Université de Tunis El Manar

Institut Pasteur de Tunis

NGS sequencing for monitoring Poliovirus strains in Patients with Primary Immunodeficiency

Sondes Haddad-Boubaker, PhD
Associate Professor in Microbiology
Laboratory of Clinical Virology
WHO Regional Reference Laboratory for Poliomyelitis, Measles& Rubella in Eastern Mediterranean Region
Institut Pasteur de Tunis

Société Tunisienne de Pahologie Infectieuse

1 st MENA Congress of Clinical Microbiology and Infectious Diseases
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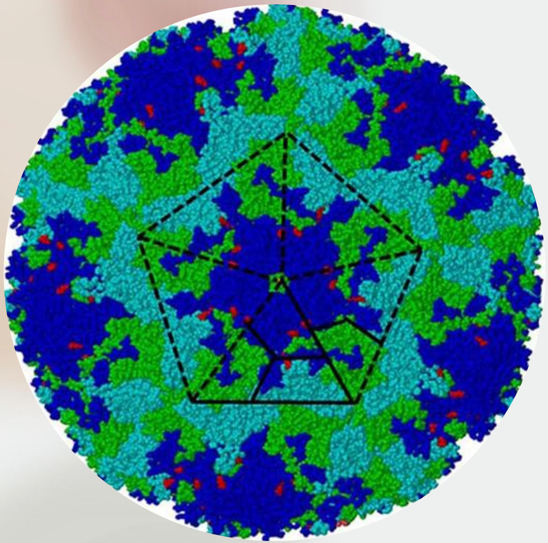


01

PV
&
Eradication challenges

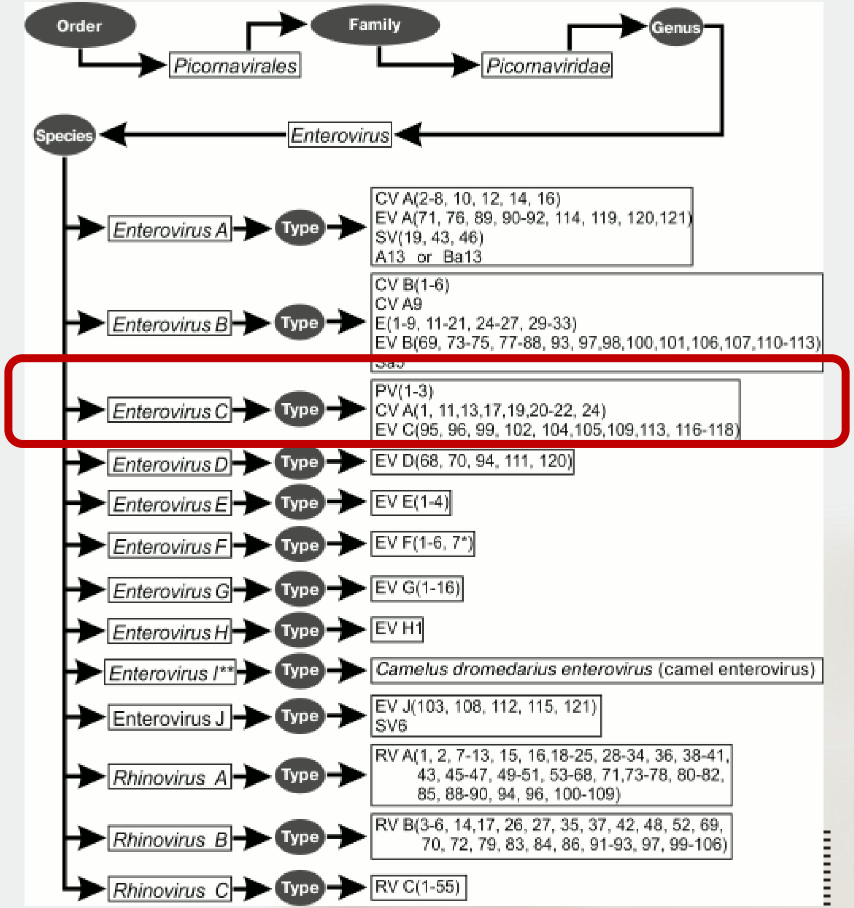
Poliovirus Classification

Family: Picornaviridae
(35 genus)

Genus: Enterovirus
(12 EV species & 3 RV)

Enterovirus C

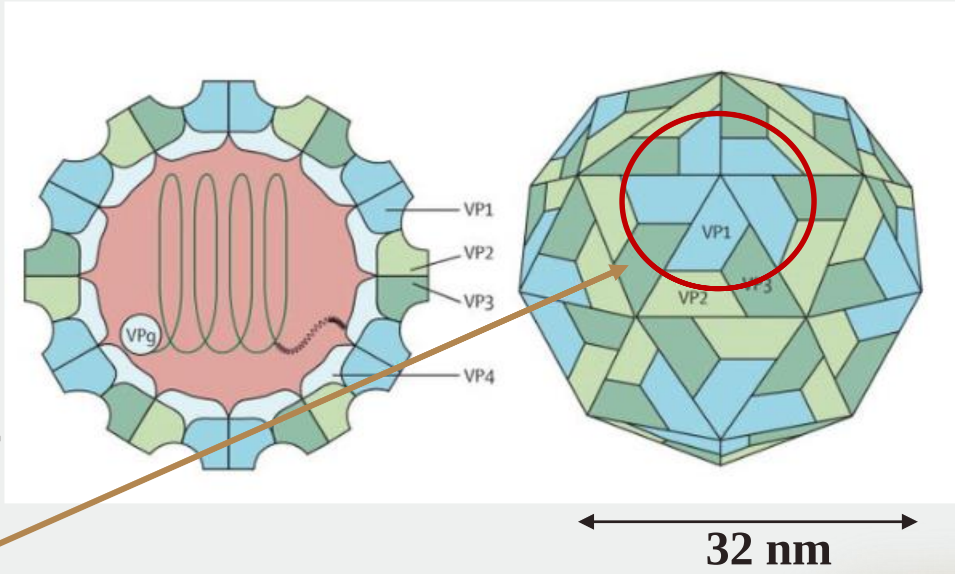
PV-1, PV-2, PV-3, CV-A21, CV-A24

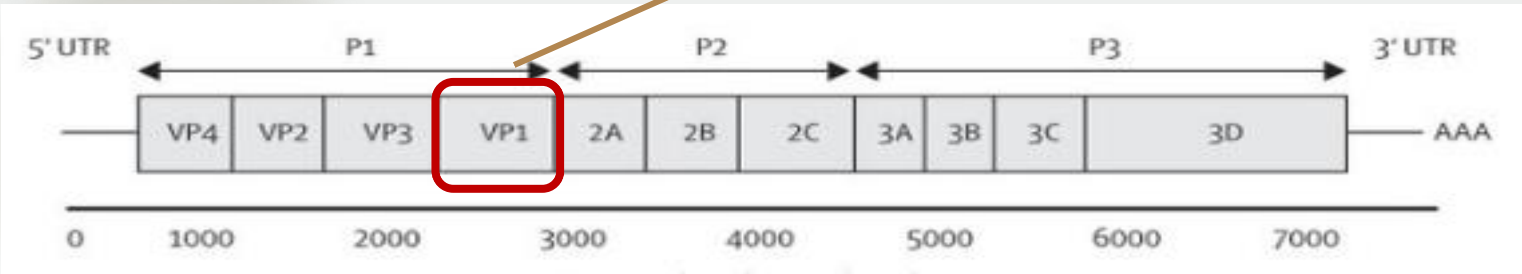


Poliovirus : Structure

Single stranded naked RNA virus

Icosahedral capsid: 60 copies of VP1, VP2, VP3, VP4





Poliovirus Eradication challenges

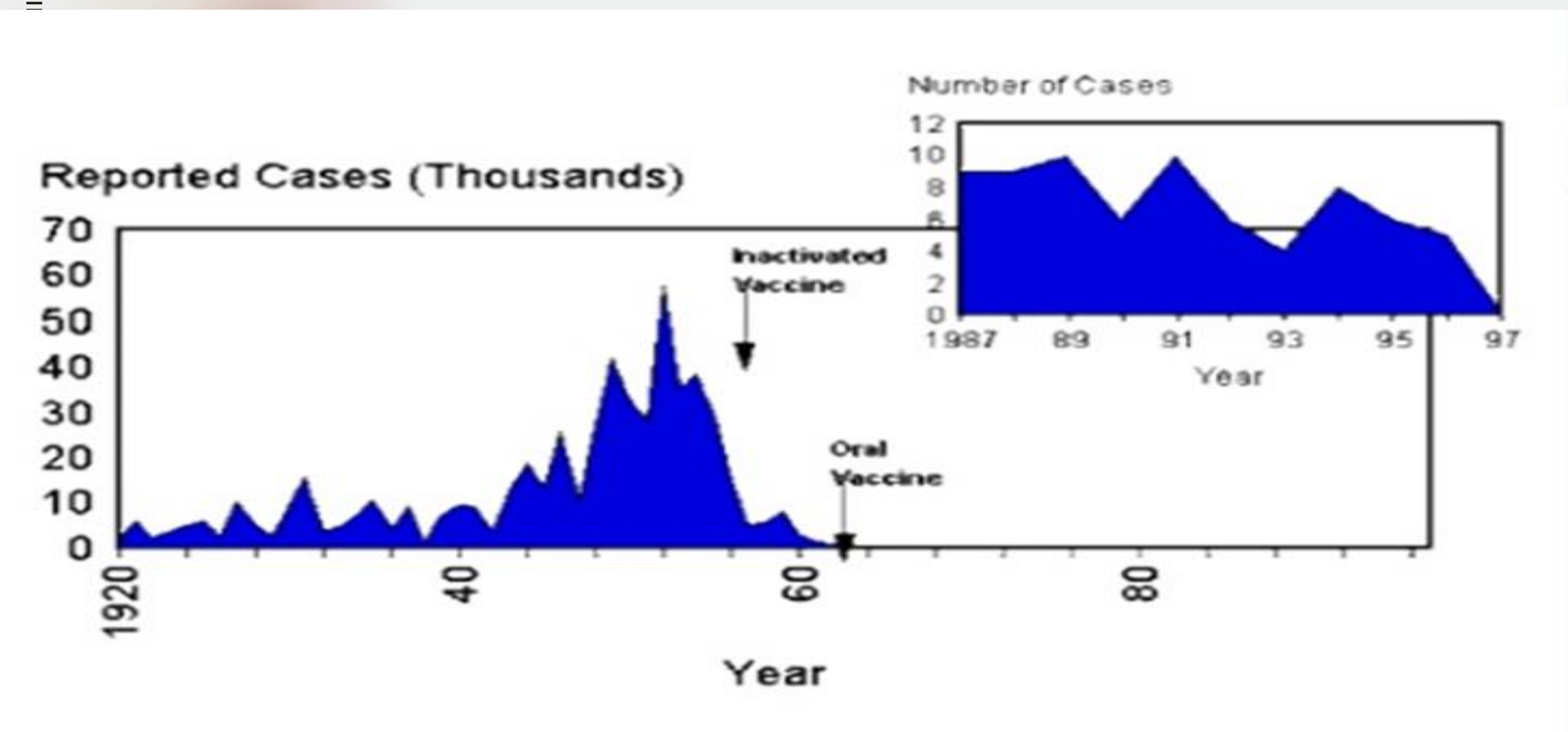
Before Polio vaccines development


Franklin Rosvelt, 1932-45


Épidémie à Newyork,1916; Unité de soins intensifs


Polio kiddies cheer news of successful vaccine., California 1955

Poliovirus Eradication challenges



Poliovirus Eradication challenges

Since 50’s

rare in developed countries

endemic in developing countries

1988

350 000 cases/ 125 countries

World Health Organization

POLIO GLOBAL ERADICATION INITIATIVE

Poliovirus Eradication challenges

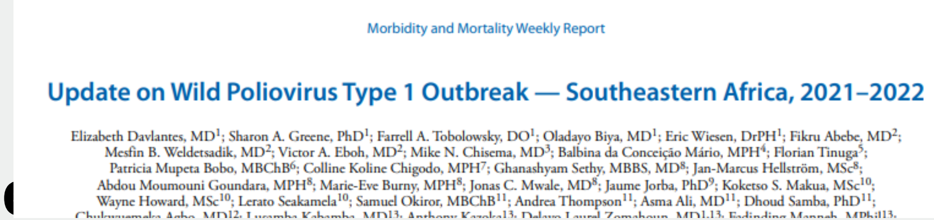
Since 2017

Source: Adapted from Global Polio Eradication Initiative (GPEI)

Poliovirus Eradication challenges

Possible importation of

WPV1: Malawi, Mozanbiqu



Morbidity and Mortality Weekly Report

Update on Wild Poliovirus Type 1 Outbreak — Southeastern Africa, 2021–2022

Elizabeth Davlantes, MD[1]; Sharon A. Greene, PhD[1]; Farrell A. Tobolowsky, DO[1]; Oladayo Biya, MD[1]; Eric Wiesen, DrPH[1]; Fikru Abebe, MD[2];
Mesfin B. Weldetsadik, MD[2]; Victor A. Eboh, MD[2]; Mike N. Chisema, MD[3]; Balbina da Conceição Mário, MPH[4]; Florian Tinuga[5];
Patricia Mupeta Bobo, MBChB[6]; Colline Koline Chigodo, MPH[7]; Ghanashyam Sethy, MBBS, MD[8]; Jan-Marcus Hellström, MSc[8];
Abdou Moumouni Goundara, MPH[8]; Marie-Eve Burny, MPH[8]; Jonas C. Mwale, MD[8]; Jaume Jorba, PhD[9]; Koketso S. Makua, MSc[10];
Wayne Howard, MSc[10]; Lerato Seakamela[10]; Samuel Okiror, MBChB[11]; Andrea Thompson[11]; Asma Ali, MD[11]; Dhoud Samba, PhD[11];

