Virology: Current research

Odoom et al., Virol Curr Res 2017, 1:1

Research Article Open Access

Environmental Surveillance for Poliovirus in Greater Accra and Eastern Regions of Ghana-2016

Odoom JK[1*], Obodai E[1], Diamenu S[2], Ahove V[2], Addo J[3], Banahene B[1], Taylor J[1], Asante-Ntim NA[1], Attiku KO[1], Dogbe M[4] and Kaluwa O[2]

[1]*Department of Virology, Noguchi Memorial Institute for Medical Research, Legon, Ghana*

[2]*World Health Organization, Country Office, Accra, Ghana*

[3]*Disease Surveillance Department, GHS, Accra, Ghana*

[4]*Regional Environment Health Services Department, Accra, Ghana*

[*]**Corresponding author:** John Kofi Odoom, Department of Virology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra, Ghana, Tel: 233 245603970; Fax: 233 302502182; E-mail: jodoom@noguchi.ug.edu.gh

Received date: February 10, 2017; **Accepted date:** March 14, 2017; **Published date:** March 20, 2017

Abstract

Introduction: As the Global Polio Eradication Initiative (GPEI) moves towards achieving the goal of polio eradication, environmental surveillance which investigates polio virus in sewage or wastewater, plays a major role in providing evidence for certification of polio-free status. Many countries in the AFRO region have added environmental surveillance to acute flaccid paralysis (AFP) surveillance but Ghana is yet to adopt and conduct environmental surveillance as a routine national activity. Here, we piloted environmental surveillance from September to December 2016 to determine silent circulation of poliovirus in some districts within Greater Accra and Eastern regions.

Objective: The study aimed to test national capacity and preparedness to undertake environmental surveillance as part of routine national surveillance activity for poliovirus.

Methods: Sewage samples collected from six selected sites within two regions were processed by grab method according to World Health Organization (WHO) guidelines on environmental surveillance. The samples were concentrated and inoculated on RD and L20B cell lines for virus isolation. Virus isolates were subjected to real-time PCR and non-polio enterovirus (NPEVs) typing.

Results: From a total of 36 sewage samples collected from the six sites, 7 (19.4%) were positive on cell lines. Of these positives, 6 (85.7%) showed growth on RD cells only and 1(14.2%) showed growth on both RD and L20B. The isolates were identified as one Coxsackie virus A 16 belonging to human enterovirus (HEV) A group, two Echovirus 6 and one each of Echovirus 13 and Echovirus 29 of HEV B, and Coxsackie virus 24 of HEV C.

Conclusion: No wild poliovirus transmission was detected in the study samples however, 7 NPEVs were isolated from 50% of the sites. Although the study in Ghana was not geographically representative, results were consistent with the enteroviruses distribution in AFP cases in the country. Our findings also indicated that environmental surveillance is feasible in Ghana and can be implemented as routine national surveillance program based on available national capacity and experience.

Keywords: Environment; Surveillance; Sewage; AFP; Regional reference polio laboratory; Ghana

Introduction

The GPEI, initiated by the World Health Organization (WHO) in 1988, is one of the most ambitious public health efforts ever attempted by man. The world is closer than ever to achieve the final goal of eradicating this devastating disease that has paralyzed millions of children worldwide. The number of cases of paralytic poliomyelitis is low, there have been no polio cases due to wild type 2 poliovirus or wild type 3 PV since October 1999 and November 2012, respectively, and only wild-type viruses of serotype 1 are now circulating in some parts of the world. Furthermore, India, where large epidemics were frequent until recently, has not seen any polio case since February 2011 and was declared polio-free on March 27, 2014 [1]. Nigeria last saw wild polio in July 2014 but recently isolated two wild polioviruses in 2016 [2]. However, several concerns and challenges remain in that, two countries, Afghanistan and Pakistan, have still not been able to interrupt circulation of indigenous wild poliovirus. There were also large outbreaks due to wild type 1 poliovirus in Kenya, Ethiopia, Iraq, and Somalia in 2014 [3,4]. Furthermore, wild type 1 poliovirus, has been found in environmental samples in Israel from February to December 2013 indicating widespread virus circulation in a population with >95% vaccine coverage with IPV and the absence of known paralytic cases [5]. This observation has highlighted the fact that poliovirus can be readily transmitted from person to person even in highly immune populations. This has also emphasized the critical role that environmental surveillance can play in detecting circulation of poliovirus in the absence of clinical cases which would prompt public health interventions to interrupt virus circulation and prevent further spread of the virus and paralytic disease. The World Health

Organization and Health Authorities of many countries have realized the importance of environmental surveillance, particularly at this stage of the GPEI, and are now working very hard to implement routine environmental surveillance procedures [6].

