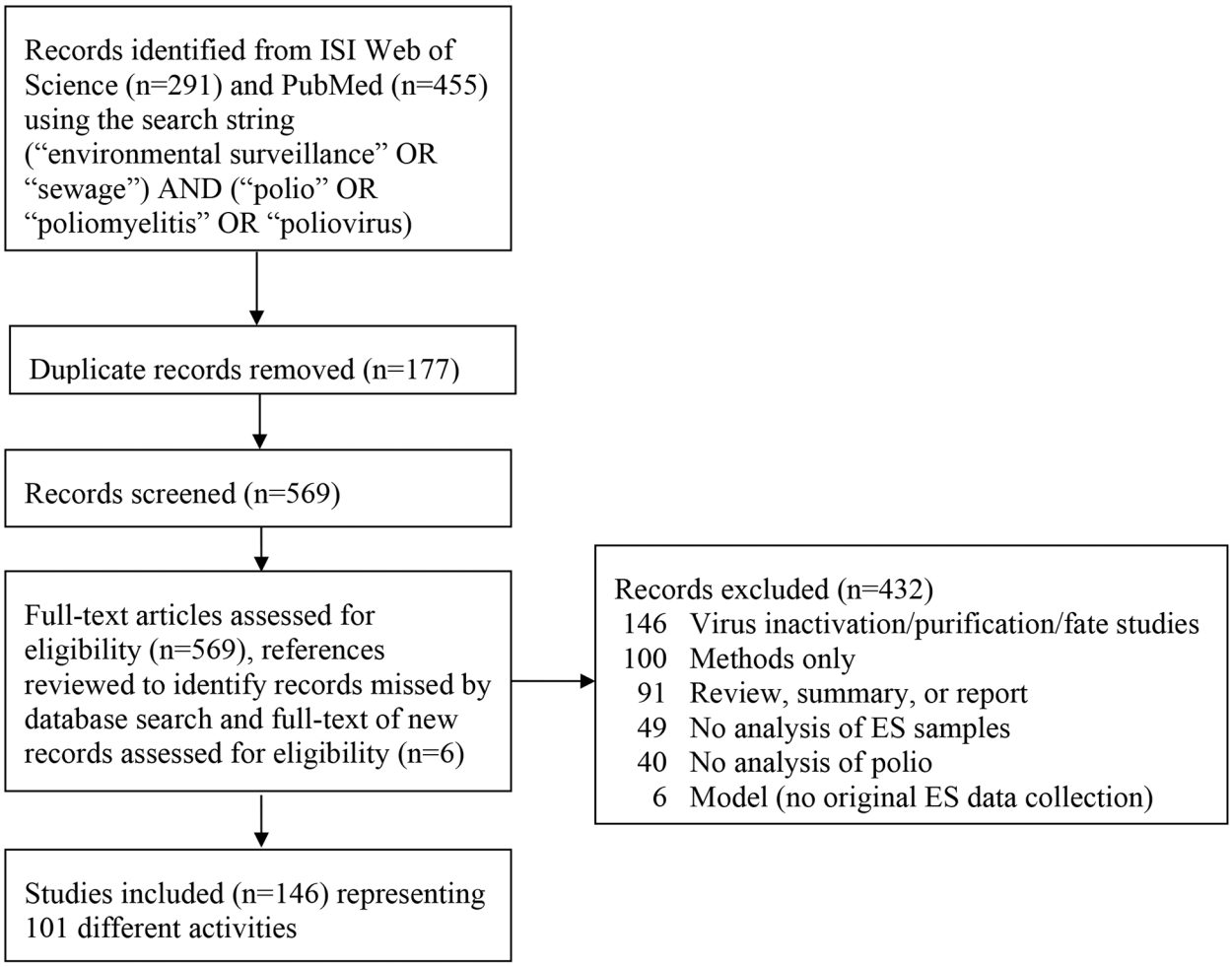

Figure 1.
Process used to identify relevant environmental surveillance studies.