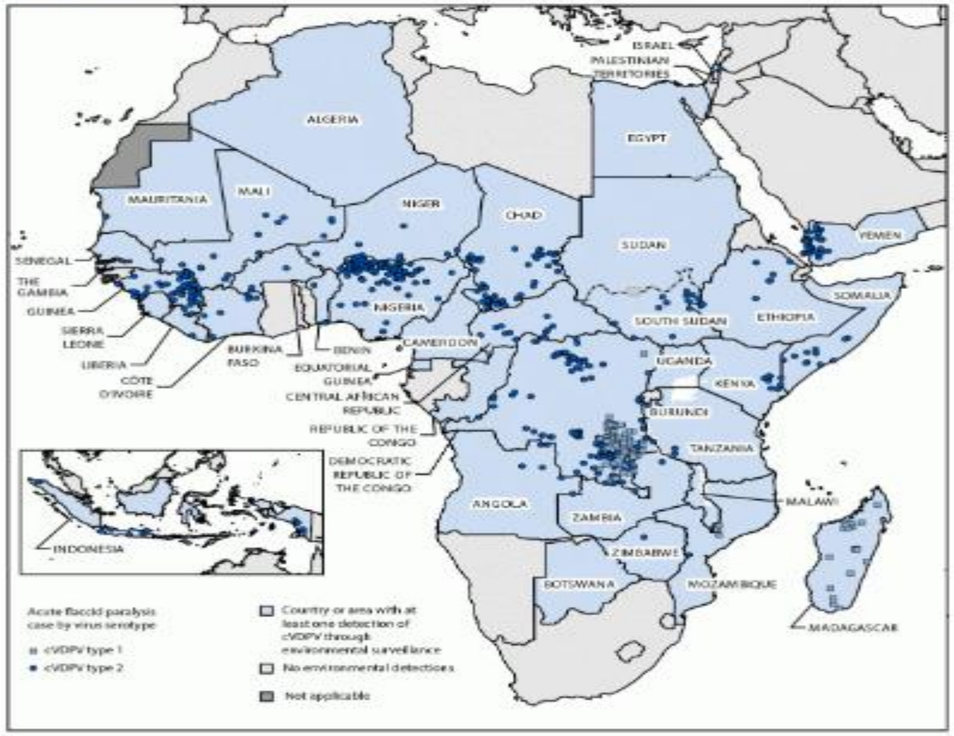
11 octobre 2022

https://www.who.int/publicat ons/i/item/global-vaccineaction-plan-2011-2020

Poliovirus Eradication challenges

VDPV/cVDPV outbreaks

FIGURE 1. Pays et zones* signalant des flambées de polio en circulation dérivées d'une souche vaccinale (N = 39) — dans le monde, janvier 2023–juin 2024 [1]

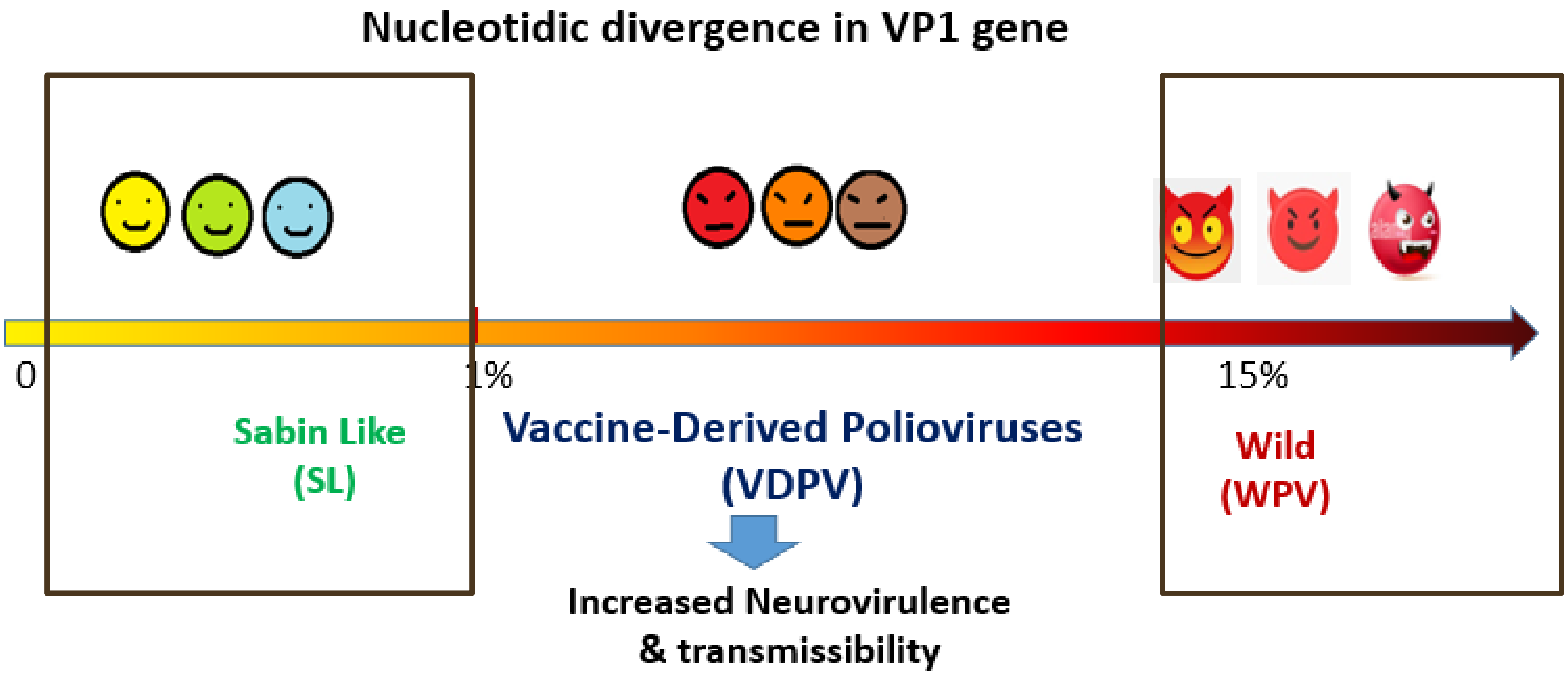


Jan 2023- June 2024: **74 outbreaks** in 39 countries

672 AFP cases

http://dx.doi.org/10.15585/mmwr.mm7341a1

Poliovirus Eradication challenges



Nucleotidic divergence in VP1 gene

0

1%

15%

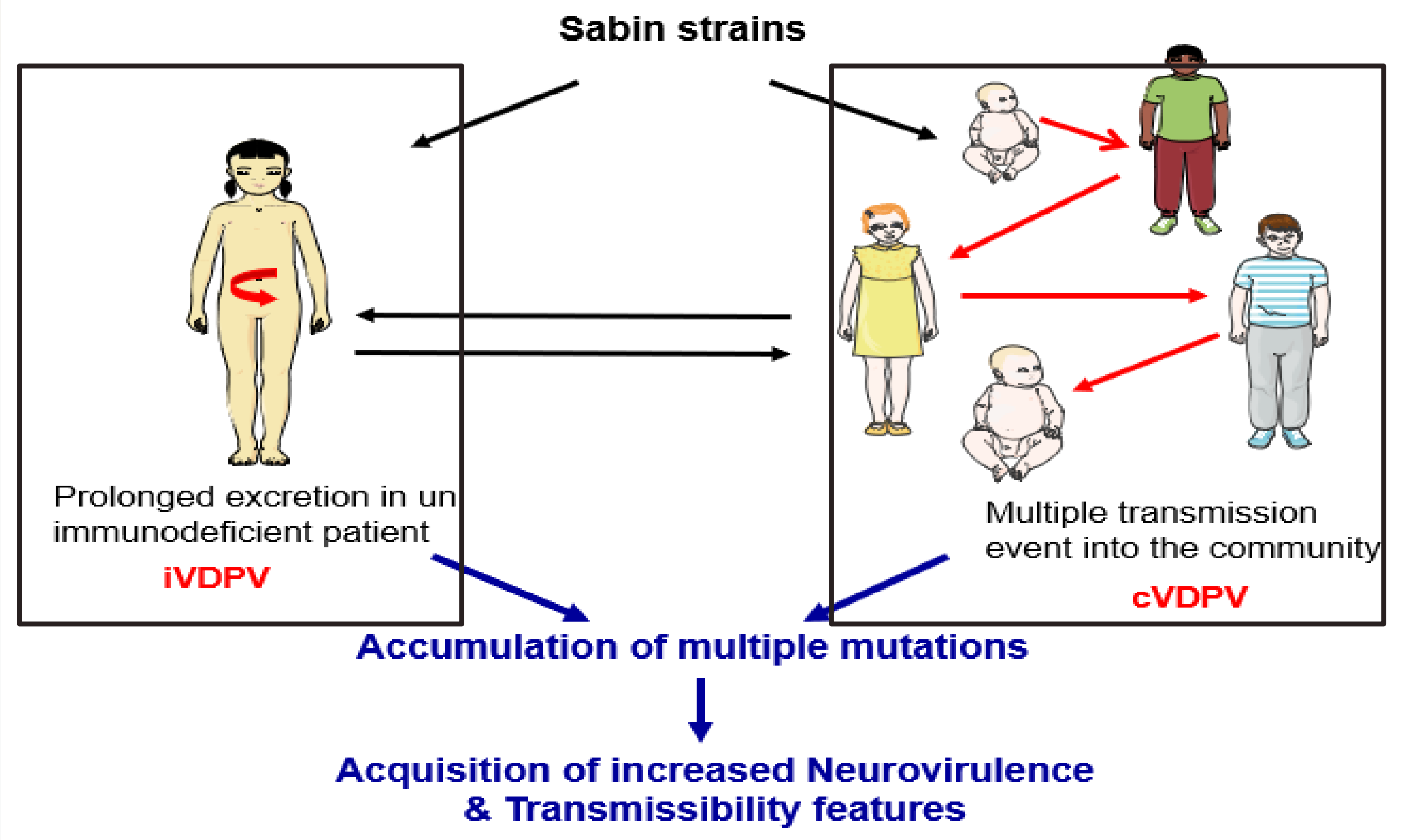
Sabin Like
(SL)

Vaccine-Derived Polioviruses
(VDPV)

Increased Neurovirulence
& transmissibility

Wild
(WPV)

Poliovirus Eradication challenges



Sabin strains

Prolonged excretion in un immunodeficient patient

iVDPV

Multiple transmission event into the community

cVDPV

Accumulation of multiple mutations

Acquisition of increased Neurovirulence & Transmissibility features

Poliovirus Eradication challenges

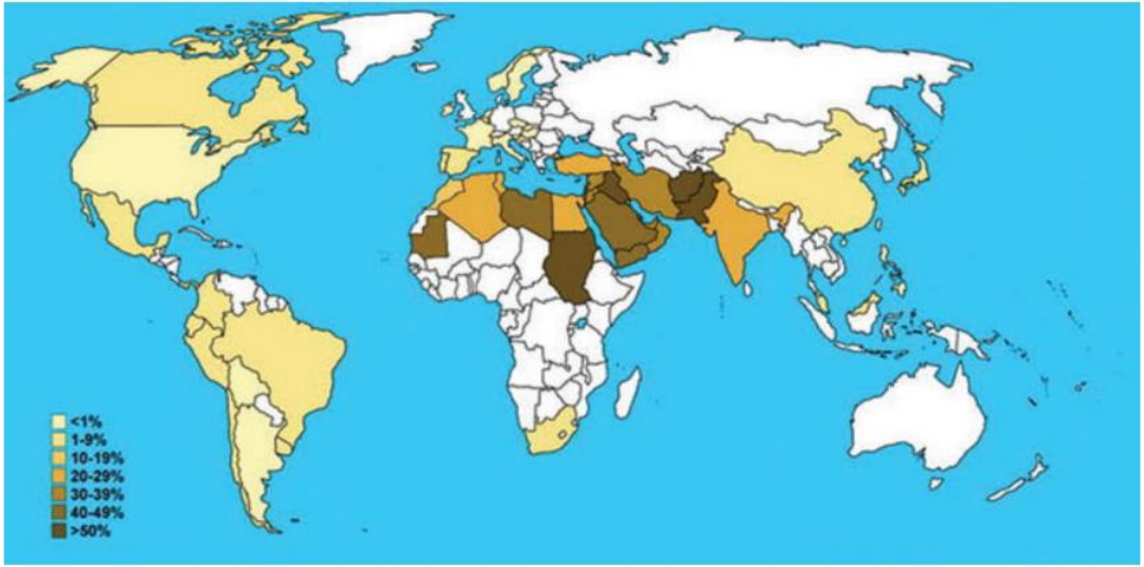


Figure 1. Available data on the worldwide consanguinity rate, with obvious high consanguinity in the Middle Eastern countries.