Several countries including Nigeria, Kenya, South Africa, Senegal, Cameroon and Madagascar perform environmental surveillance to detect circulation of wild-type polioviruses to supplement or sometimes substitute regular AFP surveillance in Africa [7,8]. Furthermore, Ethiopia, Zambia, Uganda, Cote d'Ivoire, Central African Republic and Algeria were proposed by WHO AFRO to commence environmental surveillance in 2016. Environmental surveillance is also a potential tool for the detection and monitoring of circulating vaccine-derived polioviruses. Between 1990 and 2013 several silent outbreaks due to wild-type or vaccine derived polio vaccine (VDPV) strains have been detected in Palestine [9], Egypt, [10] and Finland [11] and South Africa [12] by environmental surveillance without detection from active AFP surveillance. Irrespective of the presence or absence of clinical symptoms, poliovirus replication is considered to continue in the para-intestinal submucosal lymphatic tissue from several weeks to a few months. The virus is excreted into the faeces and shed into the environment. Apart from AFP surveillance which links poliovirus to specific individuals and permits a focused investigation of that individual and the immediate community at risk, environmental surveillance, in the form of supplementary surveillance is also becoming more crucial.

Ghana is located in areas where the GPEI relies on strategies which include high routine immunization coverage and mass vaccination campaigns with oral polio vaccine (OPV), along with continuous monitoring of wild-type virus circulation by using the laboratory-based non-polio acute flaccid paralysis (AFP) surveillance. Ghana saw the last case of WPV in November 2008 [13] at the time when the sanitation situation in some parts of the country was bad and the whole country was under reduced immunisation activity. The two most important surveillance indicators like non-polio AFP rate of 2 per 100,000 population of children under 15 years and stool adequacy of 80% were also not met by Greater Accra from 2010 to 2015 and within some districts in Eastern region though no wild poliovirus was detected. The study aimed to test national capacity and preparedness to undertake environmental surveillance as part of routine national surveillance activity for poliovirus.

Method

Selection of sampling sites

Two regions, Greater Accra and Eastern, were selected purposely for the study. Greater Accra is the capital of Ghana with a population of about 4,382,001 (estimated from the 2010 population census). Four criteria were used by a technical assessment team to select the sewer lines in relation to the region: (1) whether the region is classified as high risk for poliovirus transmission, based on existing data (ie, population density, high-risk population, sanitation, living conditions, routine immunization, and SIA coverage); (2) the presence of sewer lines that receive waste from a considerable proportion of the population in the catchment area, with a minimum amount of waste coming from other areas; (3) the absence of industrial waste in the proposed site; and (4) poor AFP performance indicators. Based on these, the Technical Assessment Team determined that 4 high-risk districts in Greater Accra region were suitable for environmental surveillance. These were; (1) Shiabu in Ablekuma sub-metro that has a population of about 213,914, (2) Agbogbloshie in Okaikoi sub-metro with a population of about 228,271, (3) Osu in the Osu-Klottey sub-metro with a population of 121,723 and (4) Legon in the Ayawaso West sub-metro with a population of 70,667. The Technical Assessment Team also obtained information on overall sanitation for Greater Accra and sewer maps of the selected sites from the Local Government Regional Environment Health Services Department and Sanitary Offices. During this feasibility assessment, it was realized that there were only few organized/functioning sewage wastewater disposal/treatment systems in Greater Accra; however, there were some non-functional sewage-pumping stations which were under renovation by the Accra Sewerage Improvement Project (ASIP) of the Accra Metropolitan Assembly (AMA).

In the Eastern region however, only one site was identified at Akosombo in the Asuogyaman district with proper open sewage channel and treatment system that serves a population of about 17,000 inhabitants. This channel receives sewage *via* 3 smaller and medium-sized sewage channels that drain residential areas. The other identified site was a drainage that runs through many areas and receives waste water from large areas within Koforidua (Figure 1).

Figure 1. Sites for sample collection in Ghana: left) inlet of sewer lines to sewage processing plant at Legon and right) drainage site at Koforidua in the Eastern region.

