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MAJOR ARTICLE

Circulation of Type 2 Vaccine-Derived Poliovirus in China in 2018–2019

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Background. China implemented the globally synchronized switch from trivalent oral poliovirus vaccine (tOPV) to bivalent OPV (bOPV) on May 1, 2016. During April 2018 to May 2019, the first outbreak caused by type 2 circulating vaccine-derived poliovirus (cVDPV2) after the switch occurred in Xinjiang and Sichuan, China.

Methods. We performed sequence analysis of VP1 and the whole genome to determine the genomic characteristics of type 2 cVDPVs, and carried out coverage surveys to assess the risk of viral propagation. Surveillance for environment and acute flaccid paralysis was intensified to enhance case ascertainment.

Results. Comparison of the complete genomes between early (Xinjiang strain) and late strains (Sichuan strains) revealed that recombination pattern and reverse mutation of attenuation sites had been fixed early, but the mutations of the neutralizing antigenic sites were introduced over the circulation. The Markov Chain Monte Carlo tree showed that the cVDPV2 initial infection was April 2016, earlier than the switch. So, we speculated that the cVDPV2 was originated from tOPV recipients and spread among children with a low level of immunity against the type 2.

Conclusions. The detection of this outbreak combined acute flaccid paralysis (AFP) surveillance with environmental surveillance (ES) indicates that ES should be expanded geographically to further complement AFP surveillance.

Keywords. cVDPV2 outbreak; eradication; surveillance; vaccination switch.

The globally synchronized removal of the type 2 component of oral poliovirus vaccine (OPV) in April 2016 will provide a significant reduction in the burden of vaccine-associated paralytic poliomyelitis, but it may increase the risk of circulating vaccine-derived poliovirus (cVDPV) outbreaks during the transition period [1]. After the switch from trivalent OPV (tOPV) (type 1, 2, and 3) to bivalent OPV (bOPV) (type 1 and 3), the number of countries where type 2 cVDPV2 have been detected rose to 26, compared to 7 before the switch [2]. During May 2016 to March 2021 after the switch, the Global Polio Laboratory Network had detected 1572 cases of cVDPV2, which were much higher than the 681 found in 2006–2016 in the world [3]. The number and geographic breadth of cVDPV2 outbreaks have exceeded the prediction of the World Health Organization (WHO) since the switch, and the probable reasons for this may be the following: (1) insufficient routine immunization coverage [4]; (2) the use of stored monovalent type 2 OPV(mOPV2) [5]; (3) the protracted cVDPV2 outbreaks from prior emergence have not been successfully controlled [6]; (4) circulating vaccine virus from previous vaccination with tOPV but low immunity against the type 2 from bOPV in the cohorts born after the switch [7]; (5) nucleotide substitutions at key neurovirulence determination sites and genetic rearrangements with human enterovirus C species et al [8].

China withdrew type 2 OPV in synchrony like other tOPV-using countries and changed the routine immunization schedule to "1 + 3", one dose of inactivated polio vaccine (IPV) followed 3 doses of bOPV. To further increase the type 2 immunity, while retaining the level of intestinal mucosal immunity against type 1 and type 3, China's polio immunization strategy has adjusted to "2 + 2" since January 1, 2020, which is 2 doses of IPV followed 2 doses of bOPV. In May 2019, an outbreak caused by cVDPV2 was found in Leibo county of Sichuan Province, China. This was the first cVDPV2 outbreak after the polio vaccination switch in China. It is interesting to note that, when we compared the 4 type 2 VDPVs isolated from the outbreak with 1 previous strain isolated from sewage sample in

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Urumqi of Xinjiang in 2018, we found that they differed from Sabin 2 by 1.4%–3.3% in nucleotides and shared 9 nucleotide substitutions in the VP1 region. Therefore, we included the strain from Xinjiang into the cVDPV2 outbreak although the distance between Xinjiang and Sichuan is approximately 2851.2 kilometers. This cVDPV2 outbreak involved longer circulation time and wider geographical scope compared with the previous VDPV outbreaks in China, which were all confined to a limited area. In addition, we captured both early strain (Xinjiang strain) and late strains (Sichuan strains) in the virus circulation, which provided us an opportunity to study the genetic changes in the cVDPV2 evolution. The discovery of this cVDPV2 outbreak by acute flaccid paralysis (AFP) surveillance combined with environmental surveillance (ES) indicates that ES can provide a scientific basis for timely detection of the spread of VDPVs as well as AFP surveillance. Before the occurrence of this cVDPV2 outbreak, China has carried out routine environmental surveillance with a sampling frequency of once a month in 9 provinces where the risk of VDPV is high, including Xinjiang but not Sichuan. After this outbreak, the number of provinces carrying out ES has been increased to 13 and the sampling frequency changed to once every 2 weeks. In this report, we described the genomic characteristics of these type 2 cVDPVs and emphasized the necessity of ES in the final stage of polio eradication.

