HHS Public Access

Author manuscript

J Virol Methods. Author manuscript; available in PMC 2021 June 21.

Published in final edited form as:

J Virol Methods. 2014 March ; 197: 25–28. doi:10.1016/j.jviromet.2013.11.017.

Identification of vaccine-derived polioviruses using dual-stage real-time RT-PCR

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Abstract

Vaccine-derived polioviruses (VDPVs) are associated with polio outbreaks and prolonged infections in individuals with primary immunodeficiencies. VDPV-specific PCR assays for each of the three Sabin oral poliovirus vaccine (OPV) strains were developed, targeting sequences within the VP1 capsid region that are selected for during replication of OPV in the human intestine. Over 2400 Sabin-related isolates and identified 755 VDPVs were screened. Sensitivity of all assays was 100%, while specificity was 100% for serotypes 1 and 3, and 76% for serotype 2. The assays permit rapid, sensitive identification of OPV-related viruses and flag programmatically important isolates for further characterization by genomic sequencing.

Keywords

Poliovirus; Real-time RT-PCR; Vaccine-derived poliovirus; VDPV

The Global Polio Eradication Initiative (GPEI) is nearing its goal of interrupting all wild poliovirus (WPV) transmission (World Health Organization, 2013). By 2012, only three countries (Pakistan, Afghanistan, and Nigeria) had never interrupted WPV transmission. Apart from residual localized WPV circulation, all other poliovirus infections worldwide are associated with administration of the live, attenuated Sabin oral poliovirus vaccine (OPV) (Sutter et al., 2013). Most OPV infections are of short duration and confer protective immunity without complication. In rare instances (~1 case per 900,000 first doses administered) phenotypic reversion of the OPV strains may lead to vaccine-associated paralytic poliomyelitis (VAPP) in susceptible OPV recipients and close contacts (Sutter et al., 2013). Vaccine-related viruses excreted by patients with VAPP show only limited sequence divergence from the parental OPV strains. However, more highly divergent vaccine-derived polioviruses (VDPVs), indicative of prolonged replication or circulation, can arise under certain conditions (Kew et al., 2005; Centers for Disease Control and

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jviromet.2013.11.017.

Prevention, 2012). Circulating VDPVs (cVD-PVs) are associated with outbreaks in settings where the rates of OPV coverage are low (Kew et al., 2005; Centers for Disease Control and Prevention, 2012). cVDPV outbreaks of all three serotypes have been described, but >80% of isolates have been type 2 cVDPVs (cVDPV2s) (Centers for Disease Control and Prevention, 2012). New cVDPV outbreaks continue to emerge as OPV coverage declines in key high-risk countries and population immunity gaps widen, particularly to type 2 (Centers for Disease Control and Prevention, 2012).

Immunodeficiency-associated VDPVs (iVDPVs) have been isolated from individuals with primary immunodeficiencies (defects in antibody production) who have had prolonged infections, some exceeding 10 years, after exposure to OPV (DeVries et al., 2011; MacLennan et al., 2004). Although fewer than 65 persons have been found since 1961 to excrete iVDPVs, they are at risk of severe paralytic disease, and may serve as a potential source of poliovirus transmission in WPV-free settings (MacLennan et al., 2004; DeVries et al., 2011). Most of the known iVDPV infections have been reported from upper- and middle-income countries where patients have better access to supportive health care. The incidence of new iVDPV infections will likely decline as more countries shift from OPV to inactivated poliovirus vaccine (IPV) (World Health Organization, 2010; Vidor and Plotkin, 2013). Ambiguous VDPVs (aVDPVs) are VDPV isolates from the environment (e.g., sewage) or from persons with no known immunodeficiency and no known link to a VDPV from other than a close contact (Centers for Disease Control and Prevention, 2012). Ambiguous VDPVs continue to be detected in the environment in some settings (possibly signaling the presence of unidentified iVDPV excretors) and in patients with acute flaccid paralysis (AFP) (in some settings signaling early cVDPV emergence) (Centers for Disease Control and Prevention, 2012). The continued emergence of VDPVs highlights the need for sensitive surveillance, including improved virologic methods, to assure early detection.