Training workshop

A 2-day workshop was organized at Noguchi Memorial Institute for Medical Research (NMIMR) Legon, on September, 2016 which brought stakeholders from the Polio Lab, Regional Environmental Health Services Department, Disease Surveillance Department, Sanitary Officers and World Health Organisation to sensitize stakeholder on the new phase of poliovirus detection. The objective was to access the feasibility of performing environmental surveillance in Ghana. Participants included those responsible for sample collection and sample processing in the polio lab. The workshop was done in four parts; showing the purpose and importance of environmental survey in the end game of polio eradication, collection of samples, processing of quality data and analyses of results. The workshop took the form of presentation, discussions, practical and hands-on training.

Sample collection procedure

From September 2016 (epidemiological week 38) to December 2016 (week 48), raw sewage samples were collected between 7:10 am and 10:40 am within a temperature range of 28°C to 31.5°C using the grab

method according to WHO approved guidelines for environmental surveillance of poliovirus. Briefly, one litre of raw sewage was collected at each of the sampling sites, either at one point in time, or, preferably, at different predetermined points to form a composite sample. Sewage or raw wastewater was collected every two weeks by lowering the bucket as closely as is safely possible into the midstream of the sewage or wastewater. One or more of the marked sample containers with the collected material were filled, using a funnel if necessary. The container(s) were sealed tightly, the outer surface wiped with disinfectant, and the container placed into a zipper bag. The wrapped sample container(s) were placed into the transport container, with frozen ice packs. During the collection, the time and temperature of collection were recorded. The relevant zipper-bag enclosed information form as well as supporting packing material as appropriate were added and the container tightly sealed. The packed specimen container was kept at 4°C and transported to the polio laboratory at NMIMR usually within 24 h.

Sample Processing in the Laboratory

Processing of environmental samples in the laboratory involves steps that may generate aerosols and as such, special precautions (processing in different rooms from AFP) were taken to avoid cross-contamination of samples. In addition separate personnel and space were assigned for the work with environmental samples and AFP samples. Processing of fluid grab samples in the laboratory was as described by Lewis and Metcalf [14]. Briefly, 500 ml of the sewage sample was pelleted at 1500 g for 20 minutes. The supernatant was taken into Erlenmeyer flask and the pH adjusted with 1N NaOH or HCl to 7-7.5. The pellet from the centrifugation was taken and stored at +4°C. To the supernatant, 39.5 ml of 22% dextran, 287 ml of 29% PEG 6000, and 35 ml of 5N NaCl was added and mix thoroughly; and kept in constant agitation for 1 h at 4°C using horizontal magnetic stirrer. Sterile conical 1 litre separation funnel was prepared per sample and attached to a stand. Grease was spread on the sliding glass surface of the valves without obstructing the holes. (Teflon valves do not require smearing). Water tightness was checked with the small volume of sterile water. The mixture from the magnetic stirrer was poured into the funnels and left overnight at 4°C. After that the valve was opened with caution to collect the entire lower layer and the interphase slowly drop-wise, into a sterile tube (usually 5-10 ml of 0.5 litre of original sample). The stored pellet was then re-suspended into the harvest. Twenty percent volume of chloroform was added to the extract and shaking vigorously for 1 min followed by centrifugation at 1500 g for 20 minutes as with faecal suspensions. The upper water phase was collected in a sterile tube and antibiotics (e.g. penicillin G and streptomycin to final concentration of 100 IU/ml and 100 mg/ml, respectively) added. One milliliter of aliquot of the extracted concentrate was taken and frozen at -20°C for potential future use.

Virus isolation, identification and characterization

Sewage samples collected from six sites within two regions in Ghana were sent to the WHO accredited Regional Reference Polio laboratory for processing. Two cell lines, RD and L20B cell lines propagated in Modified Eagle's Medium (MEM) with 10% foetal calf serum (FCS) were used for virus isolation according to WHO standard procedures. The RD cell line is of human rhabdomyosarcoma origin and allows the growth of several human enteroviruses while the L20B cells are a recombinant murine cell line genetically modified to express human poliovirus receptor and highly selective for poliovirus [15]. Both cell lines were obtained from the WHO Regional Reference Polio Laboratory in Ghana.

