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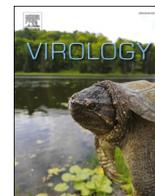
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Wide circulation of type 1 vaccine-derived poliovirus strains in clinical specimens from suspected cases of poliomyelitis, their contacts and in wastewater in Madagascar since late 2020

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ABSTRACT

Madagascar has faced three major outbreaks of vaccine-derived polioviruses (VDPVs) in recent decades, with VDPV type 1 reemerging in late 2020. Here, we report the molecular characterization of these cVDPV1 strains. WHO protocols were used for poliovirus detection in stool and wastewater samples. Molecular genotyping was based on the 5' non-coding (5'NC), VP1, and 3Dpol regions. From 2020 to 2022, 92 of 5690 stool samples and 129 of 1046 wastewater samples tested positive for cVDPV1. Genetic analysis of the VP1 gene revealed 1.3%–6.1% variability compared to the Sabin strain. Most sequences showed mutations at neurovirulence attenuation sites. Phylogenetic analysis distributed strains into four genogroups originating from Southern Madagascar. All analyzed cVDPV1 strains were recombinant, containing mutated oral polio vaccine sequences in VP1 and type C enterovirus sequences in other regions. This study demonstrated that all strains were closely related during this epidemic.

1. Introduction

Poliovirus (PV) is the causative agent of poliomyelitis, a paralytic disease affecting the nervous system (Baicus et al., 2006). PV belongs to the *Picornaviridae* family, *Enterovirus* genus, of the species *Enterovirus (EV) C*, and is divided into three serotypes: PV1, PV2 and PV3. Its virion is composed of a non-enveloped icosahedral capsid and a single-stranded positive-sense RNA genome of about 7500 nucleotides (nt) (Lentz et al., 1997). In the vast majority of cases, PV infection is inapparent or associated with mild symptoms. In very rare cases, it leads to poliomyelitis, a disease characterized by irreversible acute flaccid paralysis (AFP) that can affect all skeletal muscles. The location and extent of the paralysis vary, but the sequelae of the disease are usually severe. Paralysis is due to the destruction of motor neurons by replication of PVs if these reach the central nervous system (CNS). Poliomyelitis can thus be fatal when the paralysis affects the diaphragm muscles, in which case patients die of asphyxia (Doshi et al., 2011; Jungeblut et al.,

1951). Fortunately, poliomyelitis is among the vaccine-preventable diseases and can be stopped by the use of Sabin oral polio vaccines “OPV-Sabin” and/or Salk injectable polio vaccines “IPV-Salk”. The OPV-Sabin vaccine contains live attenuated (weakened) strains of poliovirus and is administered orally, while the IPV-Salk vaccine contains inactivated strains of poliovirus that cannot replicate and is administered intramuscularly (Ciapponi et al., 2019). Among these vaccines, the OPV-Sabin 1 vaccine was developed from the Mahoney neurovirulent strain of wild-type poliovirus type 1 (WPV1) by repeated passages in monkey tissues (Bessaud, 2021; Horaud and Albert, 1993). This vaccine strain differs from the wild type by around 56 mutated nucleotide positions scattered throughout the genome, responsible for 21 amino acid changes (Toyoda et al., 1984). Of these, six mutations, at nucleotide positions 480 of the 5' non-coding (5'NC) region, 935 of VP4, 2438 of VP3, 2795 and 2879 of VP1, and 6203 of 3Dpol, were found to be determinant sites of neurovirulence attenuation (Bouchard et al., 1995). Given that OPV-Sabin contains a live, attenuated version of PV, it

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can replicate in the human digestive tract and, on rare occasions, acquire mutations and spread to communities that are not adequately vaccinated. Mutations can accumulate over time as these viruses are transmitted from person to person, reaching the point where the strain once again acquires the ability to cause paralysis. Vaccine-derived polioviruses (VDPVs) are defined as vaccine strains that have accumulated at least 10 nucleotide substitutions ($\geq 1\%$) in the VP1 encoding sequence (about 900 nt in length) from parental OPV-Sabin 1 and 3, or 6 nucleotide substitutions ($\geq 0.6\%$) for OPV-Sabin 2. The circulation time of VDPVs is evaluated according to the number of mutations that accumulate in the VP1 region, considering that one percent of nucleotide divergence corresponds to approximately a year of circulation (Delpeyroux et al., 2013).

In 1988, the World Health Organization (WHO) adopted a worldwide polio eradication program during the World Health Assembly (WHA 41.28) (Tesfaye et al., 2020). Initially, the Global Polio Eradication Initiative (GPEI) had two tasks: mass vaccination and active surveillance of virus circulation from AFP cases to detect the presence of PV (Zeller et al., 1996; Lickness et al., 2020). Worldwide AFP surveillance was enrolled into a Global Polio Laboratory Network (GPLN) supervised by WHO (Diop et al., 2017). Then, the GPEI has implemented an environmental surveillance (ES) strategy to supplement the human AFP surveillance with enhanced sensitivity in detecting PVs in the absence of AFP cases (Asghar et al., 2014). ES thus assists in identifying residual WPVs and/or VDPVs excreted from asymptomatic sensitive population (<15 years old) (Mach et al., 2014).

To date, WPVs circulation persists in two endemic countries, Pakistan and Afghanistan (Kalkowska et al., 2023). In March 2022, an imported WPV1 was isolated in Malawi in a child under 5 years of age who developed an AFP syndrome on the November 19, 2021. This case was linked to a Pakistani strain detected in Sindh Province in 2020 (Rachlin et al., 2022; Hsu et al., 2020). This was the first imported WPV1 case in Africa since the continent was declared polio-free. WPVs are the best-known model to induce poliomyelitis, but VDPVs are another facet of PV that can cause paralytic polio and spread rapidly in the community (Minor, 2009). VDPVs have become more prevalent in most affected countries over the past decade, identified in the majority of AFP cases since 2016, due to low vaccination coverage (Lai et al., 2022).

Since the early 2000s, Madagascar has experienced three major epidemics of VDPV caused by VDPV type 2 and type 3, in 2001–2002 and 2005, respectively, and by VDPV1 in 2014–2015 (Rousset et al., 2003; Rakoto-Andrianarivelo et al., 2008; Diop et al., 2015). During these outbreaks, VDPV strains were only identified from human patients (AFP cases, contact cases and in the community). A new human case of VDPV1 was identified on the September 27, 2020 from a 4-year-old girl suspected of having AFP in the Farafangana district, in the Southeast region of Madagascar. Since then, circulating VDPV1 (cVDPV1) strains have spread across the island and have been detected in stool samples collected from AFP cases, their contacts and in the community, as well as in environmental wastewater samples. Here, we report their molecular characterization and phylogenetic relationships.

2. Materials and methods

2.1. Human sample collection

In Madagascar, AFP surveillance has been in place since 1996. Focal persons in health facilities or community informants (like traditional bone setters) are required to report every case to their respective district focal point. Stool samples are collected from suspected polio cases and/or contacts, or from the community, in one of the 3240 health centers spread across the 114 districts of Madagascar. For each stool sample collected, an AFP case investigation form (CIF) must be completed following WHO requirements (WHO, 2004).

From January 2020 to December 2022, 5690 stool samples from 1873 (two stool samples per case except for one case for which we only

received one sample, 65.8%) AFP cases, 1611 (28.3%) close contacts (one stool sample) and 334 (5.9%) healthy children (one stool sample) from the community were received and processed by the National Reference Laboratory (NRL) for poliomyelitis hosted by the Virology Unit at the Institut Pasteur de Madagascar (IPM). Extracts of stool specimens were treated with chloroform 99%–99.4% ethanol-stabilized (AnalaR NORMAPUR®ACS; Ref. 22711.290) before being inoculated onto RD and L20B cell lines (see below) following the standard operating procedures (SOPs) described in the WHO GPLN laboratory manual (WHO, 2004).

