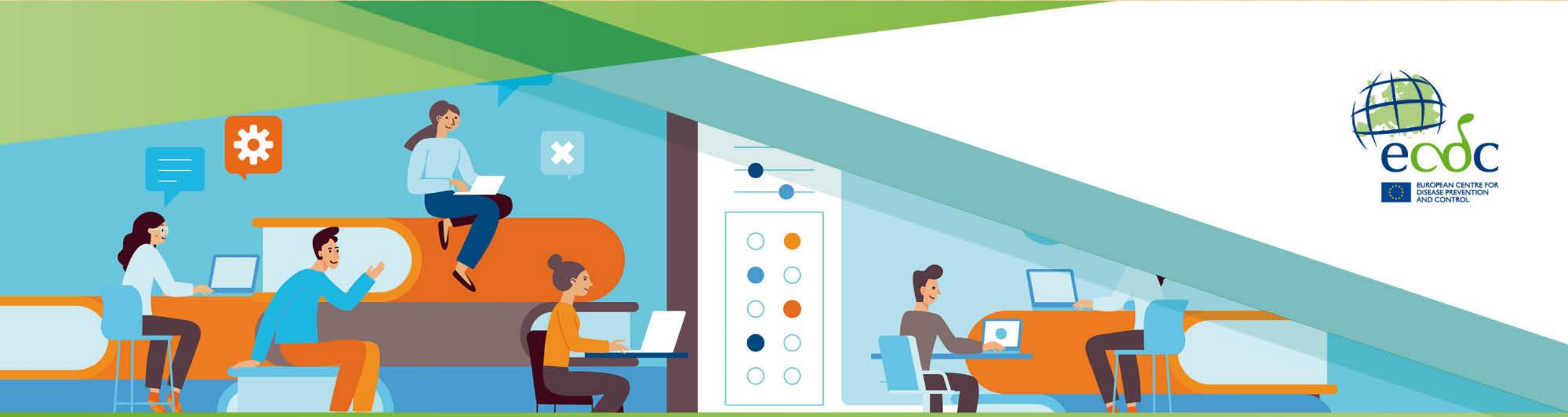


GenEpi-BioTrain Virtual Training 29 - Genomic epidemiology of mpox

The emergence of mpox

05-10-2026

Bas Oude Munnink

Aim and learning objectives

Aim:

Get insight into the circulation of different strains of mpox

Specific learning objectives

At the end of this session, you will be able to:

1. The historical context of the mpox virus outbreaks
2. How different clades of mpox are transmitted
3. Get an overview of the current distribution of different mpox lineages

Do not edit
How to change the design



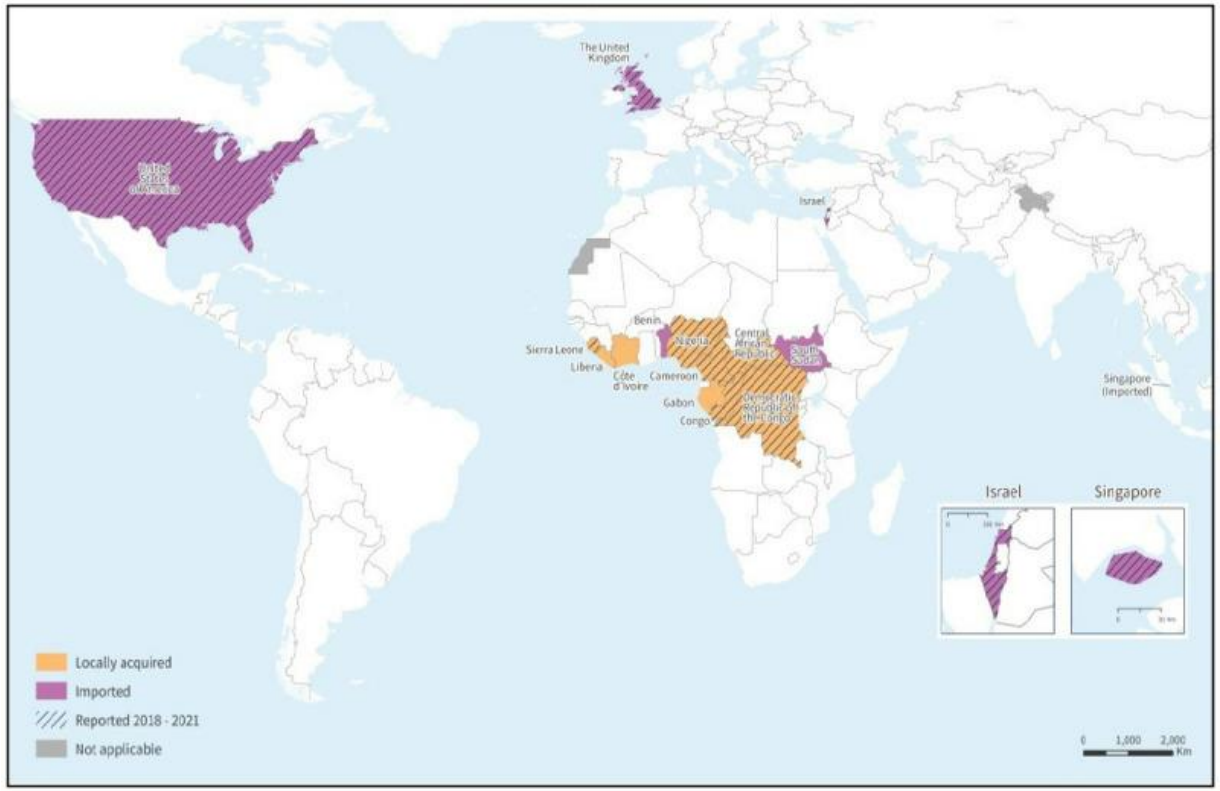
What do you know about mpox?