VDPVs are defined as having >1% nucleotide (nt) sequence divergence (i.e., ≥10 nt substitutions) from their parental Sabin strains in the ~900-nt region encoding the major capsid protein, VP1(Kew et al., 2005; Centers for Disease Control and Prevention, 2012). This definition follows from the rapid rate of poliovirus nucleotide sequence evolution of ~1% year (Yang et al., 2005; Jorba et al., 2008; DeVries et al., 2011), and is consistent with at least one year of OPV virus replication (or circulation), considerably longer than the normal period of poliovirus excretion of 4–6 weeks (Alexander et al., 1997; Kew et al., 2005). The demarcation for VDPV2s has been lowered to >0.6% nt sequence divergence in VP1 (i.e., ≥6 nt substitutions) in view of the properties of cVDPV2 isolates from the outbreaks in Nigeria and the Democratic Republic of Congo (Centers for Disease Control and Prevention, 2012; Burns et al., 2013).

Following the 2000–2001 cVDPV1 outbreak in Hispaniola (Kew et al., 2002), the Global Polio Laboratory Network (GPLN) applied two different methods to screen for VDPVs in specimens from AFP patients: (1) one molecular method, usually diagnostic RTPCR (Yang et al., 1991; Kilpatrick et al., 1996, 1998, 2009), probe hybridization (De et al., 1995), or PCR-restriction fragment length polymorphism analysis (Balanant et al., 1991) to identify polioviruses by their genetic properties and (2) one antigenic method (usually an enzyme-linked immunosorbent assay [ELISA] using specific cross-adsorbed antisera) (van der

Avoort et al., 1995) to detect antigenic differences from the OPV strains. Because most VDPVs are antigenic variants (Kew et al., 2005), vaccine-related isolates having “non-Sabin-like”, “double-reactive”, or “nonreactive” antigenic properties were flagged as candidate VDPVs and further characterized by sequencing of the VP1 capsid region. This screening procedure substantially reduced the need for VP1 sequencing to identify VDPVs and led to the recognition of the cVDPV1 outbreak in the Philippines (Shimizu et al., 2004). However, early (2005–2006) VDPV2 isolates from Nigeria retained their “vaccine-like” antigenic properties in the ELISA, such that the routine screening algorithm did not initially signal any VDPV emergence (Burns et al., 2013). An outbreak was first suspected in 2006 by the frequent isolation from AFP patients of vaccine-related poliovirus type 2, with temporal and geographic clustering in the northern states where OPV coverage rates were known to be low (Wassilak et al., 2011; Burns et al., 2013). The inability of the combined molecular and antigenic assays to identify early outbreak isolates as VDPVs underscored the need for a more sensitive diagnostic method.

Our first approach to developing an RT-PCR method to screen for cVDPVs (Kilpatrick et al., 2004) took advantage of the observation that the large majority of cVDPVs are vaccine/nonvaccine recombinants having some or all of their noncapsid sequences derived from species C human enteroviruses (Kew et al., 2002; Rousset et al., 2003; Shimizu et al., 2004; Kew et al., 2005; Centers for Disease Control and Prevention, 2012), whereas iVDPVs were usually nonrecombinant or vaccine/vaccine recombinants (Kew et al., 2005; Centers for Disease Control and Prevention, 2012). Interpretation of the RT-PCR results, however, was complicated by the occurrence of crossovers in the 3D region at the primer target sequences among some minimally divergent vaccine/vaccine recombinants, and the occurrence of a small number of nonrecombinant cVDPVs (Liang et al., 2006; Yan et al., 2010). Therefore an alternative RT-PCR method targeting capsid region “hot spots” that typically revert in cVDPVs was developed. This new screening method is to be used to identify possible VDPVs once Sabin viruses have been identified using the standard intratypic differentiation methods (Kilpatrick et al., 2009).

The primary targets for each serotype were identified by sequence analysis as those codons frequently changed in VDPVs, compared with the reference Sabin virus sequences (Martín et al., 2000; Kew et al., 2002; Shimizu et al., 2004; Yakovenko et al., 2006; Jegouic et al., 2009; Burns et al., 2013) (Fig. 1). The probes target sites encoding VP1 amino acids 97–103 for Sabin 1 (primary target: aa 99), amino acids 142–147 (primary target: aa 143) for Sabin 2, and amino acids 285–290 (primary target: aa 290) for Sabin 3 target (Fig. 1). All of the targets encode amino acids in surface determinants (Minor, 1990) that are subject to strong selection during replication in the human intestine (Yakovenko et al., 2006). Flanking PCR primers (10 pmol each) were used in conjunction with FAM-labeled TaqMan® probes (Sabin 1, 5 pmol; Sabin 2 and Sabin 3, 20 pmol) in RT-PCR mixes described previously (Kilpatrick et al., 2009). Two stages of amplification were used following the initial cDNA synthesis step, with the first stage (five cycles of 95°C for 24s, 44°C for 30s, and 60°C for 24s) performed at lower annealing and extension temperatures than the second stage (40 cycles of 95°C for 15s, 50°C for 30s, and 65°C for 24s; Fig. 2S). This dual-stage rRT-PCR method increases the sensitivity of our degenerate poliovirus intratypic differentiation (ITD) assays by >10-fold (unpublished results). The dual-stage method also increased the