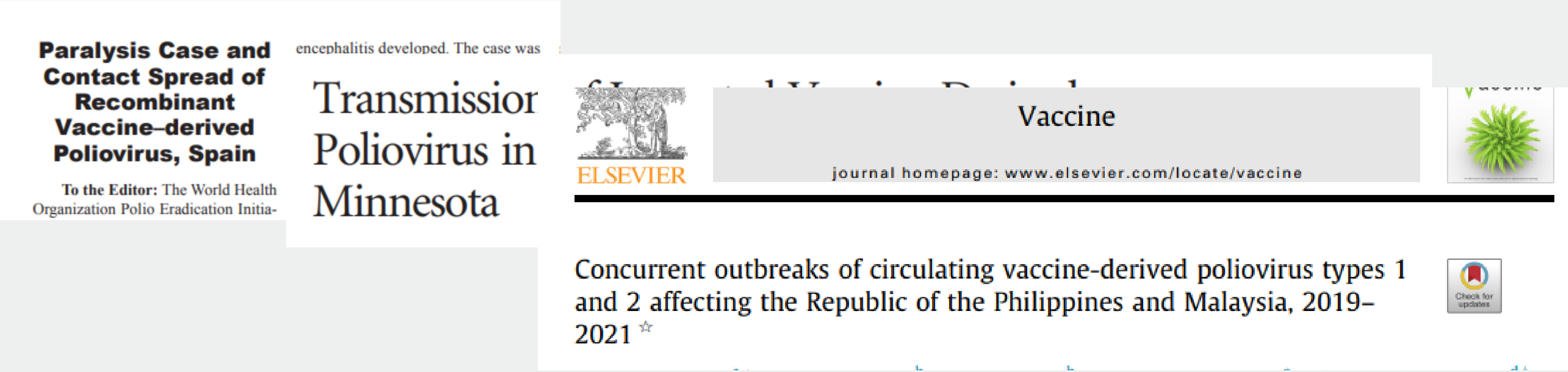
MENA region:
High rate of consanguineous marriages
High prevalence of autosomal recessive forms of PIDs.

doi: 10.1111/j.1749-6632.2011.06379.x

https://doi.org/10.1080/1744666X.2020.1801422

Poliovirus Eradication challenges

Up to date, rare community spread of iVDPV : only three iVDPV circulation



Paralysis Case and Contact Spread of Recombinant Vaccine–derived Poliovirus, Spain

To the Editor: The World Health Organization Polio Eradication Initia-

encephalitis developed. The case was

Transmissio
Poliovirus in
Minnesota

ELSEVIER

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Concurrent outbreaks of circulating vaccine-derived poliovirus types 1 and 2 affecting the Republic of the Philippines and Malaysia, 2019–2021 ☆

After wild poliovirus eradication,

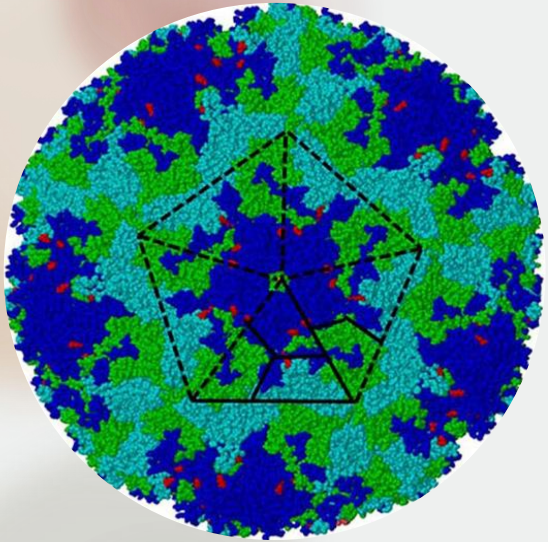
- Global reduction of poliovirus immunity
- Improvement of PID health conditions

OPEN ACCESS

Edited by:
Antonio Condino-Neto,
University of São Paulo, Brazil

Patients with Primary Immunodeficiencies Are a Reservoir of Poliovirus and a Risk to Polio Eradication

iVDPV excreters will constitute a significant risk for poliovirus re-emergence

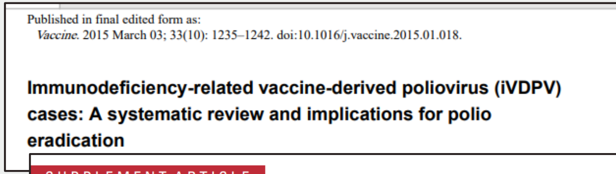


02

Increased susceptibility & persistence PV/EV in PID

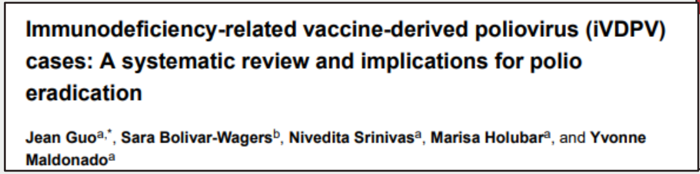
PV/EV Increased susceptibility in PID

Prolonged poliovirus excretion is associated with Primary immunodeficiency disorders (PIDs)



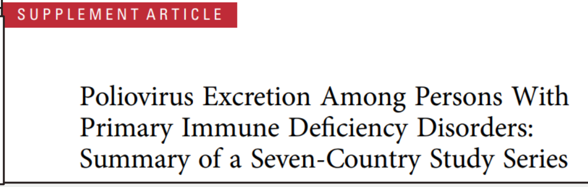
Published in final edited form as:
Vaccine. 2015 March 03; 33(10): 1235–1242. doi:10.1016/j.vaccine.2015.01.018.

Immunodeficiency-related vaccine-derived poliovirus (iVDPV) cases: A systematic review and implications for polio eradication



Immunodeficiency-related vaccine-derived poliovirus (iVDPV) cases: A systematic review and implications for polio eradication

Jean Guo[a,*], **Sara Bolivar-Wagers**[b], **Nivedita Srinivas**[a], **Marisa Holubar**[a], and **Yvonne Maldonado**[a]



SUPPLEMENT ARTICLE

Poliovirus Excretion Among Persons With Primary Immune Deficiency Disorders: Summary of a Seven-Country Study Series

Primary Antibody Deficiency: Hypogammaglobulinemia, Agammaglobulinemia, Hypergammaglobulinemia M (hyper IgM)

Combined antibody and cellular immunodeficiency disorders: Severe Combined Immunodeficiency Disorder (SCID) , Common Variable Immunodeficiency Disorder (CVID), Major Histocompatibility complex deficiencies and other disorders .

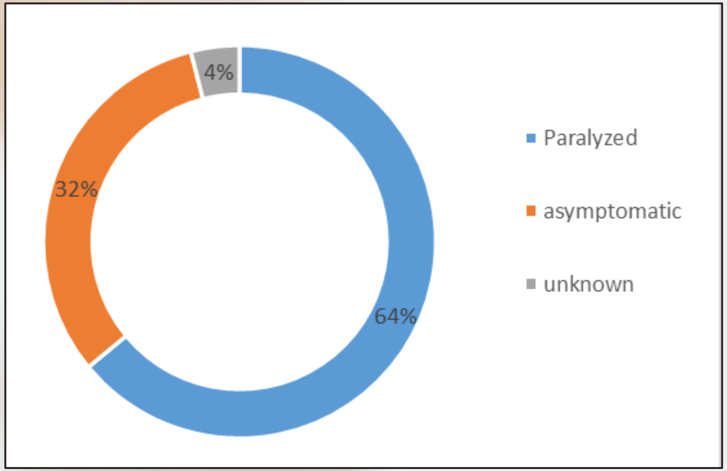
PV/EV Increased susceptibility in PID

The iVDPV excreters are classified into 3 categories:

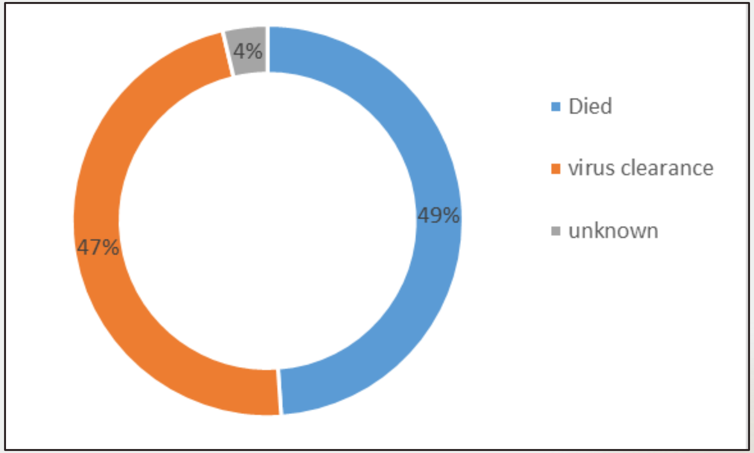
- Excreters of less than 6 months period
- Prolonged excreters when it’s comprised between 5 years and 6 months
- Chronic excreters when excretion period exceeds 5 years

PV/EV Increased susceptibility in PID

According to Macklin et al. (2020), 149 cases of iVDPV (with or without AFP) were reported to the WHO from 1961 to 2019.

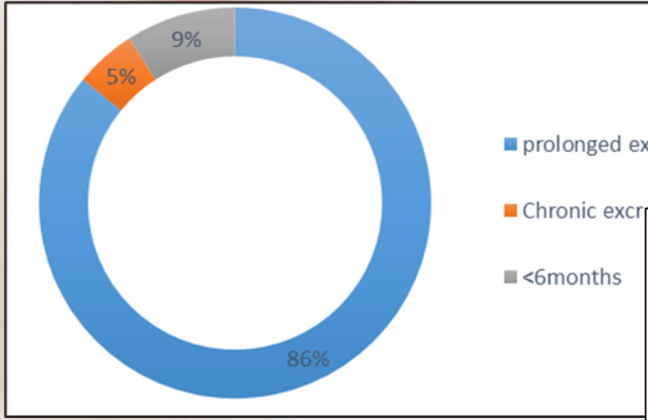


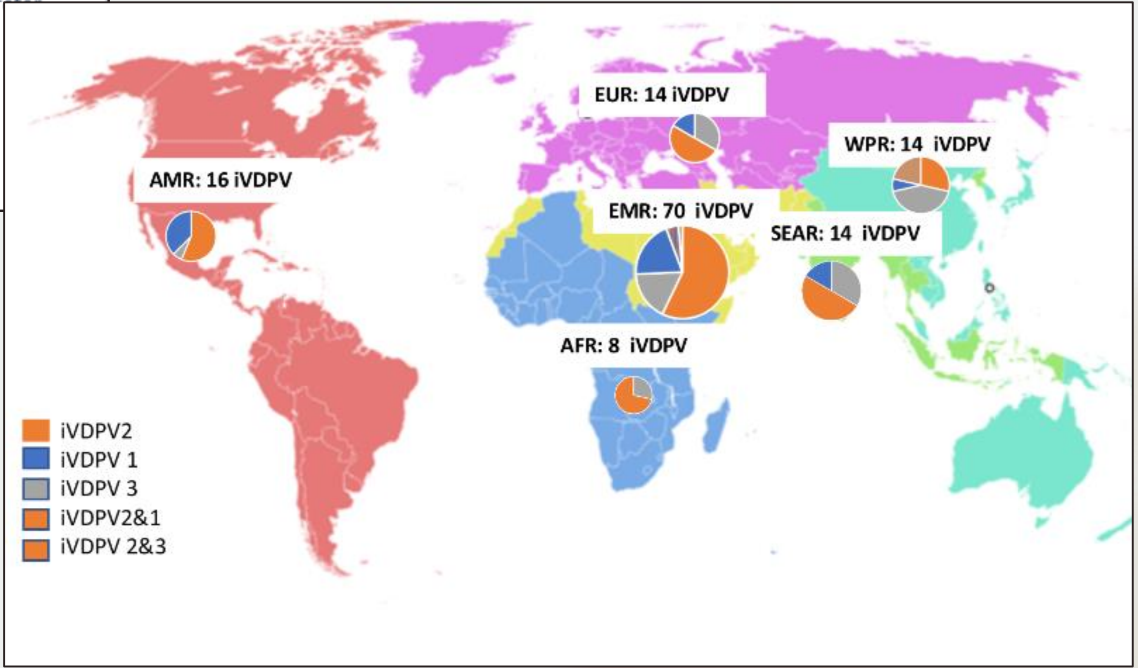
Clinical status

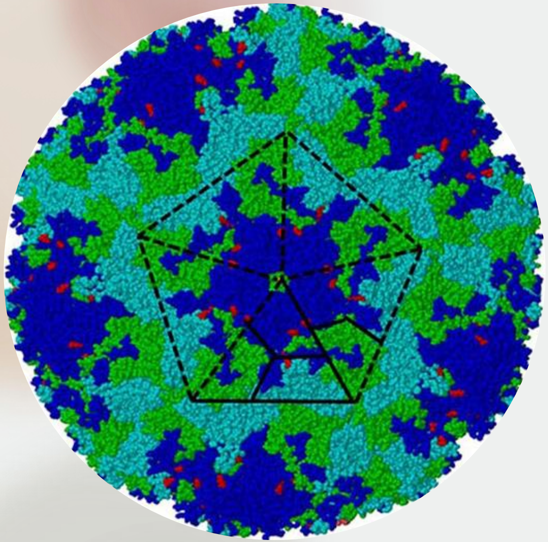


Outcome status

PV/EV Increased susceptibility in PID







03

Genetic Variability of PV/EV

Genetic variability of PV/EV

PV/EV, as with all RNA viruses, are in a constant process of evolution driven by different mechanisms: .

