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Environmental Surveillance Complements Case-Based Surveillance of Acute Flaccid Paralysis in Polio Endgame Strategy 2019–2023

Peng Chen,[a] Yao Liu,[b] Haiyan Wang,[b] Guifang Liu,[b] Xiaojuan Lin,[b] Weiyan Zhang,[b] Feng Ji,[b] Qing Xu,[b] Zexin Tao,[b] Aiqiang Xu[b]

[a]School and Hospital of Stomatology, Shandong University, and Shandong Provincial Key Laboratory of Oral Tissue Regeneration and Shandong Engineering Laboratory for Dental Materials and Oral Tissue Regeneration, Jinan, Shandong, People's Republic of China

[b]Shandong Provincial Key Laboratory of Infectious Disease Control and Prevention, Shandong Center for Disease Control and Prevention, Jinan, Shandong, People's Republic of China

Peng Chen and Yao Liu contributed equally to this article. Author order was determined both alphabetically and in order of increasing seniority.

ABSTRACT The Polio Endgame Strategy 2019–2023 has been developed. However, more effective and efficient surveillance activities should be conducted with the preparedness of emergence for vaccine-derived poliovirus (VDPV) or wild poliovirus (WPV). We reviewed the impact of the case-based acute flaccid paralysis (AFP) surveillance (1991 to 2018) and environmental surveillance (2011 to 2018) in polio eradication in Shandong province of China. Clinical characteristics of AFP cases and enterovirus (EV) investigation of research samples were assessed. During the period, 10,224 AFP cases were investigated, and 352 sewage samples were collected. The nonpolio AFP rate sustained at over 2.0/100,000 since 1997. Of 10,224 cases, males and young children experienced a higher risk of severe diseases, and 68.5% suffered lower limb paralysis. We collected 1,707 EVs from AFP cases, including 763 polioviruses and 944 nonpolio enteroviruses (NPEVs). No WPV was isolated since 1992. The AFP surveillance showed high sensitivity in detecting 143 vaccine-associated paralytic poliomyelitis (VAPP) cases and 6 VDPVs. For environmental surveillance, 217 (61.6%) samples were positive for poliovirus, and altogether, 838 polioviruses and 2,988 NPEVs were isolated. No WPV was isolated in environmental surveillance, although one VDPV2 was identified. Phylogenetic analysis revealed environmental surveillance had the capacity to detect a large scope of NPEVs. The case-based AFP surveillance will be indispensable for detecting VAPP cases and VDPV circulation in countries using oral polio vaccine. Environmental surveillance is advantageous in identifying EV circulation and responding to ongoing circulating VDPV outbreaks and should be expanded to complement the AFP surveillance.

IMPORTANCE Interrupting wild poliovirus transmission and stopping circulating vaccine-derived poliovirus (cVDPV) outbreaks have been proposed as two global goals by the World Health Organization in the Global Polio Eradication Initiative (GPEI). This analysis, based on the 28-year acute flaccid paralysis (AFP) surveillance and 8-year environmental surveillance, provides continued high-quality surveillance performance in achieving the GPEI and detecting the circulation of enterovirus. Given the ongoing cVDPV outbreaks in the world, we present the surveillance capacity of environmental surveillance in capturing enterovirus circulation. The final poliovirus (especially VDPV) elimination has become increasingly complex, and the case-based AFP surveillance alone will lead to difficulties in early detecting dynamics of poliovirus transmission and monitoring the extent of environmental circulation. This study goes beyond previous work to provide a detailed comprehensive

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Address correspondence to Zexin Tao, zexin.tao@163.com, or Aiqiang Xu, aqxuepi@163.com.

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evaluation of the enterovirus surveillance and can be used to formulate a set of implementation plan and performance indicators for environmental surveillance.

KEYWORDS acute flaccid paralysis surveillance, environmental surveillance, enterovirus, poliomyelitis, oral polio vaccine

In 2019, the World Health Organization (WHO) developed the Polio Endgame Strategy 2019–2023 (1). One of the most significant changes in the 2019–2023 strategy was a renewed focus on the updates of global goals, which referred to the interruption of transmission of wild poliovirus type 1 (WPV1) and a timely stop to all circulating vaccine-derived poliovirus (cVDPV) outbreaks. The latest polio data published by the WHO showed that the numbers of WPV cases between 2017 and 2019 were 22, 33, and 163, whereas the reported cVDPV cases were 96, 104, and 259, respectively, indicating that the cVDPV cases have exceeded the number of WPV globally (2). On 24 October 2019, World Polio Day, the WHO made an historic announcement that WPV3 had been globally eradicated as no WPV3 strains had been detected anywhere in the world since 2012 (3). For another, an even more important fact was that ongoing cVDPV outbreaks had been reported in the Philippines, Sichuan province of China, the Democratic Republic of the Congo, and many other parts of the world (2, 4, 5). Especially, most outbreaks have been attributed to VDPV2, suggesting the risk of cVDPV2 outbreaks still exists after the worldwide switch in April 2016 from trivalent oral polio vaccine (tOPV) to bivalent OPV (bOPV) (2, 6). Thus, the Global Polio Eradication Initiative (GPEI) has to confront ongoing challenges to achieve the overarching goal, and therefore there is a critical need to maintain a highly sensitive surveillance system to detect WPV, especially cVDPV.

Looking back, the case-based acute flaccid paralysis (AFP) surveillance had been proven effective for the GPEI in tandem with comprehensive immunization using OPV (7). Currently, this surveillance still shows vital significance for other infectious diseases to perform syndromic surveillance and laboratory investigation, for example, indicating that an emergence of enterovirus A71 (EV-A71) preceded the outbreaks described here. It was noted that the WPV transmission was currently limited to the two neighboring countries and that most polio cases were invisible (8, 9); moreover, silent circulation of poliovirus in the environment was becoming a serious concern (10). We had to confront whether the case-based AFP surveillance was sufficient to detect dynamics of poliovirus and monitor the extent of environmental circulation, especially in countries such as China that used OPV for a long time even after the certification.