sensitivity of non-degenerate diagnostic assays (i.e., non-inosine-containing primers/probes), including the VDPV assays, by lowering the threshold of detection values (i.e. Ct values) and increasing the overall fluorescence.

These assays are indirect, yielding a fluorescence signal only if an isolate contains unmodified Sabin strain sequences at the primer and probe sites (Fig. S1, Table S1). A VDPV isolate would be expected to be identified as Sabin-related by the standard ITD assays (Yang et al., 1991; De et al., 1995; Kilpatrick et al., 1996, 1998, 2009), as those assays target sites that are usually invariant in Sabin-related isolates, including VDPVs, but differ in WPV isolates. Sabin-related isolates that yield no amplification curve by the rRT-PCR VDPV assay would be flagged as possible VDPVs, pending confirmation by sequencing of the VP1 region.

2428 Sabin-related isolates, which included “normal” Sabin-related isolates, cVDPVs, iVDPVs, and aVDPVs, all with known VP1 sequences, representing all three poliovirus serotypes were screened. All of the 755 well-characterized VDPVs tested were correctly identified (no false-negative results) (Table 1). The frequency of false positives varies by serotype and epidemiologic setting. The type 2 VDPV assay had a false-positivity rate of ~26% (i.e., a lack of amplication in a virus with ≤0.6% divergence), while the type 1 and type 3 assays had no false positives. In the type 2 Sabin vaccine strain, VP1-Ile143 is a known attenuation site and is also known to rapidly revert in primary vaccinees, even in the absence of more extensive sequence changes. All of the viruses that gave false-positive results in the type 2 assay had an amino acid substitution in VP1–143. False positives impose an additional sequencing workload for the laboratory, but do not constitute a serious public health concern. False negatives are of greatest concern to the GPEI because they could impede timely detection of cVDPV outbreaks, as occurred in Nigeria (Wassilak et al., 2011), but the data suggest that the overall false-negative rate is well below 1% (Table 1).

Since 2009, these rRT-PCR VDPV assays have been distributed to ~80 laboratories of the GPLN as kits for VDPV screening, as part of the WHO-standard ITD algorithm (Centers for Disease Control and Prevention, 2009). Experience of GPLN virologists has allowed assessment of the assay under routine diagnostic conditions. Use of the assays [7349 Sabin-relatedisolates were screened with VDPV assays in 2012 within the GPLN (Centers for Disease Control and Prevention, 2013)] has led to the recognition of cVDPVs outbreaks in several countries, including Afghanistan (VDPV2), the Democratic Republic of Congo (VDPV2), Ethiopia (VDPV2 and VDPV3), India (VDPV2), Mozambique (VDPV1), Nigeria (VDPV2), Pakistan (VDPV2), Somalia (VDPV2), and Yemen (VDPV2) (Centers for Disease Control and Prevention, 2012). Isolates from immunodeficient patients in Argentina (iVDPV1) and the United States (iVDPV2) were flagged as VDPVs by our assays (Centers for Disease Control and Prevention, 2009; DeVries et al., 2011).

The VDPV real-time RT-PCR assays described here provide a rapid, efficient method for identifying possible VDPVs among large collections of vaccine-related isolates. Our assays offer several advantages as diagnostic tests, including: (1) demonstrated capacity to detect early emergences of cVDPVs, for which timely intervention is critical to prevent further spread; (2) ready implementation in GPLN laboratories using standardized conditions for

amplification and product detection using equipment and reagents already available in the laboratories; (3) potential for sharp reduction in workload to sequence vaccine-related isolates to screen for cVDPVs; (4) increased sensitivity (at least for detecting type 2 VDPVs) than the ELISA assay in detecting early genetic changes associated with VDPV emergence; and (5) generation of accurate results on serotype mixtures without the need for neutralizing one or more of the serotypes in the mixture, as now required when using the ELISA assay (World Health Organization, 2004).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Naomi Dybdahl-Sissoko, Deborah Moore, and Shurwern Chern for preparation of poliovirus isolates for molecular analyses; Cara Burns and Elizabeth Henderson for management of our laboratory databases; and Tary Naguib, Laila El-Bassioni, Victoria Morris-Glasgow, Hiroyuki Shimizu, Nicksy Gumede, David Bukbuk, Festus Adu, and Adekunle Adeniji for sharing the cVDPV isolates with us.