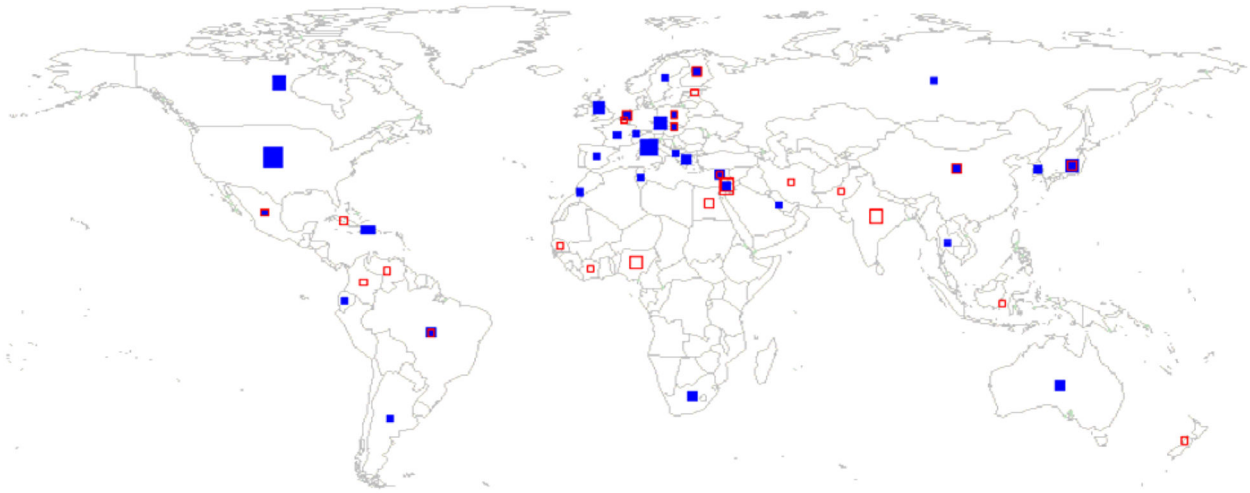

Figure 2:
Global distribution of ES activities published between 1975 and 2016 (i.e., rows in Table 1) with the area of each square proportional to the number of activities (smallest square shows a single activity and largest square (in the US) shows 9 activities. Open (red) squares indicate activities for which a GPLN laboratory performed the primary processing and filled (blue) squares indicate acitivities for which a non-GPLN laboratory performed the primary processing.

Table 1.

n the review and characteristics of the study location, timing, sampling, and laboratory methods (145 studies reflecting 101 ES

try y region, ilable)	Sample collection time period (YY or M/YY as available)	ES design						Laboratory methods					Reported polio cases[j]
		Nature of ES activity[a]	Sampling interval(s)[b]	Collection method[c]	Collected volume (by collection method or site if applicable), in liters	Sites (containing treated/ untreated sewage)[d]	Estimated catchment area population[e]	Concentration method[f]	Volume tested per culture, in ml	Effective volume tested, in liters[g]	Spiking? *h*	Virus isolation method(s)[i]	
da wa)	NR	R	W;V	CG;T	5(CG);0.06(T)	WP(PT,NS)	360,000	FAE	0.5	NR	Y	Vero	NR
noslovakia ue)	3/75–5/75	R	R	T	0.06–0.08	O(U)	50	O	5	NR	N	Vero	NR
en	67–90	S;R;T	NR	G;T	NR	WP(U)	NR	O	5	NR	N	CGM;GM;HA;HL	1
(Long d, NY)	6/76–5/77	R	M	G	95;380	WP(T)	NR	FAE+IF	0.5	NR	N	BGM	NR
salem Tel Aviv)	NR	R	NR	NR	2–3	AP(U,T)	≤ 700,000	OF	0.3	NR	N	BGM	NR
da (Laval)	8/77–8/78	R	W	NR	2–4	PS(U)	≤ 30,000	FAE+OF	5	2–4	N	Vero	0
da itoba)	5/76–4/79	R	V	T;G	0.05ml(T);2–20(G)	WP(U,PT,T);FW(T);O(T)	NR	FAE	0.2	NR	N	HA;HL;RM;Vero	NR
(Central)	77–78	R	NR	G	2 (sewage)	WP(T);O(T)	NR	FAE+OF	2 (sewage)	NR	N	BGM	NR