The mainland China introduced live, attenuated OPV to vaccinate eligible children in 1965, and implementation of the Expanded Program on Immunization (EPI), supplementary immunization activities (SIAs), and case-based AFP surveillance were then promoted (11). Shandong was the earliest province in association with the Japan International Cooperation Agency polio project to start polio surveillance enhancement in China (12), and the case-based AFP surveillance had clearly made great success as we reported a last indigenous WPV case in 1991 and Shandong was certified as polio-free in 2000. In addition, Shandong province has conducted environmental surveillance since 2008. Here, we reviewed the case-based AFP surveillance in Shandong province from 1991 to 2018 and the importance of environmental surveillance in detecting poliovirus from 2011 to 2018. These surveillance systems exhibited sufficient quality and sensitivity in identifying polio-like paralysis cases and enterovirus (EV) circulation, and the experience had proved that the expanded surveillance activities would drive poliovirus to extinction.

RESULTS

Process of polio eradication. The Shandong polio eradication process can be divided into four stages (Fig. 1). At the time of the GPEI resolution, Shandong province conducted mass OPV campaigns efficiently, and the coverage rates for routine immunization and SIAs were 98.2 to 100% and 96.8 to 99.3%, respectively (see Table S1 in the

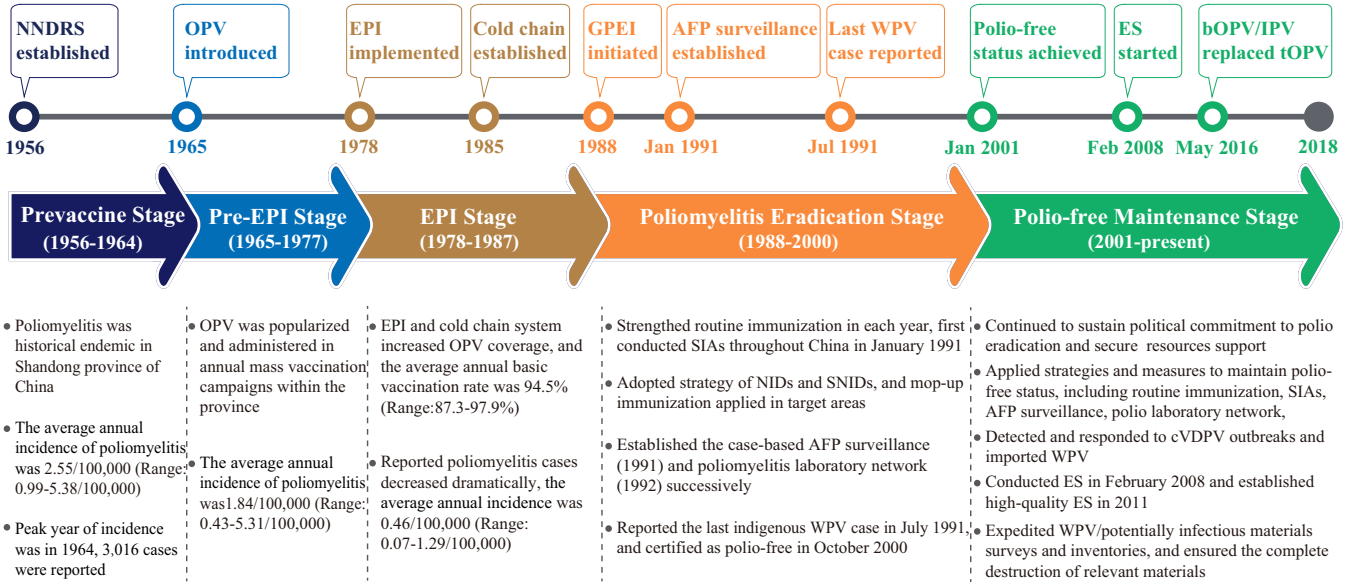

FIG 1 Timeline of poliomyelitis eradication process in Shandong province, China from 1956 to 2018. Abbreviations: AFP, acute flaccid paralysis; bOPV, bivalent oral polio vaccine; cVDPV, circulating vaccine-derived poliovirus; EPI, Expanded Program on Immunization; ES, environmental surveillance; GPEI, Global Polio Eradication Initiative; IPV, inactivated polio vaccine; NIDs, National Immunization Days; NNDRS, National Notifiable Disease Reporting System; OPV, oral polio vaccine; SIAs, supplementary immunization activities; SNIDs, Subnational Immunization Days; tOPV, trivalent oral polio vaccine; WPV, wild poliovirus.

supplemental material). It was noted that the introduction of OPV led to a stepwise reduction in poliomyelitis in Shandong province. In January 2001, Shandong province entered its polio-free maintenance stage, and a sequential immunization schedule with one dose of inactivated polio vaccine (IPV) and three doses of bOPV (types 1 and 3) was introduced in the routine immunization programs in May 2016. To investigate the circulation of poliovirus, environmental surveillance has been conducted in Shandong province of China since 2008. The above-mentioned key strategies set the stage for polio eradication in Shandong province and have been instrumental even in maintaining its polio-free status.

Case-based AFP surveillance in Shandong province. Standard indicators were regularly monitored to evaluate the sensitivity from 1991 to 2018 (Table S2). We achieved the nonpolio AFP rate every year since 1993, our laboratory showed high-quality service, and the related indicators were within the acceptable values since 1996. All of these indicators suggested that the case-based AFP surveillance in Shandong province was sustained at the WHO certification standard performance.

(i) Epidemiological characteristics of AFP cases. During the last 28 years, a total of 10,224 AFP cases were included in this study. The annual number of AFP cases reported ranged from 130 (in 1992) to 459 (in 2001), respectively. It is undeniable that the case-based AFP surveillance sensitivity was low in the first few years. For example, only 130 cases were reported through the surveillance system in 1992; however, a retrospective study conducted in Shandong province revealed 307 AFP cases were detected at that time. The case-based AFP surveillance in Shandong province was strengthened subsequently, and monthly distribution of AFP cases in each year was analyzed (Fig. 2A). In addition, the nonpolio AFP rate in the population under 15 years increased over the study period, from 1.1 AFP cases/100,000 in 1991 to 2.4/100,000 in 2018, and, in particular, the rate had remained at $>$2.0 cases/100,000 persons since 1997 (Table S2 and Fig. 2A).