2.2. Environmental wastewater distribution

Environmental surveillance (ES) has been implemented in Madagascar since August 2015. Twenty-eight functional sampling sites are distributed across seven zones: nine in Antananarivo-Renivohitra (ANT; Center, capital city), five in Mahajanga I (MAH; North-West), three in Toliary I (TOL; South-West), three in Taolagnaro (TLG; South-East), three in Antsiranana I (ATI; North); three in Toamasina I (TOA; East) and two in Antsohihy (ATS; North-West) (Fig. 1).

From January 2021 to December 2022, the ES laboratory has received and processed a total of 1046 wastewater samples, of which 396 came from ANT, 220 from MAH, 132 from TOL, 129 from TLG, 78 from ATI, 51 from TOA and 40 from ATS (Fig. 2). In January 2021, cVDPV1 was detected for the first time in wastewater samples.

2.3. Environmental wastewater sample processing

In accordance with WHO protocols, one and a half liters of raw wastewater were collected by grab method with a bucket and conserved in a sterile bottle. Samples collected according to schedule were immediately stored in a cool box with cold accumulators and shipped to the NRL. Five hundred milliliters of raw wastewater samples were

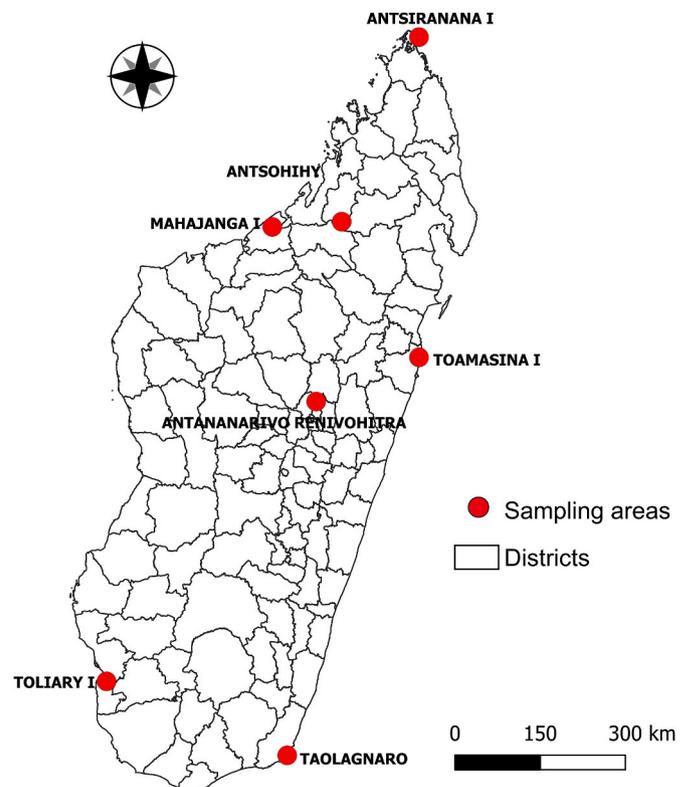


Fig. 1. Madagascar environmental surveillance collection sites: 28 sampling sites are distributed in seven provinces of Madagascar. Red dots represent the sampling areas.

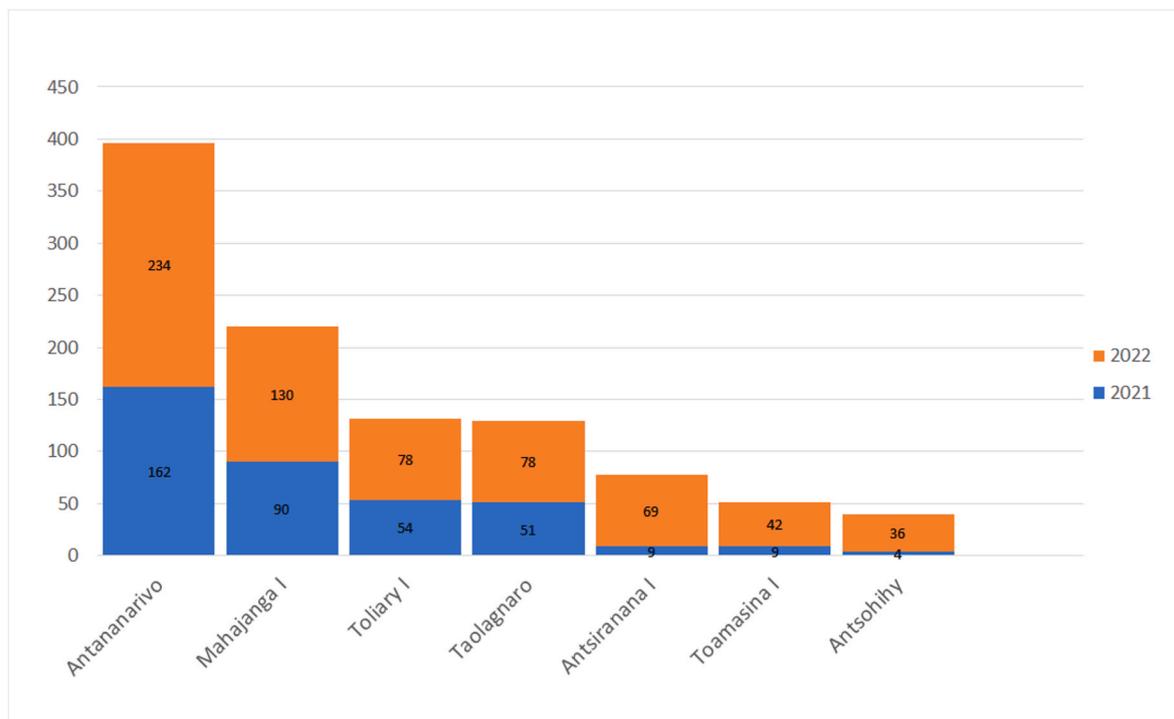


Fig. 2. Madagascar collected wastewater samples: Graphical representation of the number of environmental samples collected in the seven areas in 2021 and 2022.

concentrated (50–100-fold) by the two-phase separation method (WHO, 2015), with the remaining volume stored at -20°C until final results were available. The precipitation technique was used to separate the crude sample into an aqueous (upper) phase attracted by dextran T40 (Pharmacosmos, Ref. 551000409001). The organic (lower) phase settles to the bottom of the separatory funnel under the action of polyethylene glycol 6000 (Sigma Aldrich, Ref. 81260). This technique combines both high-speed centrifugation (1500 g; 20 min) and cold decantation ($+4^{\circ}\text{C}$, overnight). As with AFP stool samples, the concentrate was treated with chloroform (Sigma-Aldrich, Ref. 32211), 100 IU/ml of penicillin and 100 $\mu\text{g}/\text{ml}$ of streptomycin (Gibco, Ref. 15140-148), 250 $\mu\text{g}/\text{ml}$ of amphotericin B (Gibco, Ref. 15290-026) and 10 mg/ml gentamycin (Gibco, Ref. 15140-049). From the 500 ml of wastewater, final volumes of 6–15 ml of concentrates were obtained.

2.4. Cell lines and virus isolation

Human rhabdomyosarcoma (RD) and mouse fibroblast (L20B) cell lines were used in this study. L20B is a genetically engineered mouse cell line expressing the PV human receptor CD155. The two cell lines were grown as monolayers in Minimum Essential Medium (MEM) Eagle without L-Glutamine (Sigma Aldrich, Ref. M2279-1L), 10% of Fetal Bovine Serum (FBS) (Gibco, Ref. 26140-079), 1% of L-Glutamine (Sigma Aldrich, Ref. G7513), 1% of Hepes (Dominique Dutscher, Ref. L0180-100), 1% of amphotericin B (Pan Biotech, Ref. P06-01100), and 1% of penicillin-streptomycin (Gibco, Ref. 15140-122).