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noslovakia h mia)	3/79–2/80	R	M	NR	NR	S(U);O(U)	NR	O	0.2	NR	N	HL;MK	NR
Reading)	9/74–9/77	R	NR	T;G	0.015(T);5(G)	WP(U,T);FW(T)	NR	FAE;O	NR	NR	N	BGM;HA;Hep-2;HF;RM;Vero	NR
eported	10/78–3/80	R	M/2	G	1	SW(U,T)	45,000	O	NR	NR	Y	BGM	NR
noslovakia ban and 2 areas with ple sites)	9/68–12/82	S	~M	T	NR	WP(U);O(U)	NR	O	NR	NR	N	HL;Vero	NR
da treal area)	8/78–4/81	R	W;V	NR	5;100;1000	PS(U);O(U,T)	NR	FAE+OF	1;0.1;0.01	NR;6;60	N	Vero	NR
alia olk d)	9/80–11/81	T	V	G	5(water);25(effluent)	O(U,PT)	NR	O	0.2	4;20	N	Hep-2;HF;MK;Vero	NR
erlands (8 ons)	11/78–4/80	R	~0.4 M	NR	0.25–520	FW(U,PT,T);SW(U,PT,T)	NR	FAE+OF	NR	NR	Y	BGM	NR
e (Nancy)	1/83–2/83	R	NR	NR	NR	WP(U)	NR	FAE+OF	NR	NR	N	BGM	NR
(Ohio)	78–80	R	bW	NR	1.5–2	WP(U)	480,000	O	5	>0.57	N	BGM;CGM;HeLa;RD	NR
ce tsini)	1/83–3/84	R	W;bW	NR	10	WP(U)	NR	FAE	10	NR	N	BGM	NR
nd inki, 17 onal ons)	12/84–5/86	T	M-W	NR	NR	WP(U,T);AP(U)	NR	TP	0.5	NR	N	GM;Vero	9

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		Nature of ES activity[a]	Sampling interval(s)[b]	Collection method[c]	Collected volume (by collection method or site if applicable), in liters	Sites (containing treated/ untreated sewage)[d]	Estimated catchment area population[e]	Concentration method[f]	Volume tested per culture, in ml	Effective volume tested, in liters[g]	Spiking? *h*	Virus isolation method(s)[i]	
elona	79–80	S	NR	NR	NR	FW(NS)	≤ 300,000	NR	NR	NR	N	NR	NR
ain (Main l and rraq)	85–86	R	NR	NR	NR	WP(U,PT)	200,000	NR	NR	NR	N	NR	NR
(Toyoma cture)	6/83–5/85	R	M;bM	T;G	0.8(T);2(G)	FW(NS);WP(NS)	≤ 300,000	FAE	NR	NR	Y	Vero	NR
alia ney area)	2/89–4/89	R	0.6M	G	1(sewage);80 – 120(seawater)	FW(T)	NR	PP+FAE	0.5	0.04;3.2–4.8	N	BGM;Hep-2;HF;M	NR
d (Lake owieckie)	4/86–89	R	M	NR	5	SW(T)	NR	O	NR	NR	N	GM	NR
(Florida Michigan)	NR	R	NR	G	5–20	WP(U,PT,T);SW(T);O(T)	NR	IF	NA	NA	N	RT-PCR	NR
zuela e caibo)	NR	R#	M	G	10	SW(U)	≤1,000,000	FAE	0.4	NR	N	RM	NR
Northern d coast)	4/86–5/88	R	V	G	10	SW(U);FW(U)	NR	FAE+IF	NR	NR	Y	BGM	NR
(Safo	1/88–12/89	R	W	G	1	FW(NS)	180,000	PP	NR	NR	Y	HeLa;Hep-2RD;Vero	NR
mbia agena)	91	T#	V	G;T	2(G);NR(T)	FW(U)	5,300 (CO)	FAE	NR	0.2;NR	N	RD;Hep-2;PCR	0[r]
(Pisa)	5/92–4/93	R	M/2	NR	0.1 (sewage)	WP(U);O(U)	300,000	O	NR	NR	N	BGM	NR
	NR	R	NR	NR	10	FW(NS)	NR	FAE	NR	NR	N	BGM	NR

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erlands try-wide)	8/92–3/93	T[#]	V	G	0.5(sewage);180(river water)	AP(NS);FW(NS)	NR	FAE+OF	NR	NR	N	Hep-2;BGM	71
(Pesaro	1/94–9/95	R	V	G	10	FW(NS)	NR	FAE	20	10	Y	BGM;Hep-2	NR
ropolitan nes)	95–96	R	NR	NR	4–10	S(NS);O(U)	NR	PP	0.1	0.007–0.032	Y	RD;RT-PCR	NR
ria (Oyo)gun	1/96–3/97	R[#]	NR	G	1	FW(U);O(U)	NR	TP	NR	NR	N	Hep-2	NR
(Oahu,	NR	R	M;3M	NR	NR	FW(T)	NR	FAE	NR	0.6194	Y	BGM	NR
(Miyago cture)	9/96–12/98	R[#]	NR	NR	20	FW(T)	NR	FAE	1	16	N	BGM;HeLa;Hep-2;RD	NR
nia na)	1996	T	NR	NR	10	FW(NS)	NR	FAE+PP	NR	NR	N	BGM	9
Africa oria, nesburg)	1/96–10/97	R	≤ W	G	10	FW(U)	> 15,000	FAE	1	NR	N	BGM;L20B;PLC;PVK	NR
and the tinian ority try-wide)	2/89–2/95	S[#]	M/V	CG;T	1.5–2(CG);NR(T)	WP(U)	NR	O	4.5	0.20.27;NR	N	BGM;Hep-2	0
and gkok)	2/98–6/98	R	NR	G	1(sewage);5(water)	FW(U);AP(T,U)	NR	FAE	0.2	0.3–2	Y	Hep-2;RM;RT-PCR	NR
(Toyama cture)	9/93–9/95	R	M/2	G;T	1(G);0.7–08(t)	WP(NS);FW(T)	60%	FAE	NR	NR	N	MK;RD;Vero	0