ⓘ The Slido app must be installed on every computer you're presenting from

slido

Mpox historically

- First identified in 1958 in Asian monkeys employed for experiments in a laboratory in Denmark and then in 1970 in humans in Africa
- In the Democratic Republic of Congo (DRC), has continued to circulate for about 50 years in some regions of Africa, indicated as Congo Basin (CB) and West Africa (WA) where it has become endemic.
- Rare outbreaks have occurred outside endemic countries, linked to importation of the virus from endemic areas.



https://www.cdc.gov/mmwr/volumes/72/wr/mm7203a4.htm?s_cid=mm7203a4_w

Mpox during the 2000's

Outbreaks with hundreds of cases in DR Congo in the 2000s

2003

Monkeypox spreads as US public-health system plays catch-up

At the time of going to press, 81 cases (32 laboratory-confirmed) of monkeypox had been reported in the USA, a figure that is likely to increase, according to Michael Osterholm (University of Minnesota, Minneapolis, MN, USA). Unless all animal reservoirs are identified and efforts made to "contain monkeypox and, hopefully, eradicate it", he emphasises, "there is no reason why an agent like that could not become an endemic viral infection of rodents in the United States, which would be absolutely horrible".

Indeed, on July 3, the US Centers for Disease Control and Prevention (CDC) confirmed the presence of monkeypox virus in one Gambian giant rat, three dormice, and two rope squirrels that were part of an African rodent shipment from Ghana to the USA (http://www.cdc.gov/od/oc/media/pressrel/r030702.htm). The shipment is said to be the source of the US outbreak, which started with a little girl who presented at the Marshfield Clinic (Marshfield, WI, USA). "The whole thing was a big surprise. It was difficult with the few cases we had here to recognise that something was happening that involved multiple cities in multiple states", says Kurt Reed, director of Marshfield's clinical research centre. Days went by as Reed and his colleagues tried to identify the source of the girl's antibiotic-resistant infection, taking cultures and, after her mother also showed signs of illness, doing biopsies and electronic microscopy.

Rights were not granted to include this image in electronic media. Please refer to the printed journal.

A Gambian giant rat

"On June 3, we had the first indication that this was an orthopox virus. Then we heard about the Gambian rat, and things got very bizarre. That's when we finally considered that this could be an old-world virus that had somehow got into the USA", recalls Reed. "Things moved quickly from there, but it could have been figured out a lot faster if everyone had all the information up front. But we didn't. If we had known when we saw we had pox virus that there were other cases from similar exposures elsewhere, that would have prompted a public-health response."

And therein lies the rub, says Osterholm. "This is a big-picture issue now. It's not the initial hit of monkeypox or West Nile or whatever—it's the spread throughout the country", he asserts. "It's not just being able to respond initially to an outbreak, it's surveying and being sure we get it all. Monkeypox is like West Nile because we never did control it right at the beginning and now look what's happened."

The US public-health system—even with limited resources and near "burnout" as a result of the threats of bioterrorism—needs to embrace a new mindset, continues Osterholm. "We have been basing our public-health practices on the concept that the developed world dealt with the developed world, and the less developed world was just there. Now we have to think that any disease there can occur not just in travellers, but here. It's like the deer in the headlights—you're going to hit it. It's a matter now of whether you just forget about it or let it come through the windshield."

Marilynn Larkin

Outbreak of human monkeypox in Nigeria in 2017–18: a clinical and epidemiological report

Adesola Yinka-Ogunleye, Olusola Aruna, Mahmood Dalhat, Dimie Ogoina, Andrea McCollum, Yahyah Disu, Ibrahim Mamadu, Afolabi Akinpelu, Adama Ahmad, Joel Burga, Adolphe Ndoreraho, Edouard Nkunzimana, Lamin Manneh, Amina Mohammed, Olawunmi Adeoye, Daniel Tom-Aba, Bernard Silenou, Oladipupo Ipadeola, Muhammad Saleh, Ayodele Adeyemo, Ifeoma Nwadiutor, Neni Aworabhi, Patience Uke, Doris John, Paul Wakama, Mary Reynolds, Matthew R Mauldin, Jeffrey Doty, Kimberly Wilkins, Joy Musa, Asheena Khalakdina, Adebayo Adedeji, Nwando Mba, Olubunmi Oja, Gerard Krause, Chikwe Ihekweazu*, for the CDC Monkeypox Outbreak Team†*