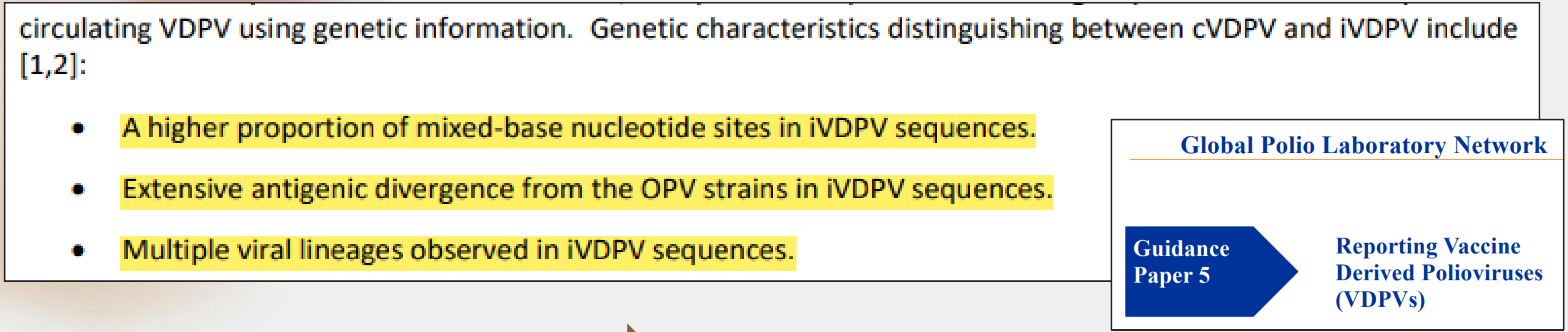
1- Generation of point mutations followed by genetic drift and selection: absence of proof-reading/repair mechanism.

Based on VP1 region, the mutation rate of WPV/ EV/ cVDPV: 1-3 10^{-2} mutations/ site/year

DOI: 10.1002/rmv

Genetic variability of PV/EV

the mutation rate of iVDPV: 2– 6 10^{-2} mutations/ site/year



circulating VDPV using genetic information. Genetic characteristics distinguishing between cVDPV and iVDPV include [1,2]:

- A higher proportion of mixed-base nucleotide sites in iVDPV sequences.
- Extensive antigenic divergence from the OPV strains in iVDPV sequences.
- Multiple viral lineages observed in iVDPV sequences.

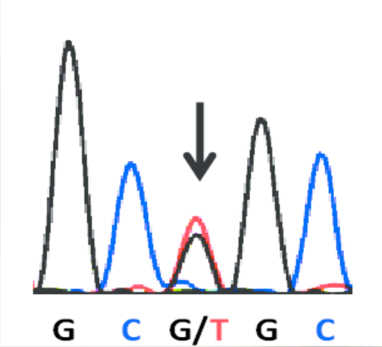
Global Polio Laboratory Network

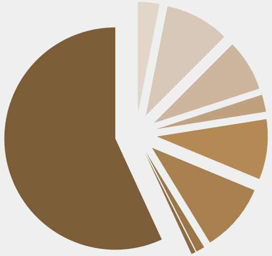
Guidance Paper 5

Reporting Vaccine Derived Polioviruses (VDPVs)

Mixed Bases +++

Intra-host diversity+++



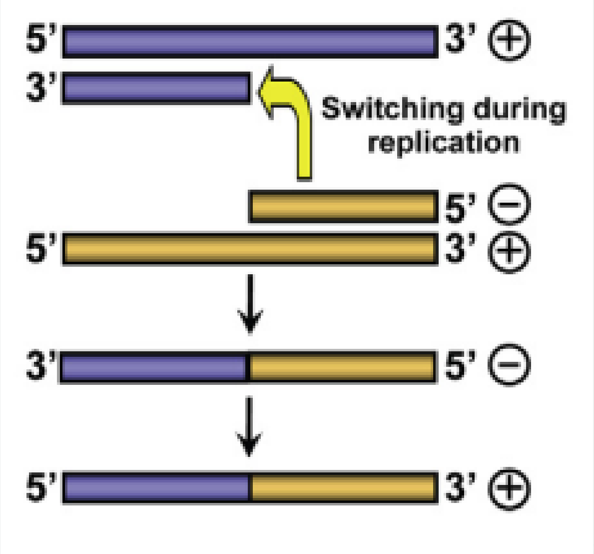


Mixed bases
43%

Genetic variability of PV/EV

Recombination:

Homologous recombination:
Both parental RNAs are homologous
The crossovers occur at homologous sites.



https://doi.org/10.1016/B978-0-12-816331-3.00002-7

After OPV vaccination, Intratypic recombinaison is frequent (20%) (Haddad-Boubaker et al, 2007)

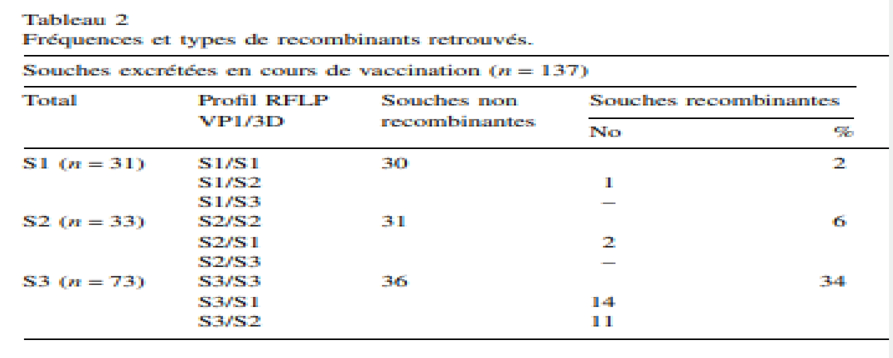


Tableau 2
Fréquences et types de recombinants retrouvés.

Souches excrétées en cours de vaccination (*n* = 137)

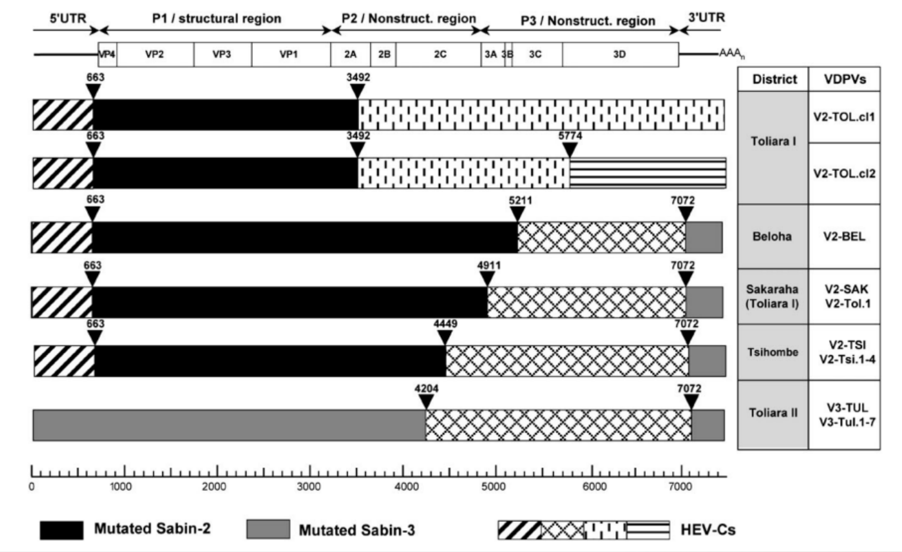
Total	Profil RFLP VP1/3D	Souches non recombinantes	Souches recombinantes	
			No	%
S1 (*n* = 31)	S1/S1	30		2
	S1/S2		1	
	S1/S3		–	
S2 (*n* = 33)	S2/S2	31		6
	S2/S1		2	
	S2/S3		–	
S3 (*n* = 73)	S3/S3	36		34
	S3/S1		14	
	S3/S2		11	

Genetic variability of PV/EV

cVDPV outbreaks

Recombinant Sabin /EV-C

coxsackievirus A (CV-A) type 13 (CV-A13) and CV-A17



District	VDPVs
Toliara I	V2-TOL.cl1
	V2-TOL.cl2
Beloha	V2-BEL
Sakaraha (Toliara I)	V2-SAK V2-Tol.1
Tsihombe	V2-TSI V2-Tsi.1-4
Toliara II	V3-TUL V3-Tul.1-7

DOI: 10.1093/infdis/jis204

Genetic variability of PV/EV

iVDPV excreters

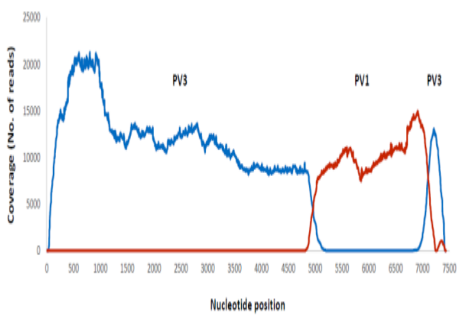
Intertypic recombination has been found occasionally in iVDPVs

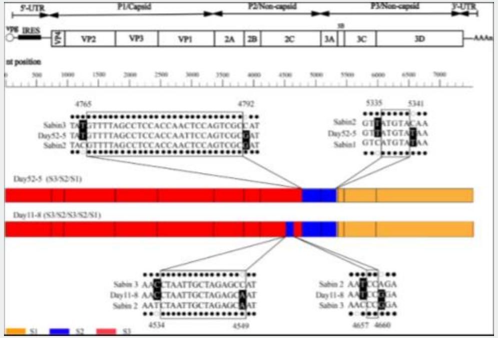
Paralysis Case and Contact Spread of Recombinant Vaccine–derived Poliovirus, Spain

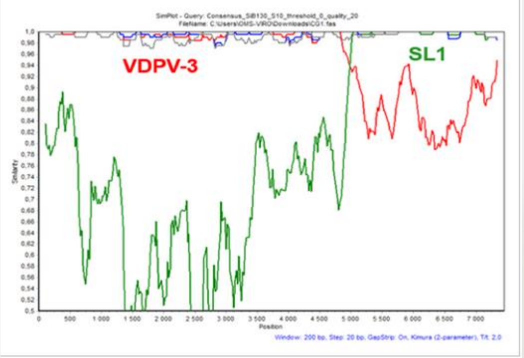
To the Editor: The World Health Organization Polio Eradication Initia-

encephalitis developed. The case was immediately considered suspicious and was therefore monitored at least monthly until the boy died. Sampling was conducted, coinciding with his visits to the hospital to receive therapy with immunoglobulin (γ globulin 0.5 g/kg). His contacts were studied, environmental surveillance was con-

iVDPV spread





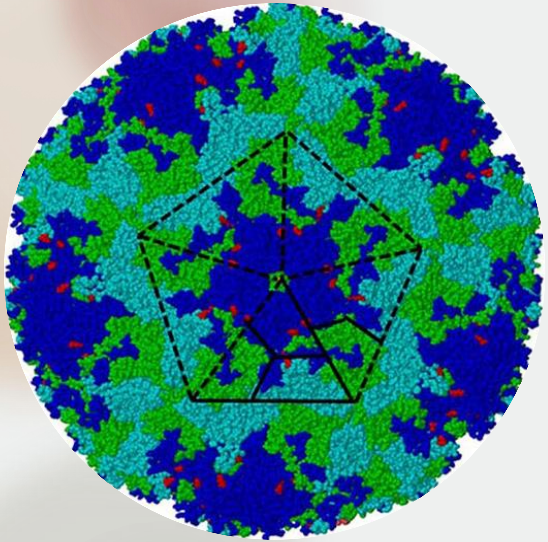


Prolonged iVDPV asymptomatic excretor

(Singanayagam et al., 2023)

Excretors with paralysis & fatal outcome

(Guo et al., 2023; Ben Salem et al., 2024)