MATERIALS AND METHODS

Virus Isolation and Serotyping

L20B (murine cell line expressing the human poliovirus receptor) and RD (human rhabdomyosarcoma cell) were used for virus isolation. The virus isolation and intratypic differentiation were carried out in accordance with the standard operating procedures of the *Polio laboratory manual*, 4th ed, made by the WHO [9]. All positive strains were subjected to intratypic differentiation directly using VP1 region sequencing.

Complete Genome Amplification

The complete genomes of 5 strains were sequenced for recombination events and attenuated sites analysis. The virus nucleotide acid was extracted by QIAamp Viral Mini Kit (QIAGEN, Valencia, CA). Two long-distance polymerase chain reaction (PCR) reactions were performed by using reverse transcription-PCR. The 2 sets of sense/antisense primer pairs 0001S48/Q8 and Y7/7500A were used to amplify 2 overlapping fragments of 3.57 kb and 5.28 kb, respectively. Sequencing was performed with primers described previously [10]. The 2 fragments were purified by using QIAquick Gel Extraction Kit (QIAGEN). Cycle sequencing reaction were marked by using BigDye Terminator version 3.0 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). After purifying by Sephadex Gel G50 (Pharmacia, Stockholm, Sweden), the labeled products were sequenced with the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Hitachi, Japan). Sequencing was bidirectional, and nucleotide position was sequenced at least once from each stand.

Phylogenetic Analysis

Sequence data were stored as standard chromatogram format files and assembled by using Sequencher program (version 5.4.5) (GeneCodes, Ann Arbor, MI). VP1 sequence alignments were performed with MEGA software (version 7.0) (Sudhir Kumar, Arizona State University, Tempe, AZ) to select the evolution model of the phylogenetic tree. TempEst software (version 1.5.3) was used to assess whether there was sufficient temporal signal in the data to proceed with phylogenetic molecular clock analysis. The phylogenetic tree was constructed by the maximum likelihood method with the TN93 + strict clock evolution model. The reliability of the tree was estimated with 1000 bootstrap replicates.

Spatiotemporal Dynamic Transmission

The initial infections of the cVDPV2 were analyzed in a phylogeographic tree constructed by Bayesian Markov Chain Monte Carlo (MCMC) analysis using the BEAST software (version 1.10.4; University of Auckland, USA) [11]. Tracer software package (version 1.7.1) [12] was used for analyzing the results of BEAST, and the phylogeographic tree was displayed and edited by using Figtree software. The SpreaD3 package (version 0.9.7) [13] was used for analysis and visualization of pathogen phylodynamic reconstructions, and the spatial diffusion rates calculated between sampling location were analyzed with Bayes factor (BF) to investigate whether the virus transmission events assessed empirically from the MCMC trees were supported statistically. The transmission event was supported by BF value>10 [14].

Nucleotide Sequence Accession Numbers

The nucleotide sequence numbers for the sequences submitted to GenBank are MZ150562 to MZ150566.

Patient Consent Statement

This study involved no human experimentation. The children's parents agreed to the use of their stool samples for research purposes and to the collection of samples by trained professional staff. All experimental protocols were approved by the National Institute for Viral Disease Prevention and Control, and all methods were carried out in accordance with the approved guidelines.