Lancet Infect Dis 2019; 19: 872–79

Published Online July 5, 2019 http://dx.doi.org/10.1016/S1473-3099(19)30294-4

See Comment page 797

*These authors contributed equally

†Members listed at end of Article

Nigeria Centre for Disease Control, Abuja, Nigeria (A Yinka-Ogunleye MPH, O Aruna FFPH, D Yahyah MPH,

Summary

Background In September, 2017, human monkeypox re-emerged in Nigeria, 39 years after the last reported case. We aimed to describe the clinical and epidemiological features of the 2017–18 human monkeypox outbreak in Nigeria.

Methods We reviewed the epidemiological and clinical characteristics of cases of human monkeypox that occurred between Sept 22, 2017, and Sept 16, 2018. Data were collected with a standardised case investigation form, with a case definition of human monkeypox that was based on previously established guidelines. Diagnosis was confirmed by viral identification with real-time PCR and by detection of positive anti-orthopoxvirus IgM antibodies. Whole-genome sequencing was done for seven cases. Haplotype analysis results, genetic distance data, and epidemiological data were used to infer a likely series of events for potential human-to-human transmission of the west African clade of monkeypox virus.

Findings 122 confirmed or probable cases of human monkeypox were recorded in 17 states, including seven deaths (case fatality rate 6%). People infected with monkeypox virus were aged between 2 days and 50 years (median

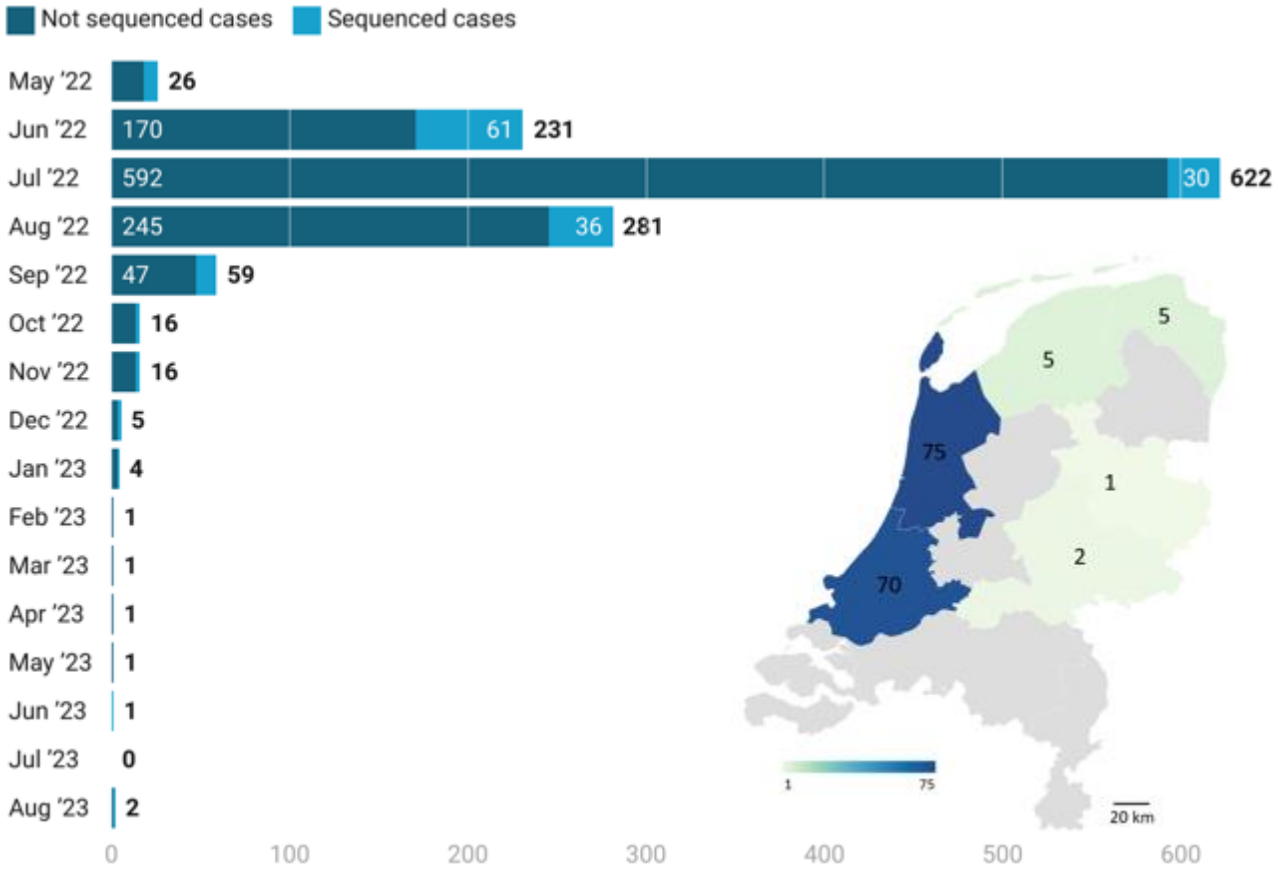
A case of imported Monkeypox in Singapore