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(Pesaro	1/98–9/02	R	M/4;Y/2	G	10	FW(PT,T)	NR	FAE	NR	NR	N	BGM	NR
and the tinian ority try-wide)	10/88	T#	NR	CG;T	1.5–2(CG);NR(T)	S(NS)	NR	O	NR	NR	N	BGM	20
(Central n)	98–05	T;S#	≤ M	CG;T	1.5–2(CG);	WP(U);AP(U)	2,400,000	PP	NR	NR	N	L20B	0
nd inki)	NR	R#	D	CG	1	WP(U)	740,000	TP;PP	0.5	0.2	N	L20B;RD	NR
(Florida)	8/97	R	S	G	2–20	FW(NS);O(NS)	NR	FAE	NA	NA	Y	RT-PCR	NR
mbai)	1/01–12/01	R#	W	G	2	PS(U);FW(U)	≤1,800,000	PP	0.1–0.5	0.18	Y	L20B	1
(3 cts in Pradesh)	12/02–1/03	T#	S	G	2	FW(NS)	NR	PP	0.1–0.5	0.18	N	L20B	6
t (8 nces)	1/01–12/02	S#	M;M/2	G	1	WP(NS);FW(NS);O(NS)	2,000,000	TP	0.5;1.2	~ 0.50.9	N	L20B;RD	8
(Havana)	97–98	R#	W	CG	1.0 (total)	S(NS)	NR	PP	0.2	NR	N	L20B;PCR	NR

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(7 ns)	94–1/98	R	NR	NR	1(raw sewage)	WP(U);FW(T);O(T)	NR	FAE	NR	NR	N	BGM	0
vaukee,	8/94–12/02	S	M	CG	1	WP(U)	600,000	OF	~ 1.5	NR	N	BGM;Caco-2;RD;RM;HL	NR
ia nn)	02 onwards	T;S#	M	T;G	NR(T);1(G)	WP(U)	500,000	TP	NR	NR	N	L20B;RD	0
dor (6 st-risk nces)	5/01–6/01	R	V	NR	1	FW(NS)	NR	PP	NR	NR	N	L20B;RD	0
(Sendai, , Osaka)	2/01–5/02	R	M;S;V	NR	0.4–80	WP(U,T)	> 36,000	PP;O	0.3	NR	N	BGM;Hep-2;RD	NR
and nican blic d-wide)	11/00–12/00	T	V	NR	1	S(NS);FW(NS);O(NS)	NR	PP	1	NR	N	L20B;RD	21
k blic try-wide)	1/03–12/04	S;T#	M/2;W	G	1	WP(U);AP(U)	≤5,400,000[aa]	TP	0.5	0.3	N	Hep-2;L20B;RD	0
a tov)	6/02	T	S	T	NR	O(U)	NR	O	NR	NR	N	RD	1*
Zealand kland, lton, ngton	11/01–4/03	T*#	W	NR	NR	WP(NS)	1,100,000	NR	NR	NR	N	L20B;TD	0
us sia, ssol)	4/03–12/03	R#	NR	G	1	WP(NS)	150,000	O	NR	NR	N	BGM;RT-PCR	NR