SOPs described in the WHO-GPLN laboratory manual were used for cell line propagation and virus culture (WHO, 2004). Inoculated monolayer cells were microscopically checked for 5 days to detect the appearance of a cytopathogenic effect (CPE). To increase the sensitivity of human AFP virus isolation, a blind passage (inoculation into the same cell line) was carried out using 0.2 ml of the supernatant of inoculated cultures that had remained negative (no CPE) into Nunc sterile flat-bottomed screw-top culture tube (Dutscher, Ref. 055054), and newly inoculated cells were then monitored for a further 5 days. Regarding virus isolation from environmental samples, at least 3 ml of wastewater concentrate were inoculated into eight cell culture flasks

(T25 cm^2 , Corning, Ref. 430168): five with L20B cells and three with RD cells. A daily microscopic monitoring was carried out to follow the evolution of CPE for a maximum of 14 days.

All viruses isolated from RD cells were systematically inoculated onto L20B cells, and the resulting PV isolates were further typed by the Intratypic Differentiation (ITD) assay (see below). Isolates showing CPE only on RD but not on L20B cells were considered non-poliovirus enteroviruses (NPEVs). However, our study focused on samples with a suspected polio result *i.e.* only those presenting CPE on the L20B cell line during the study period.

2.5. RNA extraction

RNAs from all samples (supernatant isolates) were extracted using QIAamp viral RNA mini kit (Qiagen, France) according to the manufacturer's instructions. The extracted RNA was eluted in 65 μl of RNase-free water.

2.6. Molecular characterization by the ITD assay

Quantitative ITD real time RT-PCR (rRT-PCR) assay was used to differentiate WPV and Poliovirus "Sabin-Like" (PV-SL) strains. Eleven targets were screened through a series of one quadruplex (EV + Sabin1 + Sabin2 + Sabin3), two duplex (WPV1 + Q-Beta and nOPV-1b + nOPV-2a) and three simplex (Pan-PV, PV type 2 and WPV3) rRT-PCRs, as described previously (Adedeji et al., 2012; Gerloff et al., 2018; Shaw, 2020).

The ITD rRT-PCRs were performed on an Applied Biosystems 7500 Real Time PCR System (ABI 7500, ThermoFisher Scientific, Waltham, MA) according to the ITD version 6.0 program. Each rRT-PCR mix was composed of 10 μl of qScript™ XLT One-Step rRT-PCR ToughMix (Quanta Biosciences, Beverly, MA), 1 μl of primers/probes mix (supplied by the Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA), 8 μl of RNase-free water, and 1 μl of RNA template (from culture supernatant). Thermocycling conditions were 30 min at 50°C for the reverse transcription step and 1 min at 95°C , followed by 40 cycles at 95°C for 15 s, 50°C for 45 s, and 72°C for 5 s. Fluorescence acquisition

was initiated during the hybridization and a ramp rate reduction of 25% between annealing and elongation steps was applied.

2.7. Vaccine-derived poliovirus (VDPV) assay

VDPV rRT-PCR assay was used to distinguish Sabin Like (SL) and Non-Sabin-Like (NSL) strains. Two primers and probes were used such as “S1-VDPV” and “S3-VDPV”. As the ITD assay, VDPV rRT-PCR mix was composed of 10 µl of qScript™ XLT One-Step ToughMix, 1 µl of primers/probes mix, 8 µl of RNase-free water, and 1 µl of RNA template. Thermocycling conditions were 30 min at 50 °C for the reverse transcription step and 1 min at 95 °C, followed by 40 cycles at 95 °C for 15 s, 50 °C for 45 s, and 72 °C for 5 s. Negative VDPV-SL1 or VDPV-SL3 assays were considered as non-Sabin-like and reported as PV discordant (PV disc.) for SL1 or SL3. ITD/VDPV assays thus allowed to distinguish between PV types such as WPV, PV-SL, and PV disc. or PV-NSL.

2.8. 5'NC and 3Dpol sequence amplification

To obtain the 5'NC and 3Dpol sequences, all samples with a PV disc. result were reverse transcribed with random primers p(dN)₆ (Roche Diagnostics, Ref. 11034731001) and Superscript™ II reverse transcriptase (Invitrogen, Ref. 18064022). Then, cDNA fragments were amplified by PCR using the EV2/EV3 and EUC12d/UG7A primers targeting a portion of the 5'NC and 3Dpol sequence, respectively, as previously described (Kumar et al., 2013; Oprisan et al., 2002).

2.9. Sequencing and phylogenetic analysis

The full-length coding sequence of VP1 (906 nucleotides in size from position 2480 to 3385 according to Sabin 1 reference sequence, GenBank accession number AY184219) and of partial 5'NC and 3Dpol sequences were obtained by Sanger sequencing following the procedure from CDC (Atlanta, GA, USA). Sequencing was performed in both directions (forward and reverse) with the primers used for the PCRs. Every specimen with PV2, PV-NSL or PV disc. results were mandatory sent for VP1 sequencing to the Regional Reference Laboratory (RRL) at the National Institute for Communicable Diseases (NICD), Johannesburg, South Africa. However, 5'NC and 3Dpol amplified products were sent for sequencing to GENEWIZ® from Azenta Life Sciences (Takeley, UK).

Raw sequences were edited and analyzed by using CLC main workbench v8.1 (www.qiagenbioinformatics.com). Then, multiple sequence alignments of VP1 full-length coding sequences and of partial 5'NC and 3Dpol sequences were constructed using CLC software. Pairwise sequence identity (at the nucleotide level) of the VP1 sequences was calculated with MEGA X (www.megasoftware.net) using the Maximum Composite Likelihood (MCL) estimation approach (Tamura et al., 2007). For phylogenetic analysis, a maximum-likelihood tree was constructed using MEGA X (Kumar et al., 2018), with a kimura-2 parameter model and a bootstrap of 1000 replications. The OPV-Sabin 1 (AY184219) reference strain was used to root the tree.

2.10. GenBank accession numbers

Full-length VP1 sequences (906 nt) of ninety-two human (AFP cases, contact cases and from the community) and one hundred-fourteen environmental cVDPV1 isolates described here were deposited to the GenBank database under accession numbers OR778108 to OR778199 and OQ455057 to OQ455170, respectively.

In addition, thirty-five partial 5'NC (398 nt) sequences from 16 human and 19 environmental cVDPV1 isolates and forty-one 3Dpol (842 nt) sequences from 20 human and 21 environmental cVDPV1 isolates were also deposited to the GenBank database under accession numbers OR778235 to OR778275 and OR778200 to OR778234, respectively.

3. Results

3.1. Enterovirus identification and characterization

Between 2020 and 2022, a total of 5690 stool samples were collected. Of these, 3745 (65.8%) were from suspected AFP cases, 1611 (28.3%) were from close contacts of index AFP cases, and 334 (5.9%) were from healthy children living in the community. The overall positivity rate for human EVs was 16.6% (n = 942/5690). Among AFP cases, 15.4% (n = 576/3745) were positive for EV, compared to 16.5% (n = 266/1611) of close contacts and 29.9% (n = 100/334) of healthy children (Table 1). Out of the positive EVs, 27.3% (n = 257/942) of stool samples were suspected of containing PV. This included 190 (73.9%) from AFP cases, 43 (16.7%) from contacts, and 24 (9.3%) from healthy children. The remaining 72.7% (n = 685/942) EV positive samples were NPEVs. This included 386 (56.4%) from AFP cases, 223 (32.6%) from close contacts, and 76 (11.1%) from healthy children living in the community (Table 2). Finally, out of the human samples suspected of containing PV, 36.2% (n = 93/257) gave a PV1 disc. (evocative of mutated PV1 vaccine strains) result and 59.5% (n = 153/257) were confirmed as PV-SL. In addition, 1.2% (n = 3/257) of those samples were confirmed as NPEV and 3.1% (n = 8/257) gave a non-enterovirus (NEV) result after ITD/VDPV testing (Table 3). The PV1 disc. isolates were spotted on FTA cards and sent to the RRL (NICD) for sequencing targeting the VP1 region. Ninety-two (98.9%) of them were confirmed as cVDPV1, showing from 13 (1.4%) to 50 (5.5%) nucleotide substitutions compared to the reference strain OPV-Sabin 1 (AY184219).