RESULTS

Virus Isolation and Serotyping

One cVDPV2 was isolated from a sewage sample (CHN-E18-11-XJ2018) in Urumqi of Xinjiang in 2018, and 4 cVDPV2 strains were isolated from 1 AFP case (CHN23016), 2 close contacts (CHN23018 and CHN23019), and 1 healthy child (CHN23029H) in Leibo county of Sichuan in 2019, respectively (Table 1). The AFP case was a 5-year-old boy from Leibo county of Sichuan who developed paralysis on April 25, 2019. VP1

Table 1. Details of the Five Strains Isolated in Xinjiang and Sichuan Province, China

Case	Age (Year)/Sex	Source	OPV History	Date of Onset	Date of Stool Specimen Collection	Diagnosis	No. (%) of VP1 Nucleotide Substitutions	Virus Type
CHN-E18-11-XJ2018	/	/	/	NA	April 14, 2018	/	13 (1.4)	Type2
CHN23016	5/male	AFP	3 tOPV	25 Apr 2019	May 17, 2019	Laboratory-Confirmed polio case	28 (3.1)	Type2
CHN23018	5/male	Contacts of AFP	Unknown	NA	June 14, 2019	Non-AFP	27 (3.0)	Type2
CHN23019	4/male	Contacts of case CHN23018	3 tOPV	12 June 12, 2019	June 27, 2019	Non-AFP	33 (3.7)	Type2
CHN23029H	2/female	Healthy children	3 bOPV	NA	August 18, 2019	Non-AFP	27 (3.0)	Type2

Abbreviations: AFP, acute flaccid paralysis; bOPV, bivalent OPV; NA, not applicable; OPV, oral poliovirus vaccine; tOPV, trivalent OPV.

NOTE: Sewage and stool samples from Xinjiang and Sichuan were collected in this outbreak according to the World Health Organization guidelines.

sequence alignments showed that they have 13, 28, 27, 33, and 27 nucleotide substitutions in the VP1 region compared with Sabin 2 vaccine strain (GenBank accession number AY184220), and the nucleotide substitution rates are 1.4%, 3.1%, 3.0%, 3.7%, and 3.0%, respectively. The WHO defines VDPV2 as type 2 poliovirus that differs from the Sabin 2 strain by >0.6% in the VP1 region, and they are all meet the definition and can be classified as VDPV2.

Phylogenetic Analysis

To elucidate the divergence and evolution of these cVDPV2 strains, the VP1 sequences of the following viruses were analyzed: all 5 cVDPVs strains in this study and Sichuan cVDPV2 strains isolated in 2011–2012, Nigeria(NIE) cVDPV2 strains, Democratic Republic of the Congo(RDC) cVDPV2 strains, Madagascar(MG) cVDPV2 strains, and cVDPV2 strains in Guinea(NZE). The phylogenetic tree based on the VP1 region revealed that the strains found in this outbreak can be classified as a cluster, which was independent of the cVDPV2 all over the world (Figure 1). Moreover, the strains isolated from Leibo county of Sichuan in this study were distinct from those type 2 cVDPVs isolated in Aba county of Sichuan in 2011–2012.

Recombination Events and Attenuated Sites Analysis

The complete genomes of all 5 cVDPV2 strains were analyzed by using SimPlot software (version 10.0; Systat Software Inc, San Jose, CA) to search putative recombination events (the 5 strains in this study were merged into a group named C). The results revealed that whether the Xinjiang strain of the early circulation or the Sichuan strains of the late circulation, they were all recombined with Sabin1 in the 3D region, and the recombination crossover sites were the same (nt6374–nt6381), which indicated that the recombination pattern can be fixed at the early circulation (Figure 2).

All 5 cVDPV2 strains isolated from this outbreak contained 2 nucleotide substitutions that were identified as key determinants of the attenuated phenotype of the Sabin 2 (an A-to-G reversion at nt481 in the 5'-untranslated region (UTR) and a U-to-C reversion at nt2909 encoding Ile to Thr substitution in VP1). Coinciding with the recombination pattern, the reverse mutation of attenuation sites can also be found in both early and late strains, which implicated that the reverse mutation can also be fixed at the early stage of the circulation.

Changes in Neutralizing Antigenic Sites

Comparing the amino acid sequences of the known neutralizing antigenic (NAg) sites among 5 strains in this study with Sabin 2 vaccine strain (GenBank accession number AY184220), we found that the early strain (Xinjiang strain) had no amino acid substitution, whereas the late strains (Sichuan strains) all had in NAg (Figure 3). Results showed that changes in NAg were introduced at the later stage during the circulation, which was different from the recombination pattern and the reverse mutation of attenuation sites.