From 1991 to 2018, 143 vaccine-associated paralytic poliomyelitis (VAPP) cases with poliovirus positive in stool samples were screened from AFP population, and the annual numbers of the cases are illustrated in Fig. 2B. The annual number of VAPP cases decreased year by year, and it almost disappeared once an eventual tOPV-IPV/bOPV switch was applied in Shandong province in May 2016. In addition, the annual

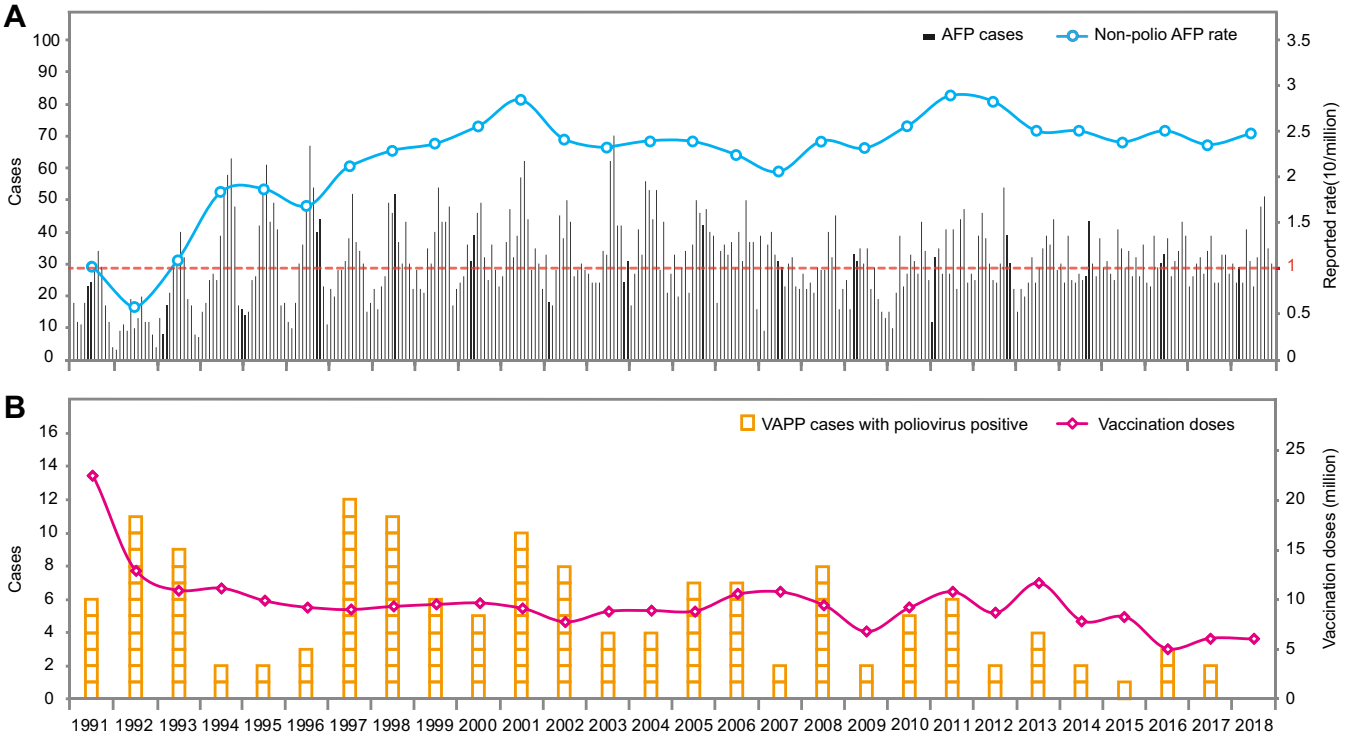

FIG 2 Data for AFP cases, VAPP cases, nonpolio AFP rates, and vaccination doses in Shandong province (1991 to 2018). (A) Monthly numbers of reported AFP cases and annual nonpolio AFP rates. The red dotted line indicates the target recommended by the WHO. (B) Annual numbers of VAPP cases positive for poliovirus and vaccination doses. A square represents one VAPP case. Abbreviations: AFP, acute flaccid paralysis; VAPP, vaccine-associated paralytic poliomyelitis.

vaccination doses in Shandong province were analyzed (Fig. 2B). In brief, we developed well-planned vaccination campaigns. In addition to routine immunization activities, we conducted two rounds of SIAs every year, except for 2009 in which the SIA was conducted only in January 2009. Beginning in 2016, the SIAs were suspended in Shandong province (see Table S1 in the supplemental material). We also tried to explore the relationship between vaccination doses and the number of VAPP cases positive for poliovirus; unfortunately, no significant correlations could be detected ($r = 0.284$, $P = 0.143$).

(ii) Clinical characteristics of AFP cases. The demographic and clinical characteristics of the AFP cases are shown in Table 1. Of 10,224 AFP cases, the median age was 2.9 years (interquartile range [IQR], 1.7 to 6.8 years) with a male/female ratio of 1.8:1. The type of limb paralysis varied from one to all four limbs. Of note, lower limb paralysis was the prominent feature, while nearly half of the AFP cases (49.5%) showed asymmetric paralysis.

AFP cases that were EV positive were analyzed separately by different parameters (Table 1). When VAPP cases with poliovirus-positive and NPEV-positive population groups were analyzed individually, the median age was 0.6 years (IQR, 0.3 to 1.2 years) and 2.4 years (IQR, 1.7 to 4.2 years), and the male/female ratios were 2.8:1 and 2.1:1, respectively. In our study, the male/female ratio showed a significant difference between EV-positive patients and EV-negative patients, which meant that the male children would be vulnerable to EV infection ($P = 0.001$). A Wilcoxon rank sum test indicated that the median age of paralysis onset in EV-positive patients was younger than in EV-negative patients ($P < 0.001$), and the same results were observed among VAPP cases with the poliovirus-positive group compared to other population groups (all P values were <0.001). However, no significant statistical difference was observed in paralysis age between the EV-A71-positive group and other NPEV-positive groups ($P = 0.309$). In addition, there were several differences between EV-positive patients

TABLE 1 Clinical characteristics of AFP cases and EV-positive cases in Shandong province, China (1991 to 2018)[a]