try y region, ilable)	Sample collection time period (YY or M/YY as available)	ES design						Laboratory methods					Reported polio cases[j]
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Africa oria, nnesburg)	01–03	R	NR	G	1	WP(NS);FW(U)	500,000[aa]	PP	1	NR	N	BGM;Hep-2;PLC	0
Korea	8/01–7/03	R	NR	NR	NR	FW(NS);WP(NS);O(T)	NR	O	1	NR	N	BGM;ICC-PCR(BGM)	NR
erlands erdam)	00–01	T[#]	NR	G	1	AP(NS)	NR	FAE+OF	NR	NR	N	Hep-2	0
ia astir)	10/00–9/01	R	NR	NR	NR	WP(U,PT,T)	NR	PP	NR	NR	N	Hep-2;L20B;RD	NR
ce ens)	5/96–10/97	R	NR	NR	1	WP(NS)	NR	TP	~ 5	NR	N	Hep-2	0
and the tinian ority district)	02	S[#]	M	CG	1.5	WP(U)	NR	PP	NR	NR	Y	BGM;L20B	NR
know)	04–10/06	S[#]	W	G	1–2	WP(NS);O(NS)	2,200,000	PP	0.2	NR	N	L20B;RD	0
(Cameron Hidalgo ies, TX)	NR	R	S	NR	1	WP(U)	NR	PP	1	NR	N	L20B;RD	NR
Sistan uchestan)	3/04–2/05	S[#]	NR	G	1	WP(U);FW(U);O(U)	> 170,000	PM;TP	0.2	NR	N	Hep-2;L20B;RD	0
us try-wide)	10/05–6/07	R	M	G	1	WP(NS)	NR	O	NR	NR	N	BGM, RD	0
erland ch)	1/04–10/06	T*	W	G	NR	WP(NS)	400,000	O	1.5	0.5	Y	L20B;RD	NR
ntina loba nce)	5/05–4/06	T*	W;W/2	G	1.5	WP(NS);AP(U)	1,000,000	PP	1	0.3	N	L20B;RD	0

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(8 cities)	1/05–12/10	S	< M	CG;G	1	WP(T,U)	7,300,000	TP	0.2–1	0.1–0.5	Y	L20B,RD	0
nd pere)	12/08–3/10	S#	NR	NR	NR	NR	NR	TP	NR	NR	N	L20B;RD	NR
l anopolis)	6/07–5/08	R	M	NR	0.005(sludge); 2(water)	WP(U)	NR	PP;FAE	NA	NA	N	ICC -PCR(Vero);PCR	NR
a (Jinan,)	2/08–12/13	S#	M;M/2	G;T	1	WP(U)	2,300,000	FAE	0.2	~0.4	Y	Hep-2;L20B;RD	3*
a (Xian)	NR	R#	NR	NR	1	WP(U);FW(NS);SW(NS)	NR	FAE	NA	NA	Y	RT-PCR	NR
us (5)	4/07–12/08	R	M	G	10	O(T)	NR	FAE	NA	NA	Y	RT-PCR	
co aba)	8/09–7/11	R	M,V	NR	1	FW(U)	< 180,000	FAE	NA	NA	Y	RT-PCR	NR
cco blanca	4/08–7/08	R	M/2	NR	0.5	WP(T,U);FW(U)	NR	PP	0.1	NA	N	ICCPCR(HEp-2;RD)	NR
t (19 nces)	00–10	S#	M;M/2	G	1	S(NS)	NR	TP	0.5	~0.9	N	L20B;RD	0
erlands	NR	R	V	G	10	PS(NS)	37,000	FAE	20	10	N	L20B	NR
(Migrants ng in a)	6/11	R	NR	NR	NR	O(U)	NR	TP	NR	NR	N	BGM;L20B	NR

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try y region, ilable)	Sample collection time period (YY or M/YY as available)	ES design						Laboratory methods					Reported polio cases[j]
		Nature of ES activity[a]	Sampling interval(s)[b]	Collection method[c]	Collected volume (by collection method or site if applicable), in liters	Sites (containing treated/ untreated sewage)[d]	Estimated catchment area population[e]	Concentration method[f]	Volume tested per culture, in ml	Effective volume tested, in liters[g]	Spiking? [h]	Virus isolation method(s)[i]	
know)	1/09–12/10	R[#]	M	NR	NR	WP(NS)	75%	TP	0.. 3	NR	N	Hep-2;Hep-2;L20;RD;RT-PCR	0
d (14 s)	1/11–12/11	S[#]	NR	NR	NR	S(U)	NR	O	0.2	NR	N	Caco-2;L20B;RD;RT-PCR	NR
ria (Kano okoto)	7/12–8/12	R[#]	NR	G	NR	S(NS)	NR	TP	0.2	NR	N	L20B;MCF-7;RD;RM	NR
ria ps)	6/10–9/12	R[#]	V	NR	1	FW(U);WP(U)	NR	PM	0.2	NR	N	L20B;RD	0
tan (4 nces, abad)	11–15	S[#]	M	G	0.5	FW(U);PS(U)	NR	TP	0.5	~0.1	N	L20B;RD	463*
co (4)	5/10–10/10	R[#]	V	G	1	WP(U);FW(U)	1,400,000	TP	NR	NR	N	L20B;RD	0
(country-)	1/13–4/14	T[#]	V	CG	1	WP(U)	2,800,000–3,200,000	PP	2	~0.15–0.30	N	L20B;RT-PCR	0
dIvoire djan area)	9/08–1/09	R[#]	W;W/2	NR	1	S(NS)	590,000	TP	0.5	NR	N	BGM;Hep- 2;L20B;RD;RT-PCR	NR

