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Short Communication

Circulating vaccine-derived polioviruses in the Extreme North region of Cameroon

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ABSTRACT

Background: The World Health Organization (WHO) poliovirus eradication program includes careful surveillance of acute-flaccid paralysis (AFP) and mass and routine immunization with oral polio vaccine (OPV). In populations with low vaccine coverage, the live-attenuated Sabin strains, OPV types 1, 2 and 3, can evolve into virulent vaccine-derived polioviruses (VDPVs) and circulate in the community. Until recently, circulating VDPVs (cVDPVs) had not been reported in Cameroon despite the fact that VDPV2 outbreaks have occurred in nearby countries.
Objectives: This study aimed to characterize virus isolates from four AFP patients infected with cVDPV2 in the Extreme North region of Cameroon in 2013.
Study design: The complete VP1 region of the four VDPV strains was sequenced and the relationships with cVDPVs from neighboring countries were investigated.
Results: All four patients were infected by cVDPV2 strains showing 1.2–2.0% nucleotide difference compared to the reference Sabin 2 VP1 sequence. Phylogenetic analysis indicated that the VDPV strains were genetically linked to cVDPV2 lineages of the recent Chad cVDPV2 outbreak.
Conclusions: The circulation of pathogenic VDPVs suggests that there are localized immunization gaps in some districts like Makary, Mada and Kolofata in Cameroon. To avoid poliomyelitis outbreaks in Cameroon, especially in the districts close to neighboring countries with ongoing cVDPV outbreaks, high polio vaccine coverage is essential.

1. Background

The Global Polio Eradication Initiative (PEI) is based on extensive vaccination campaigns with the oral polio vaccine (OPV) and efficient surveillance of acute flaccid paralysis (AFP) cases to determine the wild or vaccine-derived origin of isolated polioviruses (PVs). This strategy has led to a tremendous reduction in poliomyelitis incidence, from an estimated 350,000 in 1988 to less than 500 cases reported in 2013 (http://www.polioeradication.org/Dataandmonitoring/Poliothisweek.aspx). Trivalent OPV (tOPV) used in mass vaccination campaigns is made up of live attenuated viruses (Sabin strains of serotypes 1, 2 and 3) that are able to multiply to high titers in the gastrointestinal tract, thus inducing strong mucosal immunity. In addition to tOPV, bivalent OPV (bOPV) [composed of Sabin 1 and 3] or monovalent OPV (mOPV) [composed of Sabin 1 or 3] are used during Supplemental Immunization Activities (SIAs). bOPV, mOPV1 and mOPV3 are more effective in inducing immunity to types 1 and 3 because of the absence of interference by Sabin 2. However, low vaccine coverage allows for circulation and genetic drift of OPV strains and the emergence of pathogenic, vaccine-derived PVs (VDPVs). VDPVs are vaccine-related isolates that differ by >0.6% (VDPV serotype 2, VDPV2) or >1.0% (VDPV serotype 1 and 3, VDPV1 and VDPV3) in nucleotide (nt) identity compared to the corresponding OPV strain in the full-length sequence of the genomic region encoding the major viral capsid protein, VP1 (~900 nt) [1,2]. In addition to the

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Fig. 1. Map indicating the location of the 2013 type 2 circulating vaccine-derived polioviruses (cVDPVs) at the borders of Nigeria, Chad and Cameroon. The circles represent the cVDPVs, which are genetically linked to circulating VDPV2s originating in Chad. cVPDVs found in the Extreme North province of Cameroon originated from the districts of Makary, Mada, and Kolofata.

nt divergence criteria, circulating VDPV (cVDPV) specifically refers to VDPV strains for which epidemiologic and genetic evidence for person–person transmission in the community has been established. AFP surveillance and immunizations with OPV led to the interruption of transmission of indigenous wild PVs in Cameroon in 1999. In subsequent years, wild PV importations were reported in the Extreme North region of Cameroon [3] without re-established transmission. This part of Cameroon is a narrow region flanked by Nigeria and Chad with extensive human movement across the borders of the three countries (Fig. 1). Until recently, cVDPVs had not been reported in Cameroon despite the fact that VDPV2 outbreaks have occurred in nearby countries [2].