Parameter	AFP cases	VAPP poliovirus-positive cases	NPEV isolate cases	
			EV-A71	Other
Total no. of cases	10,224	143	45	899
Demographics				
Median age, yr (IQR)	2.9 (1.7–6.8)	0.6 (0.3–1.2)	1.9 (1.4–3.1)	2.4 (1.7–4.3)
% males	64.4 (6,581/10,224)	73.4 (105/143)	64.4 (29/45)	67.7 (609/899)
% females	35.6 (3,643/10,224)	26.6 (38/143)	35.6 (16/45)	32.3 (290/899)
% (no. of cases/total no. of cases)				
Prodrome				
Fever[b]	32.4 (2,725/8,413)	73.6 (81/110)	61.4 (27/44)	34.0 (269/792)
Diarrhea[c]	6.0 (335/5,586)	16.7 (9/54)	7.0 (3/43)	6.6 (37/557)
Limb paralysis				
Upper limbs affected	2.8 (284/10,224)	0	11.1 (5/45)	1.7 (15/899)
Lower limbs affected	68.5 (7,005/10,224)	83.9 (120/143)	68.9 (31/45)	72.6 (653/899)
Four limbs affected	22.4 (2,292/10,224)	11.2 (16/143)	17.8 (8/45)	22.0 (198/899)
Other	6.3 (643/10,224)	4.9 (7/143)	2.2 (1/45)	3.7 (33/899)
Asymmetric paralysis	49.5 (4,958/10,224)	67.1 (96/143)	55.6 (25/45)	51.6 (464/899)
Residual paralysis	24.2 (2,433/10,048)	100 (143/143)	40.0 (18/45)	21.0 (185/880)
Diagnostic classification				
Guillain-Barré syndrome	29.8 (2,926/9,816)		6.7 (3/45)	28.0 (244/873)
Encephalomyelitis/myelitis	9.4 (819/9,816)		20.0 (9/45)	7.1 (62/873)
Traumatic neuritis	3.1 (308/9,816)		2.2 (1/45)	2.4 (21/873)
Limb mononeuritis/neuritis	3.0 (296/9,816)		2.2 (1/45)	2.9 (25/873)
Polio-like paralysis	2.8 (278/9,816)	100 (143/143)		2.3 (20/873)
Myositis/myopathy	2.0 (197/9,816)			1.1 (10/873)
Transient paralysis	1.5 (146/9,816)			0.8 (7/873)
AFP with NPEV infection	1.0 (97/9,816)		28.9 (13/45)	6.8 (59/873)
Other[d]	47.3 (4,575/9,816)		40.0 (18/45)	48.7 (425/873)

[a]AFP, acute flaccid paralysis; EV, enterovirus; EV-A71, enterovirus A71; NPEV, nonpolio enterovirus; VAPP, vaccine-associated paralytic poliomyelitis.
[b]Prodromal fever was collected since 1997.
[c]Prodromal diarrhea was collected since 2004.
[d]Other diagnosed diseases included disseminated encephalomyelitis, periodic paralysis, botulinum toxin, polyneuropathy, and limb paralysis with unknown causes.

and EV-negative patients in clinical symptoms. The VAPP cases with the poliovirus-positive group and the EV-A71-positive group were more likely than the other population groups to suffer from fever on exam ($P < 0.001$). Significantly, lower limb paralysis was still the prominent feature for EV-positive patients, but the patterns of limb involvement among different groups varied ($P < 0.05$). Our study showed that asymmetric paralysis and residual paralysis in VAPP cases with the poliovirus-positive group and the EV-A71-positive group occurred more frequently than for both the EV-negative group and other NPEV-positive groups at follow-up, further highlighting plausible differences in pathogenicity. For the diagnosis at discharge, the classifications of diagnostic diseases for EV-negative patients were consistent with other NPEV-positive patients ($P = 0.053$), and Guillain-Barré syndrome was the most common diagnosis. In contrast, EV-A71-positive patients were more likely to be diagnosed as encephalomyelitis/myelitis and AFP with NPEV infection.

(iii) Poliovirus and NPEV investigation. In all, 1,707 EVs were collected from the AFP cases, including 763 poliovirus and 944 NPEV strains. All polioviruses were Sabin strains except for the last indigenous WPV1 strain isolated in 1991 (Fig. 3A). The numbers of poliovirus type 1 (PV1), PV2, and PV3 isolates were 185 (24.2%), 329 (43.1%), and 249 (32.6%), respectively. It was noted that PV2 was the predominant type in annual isolates before 2010; however, the proportions of these type strains had been decreasing since 2011. Furthermore, the case-based AFP surveillance effectively detected VDPV, and 6 VDPV strains had been isolated in 1997 (PV2, 10 nucleotides [nt]), 2007 (PV1, 9 nt; PV1, 13 nt), 2009 (PV2, 11 nt), 2011 (PV1, 10 nt), and 2017 (PV3, 13 nt), respectively. These polioviruses were all ambiguous VDPV except the virus isolated in 2017, which was identified as immunodeficiency-related VDPV.

Altogether, 944 NPEVs belonging to 53 EV types were identified with the annual number ranging between 8 in 1992 and 68 in 2003 and with a mean of 34 (IQR, 24 to