ntry ly region, ilable)	Sample collection time period (YY or M/YY as available)	ES design						Laboratory methods					Reported polio cases[j]
		Nature of ES activity[a]	Sampling interval(s)[b]	Collection method[c]	Collected volume (by collection method or site if applicable), in liters	Sites (containing treated/ untreated sewage)[d]	Estimated catchment area population[e]	Concentration method[f]	Volume tested per culture, in ml	Effective volume tested, in liters[g]	Spiking? *h*	Virus isolation method(s)[i]	
gal ar)	4/07–5/13	S#	NR	G	1	WP(PT);FW(U)	≤3,000,000	TP	0.2	NR	N	Hep-2;L20B;RD	18
esia n Y karta)	7/04–12/12	T*#	W;W/2	G	1	WP(U)	NR	TP	NR	NR	N	L20B;RD	NR
an try-wide)	7/12–12/13	S	M	NR	1	WP(U)	NR	TP	0.2	NR	N	A549;L20B;RD	NR
(Kyushu)	4/10–1/15	T*#	M	NR	1	WP(U)	190,000	FAE	0.1	NR	N	HA;Hep-2;	NR
												RD;RM;Vero	
l (Sao State)	1/14	S	2W;W/2	NR	NR	FW(NS)	NR	OF	NR	NR	N	Hep-2;L20B;RD	0
l (Rio de ro)	12/11–12/12	S#	W	CG	1	WP(NS)	<1,500,000	O	0.5	0.15	N	RD, L20B	0
um nsart)	9/14–11/14	T#	V	NR	1 (sewage)	S(NS);WP(U,T);O(NS)	NR	FAE	0.1	0.1–0.3	N	L20B;RT-PCR	NR
ia (11)	11–16	T#	M;2W;W	G	1.75	FW(U);WP(U)	NR	TP	NR	NR	N	L20B;RD	53*
k blic	12–14	R	Y	NR	1	SW(NS)	NR	TP	NR	NR	N	Hep-2;Vero;RT-PCR	NR

rch study, including pilot or ad-hoc monitoring of water quality; S = Continuous surveillance or monitoring; T = Triggered by an event (i.e., outbreak or evidence of transmission, nded poliovirus release into the environment; asterisk indicates triggered by a vaccination policy change, e.g., switch from OPV to IPV). A hash sign (#) asterisk indicates that a med the primary sample processing.

ear if twice a month or once per two months); bW=Bi-weekly (unclear if twice a week or once per two weeks); D=Day; M=Month; NR=Not reported; S=Single point in time (per nience; W=Week; Y=Year

mple; G=Grab sample; NR=Not reported ; T=Trap sample

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[d] AP=Sewage access points (not at wastewater treatment plant); FW=Flowing surface water (e.g., open drains, canals, rivers, sea, creeks); NR=Type of site not reported; NS=Not specified whether sewage source was treated; O=Other (incl. drinking or domestic use water, locally collected household or institutional sewage, central sewage collection points, mussels, soil, airplane lavatory waste tanks, aerosol samples above sewage effluent-irrigated fields, sponge tissue, swimming pool water, bore water); PS=Pumping station; PT=Partially treated or pre-treated; S=Sewage from unspecified location; SW=Standing surface water (incl. lake, pond, lagoon); T=Treated; U=Untreated (raw, incl. activated sludge); WP=Wastewater treatment plant;

[e] Total from all ES sites, with all numbers rounded to two significant digits; Lower bound (“>“) means that reported population does not include all sites; Upper bound (“≤”) means that the study reports only the total number of people living in the area, without specifying the population coverage of the sewage system; Percentage (%) means that the study only reports the population coverage of the sewage system, without specifying the total number of people living in the areas; CO=Number reflects children only; NR=Not reported