Spatiotemporal Dynamic Transmission

A Bayesian phylogeographic tree in this study was constructed by the sequences at the VP1 region of 5 type 2 cVDPV strains. The phylogeographic tree showed that the initial infection of the cVDPV2 was approximately on April 15, 2016 (95% highest posterior density F, February 11, 2016–April 20, 2016) (Figure 4). The clock rate was 0.0156, and compared with the 0.011 total substations per site per year in previous studies, it conformed to the average evolution speed of ribonucleic acid viruses [15]. The results of the BF test showed that the BF value of Sichuan to Xinjiang was 0.12, whereas the BF value of Xinjiang to Sichuan was 1631.09. Based on this, we speculated that the virus was likely to spread from Xinjiang to Sichuan (Figure 5), although there is approximately 2851.2 kilometers between the 2 areas.

DISCUSSION

This outbreak of cVDPV2 is the first time that this has been discovered in China since the polio vaccination switch. It is interesting to note that this is also the first outbreak in which we

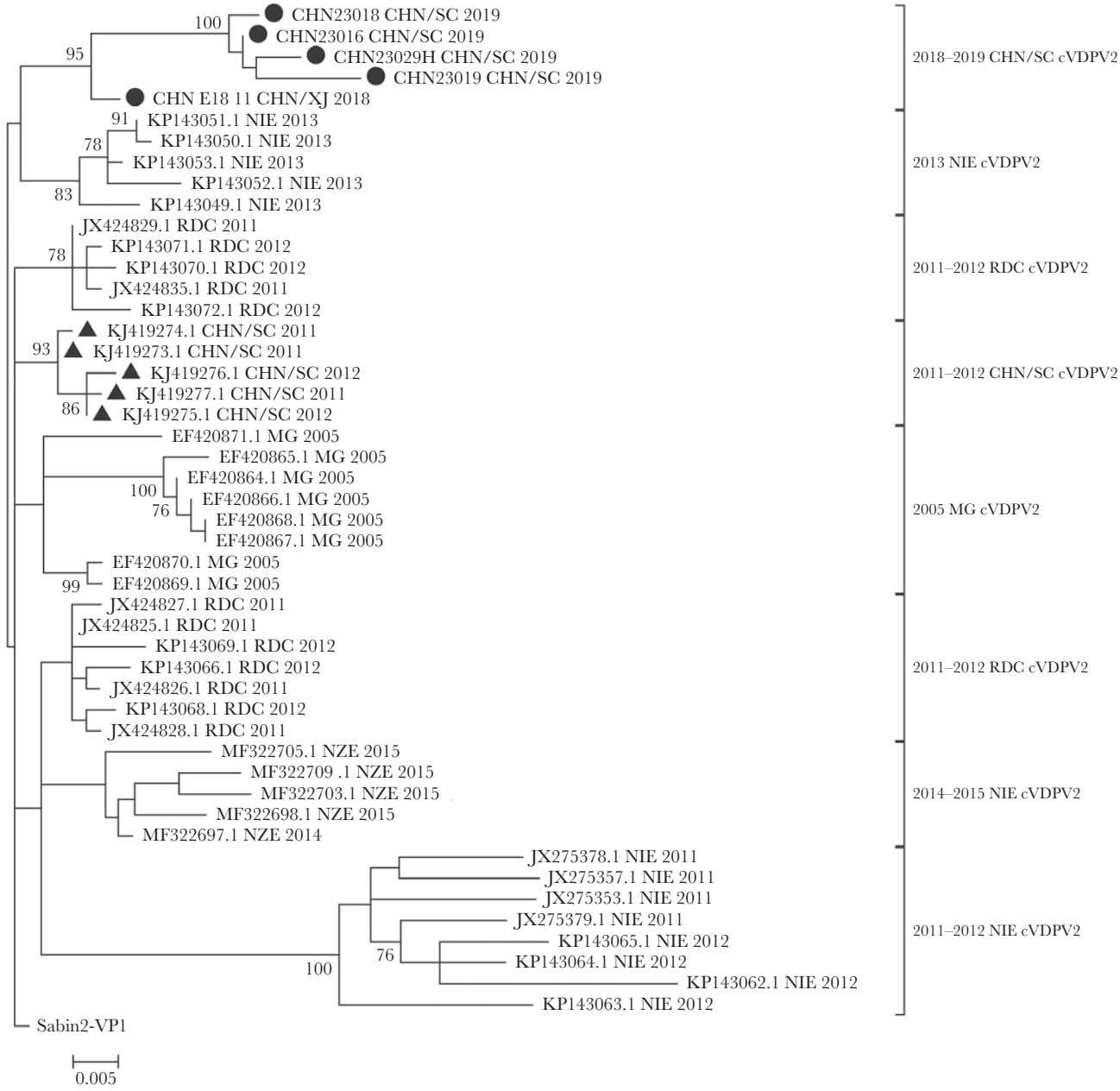

Figure 1. Maximum likelihood tree based on VP1 region between the Sichuan type 2 circulating vaccine-derived poliovirus (cVDPV2) strains and other cVDPV2 strains described previously. Bootstrap values lower than 75% are not shown at the branch nodes. ● indicated 5 strains obtained in this study; ▲ indicated the cVDPV2 strains isolated in Aba county of Sichuan in 2011–2012.