In terms of ES, 1046 wastewater samples were collected over the 2021–2022 period: 379 (36.2%) in 2021 and 667 (63.8%) in 2022, respectively. Of these, 815 (77.9%) were positive for EV. In 2021, the highest number of positive samples (48.9%; n = 159/325) was observed in Antananarivo-Renivohitra (ANT), where the number of functional sites is the highest (9 sites), followed by Mahajanga I (MAH) (24.0%; n = 78/325), the town of Toliary I (TOL) (12.6%; n = 41/325), and Taolagnaro (TLG) (10.2%; n = 33/325). The last three study areas revealed a low EV detection rate: 1.8% (6/325) for Toamasina I (TOA), 1.5% (5/325) for the town of Antsiranana I (ATI), and 0.9% (3/325) for the town of Antsohihy (ATS). In 2022, ES results showed the same trend, with a high positivity rate of 47.3% (n = 232/490) in ANT, followed by 21.2% (n = 104/490) in MAH, 9.8% (n = 48/490) in TOL, then 9.2% (n = 45/490) in TLG and finally 8.0% (n = 40/490) in the town of ATI. ATS and TOA still revealed a low EV detection rate of 2.2% (n = 11/490) for TOA and 2.0% (n = 10/490) for ATS (Table 4). Of the EV-positive samples, 425 (52.1%) were confirmed as non-polio enteroviruses (NPEVs), 238 (29.2%) were a mixture of NPEVs and suspected PVs, and 152 (18.7%) were suspected to contain only PVs (Table 5). No wild polioviruses or PV2 were detected.

3.2. VP1 sequencing

Among the PV1 disc. samples identified from human surveillance (HS), one (1.1%) isolate was confirmed as “Sabin-like” type 1 and 92 (98.9%) were cVDPV1 (4 in 2020, 47 in 2021 and 41 in 2022). Fig. 3 shows the geographic location of the VDPV1 strains detected (Fig. 3).

In the meantime, 453 PV1 disc. isolates from 208 wastewater samples (42 in 2021 and 166 in 2022) suspected of containing a PVs were sent to the RRL at the NICD for sequencing. Of these, 411 isolates (90.7%) from 129 environmental samples were confirmed as cVDPV1 (87 in 2021 and 324 in 2022) (Figs. 3), 18 (4%) were identified as PV-SL1 (8 in 2021 and 10 in 2022), five (1.1%) as PV-SL3 (all in 2021), a further 10 (2.2%) were sent to the CDC in Atlanta for genotype confirmation, while the remaining nine (2%) were non-amplifiable and reported as negative after retesting (all in 2022; data not shown).

Table 1
Human enteroviral positivity rate in Madagascar (2020–2022).

	AFP Cases		Close contacts		Community		Total	
	Stool collected	Pos. EV (%)	Stool collected	Pos. EV (%)	Stool collected	Pos. EV (%)	Total stool collected	Total Pos. EV (%)
2020	1255	143 (11.4)	1220	159 (13.0)	18	9 (50.0)	2493	311 (12.5)
<i>Antananarivo (ANT)</i>	292	28 (9.6)	172	18 (10.5)	0	0 (0.0)	464	46 (9.9)
<i>Antsiranana (ATI)</i>	109	14 (12.8)	107	9 (8.4)	0	0 (0.0)	216	23 (10.6)
<i>Fianarantsoa (FIA)</i>	182	13 (7.1)	214	21 (9.8)	0	0 (0.0)	396	34 (8.6)
<i>Mahajanga (MAH)</i>	202	29 (14.4)	210	32 (15.2)	0	0 (0.0)	412	61 (14.8)
<i>Toamasina (TOA)</i>	148	9 (6.1)	109	15 (13.8)	0	0 (0.0)	257	24 (9.3)
<i>Toliary (TOL)</i>	322	50 (15.5)	408	64 (15.7)	18	9 (50.0)	748	123 (16.4)
2021	1210	209 (17.3)	262	74 (28.2)	186	58 (31.2)	1658	341 (20.6)
<i>Antananarivo (ANT)</i>	290	38 (13.1)	21	7 (33.3)	0	0 (0.0)	311	45 (14.5)
<i>Antsiranana (ATI)</i>	112	15 (13.4)	7	3 (42.9)	19	4 (21.1)	138	22 (15.9)
<i>Fianarantsoa (FIA)</i>	206	40 (19.4)	42	3 (7.1)	0	0 (0.0)	248	43 (17.3)
<i>Mahajanga (MAH)</i>	233	33 (14.2)	87	29 (33.3)	84	26 (31.0)	399	88 (21.8)
<i>Toamasina (TOA)</i>	137	19 (13.9)	13	5 (38.5)	0	0 (0.0)	150	24 (16.0)
<i>Toliary (TOL)</i>	232	64 (27.6)	92	27 (29.3)	83	28 (33.7)	401	119 (29.2)
2022	1280	224 (17.5)	129	33 (25.6)	130	33 (25.4)	1539	290 (18.8)
<i>Antananarivo (ANT)</i>	346	50 (14.5)	7	2 (28.6)	17	6 (35.3)	368	58 (15.7)
<i>Antsiranana (ATI)</i>	88	20 (22.7)	27	8 (29.6)	20	9 (45.0)	132	37 (27.4)
<i>Fianarantsoa (FIA)</i>	238	19 (8.0)	36	5 (13.9)	19	5 (26.3)	293	29 (9.9)
<i>Mahajanga (MAH)</i>	196	39 (19.9)	5	2 (40.0)	20	2 (10.0)	221	43 (19.5)
<i>Toamasina (TOA)</i>	146	28 (19.2)	16	1 (6.3)	0	0 (0.0)	162	29 (17.9)
<i>Toliary (TOL)</i>	266	68 (25.6)	38	15 (39.5)	54	11 (20.4)	358	94 (26.3)
Total	3745	576 (15.4)	1611	266 (16.5)	334	100 (29.9)	5690	942 (16.6)

EV: Enterovirus; Pos.: Positive; Neg.: Negative.

Table 2
Human enterovirus infection results with cytopathogenic effect positive on L20B and RD cell lines from 2020 to 2022.