Increasing international travel from areas with infectious disease outbreaks poses continual risks for the global spread of emerging infectious diseases (EIDs). As

RAPID COMMUNICATION

Two cases of monkeypox imported to the United Kingdom, September 2018

The 2022 MPXV outbreak



- Sustained human-to-human spread and sexual transmission in the MSM community
- Globally >186.000 cases have been reported with several reported deaths (as of 30-09-2026)

RAPID COMMUNICATIONS

Paediatric monkeypox patient with unknown source of infection, the Netherlands, June 2022

A Marceline Tutu van Furth[1,2,*], Martijn van der Kuip[1,2,*], Anne L van Els[1,2], Lydia CR Fievez[3], Gini GC van Rijckevorsel[3,4], Anton van den Ouden[5], Marcel Jonges[4], Matthijs RA Welkers[6,7]

1. Amsterdam UMC location Vrije Universiteit Amsterdam, Emma Children's Hospital, Department of Paediatric Infectious Diseases and Immunology, Amsterdam, The Netherlands
2. Amsterdam Institute for Infection and Immunity, Infectious diseases, Amsterdam, The Netherlands
3. Public Health Service Amsterdam, Dept. of Infectious Diseases, Amsterdam, The Netherlands
4. Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands
5. Molecular Biology Systems BV, Goes, The Netherlands
6. Amsterdam UMC location AMC, University of Amsterdam, Department of Medical Microbiology and Infection Prevention, Amsterdam, The Netherlands
7. Public Health Service Amsterdam, Public Health Laboratory, Amsterdam, The Netherlands

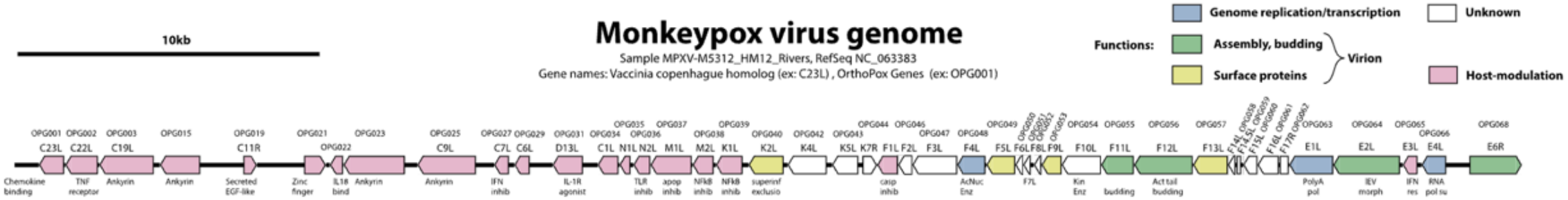
* These authors contributed equally to this work and share first authorship.

Correspondence: Matthijs Welkers (m.r.a.welkers@amsterdamumc.nl)

Schuele et al, Journal of Medical Virology, 2024

Mpox genomics

- Large genome of around 200kb encoding for around 200 different proteins
- Function of these proteins remain largely unknown
- Contains inverted terminal repeats
- Initial data suggests that the virus only changes 1-2 nucleotides per year
- Deletions and insertion are frequently observed



Metagenomic sequencing
(DNase + centrifugation)
Ct 20

Initially

Amplicon sequencing
Comparison:

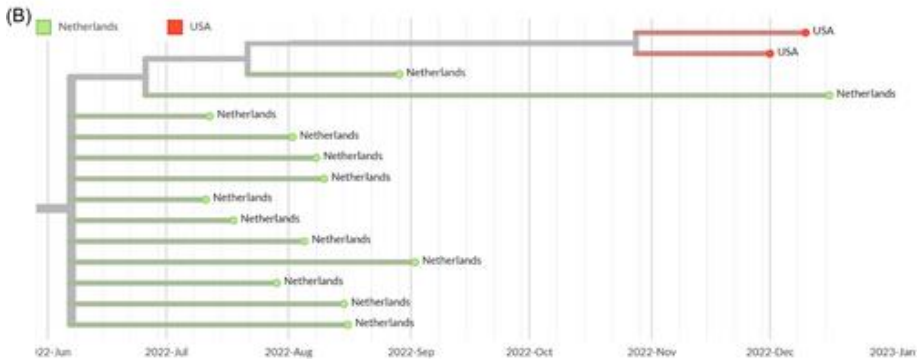
375 bp	&	2500 bp
(682 primers)		(176 primers)
Brinkmann et al.		Pedersen et al.