have isolated cVDPV2 in both the population and the environment. The finding that VDPVs circulating in the population were also isolated in environmental sewage has been reported in several countries. For example, the virus circulating in the population in the Sudan in September 2020 was also detected in sewage in Egypt. The cVDPV2 virus causing the large outbreak in the population in Afghanistan has been detected in sewage in 2 districts of the Islamic Republic of Iran, and it has also been detected in 2 AFP cases in Tajikistan [16]. These reports implicated that ES is useful in identifying VDPV circulation and responding to ongoing circulating VDPV outbreaks and may help in closing gaps in detecting polioviruses during the endgame of polio eradication.

There are many differences between this cVDPV2 outbreak and previous VDPV outbreaks found in China. For geographic scope, this outbreak involved a wider geographical range compared with previous outbreaks, which were all confined to a limited area. Except for the early strain from the sewage sample in Xinjiang, the other strains all have approximately 3% of the nucleotide substitution rate in the VP1. Compared with the previous VDPV outbreaks reported in China (Guizhou [1.0%–1.2%] [15], Guangxi [1.4%–2.2%]

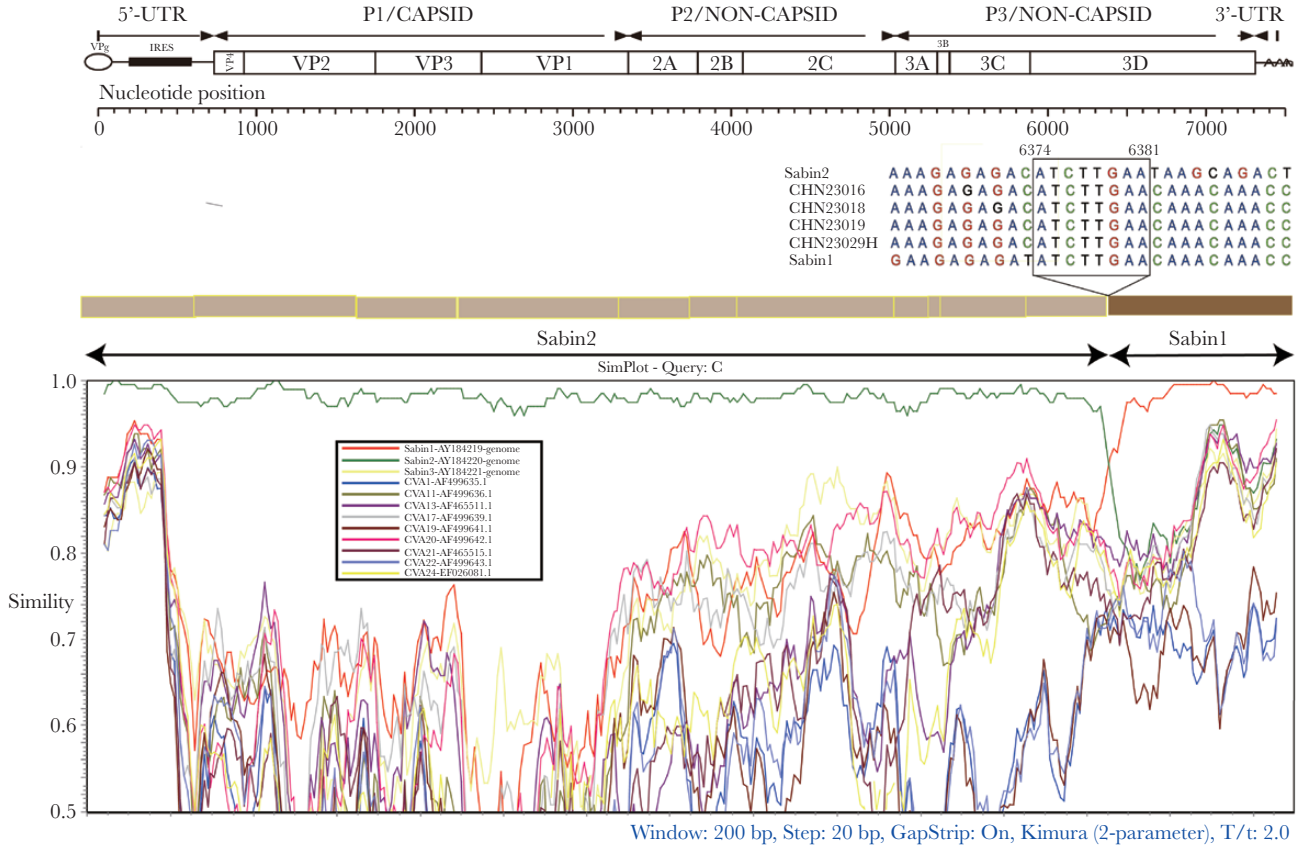

Figure 2. Recombination events predicted by SimPlot. The reference sequences of Sabin1(AY184219), Sabin2(AY184220), Sabin3(AY184220), CVA1(AF499635), CVA11(AF499636), CVA13(AF465511), CVA17(AF499639), CVA19(AF499641), CVA20(AF499642), CVA21(AF465515), CVA22(AF499643), AND CVA24(EF026081) were presented as putative parental strains.