2. Objectives

From May to August 2013, VDPV2 was isolated from stool specimens from four AFP cases originating from three districts of the Extreme North region of Cameroon. We describe the characterization of the VDPV2 originating from these patients and their relationships with cVDPVs isolated in the neighboring countries.

3. Study design

3.1. Patients

All four AFP cases originated from the Extreme North region of Cameroon. The cases from Makary district had a history of immunization with trivalent OPV while other cases had no history of immunization with OPV containing type 2 (Table 1).

3.2. Specimens, virus isolation and intratypic differentiation

Stool samples were collected from each patient within 14 days of the onset of AFP; they were stored at +4 °C and subsequently sent to Centre Pasteur of Cameroon (CPC). Virus isolation and intratypic differentiation (ITD) of the isolates by real time RT-PCR (rRT-PCR) amplification were carried out according to standard WHO procedures [4,5].

3.3. Sequencing and phylogenetic analyses

Isolates were sent to the Centers for Disease Control and Prevention (Atlanta, USA) for sequencing, according to WHO guidelines. Sequencing was performed as described previously [6]. Full-length sequences of the VP1 capsid region were compared to the reference Sabin sequence. A phylogenetic radial tree was reconstructed by the neighbor-joining (NJ) method using *MEGA* version 5 bioinformatics software [7] with the Kimura two parameter algorithm for genetic distance determination. Full-length VP1 sequences were deposited in Genbank under accession numbers KP143045–KP143072 if they had not been deposited previously.

4. Results

Virus isolation on L20B cells followed by ITD showed that all four patients were infected with strains derived from Sabin 2. The VDPV rRT-PCR screening assay flagged them for sequencing. The sequencing of the genomic region encoding the VP1 capsid protein of the isolates from these patients confirmed that they had >0.6% nucleotides (nt) divergence from the Sabin 2 VP1 sequence and were therefore classified as VDPV2s: viruses CAE1318981 (18 nt substitutions), CAE1318982 (13 nt substitutions), CAE1318983 (12 nt substitutions), and CAE1318984 (11 nt substitutions) (Table 1). PVs accumulate nt substitutions at an estimated overall rate of about 1.1% per year [8]. With 1.2–2.0% differences in nucleotide identity compared to the Sabin 2 strain, the studied VDPVs had been circulating and/or replicating for longer than 1 year.

We investigated the phylogenetic relatedness among the Cameroon VDPVs and those originating from ongoing outbreaks in neighboring countries [8–10]. VDPV sequences from Kolofata (CAE1318983 and CAE1318984) were closely related to each other at 99.9% and both were genetically linked to viruses from the recent Chad outbreak (Fig. 2). The closest matching sequences were from cVDPV2 from northern Nigeria that had been imported previously imported from Chad [11] into Nigeria. Strains from the districts of Makary and Mada were also closely related to each other (99.45% nt identity). They were genetically linked to a cVDPV2 detected in eastern N'djamena in July 2012 during an outbreak in Chad (http://www.polioeradication.org/Dataandmonitoring/Poliothisweek/Circulatingvaccinederivedpoliovirus.aspx) (Fig. 2). Overall, our findings indicated that the studied patients were infected by circulating strains genetically linked to viruses from the Chad outbreak.

5. Discussion

Multiple VDPV2 emergences have been documented in northern states of Nigeria where they have been associated with large outbreaks from 2005 to the present [9,10,12]. cVDPVs were also reported in Chad during 2012 to 2013 [13,14]. Low polio vaccine coverage is the main factor favoring the emergence and circulation of VDPVs [2,15]. The national polio vaccine coverage rate was estimated to be about 80% from 2007 to 2010 [16]. Our finding of VDPVs that have been circulating for more than 1 year in the population without detection suggests that there were immunization gaps in some districts in the Extreme North of Cameroon in 2013.

The first patient was incompletely immunized with tOPV (2 of 4 required doses) while the three others had never received tOPV containing type 2 OPV, but they had received bOPV containing