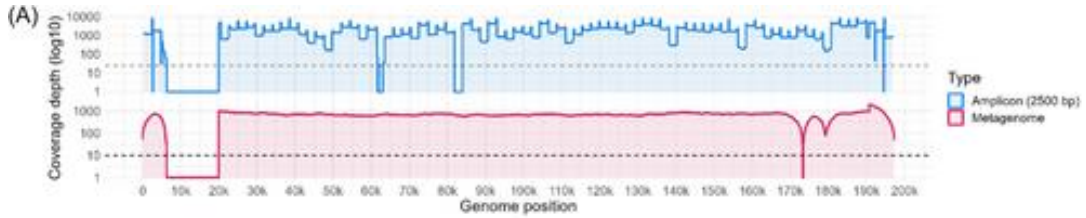
Long term

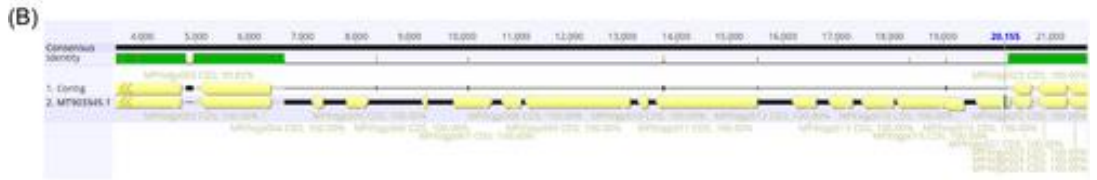
Mpox in the Netherlands

- 158 samples were sequenced during the outbreak
- Several introductions could be observed in the Netherlands followed by local transmission
- Similar to what has been described before we found a sequence with a big deletion (13,542bp encoding for 13 proteins).









Schuele et al, Journal of Medical Virology, 2024

APOBEC-3 mutations

- APOBEC3 proteins are a family of human enzymes that play a role in the body's defence against viruses and can also induce mutations in cancer cells

- They introduce mutations into the viral genome by deaminating cytosines in the viral DNA, inhibiting viral replication

- Due to the high fidelity and error correction of monkeypox virus DNA polymerase, mutations during genome replication occur at a lower rate (around one nucleotide change every 3 years) than those induced by APOBEC3 (around six nucleotide changes per year).

→ Marker of human-to-human transmission

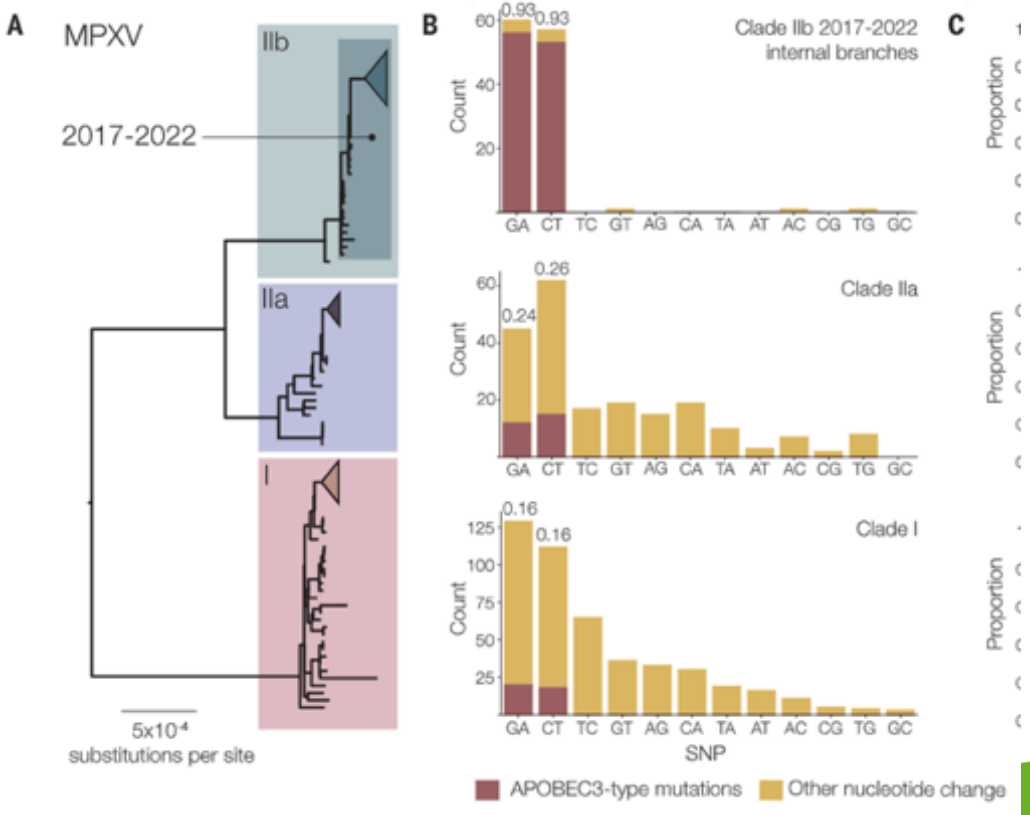
RESEARCH

MPOX

APOBEC3 deaminase editing in mpox virus as evidence for sustained human transmission since at least 2016