[17], and Sichuan [0.6%–1.0%]) [18]) before the switch, the variability in VP1 region of this outbreak was much higher. The results of BEAST analysis showed that the virus had spread in the population for approximately 3 years before the AFP case was detected, compared with 1 year in Guizhou, 1.8 years in Guangxi, and 0.8 year in Sichuan in 2011–2012, and the discovery time of this cVDPV2 outbreak was significantly delayed, implicating that it is necessary to strengthen surveillance for AFP cases to rapidly detect and interrupt the transmission of cVDPVs.

During this cVDPV2 outbreak, 252 children <6 years old in the village and surrounding towns in Sichuan were surveyed to estimate the vaccine coverage. The results showed that 53.4% had no history of polio vaccination in the local area [19], which indicated that this outbreak is related to the low vaccine coverage. The loss of type 2 antibody after the switch may be

	VP2			VP3			VP1		
	NAg3b	NAg2	NAg2	NAg3a	NAg3a	NAg3b	NAg1	NAg2	NAg3a
	71	163	268	54	70	75	86	220	286
Sabin2	W R K	DTNATNPARN	P R T	PLNLTSQR	VELSD	TAHSD	AIIEVDNDAPTKRASRLFS	STEGD	KDGLT
CHN-E18-11-XJ2018	• • •	• • • • • • • • • •	• • •	• • • • • • • •	• • • • •	• • • • •	• • • • • • • • • • • • • • • • • • •	• • • • •	• • • • •
CHN23016	• • •	• • • • • • • • • •	• • •	• • • • • • • •	• • • N •	A • • • •	• • • • • • • • • • R • • • • • • •	• • • • •	• • • • •
CHN23018	• • •	• • • • • • • • • •	• • •	• • • • • • • •	• • • N •	A • • • •	• • • • • • • • • • R • • • • • • •	• • • • •	• • • • •
CHN23019	• • •	• • • • • • • • • •	• • •	• • • • • • • •	• • • • •	A • • • •	• • • • • • • • • • R • • • • • • •	• • • • •	• • • • •
CHN23029H	• • •	• • • • • • • • • •	• • •	• • • • • • • •	• • • • •	A • • • •	• • • • • • • • • • R • • • • • • •	• • • • •	• • • • •

Figure 3. Alignment of amino acids residues of neutralizing antigenic (NAg) sites for Sabin 2, Xinjiang, and Sichuan type 2 circulating vaccine-derived poliovirus (cVDPV2) strains. The NAg1(VP1:88–106), NAg2(VP2:163–169; VP2:268–270; VP1:220–225), NAg3a (VP3:54–61; VP3:70–74; VP1:286–291), and NAg3b (VP2:71–73; VP3:75–79).