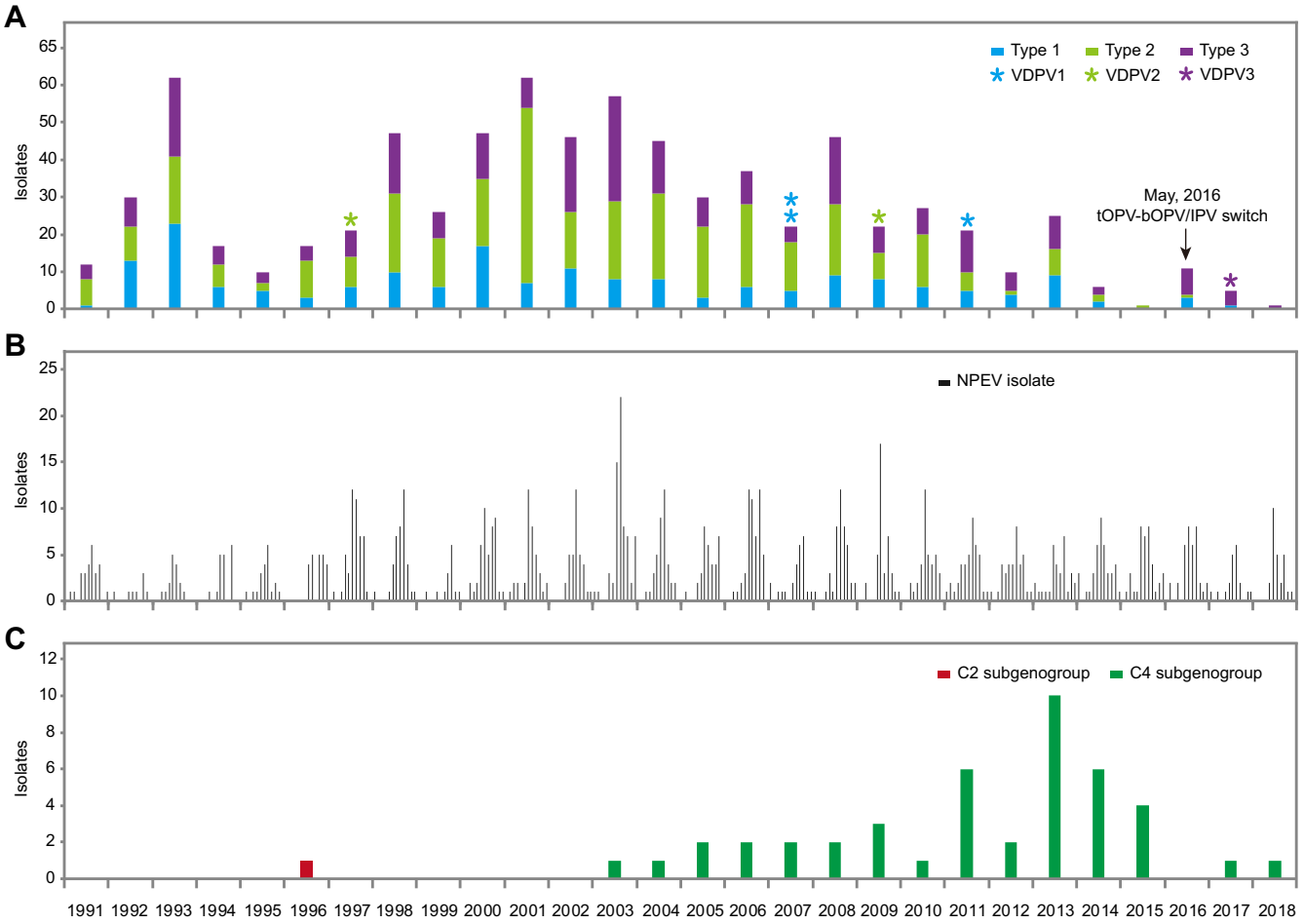

FIG 3 Numbers of polioviruses (A), NPEVs (B) and EV-A71 strains (C) isolated from the case-based AFP surveillance in Shandong province (1991 to 2018). (A) Annual numbers of three poliovirus types from 1991 to 2018. The color-coded stars indicate the numbers of three VDPV types detected from the AFP cases. The arrow indicates Shandong province achieves a switch from tOPV to bOPV and IPV in May 2016. (B) Monthly number of NPEV from January 1991 to December 2018. (C) Annual number of EV-A71 from 1991 to 2018. Abbreviations: EV-A71, enterovirus A71; NPEV, nonpolio enterovirus; VDPV, vaccine-derived poliovirus.

40). The monthly number of NPEV isolates showed strong seasonality in Shandong province, and the aggregate strain numbers had a peak every summer between 1991 and 2018 (Fig. 3B). In addition, the detection of EV-A71 isolates from the case-based AFP surveillance confirmed its great importance in monitoring EV circulation. In 1996, one EV-A71 strain belonging to C2 subgenogroup was isolated first in Shandong province, and continuous detection of C4 subgenogroup strains was observed after 2003 (Fig. 3C). This was consistent with the recent global emergence of EV-A71 as a major causative agent of hand, foot, and mouth disease (HFMD).

Environmental surveillance in Shandong province. (i) Poliovirus and NPEV investigation. Here, we report the detection of poliovirus and NPEV from environmental surveillance between January 2011 and December 2018 (Fig. 4). A total of 352 sewage samples were screened, yielding 217 (61.6%) positive samples. During these 8 years of surveillance, 838 polioviruses were identified, and the numbers of PV1, PV2, and PV3 were 268 (32.0%), 181 (21.6%), and 389 (46.4%), respectively (Fig. 4A). In addition, 2,988 NPEV strains belonging to 27 serotypes were isolated from sewage samples. We found that all polioviruses except one were Sabin-like strains with ≤8-, ≤5-, and ≤9-nt substitutions in the VP1 coding region compared to the original vaccine Sabin types 1, 2, and 3, respectively. The one poliovirus was VDPV2 collected in December 2012, which showed 7-nt substitutions in the VP1 coding region. In our study, PV2 was most frequently detected in 2011 (43.6%) and 2012 (45.5%), whereas