Briefly, suspension of sewage concentrate was prepared by adding chloroform to the concentrate in a centrifuge tube. Glass beads were added and shaken for 20 minutes. It was then span at 1500 g for 20 minutes and the supernatant transferred into two fresh tubes. One tube was stored at -20°C as a back-up. 200µl of the sewage suspension was inoculated on one 25 cm^2 flask of confluent monolayer of RD and five 25 cm^2 flasks of confluent monolayer of L20B cell lines containing serum free MEM and incubated at 36°C. Virus growth was monitored daily by microscopy for cytopathic effect (CPE) for 5 days. Samples that did not show growth after 5 days were frozen and thawed two times and re-inoculated and observed for another 5 days. Virus isolates was harvested after attaining 80-85% CPE and the infected cells frozen at -20°C. Virus isolate showing cytopathic effect on L20B cells were subjected to intratypic differentiation using real-time reverse transcriptase polymerase chain reaction (rRT-PCR PCR) kit version 4.0 (provided by Centers of Disease Control and Prevention, Atlanta, USA) for the intratypic differentiation (ITD) of polioviruses to determine the serotype and the origin while virus isolates that showed growth on RD cells only were subjected to non-polio typing using WHO antisera provided by the National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands [16].

Data Analysis

The MS excel data base was imported into SPSS version 16 and analysed. Univariable analysis of case investigation and administrative data by person, place and time were expressed as frequency distributions, percentages and charts.

Ethical Issues

Ethical approval for the study was waived by the Institutional Review Board of the Noguchi Memorial Institute for Medical Research, ID number 1577. Approval was also given by the Surveillance Department of the Ghana Health Service.

Results

A total of 36 sewage samples from five sewage sites and one drainage waste water in six districts within two regions were collected from September to December 2016 for analysis. In all cases, the characteristics of sewage samples, time and temperature, were documented (Table 1). Samples were collected from 7:10 to 10:40 within a temperature range of 28.6°C to 31°C. From the 36 samples collected from the six sites, 7 (19.4%) were positive on cell lines. Of these, 6 (85.7%) showed growth on RD cells only and 1 (14.3) show growth on both RD and L20B. Two isolates each were harvested on epidemiological weeks 38 and 48 while three isolates were harvested on week 42. From Table 1, all seven positive isolates were collected in the morning between 8:37 and 9:90 within the temperatures of 29.5°C and 30.6°C.

Of the 7 isolates, three were identified in Agbogbloshie in the Ablekuma sub-metro, two isolates in Koforidua district within the Eastern region and one from Osu in the Osu Klotey sub-metro in Greater Accra. Figure 2 shows the epidemiological weeks within which all the isolates were harvested. No isolate was obtained during epidemiological weeks 40, 44 and 46.

Collection site	Time/temp	Epidemiological week					
		wk 38	wk 40	wk 42	wk 44	wk 46	wk 48
Shiabu	Time	7:50	7:55	7:40	7:10	7:20	7:15
	Temp (°C)	30.9	31.0	28.7	29.5	29.7	29.0
Agbog bloshie	Time	8:40	8:41	8:43	8:41	8:35	8:37
	Temp (°C)	30.6	29.2	29.7	28.7	29.2	29.5
Osu	Time	9:20	9:10	9:27	9:38	9:37	9:15
	Temp (°C)	30.4	30.7	30.4	30.7	30.1	29.6
Legon	Time	10:30	10:40	10:17	10:20	10:36	10:16
	Temp (°C)	31.5	30.4	30.5	31.0	30.4	29.8
Koforidua	Time	9:30	9:30	9:10	9:40	9:35	9:27
	Temp (°C)	29.9	28.5	28.7	30.6	29.4	29.8
Akosombo	Time	9:05	9:00	9:10	9:10	9:25	9:15
	Temp (°C)	28.6	30.3	30.5	30.2	30.8	30.2

Table 1: Time and temperature readings recorded during sample collection.

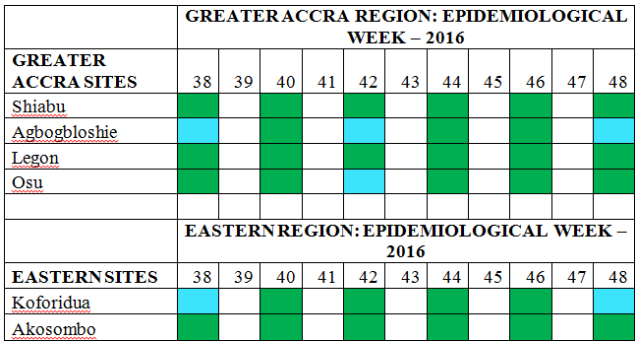

Figure 2: Distribution of enterovirus within Greater Accra and Eastern region in the epidemiological weeks. Colors indicates Green- Negative, White- Non Scheduled and Blue- NPEV.