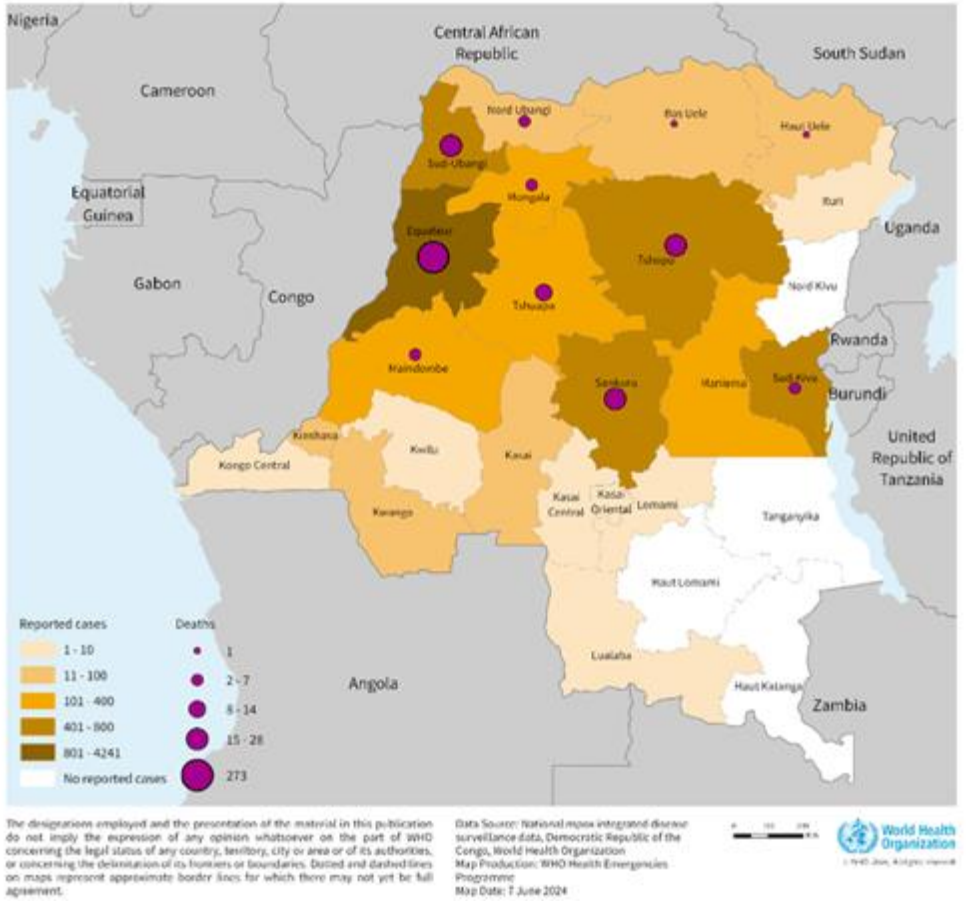
Áine O'Toole[1*], Richard A. Neher[2], Nnaemeka Ndodo[3], Vitor Borges[4], Ben Gannon[5], João Paulo Gomes[4,6], Natalie Groves[7], David J. King[8], Daniel Maloney[1], Philippe Lemey[9], Kuiama Lewandowski[8], Nicholas Loman[7,10], Richard Myers[7], Ifeanyi F. Omah[1,11], Marc A. Suchard[12], Michael Worobey[13], Meera Chand[7,14], Chikwe Ihekweazu[3], David Ulaeto[7]†, Ifedayo Adetifa[3]†, Andrew Rambaut[1*†]

TC→TT / GA→AA mutations



Mpox outbreak in Congo

- The World Health Organization (WHO) revealed that the Democratic Republic of the Congo (DRC) was experiencing its largest, most deadly outbreak of mpox ever.
- In 2023 the DRC reported a total of 14,626 suspected mpox cases including 654 deaths.
- Mpox cases are also diagnosed in regions where it previously was not detected.