	AFP Cases		Close contacts		Community		Total		
	Susp. PV (L20B +)	NPEV (RD +)	Susp. PV (L20B +)	NPEV (RD +)	Susp. PV (L20B +)	NPEV (RD +)	Total Susp. PV (L20B +)	Total NPEV (RD +)	Total Pos. EV
2020	11 (45.8)	132 (46.0)	13 (54.2)	146 (50.9)	0 (0.0)	9 (3.1)	24 (7.7)	287 (92.3)	311
<i>Antananarivo (ANT)</i>	0 (0.0)	28 (62.2)	1 (100.0)	17 (37.8)	0 (0.0)	0 (0.0)	1 (2.2)	45 (97.8)	46
<i>Antsiranana (ATI)</i>	1 (100.0)	13 (59.1)	0 (0.0)	9 (40.9)	0 (0.0)	0 (0.0)	1 (4.3)	22 (95.7)	23
<i>Fianarantsoa (FIA)</i>	6 (85.7)	7 (25.9)	1 (14.3)	20 (74.1)	0 (0.0)	0 (0.0)	7 (20.6)	27 (79.4)	34
<i>Mahajanga (MAH)</i>	0 (0.0)	29 (47.5)	0 (0.0)	32 (52.5)	0 (0.0)	0 (0.0)	0 (0.0)	61 (100.0)	61
<i>Toamasina (TOA)</i>	0 (0.0)	9 (37.5)	0 (0.0)	15 (62.5)	0 (0.0)	0 (0.0)	0 (0.0)	24 (100.0)	24
<i>Toliary (TOL)</i>	4 (26.7)	46 (42.6)	11 (73.3)	53 (49.1)	0 (0.0)	9 (8.3)	15 (12.2)	108 (87.8)	123
2021	94 (69.6)	115 (55.8)	27 (20.0)	47 (22.8)	14 (10.4)	44 (21.4)	135 (39.6)	206 (60.4)	341
<i>Antananarivo (ANT)</i>	4 (100.0)	34 (82.9)	0 (0.0)	7 (17.1)	0 (0.0)	0 (0.0)	4 (8.9)	41 (91.1)	45
<i>Antsiranana (ATI)</i>	4 (57.1)	11 (73.3)	0 (0.0)	3 (20.0)	3 (42.9)	1 (6.7)	7 (31.8)	15 (68.2)	22
<i>Fianarantsoa (FIA)</i>	19 (100.0)	21 (87.5)	0 (0.0)	3 (12.5)	0 (0.0)	0 (0.0)	19 (44.2)	24 (55.8)	43
<i>Mahajanga (MAH)</i>	20 (55.6)	13 (25.0)	11 (30.6)	18 (34.6)	5 (13.9)	21 (40.4)	36 (40.9)	52 (59.1)	88
<i>Toamasina (TOA)</i>	9 (90.0)	10 (71.4)	1 (10.0)	4 (28.6)	0 (0.0)	0 (0.0)	10 (41.7)	14 (58.3)	24
<i>Toliary (TOL)</i>	38 (64.4)	26 (43.3)	15 (25.4)	12 (20.0)	6 (10.2)	22 (36.7)	59 (49.6)	60 (50.4)	119
2022	85 (86.7)	139 (72.4)	3 (3.1)	30 (15.6)	10 (10.2)	23 (12.0)	98 (33.8)	192 (66.2)	290
<i>Antananarivo (ANT)</i>	9 (81.8)	41 (87.2)	0 (0.0)	2 (4.3)	2 (18.2)	4 (8.5)	11 (19.0)	47 (81.0)	58
<i>Antsiranana (ATI)</i>	13 (81.3)	7 (33.3)	0 (0.0)	8 (38.1)	3 (18.8)	6 (28.6)	16 (43.2)	21 (56.8)	37
<i>Fianarantsoa (FIA)</i>	8 (100.0)	11 (52.4)	0 (0.0)	5 (23.8)	0 (0.0)	5 (23.8)	8 (27.6)	21 (72.4)	29
<i>Mahajanga (MAH)</i>	14 (87.5)	25 (92.6)	2 (12.5)	0 (0.0)	0 (0.0)	2 (7.4)	16 (37.2)	27 (62.8)	43
<i>Toamasina (TOA)</i>	9 (90.0)	19 (100.0)	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (34.5)	19 (65.5)	29
<i>Toliary (TOL)</i>	32 (86.5)	36 (63.2)	0 (0.0)	15 (26.3)	5 (13.5)	6 (10.5)	37 (39.4)	57 (60.6)	94
Total	190 (73.9)	386 (56.4)	43 (16.7)	223 (32.6)	24 (9.3)	76 (11.1)	257 (27.3)	685 (72.7)	942

Susp.: Suspected; PV: Poliovirus; Pos.: positive; NPEV: Non-polio enterovirus; L20B: murine cell line with CD155 receptor; RD: Human rhabdomyosarcoma cell line.

Table 3
RT-qPCR ITD/VDPV results from human specimens (2020–2022).

	PV1 disc.	PV-SL	NPEV	NEV	Total Susp. PV
2020	2 (8.3)	19 (79.2)	2 (8.3)	1 (4.2)	24 (9.3)
<i>Antananarivo (ANT)</i>	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (0.4)
<i>Antsiranana (ATI)</i>	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (0.4)
<i>Fianarantsoa (FIA)</i>	0 (0.0)	5 (71.4)	2 (28.6)	0 (0.0)	7 (2.7)
<i>Mahajanga (MAH)</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Toamasina (TOA)</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Toliary (TOL)</i>	2 (13.3)	13 (86.7)	0 (0.0)	0 (0.0)	15 (5.8)
2021	52 (38.5)	82 (60.7)	1 (0.7)	0 (0.0)	135 (52.6)
<i>Antananarivo (ANT)</i>	0 (0.0)	4 (100.0)	0 (0.0)	0 (0.0)	4 (1.6)
<i>Antsiranana (ATI)</i>	5 (71.4)	2 (28.6)	0 (0.0)	0 (0.0)	7 (2.7)
<i>Fianarantsoa (FIA)</i>	0 (0.0)	19 (100.0)	0 (0.0)	0 (0.0)	19 (7.4)
<i>Mahajanga (MAH)</i>	24 (66.7)	12 (33.3)	0 (0.0)	0 (0.0)	36 (14.0)
<i>Toamasina (TOA)</i>	0 (0.0)	9 (90.0)	1 (10.0)	0 (0.0)	10 (3.9)
<i>Toliary (TOL)</i>	23 (39.0)	36 (61.0)	0 (0.0)	0 (0.0)	59 (23.0)
2022	39 (39.8)	52 (53.1)	0 (0.0)	7 (7.1)	98 (38.1)
<i>Antananarivo (ANT)</i>	3 (27.3)	5 (45.5)	0 (0.0)	3 (27.3)	11 (4.3)
<i>Antsiranana (ATI)</i>	10 (62.5)	6 (37.5)	0 (0.0)	0 (0.0)	16 (6.2)
<i>Fianarantsoa (FIA)</i>	2 (25.0)	5 (62.5)	0 (0.0)	1 (12.5)	8 (3.1)
<i>Mahajanga (MAH)</i>	10 (62.5)	6 (37.5)	0 (0.0)	0 (0.0)	16 (6.2)
<i>Toamasina (TOA)</i>	0 (0.0)	8 (80.0)	0 (0.0)	2 (20.0)	10 (3.9)
<i>Toliary (TOL)</i>	14 (37.8)	22 (59.5)	0 (0.0)	1 (2.7)	37 (14.4)
Total	93 (36.2)	153 (59.5)	3 (1.2)	8 (3.1)	257 (100.0)

PV1 Disc.: Poliovirus type 1 discordant; **PV-SL:** Poliovirus Sabin Like; **NPEV:** Non-polio enterovirus; **NEV:** Non enterovirus.

Table 4
Enteroviral wastewater positivity rate (2021–2022).

	Pos. EV (%)	Neg. EV (%)	Total
2021	325 (85.8)	54 (14.2)	379
<i>Antananarivo-Renivohitra (ANT)</i>	159 (48.9)	3 (0.9)	162 (42.7)
<i>Mahajanga I (MAH)</i>	78 (24.0)	12 (3.7)	90 (23.7)
<i>Toliary I (TOL)</i>	41 (12.6)	13 (4.0)	54 (14.2)
<i>Taolagnaro (TLG)</i>	33 (10.2)	18 (5.5)	51 (13.5)
<i>Antsiranana I (ATI)</i>	5 (1.5)	4 (1.2)	9 (2.4)
<i>Toamasina I (TOA)</i>	6 (1.8)	3 (0.9)	9 (2.4)
<i>Antsohihy (ATS)</i>	3 (0.9)	1 (0.3)	4 (1.1)
2022	490 (73.5)	177 (26.5)	667
<i>Antananarivo-Renivohitra (ANT)</i>	232 (47.3)	2 (1.1)	234 (35.1)
<i>Mahajanga I (MAH)</i>	104 (21.2)	26 (14.7)	130 (19.5)
<i>Toliary I (TOL)</i>	48 (9.8)	30 (16.9)	78 (11.7)
<i>Taolagnaro (TLG)</i>	45 (9.2)	33 (18.6)	78 (11.7)
<i>Antsiranana I (ATI)</i>	40 (8.2)	29 (16.4)	69 (10.3)
<i>Antsohihy (ATS)</i>	10 (2.0)	32 (18.1)	42 (6.3)
<i>Toamasina I (TOA)</i>	11 (2.2)	25 (14.1)	36 (5.4)
Total	815 (77.9)	231 (22.1)	1046

EV: Enterovirus; **Pos.:** Positive; **Neg.:** Negative.

Table 5
Enteroviral wastewater isolation results per collection area (2021–2022).