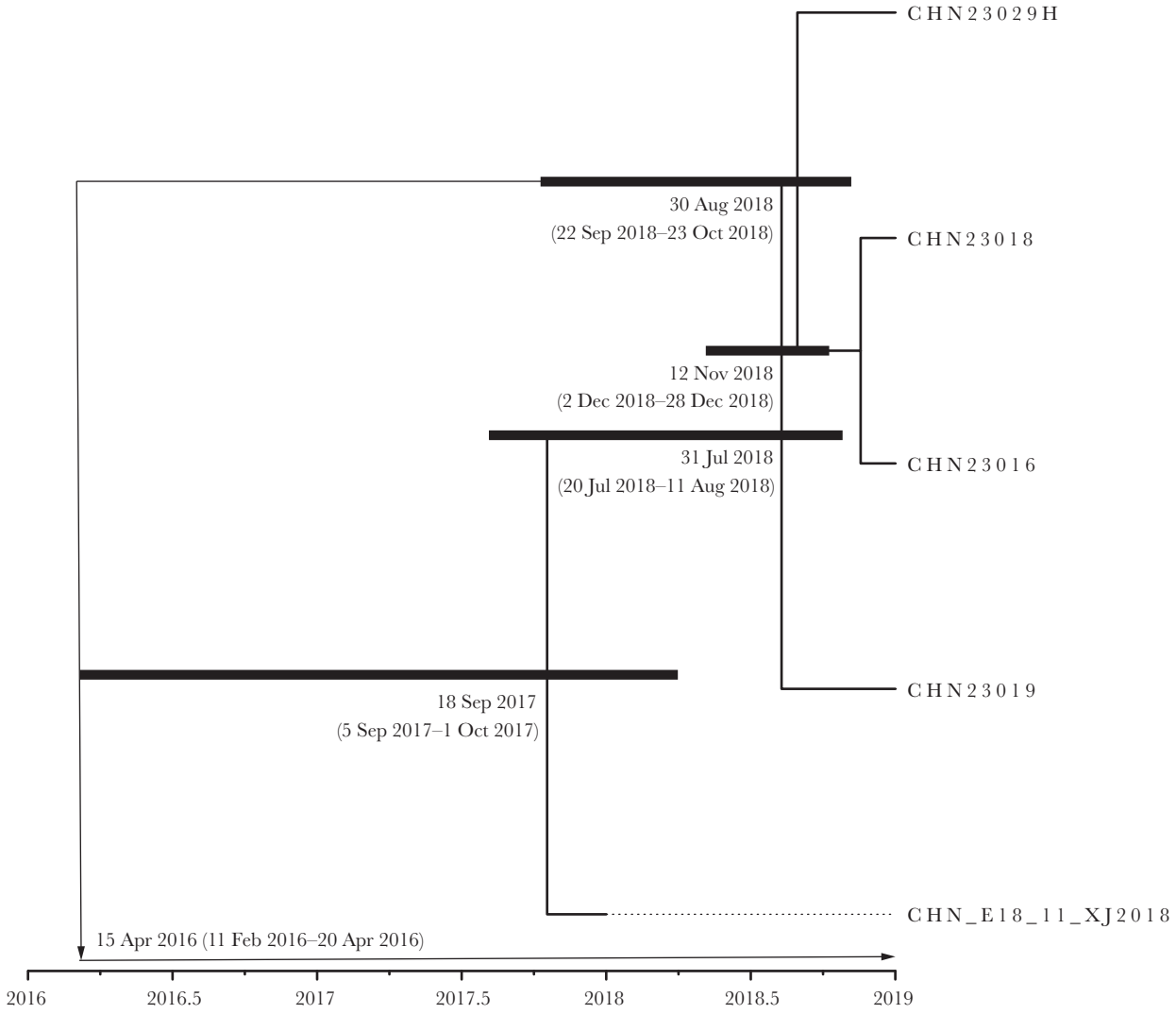

Figure 4. Bayesian Markov Chain Monte Carlo tree based on the complete VP1 region of 5 strains in this outbreak. The date of the divergence of different lineages were estimated by assuming a strict molecular clock.

another reason for this outbreak. A serological investigation performed by Yan et al [20] showed that the switch can provide a high-level immunity against types 1 and 3 but not against type 2, indicating a high risk of VDPV2 emergence and transmission. Moreover, a population-based serological study performed in Pakistan also confirmed this finding [21]. The complete VP1 phylogeographic tree showed that the initial infection of the cVDPV2 occurred on approximately April 15, 2016, which is earlier than the switch. In China, the switch came into effect in May 1, 2016, and all of the remaining stocks of tOPV have been destroyed. Meanwhile, OPV2 and type 2 potential infectious materials were contained, suggesting that theoretically there is no type 2 poliovirus in China after the switch. In addition, the mOPV2 that was used to respond to VDPV2 outbreaks, as recommended by the WHO, was never used in China. Therefore, we speculated that the cVDPV2 found in this study originated from previous tOPV recipients and spread among children with low levels of immunity against the type 2, which was probably due to the low immunization coverage in local area and the loss of type 2 antibody after the switch. To summarize, the insufficient immunization coverage and low-level immunity against the type 2 were the most important factors for this cVDPV2 outbreak.