https://www.who.int/emergencies/disease-outbreak-news/item/2024-DON522

Mpox outbreak in DRC

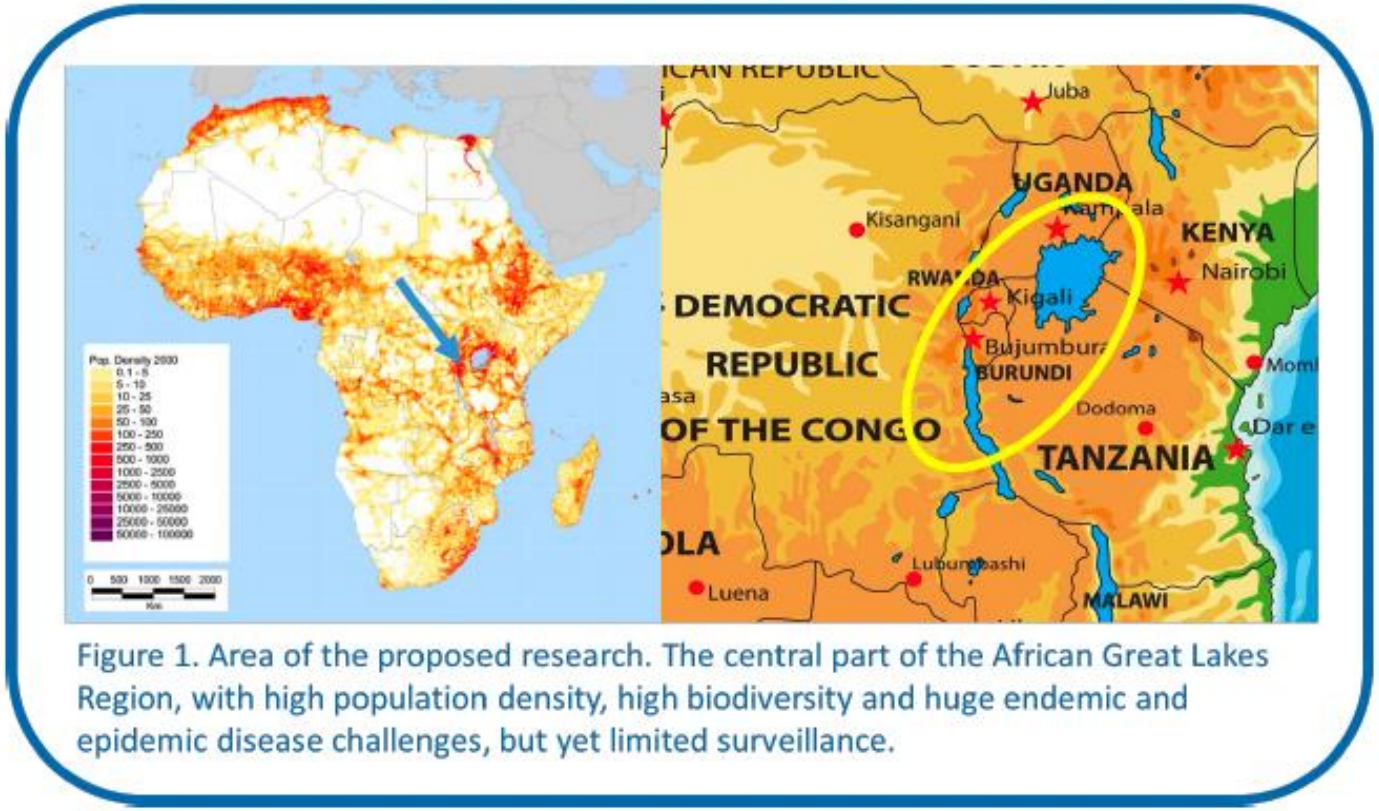


Figure 1. Area of the proposed research. The central part of the African Great Lakes Region, with high population density, high biodiversity and huge endemic and epidemic disease challenges, but yet limited surveillance.



Investigation of a Mpox Clade I outbreak in Kamituga, a mining city located in the eastern region of the Democratic Republic of the Congo.

Epi data and contact tracing

Table 1 – Characteristics of the cohort.