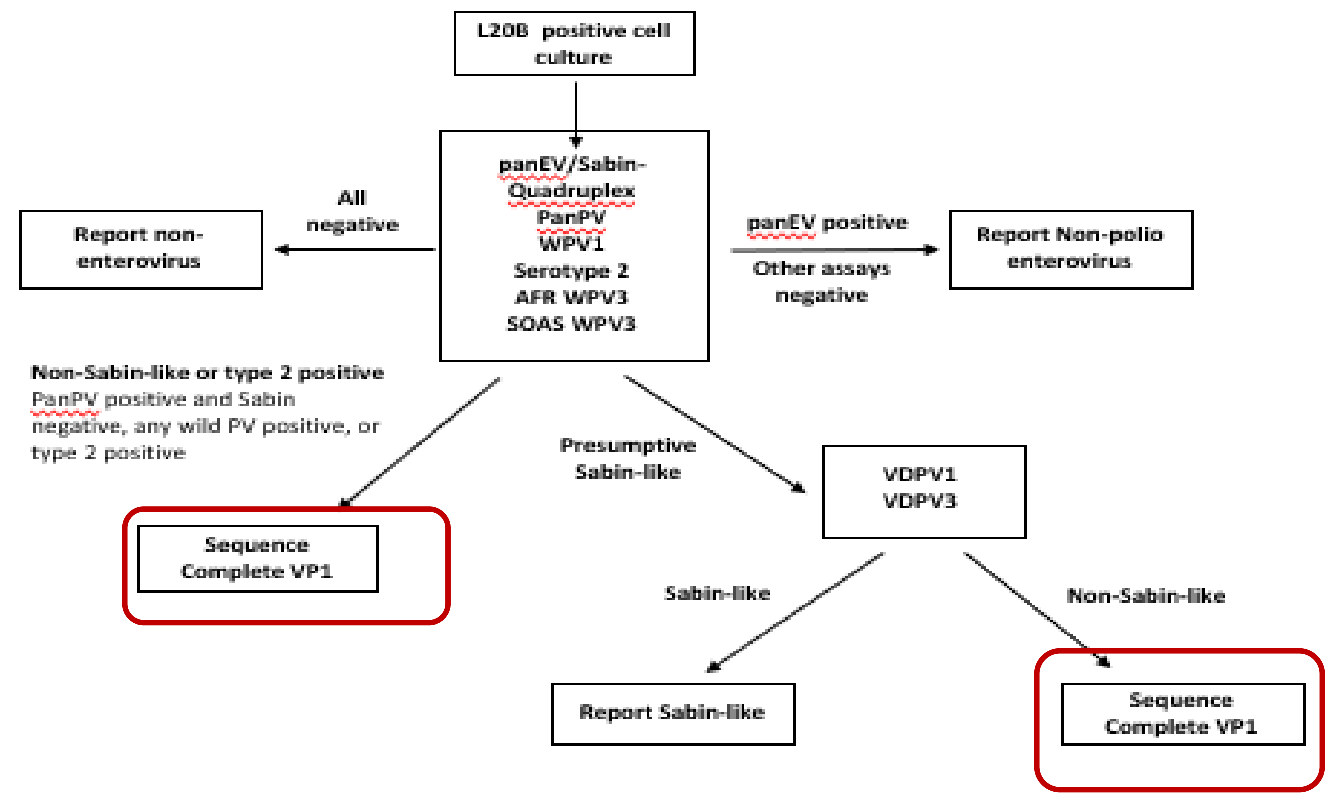


04

Apport of NGS sequencing

Apport of NGS sequencing

Global Polio Laboratory Network



L20B positive cell culture

panEV/Sabin-Quadruplex
PanPV
WPV1
Serotype 2
AFR WPV3
SOAS WPV3

All negative

Report non-enterovirus

panEV positive

Other assays negative

Report Non-polio enterovirus

Non-Sabin-like or type 2 positive

PanPV positive and Sabin negative, any wild PV positive, or type 2 positive

Sequence Complete VP1

Presumptive Sabin-like

VDPV1
VDPV3

Sabin-like

Report Sabin-like

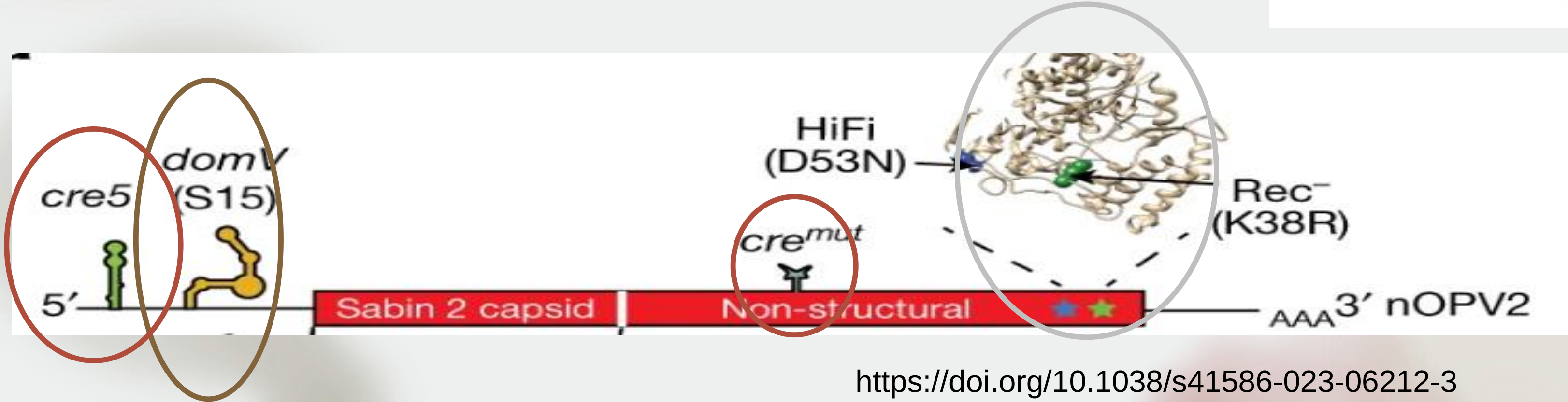
Non-Sabin-like

Sequence Complete VP1

Apport of NGS sequencing

- COVID-19 pandemic: Democratisation of NGS sequencing
- PV2 identification (cVDPV2, SL2, nOPV2)

nOPV2: modified Sabin 2 strain



https://doi.org/10.1038/s41586-023-06212-3

Apport of NGS sequencing

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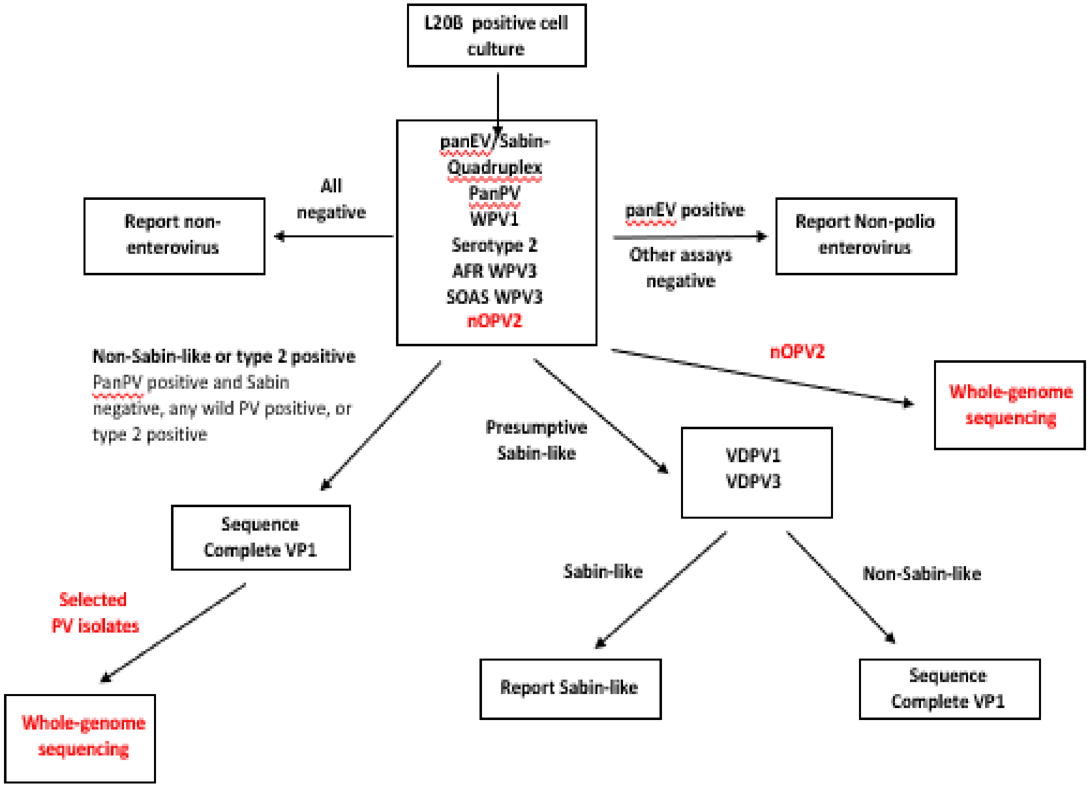
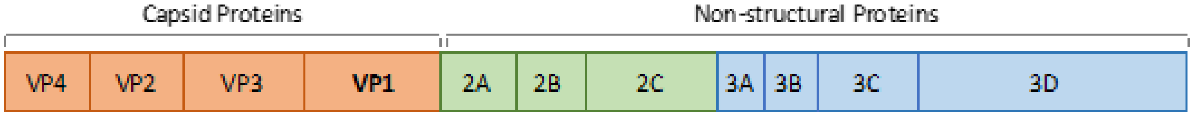
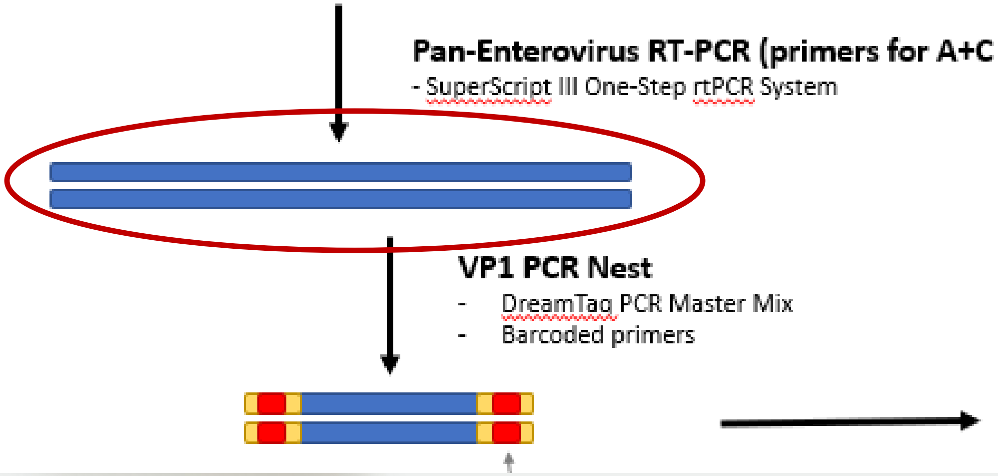


Polio Sequencing Consortium

Our PCR strategy





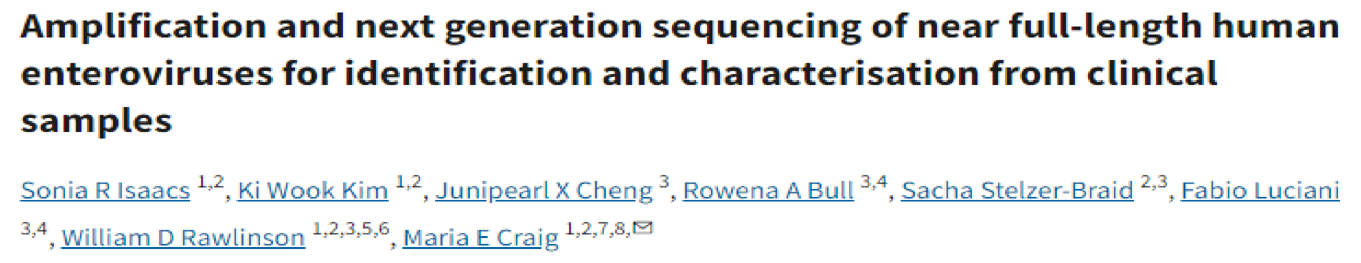
Pan-Enterovirus RT-PCR (primers for A+C enteroviruses, inc. nOPV2)

- SuperScript III One-Step rtPCR System

VP1 PCR Nest

- DreamTaq PCR Master Mix
- Barcoded primers

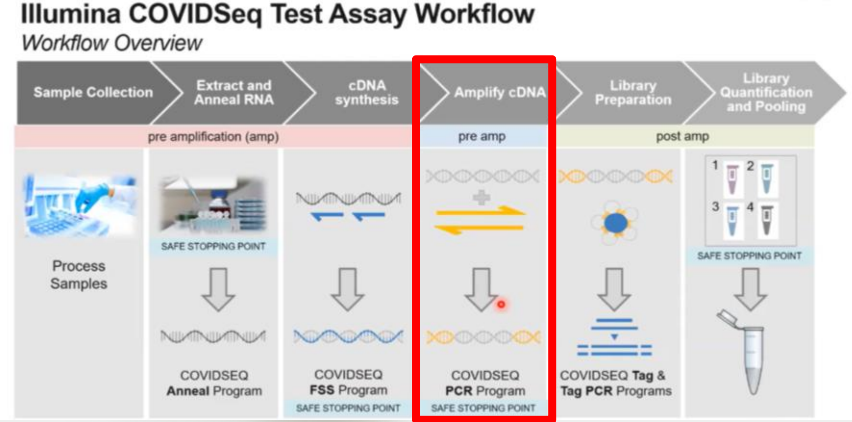
Apport of NGS sequencing



Amplification and next generation sequencing of near full-length human enteroviruses for identification and characterisation from clinical samples

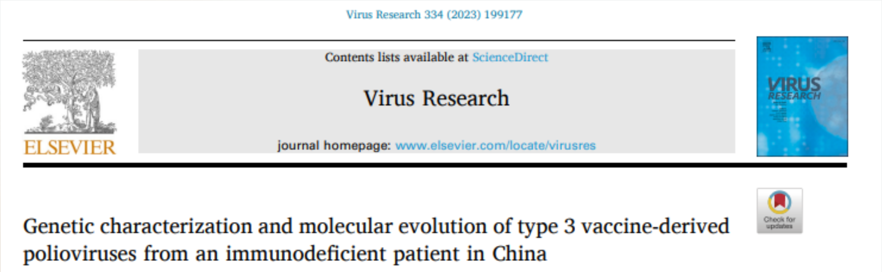
Sonia R Isaacs [1,2], Ki Wook Kim [1,2], Junipearl X Cheng [3], Rowena A Bull [3,4], Sacha Stelzer-Braid [2,3], Fabio Luciani [3,4], William D Rawlinson [1,2,3,5,6], Maria E Craig [1,2,7,8,✉]

Conception specific primers pools nOPV2/ PV/EV:



Illumina COVIDSeq Test Assay Workflow

Workflow Overview



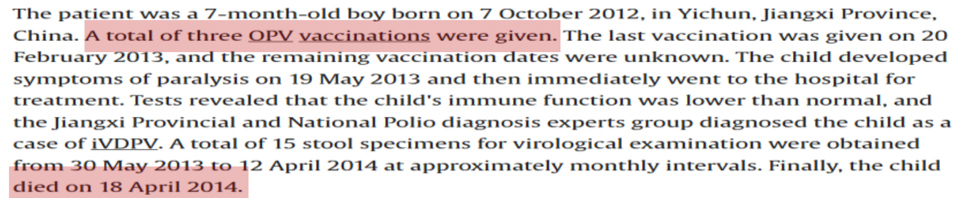
Virus Research 334 (2023) 199177

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Genetic characterization and molecular evolution of type 3 vaccine-derived polioviruses from an immunodeficient patient in China