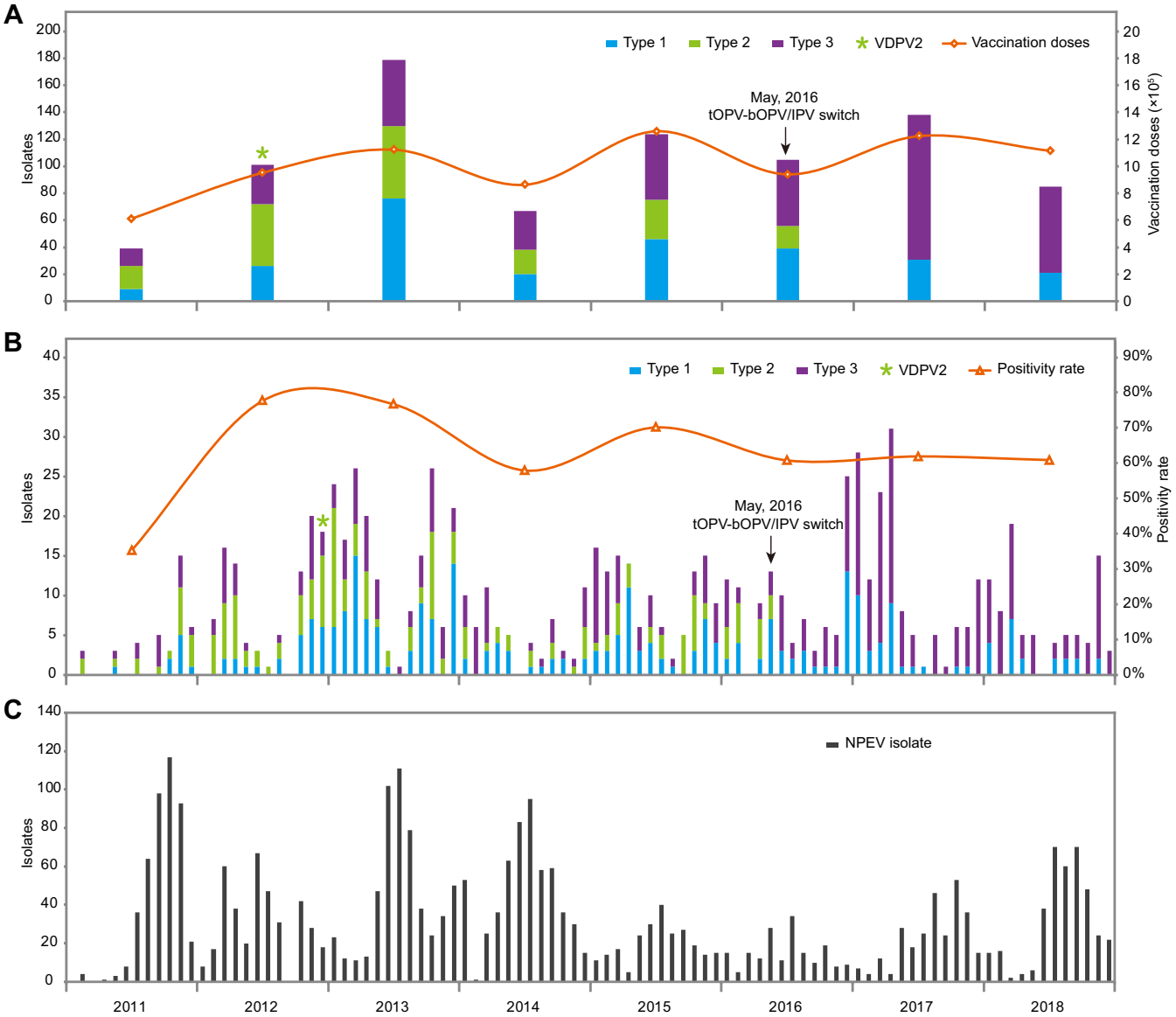

FIG 4 Poliovirus and NPEV isolated from the environmental surveillance in Shandong province (2011 to 2018). (A) Annual numbers of three poliovirus types and vaccination doses inoculated at three environmental surveillance sites from 2011 to 2018. A color-coded star indicates one VDPV2 is detected from a sewage sample. The arrow indicates Shandong province achieves a switch from tOPV to bOPV and IPV in May 2016. (B) Monthly distribution of three poliovirus types in environmental surveillance. The solid brown line with triangles indicates the positivity rate of poliovirus in sewage samples every year. The green star indicates one VDPV2 was isolated from sewage samples in December 2012. (C) Monthly distribution of NPEV in environmental surveillance. Abbreviations: NPEV, nonpolio enterovirus; bOPV, bivalent oral polio vaccine; IPV, inactivated polio vaccine; tOPV, trivalent oral polio vaccine; VDPV, vaccine-derived poliovirus.

PV3 was found increasing dramatically from 2013 to 2018. The number of poliovirus strains detected from environmental surveillance corresponded to immunization campaigns; the total amounts of vaccination doses every year in Shandong province are presented in Fig. 4A. A Spearman correlation test showed that the polioviruses were positively correlated with the vaccination doses inoculated in environmental surveillance sites ($r = 0.810$, $P = 0.015$), which suggested that environmental surveillance exhibited sufficient sensitivity in detecting poliovirus circulation.

The monthly distributions of three poliovirus types and NPEVs are illustrated in Fig. 4B and C. Interestingly, we observed that poliovirus peaked in winter and spring, whereas NPEVs were more frequently detected in summer and autumn. In May 2016, Shandong province achieved the tOPV-IPV/bOPV switch, and the absence of PV2 in sewage samples since June 2016 indicated its rapid disappearance after the withdrawal

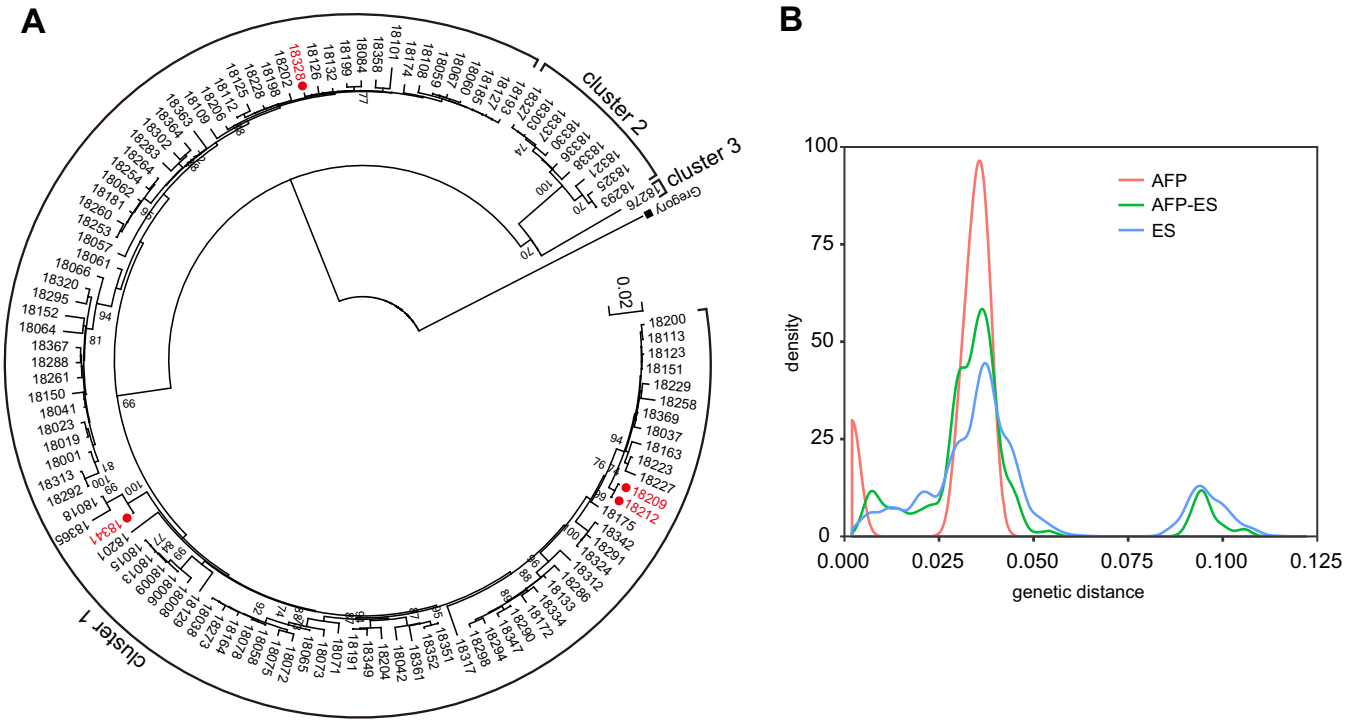

FIG 5 Phylogenetic relationship and genetic divergence of E-11 from AFP surveillance and environmental surveillance in Shandong province in 2018. (A) Phylogenetic tree of E-11 based on complete VP1 sequences. Circles indicate strains detected from AFP cases; squares indicate prototype strains. (B) Density curve of the genetic distance for E-11. The red line indicates the genetic divergence of strains from AFP cases, the blue line indicates the genetic divergence of strains from sewage, and the green line indicates the genetic divergence of strains between cases and sewage. Abbreviations: AFP, acute flaccid paralysis; E-11, echovirus 11; ES, environmental surveillance.

of OPV2. These results further emphasized that environmental surveillance is highly sensitive for detecting poliovirus relative to the case-based AFP surveillance, and the positivity rate of poliovirus obtained from environmental surveillance (35.4 to 77.8%) was significantly higher than that from the case-based AFP surveillance in Shandong province (Fig. 4B).