The detection of NPEVs in three of the six sites indicates that the procedure was sensitive and could detect wild poliovirus if it was present. No reasons however, was found for the other three sites where no isolation was made but the sensitivity of these sites could be tested to determine their inclusion or exclusion from the sampling site.

When the L20B isolate was subjected to intratypic differentiation using the rRT-PCR, no poliovirus was detected. All the isolates were then subjected to non-polio enterovirus typing using the antisera from RIVM. Of the 7 isolates, 2 were Echo 6 (28.5%) and Echo 13 (28.5%), and one Echo 29 (14.3%) all belonging to the human enterovirus B group. One each of Cox A16 (from L20B)(14.3%) and Cox A24 (14.3%) belonging to human enterovirus A and C respectively were also identified (Figure 3).

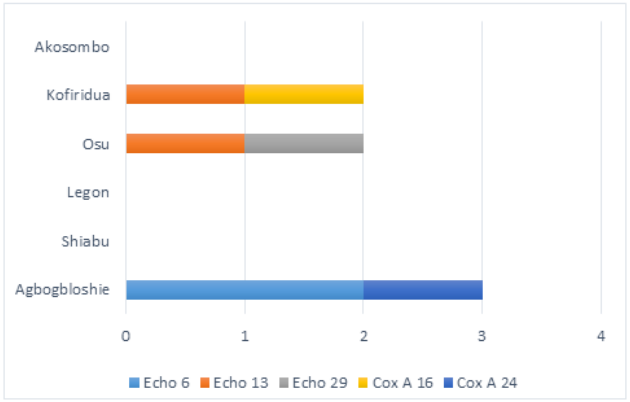

Figure 3: Distribution of non-polio enteroviruses isolated from the environmental sites.

Discussion

In the context of poliomyelitis eradication, a joint sentinel surveillance and laboratory network for surveillance of enteroviruses was piloted in two regions of Ghana to consider the possibility of integrating environmental surveillance into routine poliovirus surveillance. A variety of enteroviruses belonging to 3 of the four human enterovirus groups were detected in the collected samples with isolation rate of 19.4%. The rate found in our study was consistent with the study in Fars province in Iran (22.9%) [17] but lower compared to similar studies conducted in Dakar (79.7%) [18], Abidjan (53.5%) [19] and Iran (65.2%) [20]. On site to site bases, sewage samples concentration from Agbogbloshie and Koforidua revealed 50% and 33% isolation rate of enteroviruses which confirms efficiency of

method. Monitoring circulating enteroviruses is important due to individual serotypes which may have different temporal patterns of circulation and changes in predominant serotypes that can be accompanied by outbreaks. The data are important for interpreting tendencies of enteroviral diseases, such as aseptic meningitis, by associating them with circulating serotypes and can be helpful for studying the association of enteroviruses with clinical manifestations [17]. Five different serotypes of enteroviruses (E6, E13, E29, Cox A16 and Cox A24) were found in the collected samples with E6 and E 13 being the most. Echovirus 6 from environmental surveillance has been found circulating in regions in China where there is no specialized HEV surveillance system [21]. The identified echoviruses are known to cause mild gastro intestinal and respiratory illness, myocarditis and encephalitis mainly in children less than 5 years old [22,23]. E6 and Cox A16 are major pathogens associated with foot and mouth disease as well as febrile illnesses in neonates [24,25]. Cox-A24 is a major etiological agents involved in acute hemorrhagic conjunctivitis (AHC) outbreaks worldwide, the first AHC outbreak was described in 1969 in Ghana, West Africa, and was called Apollo disease [26]. Since this first reports, the infection has been described in numerous other countries, (China, India, Egypt, Cuba, Singapore, Taiwan, Japan, Pakistan, Thailand and United States). Massive outbreaks of AHC periodically occur in tropical areas and involve large populations [27-31].