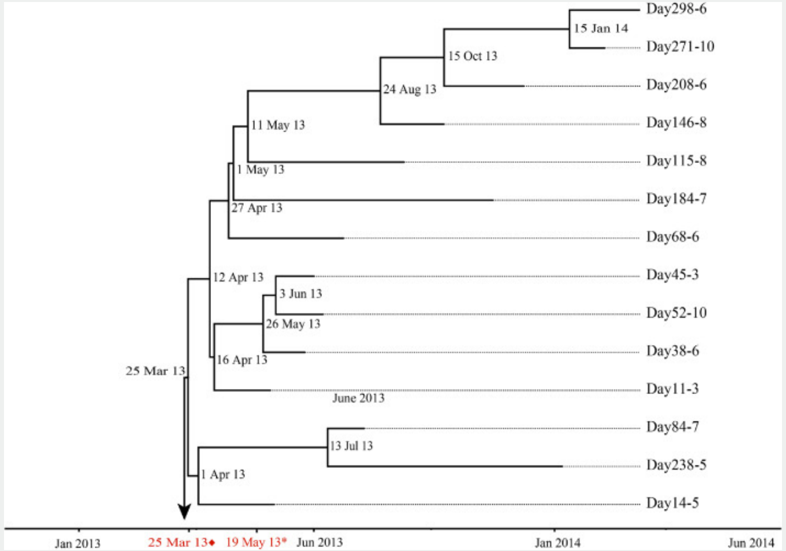


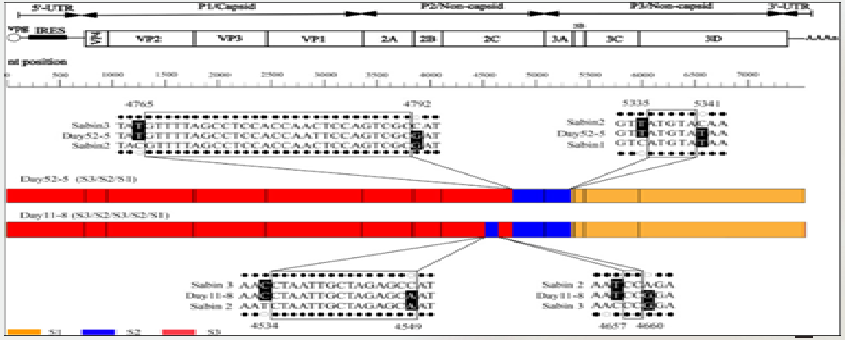
The patient was a 7-month-old boy born on 7 October 2012, in Yichun, Jiangxi Province, China. A total of three OPV vaccinations were given. The last vaccination was given on 20 February 2013, and the remaining vaccination dates were unknown. The child developed symptoms of paralysis on 19 May 2013 and then immediately went to the hospital for treatment. Tests revealed that the child's immune function was lower than normal, and the Jiangxi Provincial and National Polio diagnosis experts group diagnosed the child as a case of iVDPV. A total of 15 stool specimens for virological examination were obtained from 30 May 2013 to 12 April 2014 at approximately monthly intervals. Finally, the child died on 18 April 2014.

Multiple variants
and lineages have been diverged

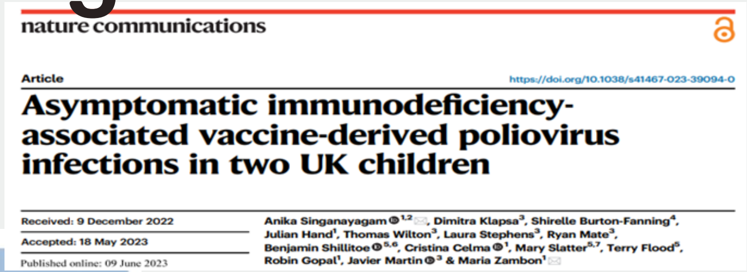
All iVDPVs were vaccine recombinants,

Modification in Ag sites all along the genome





Apport of NGS sequencing


nature communications

Article https://doi.org/10.1038/s41467-023-39094-0

Asymptomatic immunodeficiency-associated vaccine-derived poliovirus infections in two UK children

Received: 9 December 2022

Accepted: 18 May 2023

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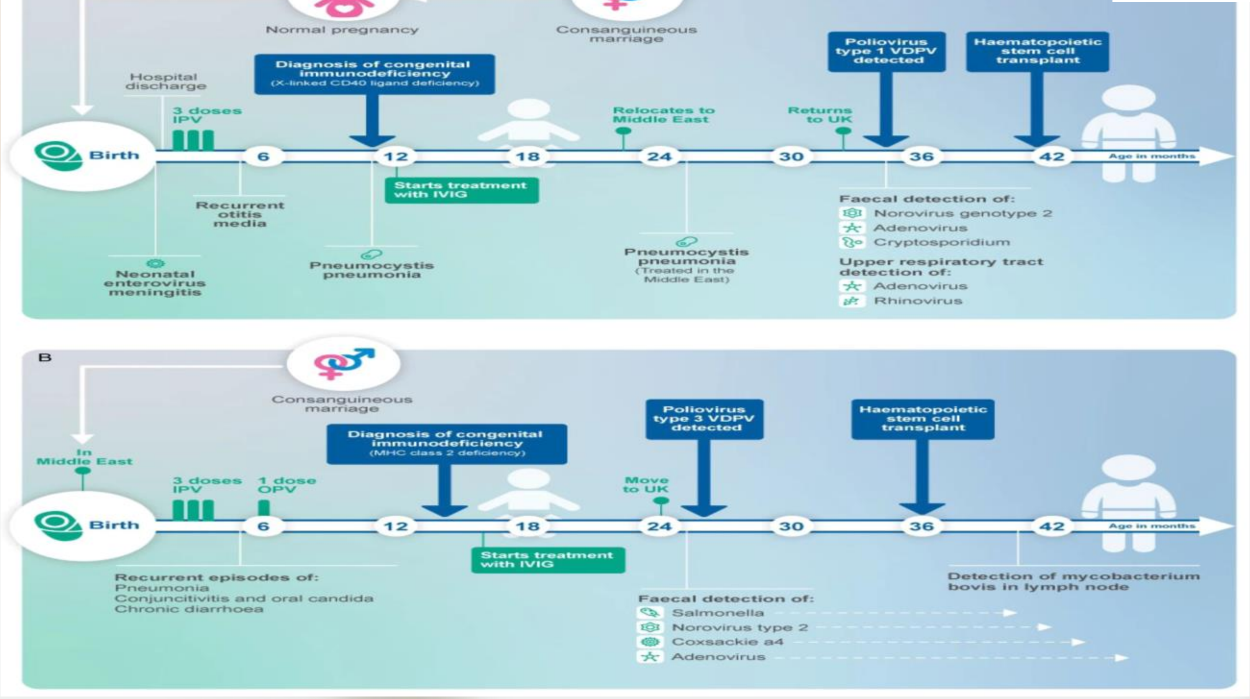
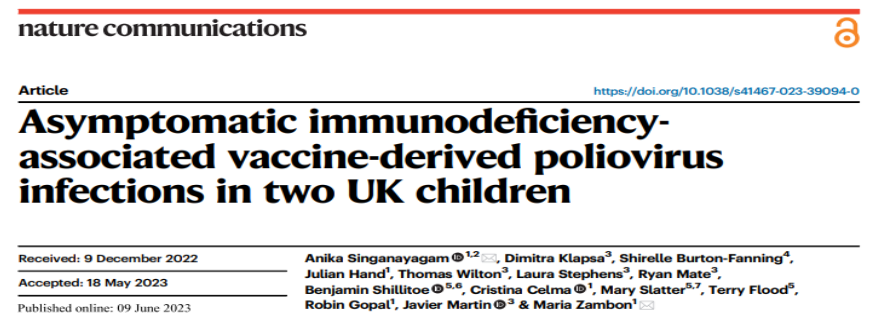


6 weeks & 3 months excretion periods


nature communications

Article

https://doi.org/10.1038/s41467-023-39094-0

Asymptomatic immunodeficiency-associated vaccine-derived poliovirus infections in two UK children

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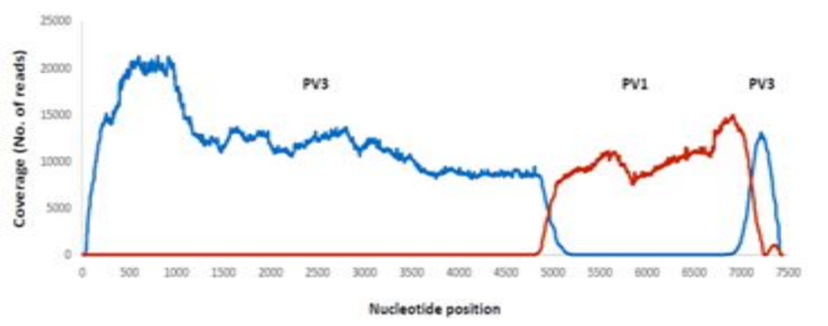
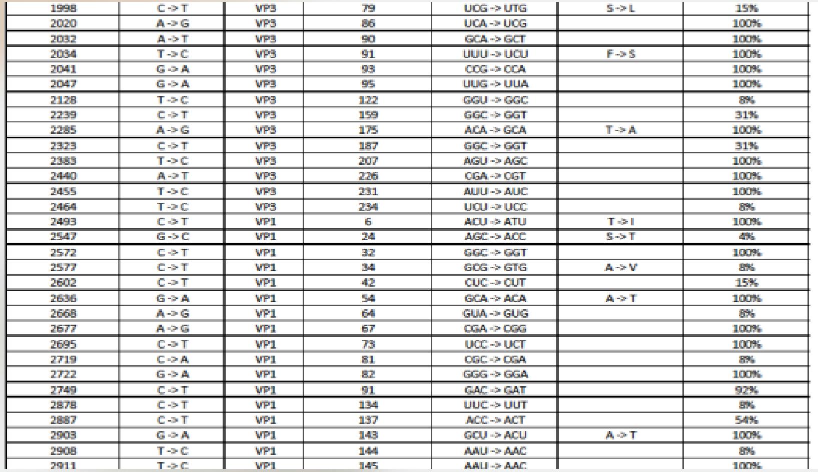
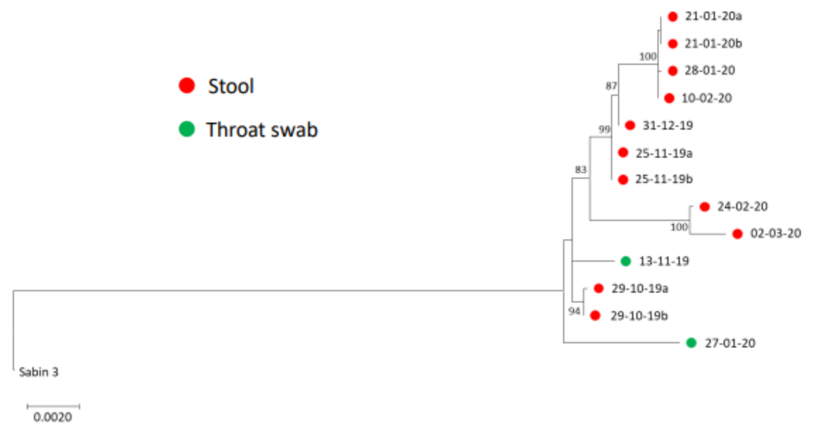
Anika Singanayagam[1,2], Dimitra Klapsa[3], Shirelle Burton-Fanning[4], Julian Hand[1], Thomas Wilton[3], Laura Stephens[3], Ryan Mate[3], Benjamin Shillitoe[5,6], Cristina Celma[1], Mary Slatter[5,7], Terry Flood[5], Robin Gopal[1], Javier Martin[3] & Maria Zambon[1]

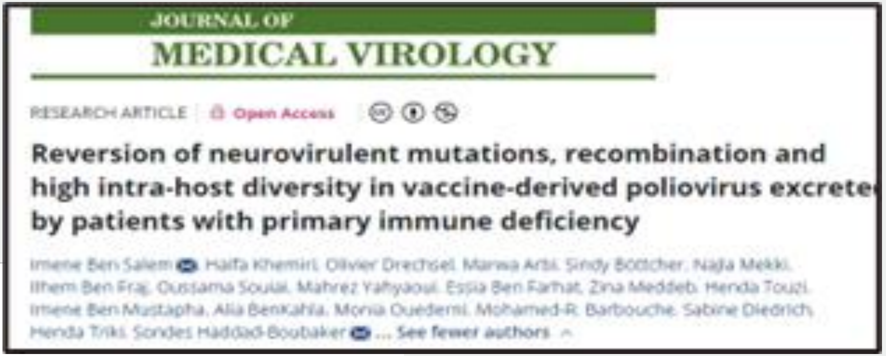




1998	C -> T	VP3	79	UCG -> UTG	S -> L	15%
2020	A -> G	VP3	86	UCA -> UCG		100%
2032	A -> T	VP3	90	GCA -> GCT		100%
2034	T -> C	VP3	91	UUU -> UCU	F -> S	100%
2041	G -> A	VP3	93	CCG -> CCA		100%
2047	G -> A	VP3	95	UUG -> UUA		100%
2128	T -> C	VP3	122	GGU -> GGC		8%
2239	C -> T	VP3	159	GGC -> GGT		31%
2285	A -> G	VP3	175	ACA -> GCA	T -> A	100%
2323	C -> T	VP3	187	GGC -> GGT		31%
2383	T -> C	VP3	207	AGU -> AGC		100%
2440	A -> T	VP3	226	CGA -> CGT		100%
2455	T -> C	VP3	231	AUU -> AUC		100%
2464	T -> C	VP3	234	UCU -> UCC		8%
2493	C -> T	VP1	6	ACU -> ATU	T -> I	100%
2547	G -> C	VP1	24	AGC -> ACC	S -> T	4%
2572	C -> T	VP1	32	GGC -> GGT		100%
2577	C -> T	VP1	34	GCG -> GTG	A -> V	8%
2602	C -> T	VP1	42	CUC -> CUT		15%
2636	G -> A	VP1	54	GCA -> ACA	A -> T	100%
2668	A -> G	VP1	64	GUA -> GUG		8%
2677	A -> G	VP1	67	CGA -> CGG		100%
2695	C -> T	VP1	73	UCC -> UCT		100%
2719	C -> A	VP1	81	CGC -> CGA		8%
2722	G -> A	VP1	82	GGG -> GGA		100%
2749	C -> T	VP1	91	GAC -> GAT		92%
2878	C -> T	VP1	134	UUC -> UUT		8%
2887	C -> T	VP1	137	ACC -> ACT		54%
2903	G -> A	VP1	143	GCU -> ACU	A -> T	100%
2908	T -> C	VP1	144	AAU -> AAC		8%
2911	T -> C	VP1	145	AAU -> AAC		100%