(ii) Phylogenetic and sequence analysis. To analyze the phylogenetic relationships of NPEV obtained from AFP surveillance and environmental surveillance, the most common NPEV serotypes (echovirus 11 [E-11]) isolated in 2018 were selected for the further study. The Shandong E-11 sequences were segregated into three distinct clusters (Fig. 5A). The phylogenetic analysis showed that 91.2% (103/113) of E-11 sequences in Shandong province formed cluster 1 (including four sequences from AFP surveillance) and that nine sequences were located in cluster 2 and only 1 sequence was located in cluster 3 (Fig. 5A). Homologous comparison showed E-11 strains in cluster 1 and cluster 2 had 93.2 to 99.8% (95.2 to 100% amino acid sequence identities) and 97.2 to 99.8% (97.9 to 100% amino acid sequence identities) VP1 nucleotide sequence identities among themselves and that the overall mean p-distance values were 0.0072 and 0.0140, respectively. Obviously, the case-based AFP surveillance and environmental surveillance in Shandong province had sufficient capacity in detecting NPEV circulation as sequences from AFP cases showed close genetic relationship with many environmental surveillance sequences. Homologous comparisons of E-11 were conducted, and the results indicated that strains from environmental surveillance had higher genetic divergence than those from AFP cases. The levels of genetic divergence of E-11 strains from AFP surveillance and environmental surveillance were 0.2 to 3.8% and 0.2 to 12.2%, respectively, and 0.4 to 10.6% genetic divergence was observed when environmental surveillance strains were compared to clinical strains, which further emphasized that environmental surveillance seemed to be more advantageous in identifying a large scope of NPEVs than case-based AFP surveillance. The proportion

of each genetic distance value in the pairwise comparison was calculated and illustrated as the density curve in Fig. 5B.

DISCUSSION

Poliomyelitis is historically endemic in China. All polio cases were initially reported every year through the National Notifiable Disease Reporting System, which was established in 1953 (11). With the initiation of the GPEI, this channel limited the notification of polio since many cases were underreported, and most cases lacked laboratory confirmation. Accordingly, China introduced a case-based virus surveillance system based on identification and investigation of AFP cases for the complete polio eradication in 1990 (13). The case-based AFP surveillance was accepted as the precedent of syndrome surveillance in communicable disease surveillance activities in China and implemented as a key strategy for GPEI in tandem with comprehensive immunization using OPV. Until 1991, the case-based AFP surveillance was systematically established in China.

In the past 30 years of GPEI, Shandong has made remarkable progress in eradicating indigenous poliomyelitis and maintaining its polio-free status. It is important to note that the aforementioned strategies have achieved effective poliovirus containment in Shandong province for over a decade. Our analysis showed a significant increase in reported AFP cases in Shandong province during 1991 through 2018, demonstrating the persistence of high-quality AFP surveillance in detection of polio-like cases. In particular, the nonpolio AFP rates have reached a steady state at over 2.0 cases/100,000 children aged $<$15 years since 1997, suggesting the case-based AFP surveillance not only goes beyond the WHO requirements but also sustains higher sensitivity than other countries (14, 15). Also, the demographic and clinical characteristics of AFP cases were analyzed, and the findings that the younger age and residual paralysis in EV-A71-positive group closely resembled those detected in VAPP cases implied higher transmissibility and pathogenicity for EV-A71 than the other NPEV types. Moreover, a comprehensive picture of the dynamics of EV epidemiology also indicated that accessibility to high-quality laboratory services in Shandong province enabled better detection of the emergent and unexpected circulation of other EV types.

As the first syndrome surveillance system established in China, the case-based AFP surveillance in Shandong province indicated an emergence and significant increase of EV-A71 among AFP cases since 2003 through virological investigation, preceding the HFMD outbreak to illustrate the temporal circulation of EV-A71 in the population (16). Similarly, recent reports showed that clusters of AFP associated with EV-D68 have been recognized worldwide (17–19). Although the EV-D68 is known to be associated with respiratory disease, establishing the corresponding syndrome surveillance system can still respond quickly to identify emergence of new cases and conducting virological surveillance will be of great significance to monitor EV-associated outbreaks.

Polio-associated disease associated with OPV use is now better understood as a new risk in a polio-free world. In rare instances, OPV can cause VAPP, but more seriously, this live-attenuated vaccine will lead to outbreaks of cVDPV. Researchers have also documented that more countries have suffered a polio outbreak due to a cVDPV (especially cVDPV2) than due to a WPV (2, 4, 5), especially since the global cVDPV cases have exceeded the number of WPV cases in recent years (2). These findings reaffirmed that a larger eradication effort should be made to rapidly address this risk and that special surveillances that focus on the virus evidence will be necessary to achieve the GPEI. Recognizing the challenges that detracted from achieving sufficient sensitivity in case-based AFP surveillance, the WHO has proposed environmental surveillance for the timely detection of vaccine-related poliovirus. In practice, however, only 15 countries conducted environmental surveillance in routine surveillance activities, while most countries have yet to start environmental surveillance due to various barriers. Recently, the identification of WPV importation and VDPV emergence in polio-free countries has demonstrated the value of environmental surveillance in detecting poliovirus in the absence of polio-related AFP cases (20–23). Shandong is one of the few provinces

maintaining environmental surveillance in China, and our environmental surveillance strategy searches for EV on sewage have also provided evidence in investigating poliovirus circulation and identifying VDPV emergence (24–26); moreover, enhanced environmental surveillance will be of great importance in tracing the transmission pathways of circulating NPEV (27).