Unlike this study, several countries have detected Sabin strains, VDPVs and WPVs in environmental surveillance even where no AFP cases were reported. Here, no such viruses were found even though Sabin strains are frequently found in the normal AFP surveillance. The two cell lines, RD and L20B, whose sensitivity are regularly tested, would have isolated these viruses if they were present. Our findings have also demonstrated the feasibility of conducting environmental surveillance in Ghana and the results could guide programmatic public health responses to polioviruses by the national AFP surveillance. The study was not without limitations: although the ability to isolate poliovirus from waste water in an endemic area has been demonstrated a number of times, much less is known about the sensitivity of these procedures for detecting low levels of poliovirus circulation. As a result we do not know if our inability to detect enterovirus in the three sites was due to inappropriateness of sites or no circulation of enterovirus. Furthermore, some lessons including how to collect good sewage, how to process and analyze samples in the lab were learnt. Moreover, technical team for site sampling scouting and capacity building have been established and is now available in the country.

Conclusion

This study is the first one depicting enteroviruses detection in sewage specimens in Ghana. Even though no WPV transmission was detected in the environmental surveillance samples from the 2 regions, non-polio enteroviruses were isolated. The absence of Sabin and wild poliovirus in the environmental samples from the 6 high-risk districts has tremendously increased the confidence of the polio eradication program regarding the absence of transmission of indigenous or imported WPV in Ghana. The pilot project has also built national capacity for environmental surveillance and prepared the program for roll out as a routine activity.

Author contributions

JKO was involved in the design, sample processing and analysis and manuscript writing, EO participated in data analysis and manuscript writing, SD and VA were involved in the design, training, editing and manuscript writing, JA and MD participated in data analysis and editing of manuscript, BB and TJ were involved in sample processing and writing of manuscript, NAAN was involved in sample analysis and manuscript writing, KOA and OK participated in data analysis and interpretation. All authors read and approved the final manuscript.

Acknowledgement

We thank WHO Country Office for initiating and supporting the pilot study. We also thank WHO AFRO for providing equipment in the course of the study. We are most grateful to the Disease Surveillance Department and the Regional Environmental Health Services Department of the Ministry of Local Government for providing immense support in scouting for sites and providing sewer maps for the work. Ms Deborah Pratt and Linda Boatemaa for providing technical assistance for sewage and wastewater concentration in the laboratory.

Competing Interest

The authors declare that they have no competing interest.

References

1. Siddique AR, Singh P, Trivedi G (2016) Role of Social Mobilization (Network) in Polio Eradication in India. Indian Pediatr 53: S50-S56.
2. Zoakah AI, Adebiyi OA, Ashikeni M, Makinde OA (2016) Wild poliovirus in Nigeria. The Lancet Infec Dis 16: 1224.
3. Sheikh MA, Makokha F, Hussein MA, Mohamed G, Mach O, et al. (2014) Combined Use of Inactivated and Oral Poliovirus Vaccines in Refugee Camps and Surrounding Communities - Kenya, December 2013. Wkly Epidemiol Rec 63: 237-241.
4. Global Polio Eradication Initiative (2014) Data and monitoring. Geneva, Switzerland: World Health Organization.
5. Moran-Gilad J, Kaliner E, Gdalevich M, Grotto I (2016) Public health response to the silent reintroduction of wild poliovirus to Israel. J Clin Microbiol and Infec 22: S140-S145.
6. World Health Assembly. Plan of Action for Global Polio Eradication. Resolution WHA 41: 28.
7. Weldegebriel G, Adeneji A, Gasasira1 A, Okello D, Elemuwa C, et al. (2015) Environmental Surveillance for Poliovirus in Polio High Risk States of Nigeria, 2011- 2012 . Sci J Pub Hlth 3: 655-663.
8. Muluh TJ, Hamisu AW, Craig K, Mkanda P, Andrew E, et al. (2016) Contribution of Environmental Surveillance Toward Interruption of Poliovirus Transmission in Nigeria, 2012–2015. J Infect Dis 213: S131-S135.
9. Manor Y, Handsher R, Halmut T, Neuman M, Bobrov A, et al. (1999) Detection of poliovirus circulation by environmental surveillance in the absence of clinical cases in Israel and the Palestinian authority. J Clin Microbiol 37:1670-1675.
10. Blomqvist S, El Bassioni L, El Maamoon Nasr EM, Paananen A, Kaijalainen S, et al. (2012) Detection of imported wild polioviruses and of vaccine-derived polioviruses by environmental surveillance in Egypt. Appl Environ Microbiol 78: 5406-5409.
11. Roivainen M, Blomqvist S, al-Hello H, Paananen A, Delpeyroux F, et al. (2010) Highly divergent neurovirulent vaccine-derived polioviruses of all three serotypes are recurrently detected in Finish sewage. Euro surveill 15: 19566.
12. Pavlov DN, Van Zyl WB, Van Heerden J, Grabow WO, Ehlers MM (2005) Prevalence of vaccine-derived polioviruses in sewage and river water in South Africa. Water Res 39: 3309-3319.
13. Odoom JK, Forrest I, Dunn C, Osei-Kwasi M, Obodai E, et al. (2012) Interruption of poliovirus transmission in Ghana: molecular

epidemiology of wild-type 1 poliovirus isolated from 1995-2008. J Infec Dis 206: 1111-1120.