Studies have shown that vaccine and/or nonvaccine recombination can often be observed in cVDPVs, and the recombination between VDPV and human enteroviruses C species (HEV-C) may promote the emergence of cVDPV [22]. For example, all cVDPVs described underwent recombination with HEV-C in Hispaniola, Egypt, Philippines, and Madagascar [23]. Thus far, the recombination of polioviruses and HEV-C has not been found in China. The previous reports in China assumed that the absence of HEV-C recombinants among the Guangxi cVDPV1 [17] and Sichuan cVDPV2 in 2011–2012 [18] and the nonrecombinant cVDPV1 from the 2004 Guizhou

Figure 5. The spatial diffusion of type 2 circulating vaccine-derived poliovirus (cVDPV2) virus on the standard map of China. This map comes from the standard map service system, China (http://bzdt.ch.mnr.gov.cn).

outbreak [15] was due to limited VDPV circulation. However, in this study, we found that the virus isolated from this outbreak did not recombine with other HEV-C, although they involved a wider geographical range (Xinjiang and Sichuan are 2851.2 kilometers apart) and longer circulation time. We speculated that the HEV-C carriage rate in the local region in Sichuan may be low and cannot provide the donor sequences.

The 481 A-to-G change in 5'-UTR and the amino acid substitution Ile143Thr in VP1 are well known to be the 2 major determinants of the attenuated phenotype of Sabin 2 [24]. In this study, 2 nucleotide substitutions at the nt481 (A-to-G) and nt2909 (U-to-C) were all found in the 5 strains, and cVDPVs reported previously in China also have reverse mutation at the 2 sites, which implied that the reverse mutation of the attenuation sites was fixed at the early stage of the circulation. From the results of changes in NAg, we noted that the mutations of the NAg sites were introduced over the circulation, which increased the risk of immune escape.

CONCLUSIONS

This outbreak have posed a great threat to the public health, we have immediately implemented effective measures to prevent the circulation of VDPV2, such as monitoring healthy children, conducting retrospective searches for AFP cases, and enhancing environmental surveillance for poliovirus. In addition, we have assessed polio vaccine coverage, and conducted 2 rounds of polio booster immunization for children in Leibo county and its areas. The discovery of this cVDPV2 outbreak by AFP surveillance combined with ES indicates that ES can provide a scientific basis for timely detection of the spread of VDPVs as well as AFP surveillance. The final poliovirus elimination has become increasingly complex while cVDPVs have emerged as a key challenge at the final stage of eradication [25], especially for many countries using OPV, where a series of social factors will affect the epidemic of cVDPVs. The AFP surveillance alone will lead to difficulties in the early detection dynamics of poliovirus transmission, so WHO has proposed ES for the timely detection of poliovirus. However, only 15 countries conduct ES in routine surveillance activities at this time, and most countries temporarily do not due to various barriers [26]. Before the occurrence of this cVDPV2 outbreak, China carried out routine environmental surveillance with a sampling frequency of once a month in 9 provinces where the risk of VDPV is high, including Xinjiang but not Sichuan. After this outbreak, the number of provinces carrying out ES has been increased to 13 and the sampling frequency has changed to once every 2 weeks. Given the ongoing cVDPV outbreaks in the world, ES presents an even

greater advantage in verifying cVDPVs and should be expanded geographically to further complement AFP surveillance.

Acknowledgments

Author contributions. H. Z. prepared the manuscript and all authors reviewed and approved it. D. Y., W. T., and W. X. contributed to the study design. X. M., H. T., C. W., N. C., W. K., Q. W., Q. Q., Y. L., Q. M., Q. Y. contributed to the samples collection. Y. Z., S. Z., J. L., D. W., X. L., and H. Z. contributed to the virus isolation and serotyping. H. Z. and J. X. contributed to the data analysis.

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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