	Susp. PV (%)	NPEV (%)	Susp. PV + NPEV (%)	Pos. EV (%)
2021	57 (17.5)	177 (54.5)	91 (28.0)	325
<i>Antananarivo-Renivohitra (ANT)</i>	25 (43.9)	86 (48.6)	48 (52.7)	159
<i>Mahajanga I (MAH)</i>	21 (36.8)	33 (18.6)	24 (26.4)	78
<i>Toliary I (TOL)</i>	8 (14.0)	22 (12.4)	11 (12.1)	41
<i>Taolagnaro (TLG)</i>	2 (3.5)	25 (14.1)	6 (6.6)	33
<i>Antsiranana I (ATI)</i>	0 (0.0)	4 (2.3)	1 (1.1)	5
<i>Toamasina I (TOA)</i>	0 (0.0)	5 (2.8)	1 (1.1)	6
<i>Antsohihy (ATS)</i>	1 (1.8)	2 (1.1)	0 (0.0)	3
2022	95 (19.4)	248 (50.6)	147 (30.0)	490
<i>Antananarivo-Renivohitra (ANT)</i>	53 (55.8)	98 (39.5)	81 (55.1)	232
<i>Mahajanga I (MAH)</i>	18 (18.9)	50 (20.2)	36 (24.5)	104
<i>Toliary I (TOL)</i>	8 (8.4)	27 (10.9)	13 (8.8)	48
<i>Taolagnaro (TLG)</i>	6 (6.3)	33 (13.3)	6 (4.1)	45
<i>Antsiranana I (ATI)</i>	10 (10.5)	21 (8.5)	9 (6.1)	40
<i>Toamasina I (TOA)</i>	0 (0.0)	11 (4.4)	0 (0.0)	11
<i>Antsohihy (ATS)</i>	0 (0.0)	8 (3.2)	2 (1.4)	10
Total	152 (18.7)	425 (52.1)	238 (29.2)	815

Susp.: Suspected; **PV:** Poliovirus; **Pos.:** positive; **NPEV:** Non-polio enterovirus.

3.3. Sequence analysis

As mentioned, OPV-Sabin 1 is derived from the wild-Mahoney poliovirus type 1 (WPV1-Mahoney) strain (V0119) after several passages in monkey tissue cultures. OPV-Sabin 1 differs from WPV1-Mahoney by 56 nucleotide substitutions leading to 21 amino acid changes. Six of them are known to be responsible for attenuating neurovirulence and considerably reducing viral replication in the nervous system, four of which are located in the 5'NC, VP1 and 3Dpol regions. Sequence analysis of cVDPV1 isolates from human and environmental strains based on these three genomic regions revealed that all 35 sequences (5'NC), 104 of the 206 sequences (VP1), and all 41 sequences (3Dpol) were mutated at these neurovirulence attenuation sites and reverted to a wild-type genotype similar to that of the WPV1-Mahoney strain (Fig. 4). Regarding isolates for which the three regions were sequenced, their combined analysis revealed that all but one were recombinant viruses between mutated sequences of the parental OPV-Sabin 1 in the VP1 region and other type C enteroviruses. Coxsackievirus A20 (CVA20), PV1, PV3 and EV-C99 sequences were identified only in the 5'NC region, CVA13 sequences only in the 3Dpol region, while CVA11 sequences were identified in both. Whole genome sequencing (WGS) of four representative cVDPV1 strains revealed a recombination breakpoint located between nucleotide positions 3400 and 3500 (data not shown).

Of all these type C enterovirus sequences, the only exception was an Echovirus 11 sequence, belonging to the type B species, identified in the 3Dpol region (Supplemental Table S1).

Regarding the 5'NC region, position 480, which is the main determinant of attenuation, was reverted in all 35 sequences generated from 16 human AFP cases and 19 sequences from environmental wastewater isolates (GenBank ref. OR778200 to OR778234) (Fig. 4A). For VP1, position 2795 was reverted in 51 (55.4%) human and 53 (46.5%) environmental isolates and acquired the A to G mutation leading to the T106A substitution. This substitution in the VP1 region contributes to enhanced binding of the PV1 viral particle to host cells, thereby increasing capsid stability (Adeyemi et al., 2019; Couderc et al., 1996). It is worth noting that no sequences were mutated at position 2,879, the second site of neurovirulence attenuation located in VP1 (Fig. 4B). In addition, four other nucleotide substitutions at positions 2545 (G > A),

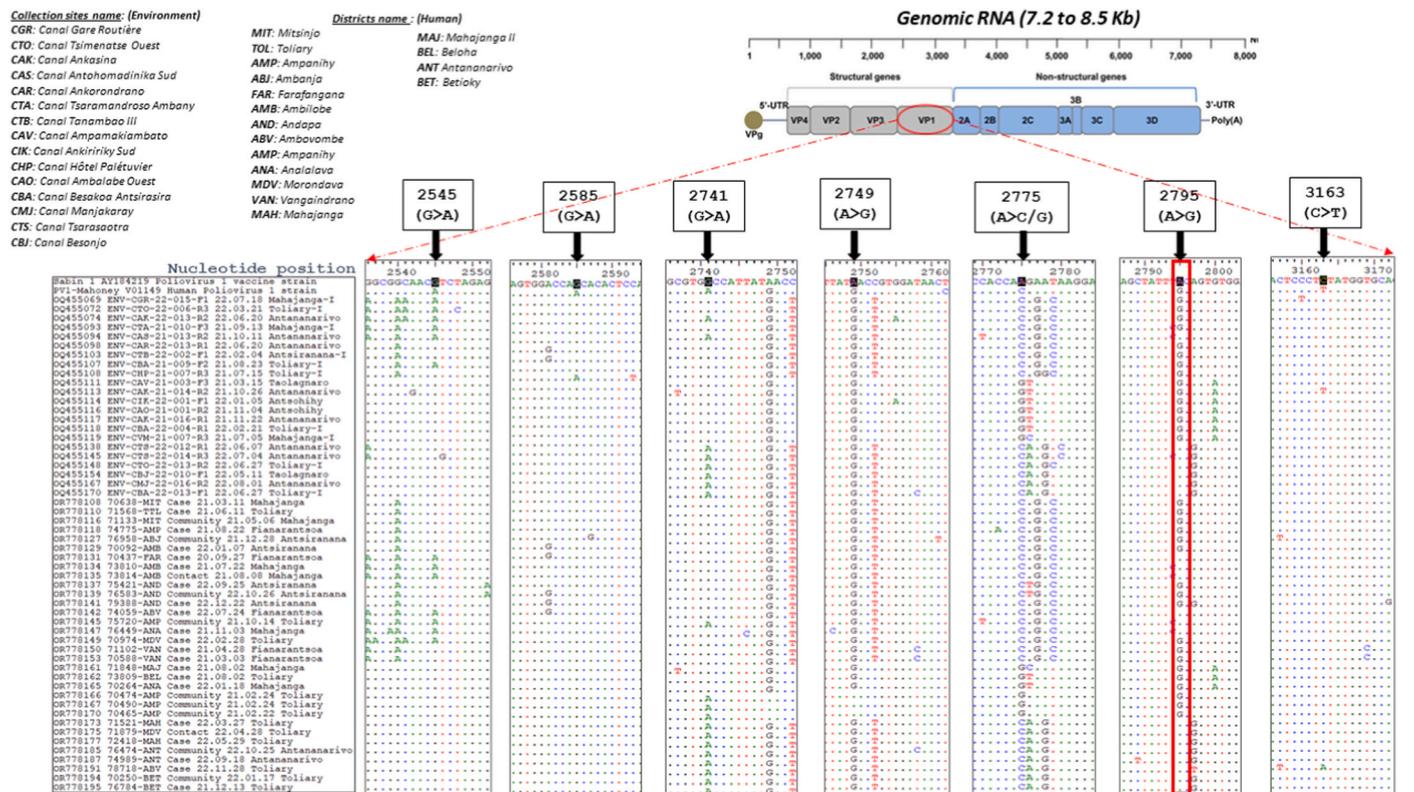


Fig. 5. Analysis of neurovirulence sites located on the VP1 gene: Sequence comparison of the Sabin1 vaccine strain (AY84219) and the wild-type PV1 strain (V01149) with selected human and environmental cVDPV1 sequences. Nucleotide positions 2545; 2585; 2741; 2749; 2775; 2795 and 3163 are highlighted. Position 2,795, whose A2,795G mutation is responsible for the T106A substitution is highlighted by a red rectangle.