Apport of NGS sequencing

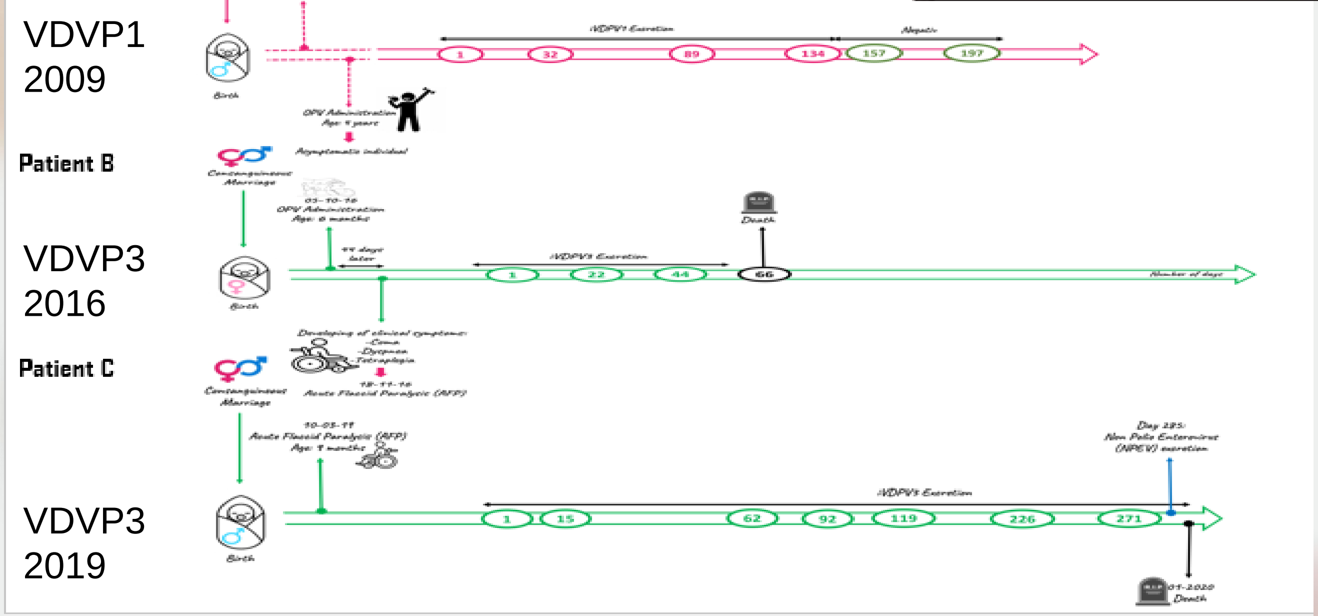


JOURNAL OF
MEDICAL VIROLOGY

RESEARCH ARTICLE | Open Access

Reversion of neurovirulent mutations, recombination and high intra-host diversity in vaccine-derived poliovirus excrete by patients with primary immune deficiency

Imene Ben Salem, Haifa Khemiri, Olivier Drechsel, Marwa Arbi, Sindy Böttcher, Najla Mekki, Ilhem Ben Fraj, Oussama Souiai, Mahrez Yahyaoui, Essia Ben Farhat, Zina Meddeb, Henda Touzi, Imene Ben Mustapha, Alia BenKahla, Monia Ouederni, Mohamed-R. Barbouche, Sabine Diedrich, Henda Triki, Sondes Haddad-Boubaker ... See fewer authors ^



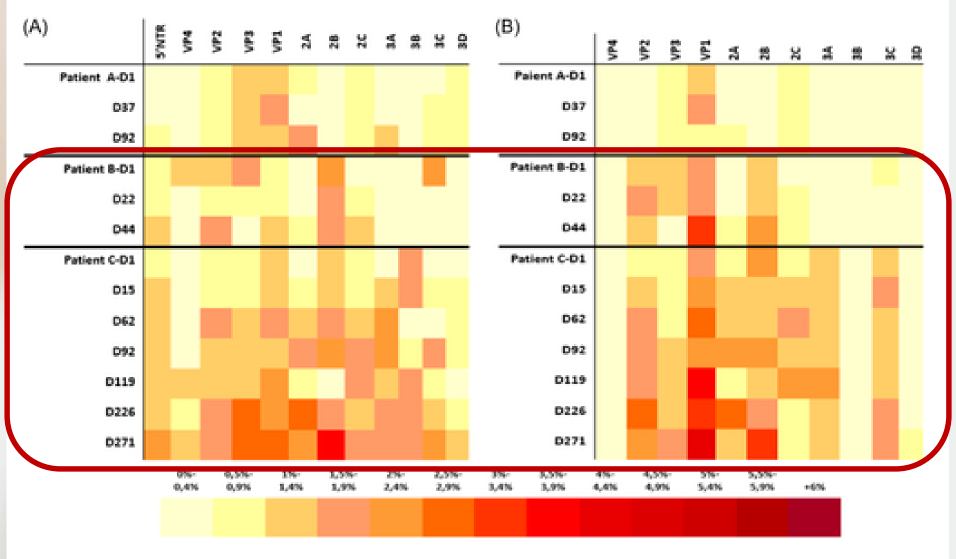
VDVP1
2009

VDVP3
2016

VDVP3
2019

SCAN ME

Apport of NGS sequencing



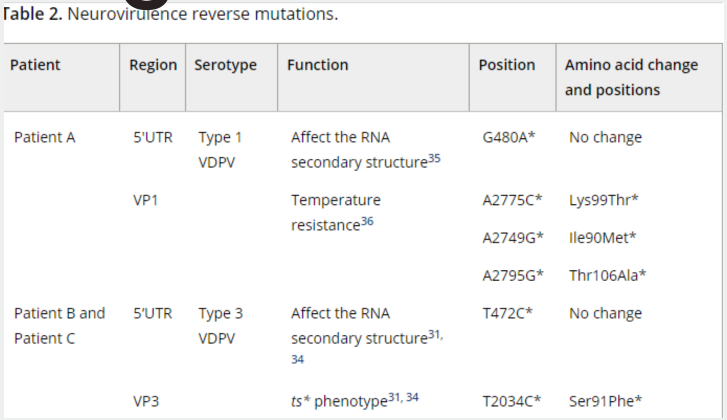
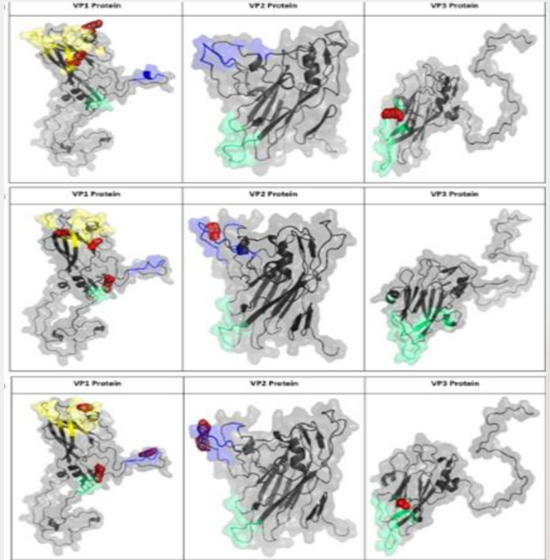


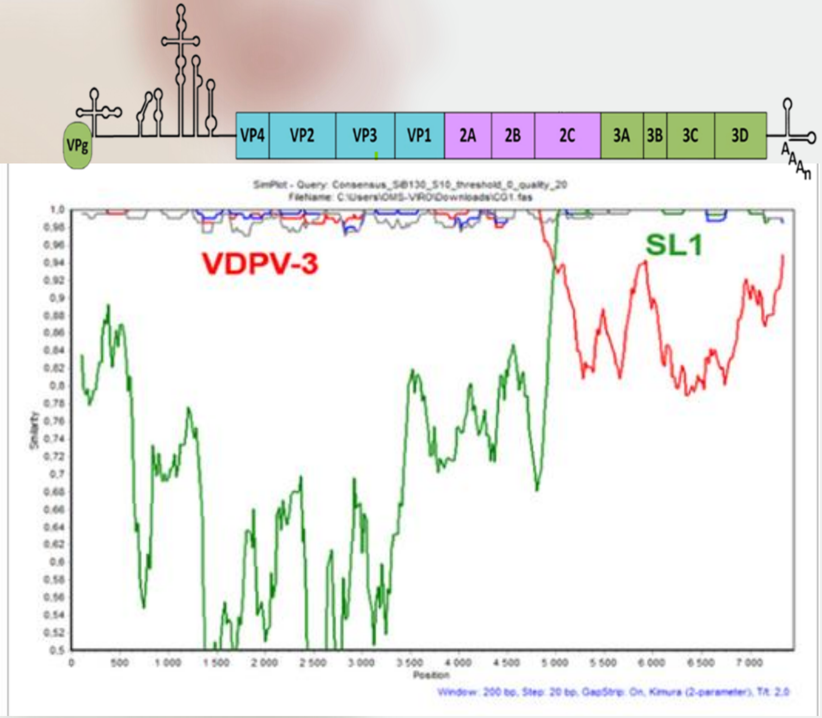
Table 2. Neurovirulence reverse mutations.

Patient	Region	Serotype	Function	Position	Amino acid change and positions
Patient A	5'UTR	Type 1 VDPV	Affect the RNA secondary structure[35]	G480A*	No change
	VP1		Temperature resistance[36]	A2775C*	Lys99Thr*
				A2749G*	Ile90Met*
				A2795G*	Thr106Ala*
Patient B and Patient C	5'UTR	Type 3 VDPV	Affect the RNA secondary structure[31, 34]	T472C*	No change
	VP3		ts* phenotype[31, 34]	T2034C*	Ser91Phe*

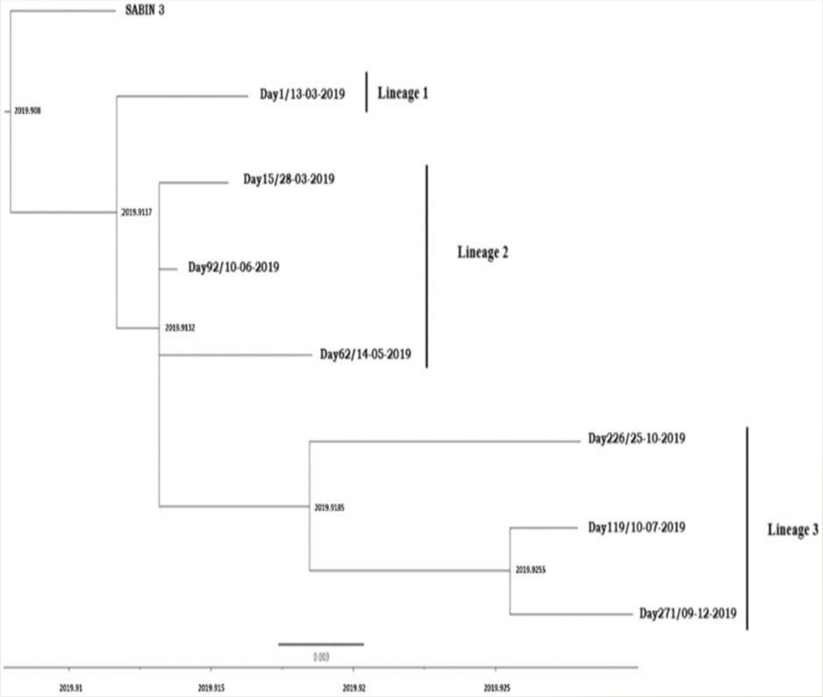


Apport of NGS sequencing

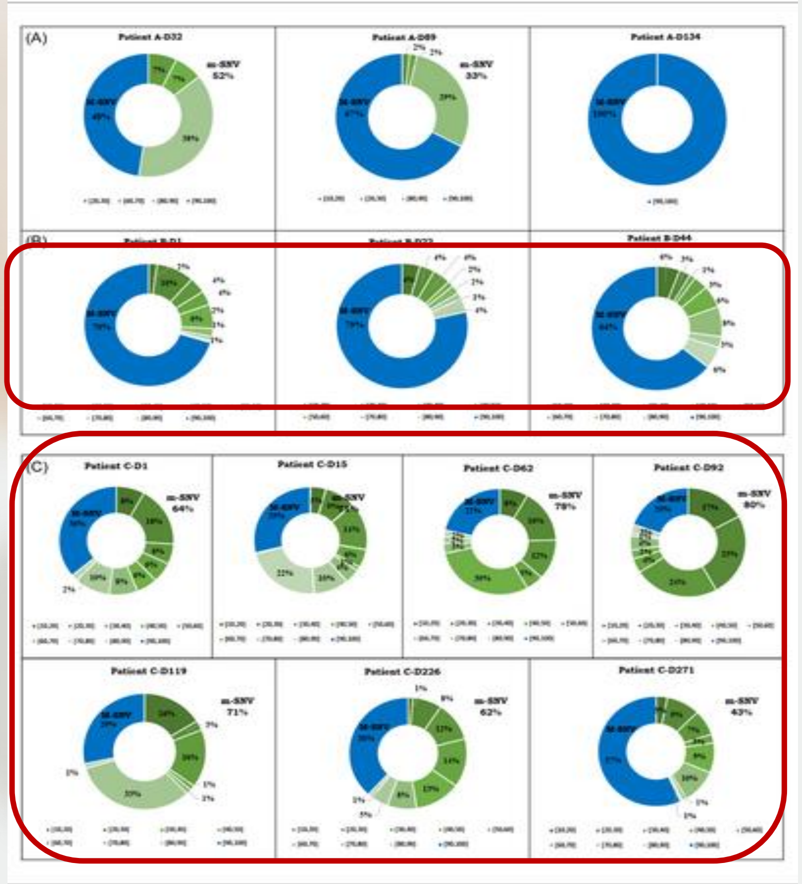
Patient 2



Patient 3



PV/EV surveillance of PID in Tunisia



Patient 1

Patient 2

Patient 3

Possible role of genetic and intra-host diversity in the prolonged virus shedding as well as the genesis of virulent strains.