[f] FAE=Filter adsorption/elution or ultrafiltration; IF=Inorganic flocculation; PM=Pellet method; PP=Precipitation using polyethylene glycol; O=Other (including extraction/separation through chloroform treatment, centrifugation, freezing, and thawing; ultrasonic waterbath and centrifugation, protein precipitation, oyster homogenate adsorption with two elution and concentration steps, beef extract elution only, aluminum sulfate coagulation, virus adsorption-enumeration, hollow fiber concentration/dialysis, precipitation using SiO2, silica adsorption); OF=Organic flocculation; TP=Two-phase separation

[g] Defined as the fraction of the concentrated sample tested for presence of poliovirus, multiplied by the volume processed for concentration. For example, if a study collected 1 liter of raw sewage, concentrated half of it to a final volume of 30 ml and inoculated 1 ml of the concentrated into 6 flasks with different cell lines, then the effective volume equals (6/30)×0.5=0.1 liter. NR indicates that the study report(s) included insufficient information to determine the effective volume. NA indicates that we could not determine a meaningfully comparable effective volume in the context of studies that performed direct detection using PCR techniques.

[h] Affirmative (Y) only if the study reports the results of seeding poliovirus to the sample prior to and recovery after, the concentration step (otherwise N). See text for studies that report experiments attempting to isolate poliovirus introduced directly into the sewage system.

[i] A549=Human lung adenocarcinoma epithelial; BGM=Buffalo green monkey kidney; Caco-2=Human adenocarcinoma of the colon; CGM=Cynomolgus green monkey kidney; GM=Green monkey (not further specified) kidney; ICC-PCR=Integrated cell culture PCR, with cell line indicated in parentheses; HA=Human amnion; HeLa=Human cervical cancer; Hep-2= Human epithelial type 2; HF=Human embryonic fibroblast; HFS=Human foreskin; HL=Human embryonic lung; L20B= Mouse cell line expressing the human poliovirus receptor; M=Monkey kidney (not further specified); MCF-7=Human mammary gland adenocarcinoma; NR=Not reported; PCR = Direct detection using polymerase chain reaction; PLC=Human primary liver carcinoma; PVK=Primary vervet kidney; RD=Human rhabdomyosarcoma; RM=Rhesus monkey kidney; RT-PCR=Direct detection using real time reverse transcriptase PCR; Vero=Vero (may include primary BC-S-1 and continuous African green monkey kidney cell lines)

[j] Numbers reflect paralytic polio cases reported during the time of the study from the catchment area, which typically reflect upper bounds because we cannot determine if all reported cases resided in the catchment area during the time of the study (asterisk means that number include at least one polio case associated with a vaccine-derived poliovirus); NR=not reported

[k] Sattar and Westwood 1977 described the isolation of a serotype 1 wild poliovirus, Sattar and Westwood 1976 compared the virus recovery rate for different eluents for the same ES site, Sattar and Westwood 1978 investigates differences in isolation rates at different sewage treatment stages

[l] Böttiger 1978 described the ES activity and ability to detect OPV viruses deliberately introduced in the sewage system, Böttiger et al. 1979 applied the approach to investigate transmission around a reported clinical case, Bottiger and Herrstrom 1992 reviewed the entire ES experience from 1967–1990

[m] Includes a discussion of the feasibility of poliovirus as an indicator of water quality (Payment et al. 1979b) and a report of polioviruses and other enteroviruses detected in sewage samples (Payment et al. 1979a)

[n] Matyasova and Koza 1982 and Matyasova and Koza 1985 provided genetic characterization of a subset of isolates reported in Zdrazilek et al. 1982

[o] Payment 1981 reported poliovirus isolation from both raw and treated drinking water at a water treatment plant, Payment et al. 1983 reported poliovirus isolation from two sewage treatment plants in the same area

[p] Poyry et al. 1988 details the ES findings, Hovi et al. 1986 described the outbreak, including environmental detections, Huovilainen et al. 1988 provided antigenic characterizations of clinical and environmental isolates

[q] Carducci et al. 1995 reported the ES study of sewage and activated sludge aerosols, Muscillo et al. 1997b further characterized a serotype 3 poliovirus aerosol isolate

[r] However, 3 polio cases reported in adjacent areas (Tambini et al. 1993)

[s] Muscillo et al. 1997a reported the ES study of seawaters, Muscillo et al. 1999 further characterized a serotype 3 OPV-related isolate

[t] Kittigul et al. 2000 described results of ES study, Tansuphasiri et al. 2000 evaluated the laboratory methods used