The sequences were divided into four distinct, highly supported genogroups, named 'MAD-SUO-1', 'MAD-ANO-1', 'MAD-ANO-2' and 'MAD-SUE-1', respectively. For the sake of clarity, with the exception of MAD-SUO-1, not all the sequences obtained are represented in the phylogenetic tree in Fig. 6. Nevertheless, the sequences used to construct the phylogenetic tree are geographically and temporally representative of all cVDPV1 strains confirmed in Madagascar from 2020 to 2022. The MAD-SUO-1 genogroup consisted solely of human sequences obtained from one AFP case and three healthy children living in the community, collected between December 2020 and February 2021 in the Ampanihy district (Toliary province, South). Sequences belonging to this genogroup showed a nucleotide divergence of 1.66–1.99% from the OPV-Sabin 1 reference strain. The MAD-ANO-1 genogroup, supported by a bootstrap value of 82, was composed of sequences from AFP cases and from the community from the provinces of Mahajanga (Northwest) and Toliary (South). Moreover, environmental strain sequences were from five districts (Antsohihy, Mahajanga I, Antananarivo, Toliary I and Taolagnaro) collected between March 2021 and April 2022. Pairwise comparison of MAD-ANO-1 sequences revealed a divergence of 1.32–3.53% at the nucleotide level from the Sabin 1 reference strain. The third genogroup, MAD-ANO-2, presented a nucleotide sequence divergence ranging from 3.42 to 6.07% from the Sabin 1 reference strain. Overall, sequences from AFP cases, index contacts and healthy children living in the community from the provinces of Antananarivo, Fianarantsoa and Toliary, as well as sequences from environmental samples collected in the districts of Antananarivo, Toliary I and Taolagnaro, between December 2021 and December 2022, belonged to this genogroup. Finally, the first and last AFP cases confirmed as cVDPV1 between 2020 and 2022 belonged to the MAD-SUE-1 genogroup, whose sequences as a whole exhibited a nucleotide divergence of 1.77%–5.78% compared to OPV-Sabin 1. Of the four genogroups, MAD-SUE-1 was the most geographically widespread, affecting the majority of Madagascar's

provinces, with samples collected between September 2020 and December 2022. Sequences were from AFP cases, close contacts and healthy children from the provinces of Antsiranana, Mahajanga, Antananarivo, Fianarantsoa, Toliary as well as from environmental samples collected in the districts of Antsiranana I, Mahajanga I, Antananarivo and Toliary I (Fig. 6).

4. Discussion

The identification of the first strain of VDPV1 of the current epidemic was confirmed in week 39 of 2020 from an AFP case discovered in Southeastern Madagascar, in the Farafangana district. This case "MAD-SUE-FAR-20-460" (corresponding to "OR778131_70437-FAR_Case_2020.09.27_Fianarantsoa" in Fig. 6), concerned a 4-year-old girl from the village of Bedinga, has been paralyzed since September 27, 2020. She was suffering from pain in her left arm and leg and severe malnutrition, as recorded on the "CIF" standard form for suspected AFP cases. Since then, the epidemic has spread throughout the country within the community and the environment. Indeed, 92 "human" isolates of cVDPV1 were detected from 67 children, of which 30 AFP cases and 37 healthy carriers geographically distributed over 22 districts, and 411 "environmental" cVDPV1 strains were isolated from 129 wastewater samples collected from 23 sites in six zones.

To consider that the vaccine strain has derived into a VDPV1, at least 10 nucleotide substitutions (>1%) must accumulate in the entire VP1 gene for OPV-Sabin 1 (Liang et al., 2006). Additionally, Jorba et al. evaluated the circulation time of strains based on a molecular timeclock taking into account the number of mutations in the genomic region coding for the VP1 capsid protein (Jorba et al., 2008). Based on this criterion, a 1% nucleotide divergence is considered to correspond to a year of circulation in nature. The VP1 sequences obtained from wastewater samples collected between 2021 and 2022 showed 12 to 55

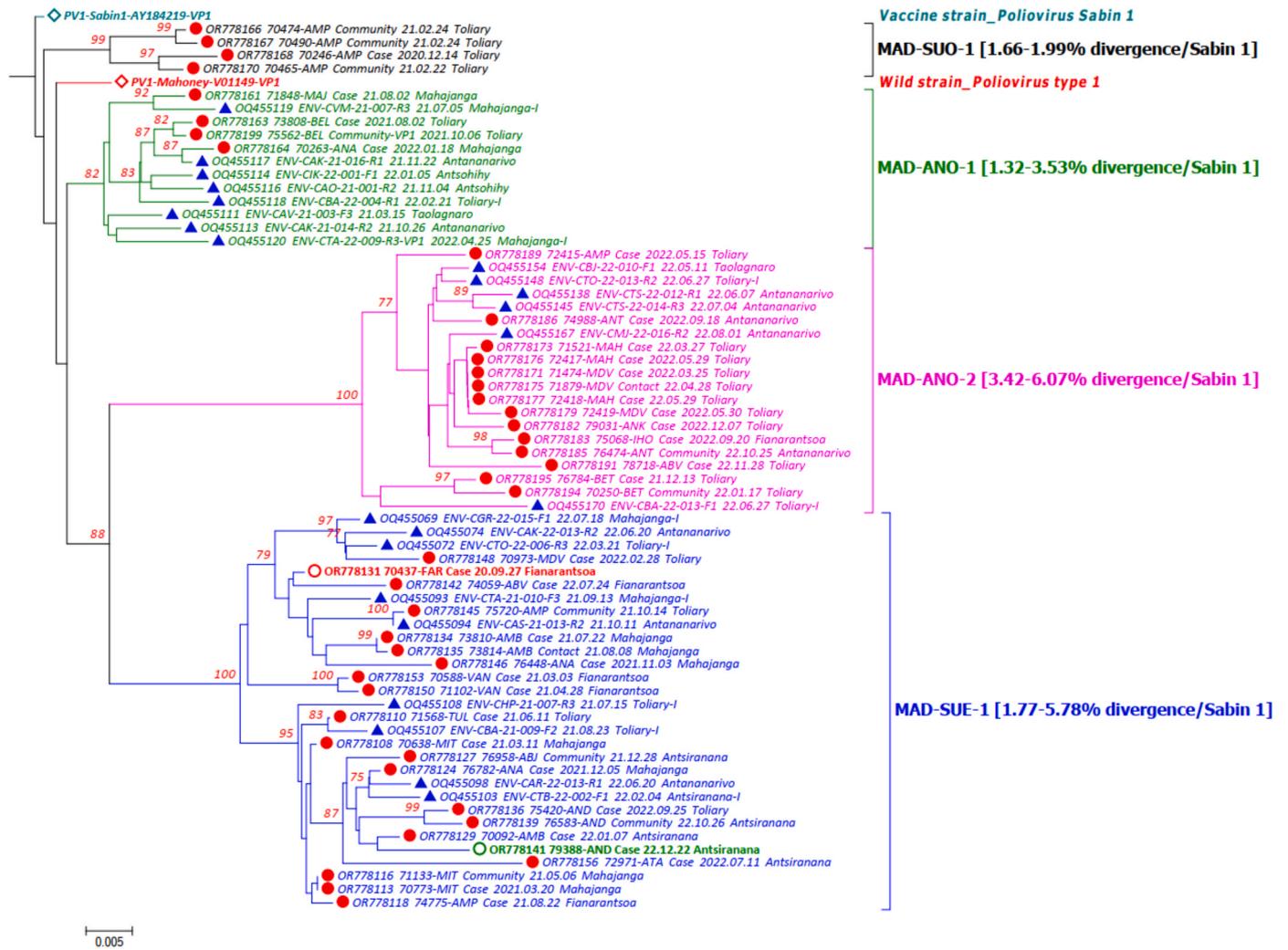


Fig. 6. Phylogenetic tree of cVDPV1 strains from human and environmental samples. Maximum likelihood phylogenetic tree of cVDPV1 strains based on the complete coding sequence of VP1. The phylogenetic tree shows the four identified genogroups with their respective rates of divergence in comparison to the Sabin1 vaccine reference strain (AY84219). The first case of VDPV1 is shown as an open red circle, while the last case is shown as an open green circle. The blue triangles represent environmental cVDPV1 sequences and the red circles represent human sequences from AFP cases, their contacts and healthy children in the community. Support for nodes was provided by bootstrap analysis. Only resolved nodes with a bootstrap value greater than 0.75 are indicated (in red). The tree is drawn to scale with branch lengths measured in the number of substitutions per site. Sequences are color-coded according to their genogroup. The different well-supported genogroups are indicated on the right-hand side.