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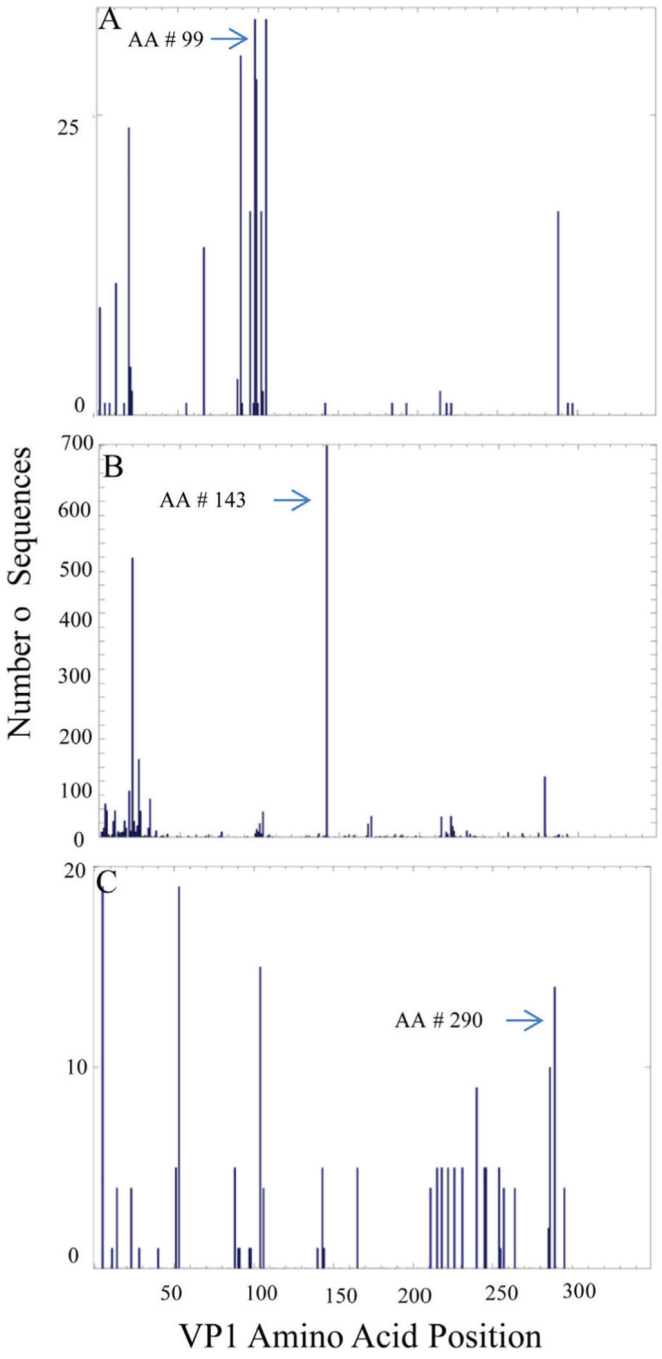

Fig. 1.

Frequency of changes at each VP1 amino acid position for 34 VDPV1s (A), 702 VDPV2s (B), and 19 VDPV3s (C). The TaqMan probes targeted the identified amino acid as well as amino acids either upstream or downstream to the primary target, depending on the serotype.

Table 1

VDPVs detected by RT-PCR assay.[a]

	Sequence +	Sequence −
A		
S1VDPV assay +	34	0
S1VDPV assay −	0	390
B		
S2VDPV assay +	702	217
S2VDPV assay −	0	688
C		
S3VDPV assay +	19	0
S3VDPV assay −	0	595

[a]A total of 2428 Sabin virus isolates were screened. The viruses that were flagged as possible VDPV due to a positive assay result (i.e. no signal for the VP1 target) were sequenced to confirm whether they are VDPVs (i.e., Sequence +). The assays were 100% specific and had a sensitivity of 100% for S1 and S3, and 76% for S2.