[u] Matsuura et al. 2000 described the ES study and a genetic analysis of isolated Sabin-like viruses, Yoshida et al. 2000 and Yoshida et al. 2002 performed mutant analysis on serotype 3 and 2 viruses, respectively, to determine potential neurovirulence, Horie et al. 2002a and Horie et al. 2002b characterized isolated serotype 1 viruses with respect to antigenicity, neurovirulence, and temperature sensitivity

[v] Pianetti et al. 2000 reported results from regular seawater sampling in 1998, Pianetti et al. 2004 added a grain size analysis for two sampling rounds in 2000

[w] Shulman et al. 2000b provided a first report of a highly-diverged serotype 2 vaccine-derived poliovirus detection in 1998, Shulman et al. 2006a and Shulman et al. 2006bprovided further characterization of the isolates, described a genetically unrelated highly-diverged serotype 2 vaccine-derived poliovirus detection in 2004–2005, reported on a upstream search for the infected individual(s), examined the sequence of other isolated OPV-related viruses, and studied the immunity of the population to these viruses, Shulman et al. 2011 studied the *in vitro* effect of an antiviral on the isolated strains.

[x] El Bassioni et al. 2003 described the ES activities and results for 2001, Hovi et al. 2005 updated with results through 2002 and methodological findings, Blomqvist et al. 2010 genetically characterized serotype 2 and 3 OPV recombinants detected by the ES system in 2005 and 2007

[y] Blomqvist et al. 2004 reported a serotype 3 vaccine-derived poliovirus detected by ES in 2002, Al-Hello et al. 2013 updated this with information from subsequent detections in 2008–10 indicating both serotype 2 and 3 vaccine-derived polioviruses linked to the 2002 isolation

[z] Klement et al. 2013 described the ES experience from 2001–2011, Černáková et al. 2005reported the detection of a serotype 2 vaccine-derived poliovirus in 2004, Hovi et al. 2013a and Hovi et al. 2013b further characterized the isolated vaccine-derived polioviruses and attempts to identify the source

[aa] Upstream search for excreting individual(s) detected vaccine-derived poliovirus in sites with wastewater originating from 530 inhabitants

[ab] Paximadi et al. 2008 retrospectively characterized recombination sites of ES isolates reported by and Papaventsis et al. 2005 and Dedepsidis et al. 2007

[ac] Pavlov et al. 2005 provided sequencing results for the isolated reported in Pavlov 2006

[ad] Figure reflects accumulated reported population sizes from each wastewater treatment plant, although “Together these plants treat domestic and industrial sewage for approximately 3 500 000 people” (Pavlov et al. 2005, p. 3311)

[ae] Battistone et al. 2014b summarized the detection of poliovirus and other viruses from 2005–2008, Cesari et al. 2010 and Pellegrinelli et al. 2013 reported results from selected sites, Battistone et al. 2014a reported results from 3 sites (including 1 not reported in related studies) from both sewage inlets and effluents

[af] Wang et al. 2014 reviewed the ES findings from 2008–2012, Tao et al. 2010 reported the detection of a type 2/3 recombinant OPV-related virus, Tao et al. 2013 reported the detection of a serotype 2 vaccine-derived poliovirus through the same ES system, Tao et al. 2016 summarized the results for 2013 and explored factors affecting sensitivity of the concentration method

[ag] Troy et al. 2011 reported the results of both stool and sewage sampling following an OPV campaign in 2009, Troy et al. 2012 reported a longer episode of OPV-related virus detection in sewage following an OPV campaign in 2010 and 2011

[ah] Wieczorek et al. 2013 described the detection of polioviruses in sewage, Kuryk et al. 2014 provided genetic characterization of the poliovirus isolates

[ai] Faleye and Adeniji 2015 examined cell line differences and further characterizes isolates collected by the two ES activities described in Adeniji and Faleye 2014a and Adeniji and Faleye 2014b

[aj] Alam et al. 2014 described the ES activities and findings from 2011–2013, Alam et al. 2016 described the findings from 2013–2015 in the context of a genetic analysis of all clinical and ES isolated in Pakistan during this period

[ak] Shulman et al. 2014b described the silent serotype 1 wild poliovirus transmission event detected by ES, Manor et al. 2014described the modified laboratory processing algorithm to accommodate the large volume of samples, Shulman et al. 2014a described the molecular epidemiology of the event, Shulman et al. 2015 described antigenic and phenotypic properties of the isolates

[al] Johnson Muluh et al. 2016 reviewed the experience with and public health response to ES detection in 11 states, Etsano et al. 2016 described the detection of and response to an orphan serotype 2 serotype circulating vaccine-derived poliovirus, Hamisu et al. 2016 described the ES strategy to improve surveillance quality in conflict-affected areas