nucleotide substitutions (1.3–6.1% nucleotide divergence). The level of nucleotide divergence in human cVDPV1 strains was similar, ranging from 1.4 to 5.8%. This indicates that both human and environmental cVDPV1 strains have been circulating for 16–73 months, in other words since 2016–2017.

Phylogenetically, human and environmental cVDPV1 VP1 sequences are grouped into four and three genogroups, respectively, corresponding to MAD-SUO-1 (containing only sequences from humans), MAD-ANO-1, MAD-ANO-2, and MAD-SUE-1. The phylogenetic analysis shows that all cVDPV1 in Madagascar are derived from the bivalent OPV (bOPV) vaccine (Sabin 1 and 3) in use throughout the island. All the sequences identified are different from each other and from those already published, ruling out the possibility of importation. In addition, the geographical spread of cVDPV1 is confirmed by the genetic closeness of human and environmental sequences scattered across different districts and provinces of Madagascar. Indeed, closely related cVDPV1 strains were detected in the Southern province of Fianarantsoa and in Mahajanga in the North, through environmental surveillance. Furthermore, comparison of these sequences shows that the strains have spread from South to North, East to West and across the center of the country.

Genogroup MAD-SUO-1 consists only of cVDPV1 strains from human AFP cases and their contacts living in the community detected in the Ampanihy district in the Southern region of Madagascar from December 2020 to February 2021. The rapid disappearance of this genogroup can be interpreted as a limited human-to-human circulation over a short period of time. Despite several vaccination campaigns against this epidemic, strains belonging to the MAD-SUE-1 and MAD-ANO-2 genogroups were still detected at the end of 2022 and even in 2023 (data not shown).

Of the two known attenuation sites in VP1, around half the sequences from human and environmental samples carried the 2795 (A > G) leading to T106A mutation, but none the 2879 (T > C) leading to F134L substitution. The T106A substitution promotes virus replication in the CNS. We also observed mutations at four other positions, all of which were reverted to wild-type, close to antigenic sites involved in neutralization by host antibodies, which could play a role in immune escape and virus survival (Yakovenko et al., 2006). Sequence analyses of the partial 5'NC region and 3Dpol region, which encodes the RNA dependent RNA polymerase enzyme, also revealed a reversion of their neurovirulence attenuation sites (Tardy-Panit et al., 1993). The G480A

mutation in the 5'NC region reverts to the non-polio enterovirus (NPEV) sequence IRES cloverleaf, known to facilitate viral replication in the CNS. Consequently, isolates that acquire this mutation exhibit enhanced translational efficiency and neurovirulence (Malnou et al., 2004). In addition, the H73Y substitution in the 3Dpol has been shown to increase viral replication (Paul et al., 2000). However, whereas the VP1 mutations were acquired through point mutations, those in the 5'NC and 3Dpol regions occurred through inter-typical recombination events with other C-type enteroviruses. Indeed, sequences of serotypes CVA11, CVA20, PV1, PV3 and EV-C99 were identified in the 5'NC region and sequences of serotypes CVA11 and CVA13 in the 3Dpol region, all belonging to the non-polio EV-C species (Supplementary Table 1). The 5'NC sequences presented from 94.0 to 100% nucleotide identity, and those of the 3Dpol region from 87.1 to 100% nucleotide identity, with sequences previously isolated during an epidemic in Madagascar in 2011 (Razafindratsimandresy et al., 2022; Bessaud et al., 2011). In addition, a sequence of the 3Dpol region corresponded to a B-type enterovirus showing 87.9% of nucleotide identity with an Echovirus 11 strain identified in Italy in 2013. The 3Dpol region sequence of the "71130-MIT_Community-3Dpol_2021.05.06_Mahajanga" strain in the Supplemental Table S1 indicates the possibility of co-infection between the EV-B and C species. Further NGS analysis may be required to distinguish the sequences of the two viruses. Regardless of the underlying mechanism, the mutations identified at these sites are likely to have increased neurovirulence and conferred native transmissibility to the cVDPV1 strains here characterized (Lu et al., 1996), explaining the ease with which they are transmitted and circulate in a population with a low level of immunity (Khetsuriani et al., 2017).

This is the fourth VDPV epidemics to occur in Madagascar since the early 2000s, but the first time that a VDPV epidemic has reached such a large geographical scale. Since 2000, in addition to routine immunization, 18 national and 11 sub-national supplementary immunization activities (Supplemental Table S2) have been implemented to achieve polio eradication (Delpeyroux et al., 2013; Rakoto-Andrianarivelo et al., 2008; Diop et al., 2015; Razafindratsimandresy et al., 2022; Nimpa et al., 2021). Despite all these efforts, the immunity of the population, particularly that of targeted children, has remained low, far below the 90% coverage target, contributing to the reappearance of VDPVs (Supplemental Table S3). The low vaccination coverage rate observed from year to year, which also varies from region to region, can be explained by the reluctance of parents to vaccinate their children, but also by the difficulty of access to certain remote regions. In addition, by limiting routine vaccination for months on end due to nationwide movement restrictions, the COVID-19 pandemic has certainly also contributed to the current epidemic. Finally, in recent years, the extreme climatic conditions encountered in the South of the country - famine due to drought and cyclones - have led to a massive migration of the population to other, less affected regions. The combination of all these factors may explain the widespread distribution of cVDPV1 strains in the country since 2020.

5. Conclusion

This study provides the first molecular data on cVDPV1 strains isolated from humans and wastewater that have been circulating in Madagascar since late 2020. It highlights the close phylogenetic relationships between strains isolated from the environment and those from human AFP cases and their contacts. Sequencing of VP1 revealed the emergence and spread of four distinct phylogenetic genogroups, all originating in Southern Madagascar. In addition, all strains were recombinant between mutated parental sequences of OPV-Sabin 1 and sequences of other type B and C enteroviruses. Acquisition of mutations and inter-typical genetic recombination are two mechanisms used by polioviruses to evade host immune responses. This genetic plasticity also contributes to the increase in viral replication and their evolution.

This persistent cVDPV1 epidemic reflects a low childhood polio

vaccination profile in the Malagasy population, due to a certain reluctance to vaccinate, for several reasons such as the repetitiveness of vaccination campaigns, certain beliefs and in particular the belief that vaccination can make you sick and kill. These results highlight the need to review the vaccination status of the Malagasy population at national level. Active case-finding and rapid mass vaccination responses are also essential to break transmission chains as quickly as possible.

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Data availability

All data generated and analyzed during this study are included in this published article.

CRediT authorship contribution statement

Jonhson Raharinantoanina: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Marie-Line Joffret:** Writing – review & editing, Visualization, Validation. **Maël Bessaud:** Writing – review & editing, Visualization, Validation. **Danielle Aurore Doll Rakoto:** Visualization, Validation. **Philippe Dussart:** Visualization, Validation. **Vincent Lacoste:** Writing – review & editing, Visualization, Validation, Supervision. **Richter Razafindratsimandresy:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.virol.2024.110253>.

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