Given the ongoing cVDPV outbreaks in the world, case-based AFP surveillance capacity is insufficient for detecting all Sabin viruses, Sabin-like viruses, and VDPVs. Environmental surveillance that focuses on virus evidence presents an even greater advantage in verifying the lack of circulating poliovirus and should be expanded geographically to further complement case-based AFP surveillance. Especially for many OPV-using countries, a series of social factors (such as population density, habits and customs, sanitary conditions, and poverty) will also affect the epidemic of VDPV and present challenges for the timely prevention of cVDPV outbreaks. In the 2019–2023 strategy, environmental surveillance was recommended as a crucial aspect of the final poliovirus elimination and should be implemented in high-risk areas and in OPV-using countries. Furthermore, a set of practical implementation plans for environmental surveillance should be formulated and agreed in accordance with both routine surveillance and emergency surveillance, which included details of the sampling sites (population sizes and geographical attributes to be represented), type and characteristics of sewage and treatment systems, the provision of personal training, and available laboratory resources (laboratory space, equipment, and reagent support). Also, the sensitivity of environmental surveillance has not been well characterized, and a series of indicators may be developed based on the experience of case-based AFP surveillance. Consequently, an appropriate mix of surveillance strategies for GPEI will lay the groundwork for both the achievement of goals in the 2019–2023 strategy and the establishment of a sustainable polio-free world in the future.

Our study was conducted on a 28-year analysis of the case-based AFP surveillance in Shandong province. The study limitations included the retrospective nature of the data, since the presentations of many parameters may be inconsistent or incomplete in the database histories. In addition, the case-based AFP surveillance was established mainly to monitor polio eradication, which probably omitted some clinical characteristics as the case information was collected.

Conclusions. With the success of GPEI in sight and new goals presented in the Polio Endgame Strategy 2019–2023, we reviewed here the case-based AFP surveillance and virological investigation in Shandong province between the years of 1991 and 2018 and evaluated the performance of this syndromic surveillance to detect polio-like cases and poliovirus transmission. Although case-based AFP surveillance plays an important role in the GPEI and will be indispensable to finally reach the intended goals, the syndrome surveillance capacity is insufficient for detecting all poliovirus (especially VDPV). Environmental surveillance that focuses on the virus evidence presents an even greater advantage in verifying the lack of circulating poliovirus and should be expanded geographically to further complement case-based AFP surveillance by strengthening the overall sensitivity of poliovirus surveillance.

MATERIALS AND METHODS

Data collection. Case-based AFP surveillance was performed systematically in Shandong province since 1991. All AFP cases were defined by the WHO guideline, and every clinical case was reported by hospitals to county centers for disease control and prevention (CDCs) within 2 days of case detection. After the report, county-level CDC staff were responsible for case investigation and data input. In addition, rigorous follow-up was required to verify whether there was residual paralysis at 60 days after the onset of motor deficit. Finally, investigation forms were sent to provincial CDCs for data analysis and feedback. In addition, two adequate stool samples were collected from each case at least 24 h apart and within 14 days of paralysis onset. On arrival in the laboratory, stool samples were carefully checked and processed according to the WHO-recommended protocol (28). All supernatants were frozen at −20°C for cell inoculation. We collected data from admitted children $<$15 years of age with AFP, as reported through a real-time AFP surveillance system from clinical and epidemiological investigations between 1 January 1991 and 31 December 2018. After data cleaning, a series of indicators were used to evaluate the quality of the case-based AFP surveillance.

Environmental surveillance was conducted in Shandong province since 2008, and three wastewater treatment plants in the surveillance cities (Jinan, Linyi, and Yantai) were selected as the sampling sites. All sewage samples were collected monthly in Jinan and Yantai and semimonthly in Linyi during the study period. Approximately 1 liter of flowing water was collected at the inlet collector canals of the wastewater treatment plants by grab sampling method in the afternoon between 2:00 and 3:00 p.m. The sewage samples were transported to the laboratory on ice and were stored (<24 h) and processed at 4°C. As described previously, all samples were concentrated by the membrane adsorption/elution method, and the pH of the elution fluid (3% beef extract solution) was set to 9.0, which was demonstrated to increase the sensitivity (24, 29). The supernatant was filtered through a 0.22-μm filter and was frozen at −20°C for cell inoculation.

Enterovirus identification. As described previously, EV isolations were carried out in RD, Hep-2, and L20B cell lines. A total of 200 μl of treated supernatant was added to each of the cell culture tubes (12 tubes of each cell line for one sewage sample). After two 7-day examinations, the cells in the tube would be collected if a complete cytopathic effect was obtained, and reverse transcription-PCR (RT-PCR) was performed to amplify the entire VP1 coding regions of polioviruses and nonpolio enteroviruses (NPEVs) with specific primer pairs (27, 30, 31). For poliovirus identification, virus serotype and intratypic differentiation were simultaneously determined with real-time RT-PCR assays (32). Furthermore, VDPV was determined following the standards for the diagnosis for poliomyelitis (33). Molecular typing method was performed to identify the serotypes of NPEVs using a BLAST search, and the criterion used for this method was as described by Oberste et al. (34).

Phylogenetic analysis. Phylogenetic tree was constructed with MEGA v6.0, using the neighbor-joining method after estimation of genetic distance using the Kimura two-parameter method (35). The reliability of the phylogenetic tree was assessed by bootstrap with 1,000 duplicates, and the transition/transversion rate was set at 2.0.

Statistical analysis. To identify characteristics that varied among different population groups, one-way analysis of variance was used to compare continuous variables, and *post hoc* analysis was conducted with a Fisher least-significant-difference test. A Wilcoxon rank sum test was used if the data failed to obey the normal distribution or homogeneity of variance. Correlations among vaccination doses with vaccine-associated paralytic poliomyelitis (VAPP) cases and polioviruses from environmental surveillance were tested by the Spearman rank correlation test. Categorical variables were analyzed using the chi-square test. Two-tailed P values of <0.05 were considered statistically significant.

Ethics statement. Ethical approval was given by the Ethics Review Committee of Shandong Center for Disease Control and Prevention, and the methods were carried out in accordance with the principles of the Declaration of Helsinki. Written informed consents for the use of clinical samples of AFP cases were obtained from all subjects (the legal guardians of the patients and contacts).

Data availability. The complete VP1 sequences of 113 E-11 strains described in this study have been deposited in the GenBank database under accession numbers MT461165 to MT461277.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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