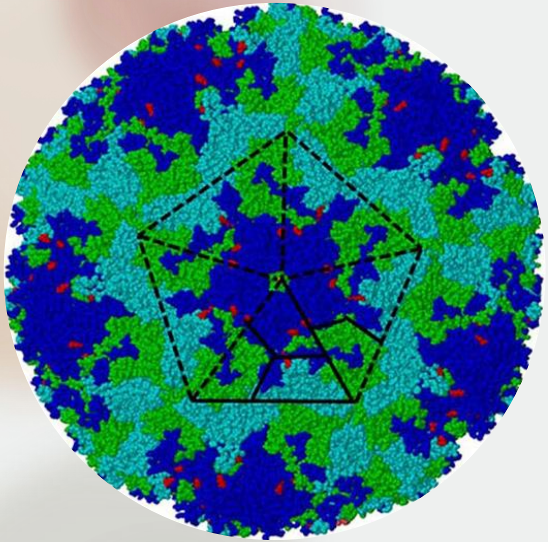
Apport of NGS sequencing

Complete genome characterization provides valuable information

for the patient management and treatment

as well as rapid detect of potential epidemic strains

establishment of an efficient public health responses for any VDPV emergence and spread.



05

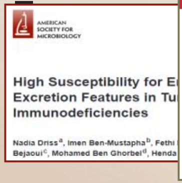
PV/EV surveillance of PID in Tunisia

PV/EV surveillance of PID in Tunisia

- Last Paralytic case due to WPV: 1992
- Last detection in asymptomatic case: 1994
- No epidemic due to cVDPV
- No WPV importation but it is still possible
- Good vaccination covarege & performant AFP surveillance system
- No regular surveillance of poliovirus/enterovirus excretion in ID patients in Tunisia, until November 2021

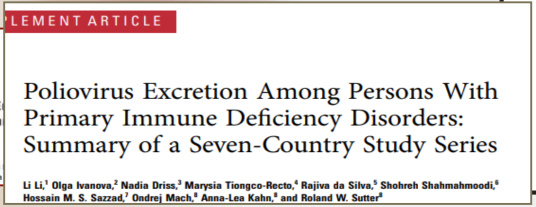
PV/EV surveillance of PID in Tunisia

Integration of PID excretor surveillance with AFP surveillance


AMERICAN SOCIETY FOR MICROBIOLOGY

High Susceptibility for E
Excretion Features in Tu
Immunodeficiencies

Nadia Driss[a], Imen Ben-Mustapha[b], Fethi
Bejaoui[c], Mohamed Ben Ghorbel[d], Henda


LEMENT ARTICLE

Poliovirus Excretion Among Persons With Primary Immune Deficiency Disorders: Summary of a Seven-Country Study Series

Li Li,[1] Olga Ivanova,[2] Nadia Driss,[3] Marysia Tiongco-Recto,[4] Rajiva da Silva,[5] Shohreh Shahmahmoodi,[6] Hossain M. S. Sazzad,[7] Ondrej Mach,[8] Anna-Lea Kahn,[8] and Roland W. Sutter[8]

National Plan for Polio virus Surveillance among Primary Immunodeficiency (PID) patients

2003

2012

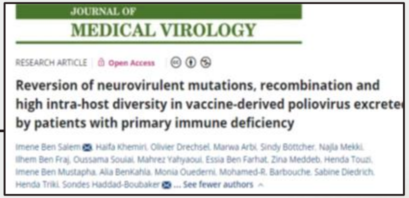
2014

2019

2021

Janvier 2024

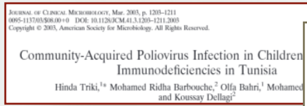
COVID-19


JOURNAL OF
MEDICAL VIROLOGY

RESEARCH ARTICLE | Open Access

Reversion of neurovirulent mutations, recombination and high intra-host diversity in vaccine-derived poliovirus excrete by patients with primary immune deficiency

Imene Ben Salem, Haifa Khemiri, Olivier Drechsel, Marwa Arbi, Sindy Böttcher, Najla Mekki, Ilhem Ben Fraj, Oussama Soulai, Mahrez Yahyaoui, Essia Ben Farhat, Zina Meddeb, Henda Touzi, Imene Ben Mustapha, Alia BenKahla, Monia Ouederni, Mohamed-R. Barbouche, Sabine Diedrich, Henda Triki, Sondes Haddad-Boubaker ... See fewer authors


Journal of Clinical Microbiology, Mar. 2003, p. 1203–1211 Vol. 41, No. 3
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.3.1203–1211.2003
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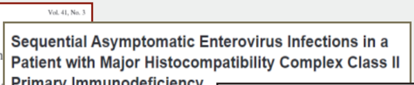
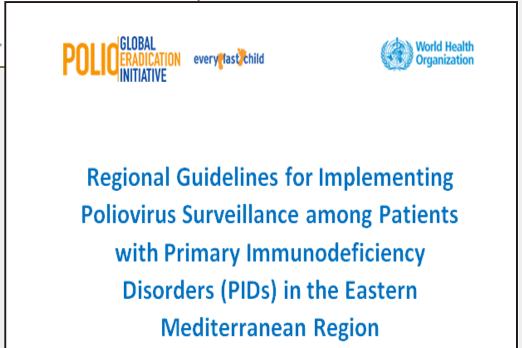
Community-Acquired Poliovirus Infection in Children
Immunodeficiencies in Tunisia

Hinda Triki,[1*] Mohamed Ridha Barbouche,[2] Olfa Bahri,[1] Mohamed
and Koussay Dellagi[2]


Sequential Asymptomatic Enterovirus Infections in a Patient with Major Histocompatibility Complex Class II Primary Immunodeficiency

Nadia Driss[a], Fethi Mellouli[b], Ahlem Ben Yahia[a],
Henda Triki[a], Mohamed Bejaoui[b]


POLIO GLOBAL ERADICATION INITIATIVE
every last child
World Health Organization

Regional Guidelines for Implementing Poliovirus Surveillance among Patients with Primary Immunodeficiency Disorders (PIDs) in the Eastern Mediterranean Region

Nov 2021:
PID surveillance
WHO Pilot Project

PID line list

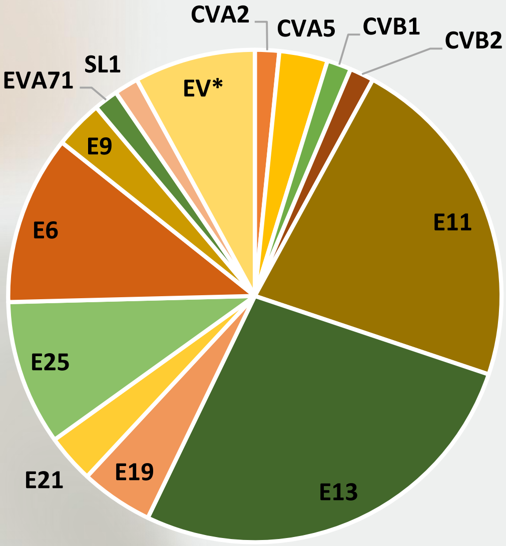
As of 19 May 2025

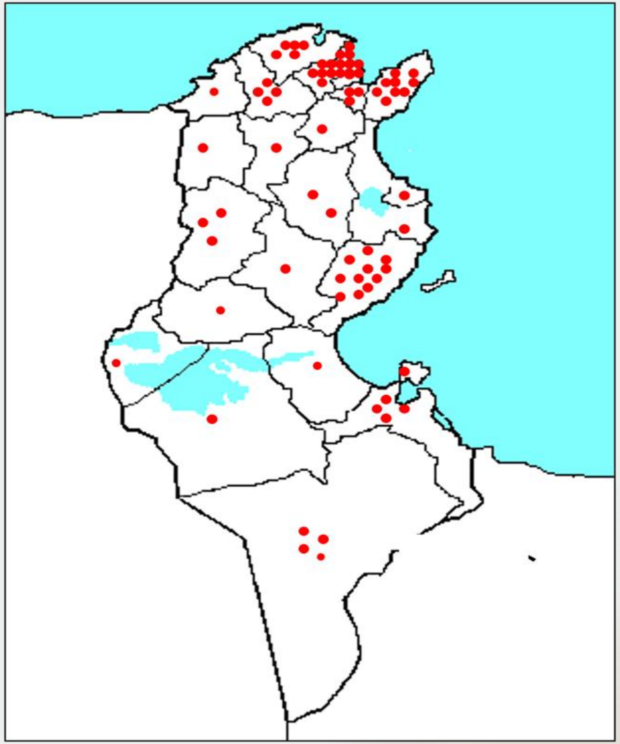
Included patients: 144

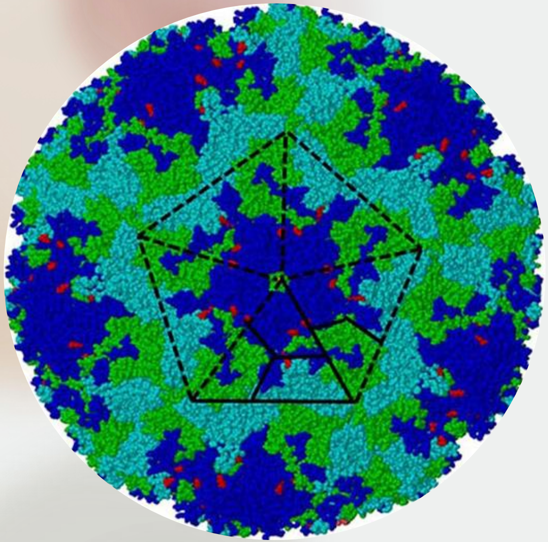
Patients refusing sampling: 4

Died patients: 11

Transplanted patients: 9







06

Conclusion

- The prolonged excretion and evolution of PVs in patients with Primary Immunodeficiency (PID) pose major concerns for the Global Polio Eradication Initiative (GPEI).
- in-depth genetic characterization of complete genomes of the excreted PV/EV strains adds new genomic data and knowledges, on the limited global PV/EV excretion.
- Studies showed Huge genomic diversity, Multiple variants and lineages that have been diverged for rapid adaptation to new environments and resistance to host immune stress.
- Complete genome characterization provides valuable information for the patient management and treatment as well as establishment of an efficient public health responses for any VDPV emergence and spread.

Acknowledgments

World Health Organization

ROBERT KOCH INSTITUT

NIBSC
Confidence in Biological Medicines

WHO: HQ- EMRO – WR-TUN

Collaborators

Surveillance team – MOH

Nadia Driss; Mehrez Yahyaoui; Essia Hmida; Ines Ben Mrad

Immunology team –IPT

Najla Mekki; Imen Ben Mustafa; Ridha Barbouche

Virology team –IPT

Henda Touzi ; Zina Meddeb; Imen Ben Salem; Haifa Khemiri; Henda Triki


рахмат
danke
謝謝
ngiyabonga
شكراً جزيلاً
Баярлалаа
mersi
kia ora
barka
spas
welalin
tack
teşekkür ederim
mahalo
tapadh leat
спасибо
faafetai lava
vinaka
спасибі
blagodaram
dank je
misaotra
paldies
grazzi
matondo
thank you
gracias
хвала
kiitos
dankie
nanni
nandi
dhanyavad
mauruuru
köszönöm
aciu
dankon
akun
asante
manana
obrigada
tenki
murakoze
bedankt
bayarlalaa
gracie
hvala
enkosi
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дякую
mamnun
chokrane
dziękuję
chnorakaloutioun
gratias ago
gràcies
sulpáy
go raibh maith agat
taiku
sobodi
dekuji
sağolun
sukriya
kop khun krap
obrigado
arigatô
takk
dakujem
trugarez
mesi
didi madloba
najis tuke
ありがとう
tanemirt
grazie
rahmet
kam sah hamnida
rahmat
terima kasih
ευχαριστώ
diolch
dhanyavadagalu
shukriya
merce
мерси
তোমাকে ধন্যবাদ
감사합니다
xiexie
merci

Pleconaril antiviral is a 3C protease inhibitor, developed by Pfizer [58] against picornaviruses including most Enteroviruses and Rhinoviruses.

The safety of Pleconaril was proved in adults [60] and pediatric population such as children [61] and neonates [62-63] for enteroviral and Rhinoviral treatment. Nevertheless, pleconaril resistant strain against Echovirus 11 was identified in immunocompromised patient and it was also resistant to intravenous immunoglobulin, used in combined treatment [64]. Thus, further investigation was suggested since multiple factors such as immunity status may play a role in the development of resistant strains [64].