14. Lewis GD, Metcalf TG (1988) Polyethylene glycol precipitation for recovery of pathogenic viruses, including hepatitis A virus and human rotavirus, from oyster, water, and sediment samples. Appl Environ Microbiol 54: 1983-1988.
15. Pepkin PA, Wood DJ, Racaniello VR, Minor PD (1993) Characterization of L cells expressing the human poliovirus receptor for the specific detection of polioviruses *in vitro*. J Virol Methods 41: 333-340.
16. (2004) Manual for the virological investigation of poliomyelitis. WHO.
17. Kargar M, Sadeghipour S, Nategh R (2009) Environmental surveillance of non-polio enteroviruses in Iran. J Virol 6: 149.
18. Ndiaye AKK, Diop AM, Diop OM (2014) Environmental surveillance of poliovirus and non-polio enterovirus in urban sewage in Dakar, Senegal (2007-2013). Pan Afrcn Med J 19: 243.
19. Momou KJ , Akoua-Koffi C, Akre DS, Adjogoua EV, Tie´oulou L, et al. (2012) Detection of enteroviruses in urban wastewater in Yopougon, Abidjan. Pathol Biol (Paris) 60: e21-e26.
20. Hamed Khodaei S, Kargar M, Tabatabaei H, Sarijlou M, Nategh R (2008) Environmental Surveillance of Polio and Non-Polio Enteroviruses in Sistan and Balouchestan Province Iranian. Iranian J Pub Hlth 37: 127-133.
21. Tao Z, Song Y, Wang H, Zhang Y, Yoshida H, et al. (2012) Intercity Spread of Echovirus 6 in Shandong Province, China: Application of Environmental Surveillance in Tracing Circulating Enteroviruses. Appl Environ Micro 78: 6946-6953.
22. Stanway G, Joki-Korpela P, Hyypiä T (2000) Human parechoviruses-biology and clinical significance. Rev Med Virol 10: 57-69.
23. Joki-Korpela P, Hyypiä T (2001) Parechoviruses, a novel group of human picornaviruses. Ann Med 33: 466-471.
24. Zeng H, Lu J, Zheng H, Yi L, Guo X, et al. (2015) The Epidemiological Study of Coxsackievirus A6 revealing Hand, Foot and Mouth Disease Epidemic patterns in Guangdong, China. Sci Rep 5: 10550.
25. Mao Q, Wang Y, Yao X, Bian L, Wu X, et al. (2014) Coxsackievirus A16 Epidemiology, diagnosis, and vaccine. Vaccin Immunother 10: 360-367.
26. Brandful JA, Takeda N, Yoshii T, Miyamura K, Mingle JA, et al. (1991) A study of the evolution of coxsackievirus A24 variant in Ghana by viral RNA fingerprinting analysis. Res Virol 142: 57-65.
27. Guidelines for environmental surveillance of poliovirus circulation. WHO/V&B/03.03: March 2003.
28. Lévêque N, Huguet P, Norder H, Chomel JJ (2010) Enteroviruses responsible for acute hemorrhagic conjunctivitis. Med Mal Infect 40: 212-218.
29. Shukla D, Kumar A, Srivastava S, Dhole TN (2013) Molecular identification and phylogenetic study of coxsackievirus A24 variant isolated from an outbreak of acute hemorrhagic conjunctivitis in India in 2010. Arch Virol 158: 679-84.
30. Aubry C, Gautret P, Nougairede A, Dussouil AS, Botelho-Nevers E, et al. (2012) 2012 outbreak of acute haemorrhagic conjunctivitis in Indian Ocean Islands: identification of Coxsackievirus A24 in a returned traveler. Euro surveill 17: 20185.
31. Khan A, Sharif S, Shaukat S, Khan S, Zaidi S (2008) An outbreak of acute hemorrhagic conjunctivitis (AHC) caused by coxsackievirus A24 variant in Pakistan. Virus Res 137: 150-152.