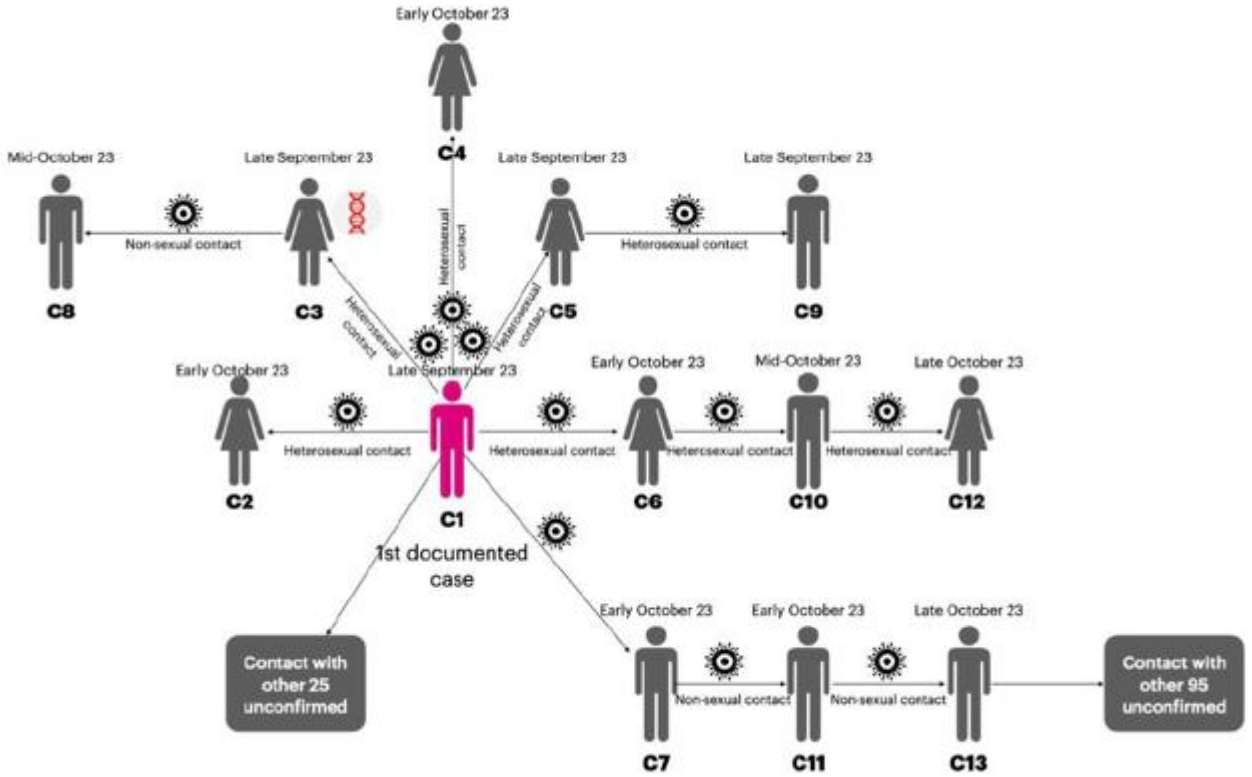
		N	Female	Male	*P* value (Chi-square test)
Viral load ct value	< 25	14	-	-	-
	>25 and <35	5	-	-	-
	>35	7	-	-	-
Confirmed case/ Negative case/ Pending case		37/2/12	21/1/4	16/1/8	-
Age (mean; median; Q1-Q3; min-max)		-	19.7; 20; 17-21; 1-59	24.3; 23; 18.5-30.5; 15-36	-
Sexual Orientation					
Heterosexual		47	22	25	-
Homosexual		0	0	0	-
Bisexual		2	2	0	-
More than one sexual partner within the last 6 months (yes)		31	16	15	-
Profession/occupation					
Professional sex worker		24	19	5	-





Epidemiology, clinical characteristics, and transmission patterns of a novel Mpox (Monkeypox) outbreak in eastern Democratic Republic of the Congo (DRC): *an observational, cross-sectional cohort study*

Leandre Murhula Masirika[1,2], Jean Claude Udahemuka[3], Pacifique Ndishimye[4,5], Gustavo Sganzerla Martinez[6,7], Patricia Kelvin[6,7], Maliyamungu Bubala Nadine[8], Bilembo Kitwanda Steeven[8], Franklin Kumbana Mweshi[8], Léandre Mutimbwa Mambo[9], Bas B. Oude Munnink[10], Justin Bengehya Mbiribindi[11], Freddy Belesi Siangoli[11], Trudie Lang[12] Jean M. Malekani[13], Frank M. Aarestrup[14], Marion Koopmans[10], Leonard Schuele[10], Jean Pierre Musabyimana[4,15], Brigitte Umutoni[5,16], Ali Toloue Ostadgavahi[6], Benjamin Hewins[6], Mansi Dutt[6,7], Anuj Kumar[6,7], , Jean-Paul Kabemba Lukusa[17], Christian Gortazar[2], David J Kelvin[6,7,18*], Luis Flores[1,19,20]

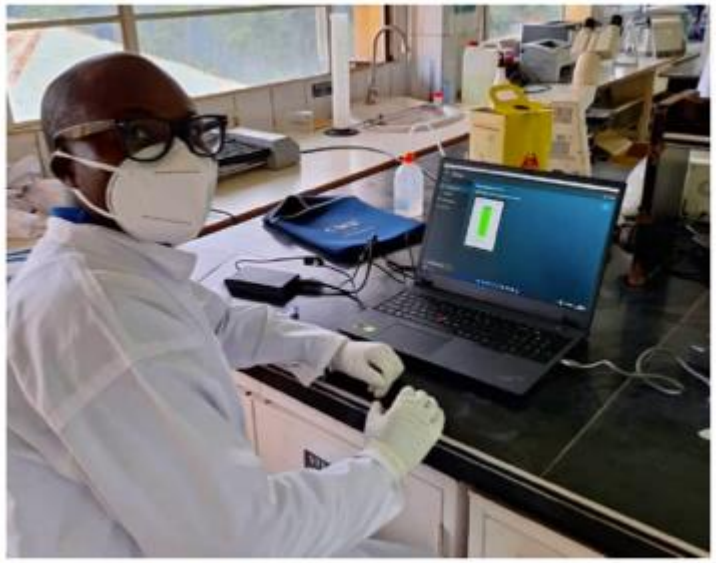


https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2024.29.11.2400106

On site training for amplicon-based sequencing for mpox

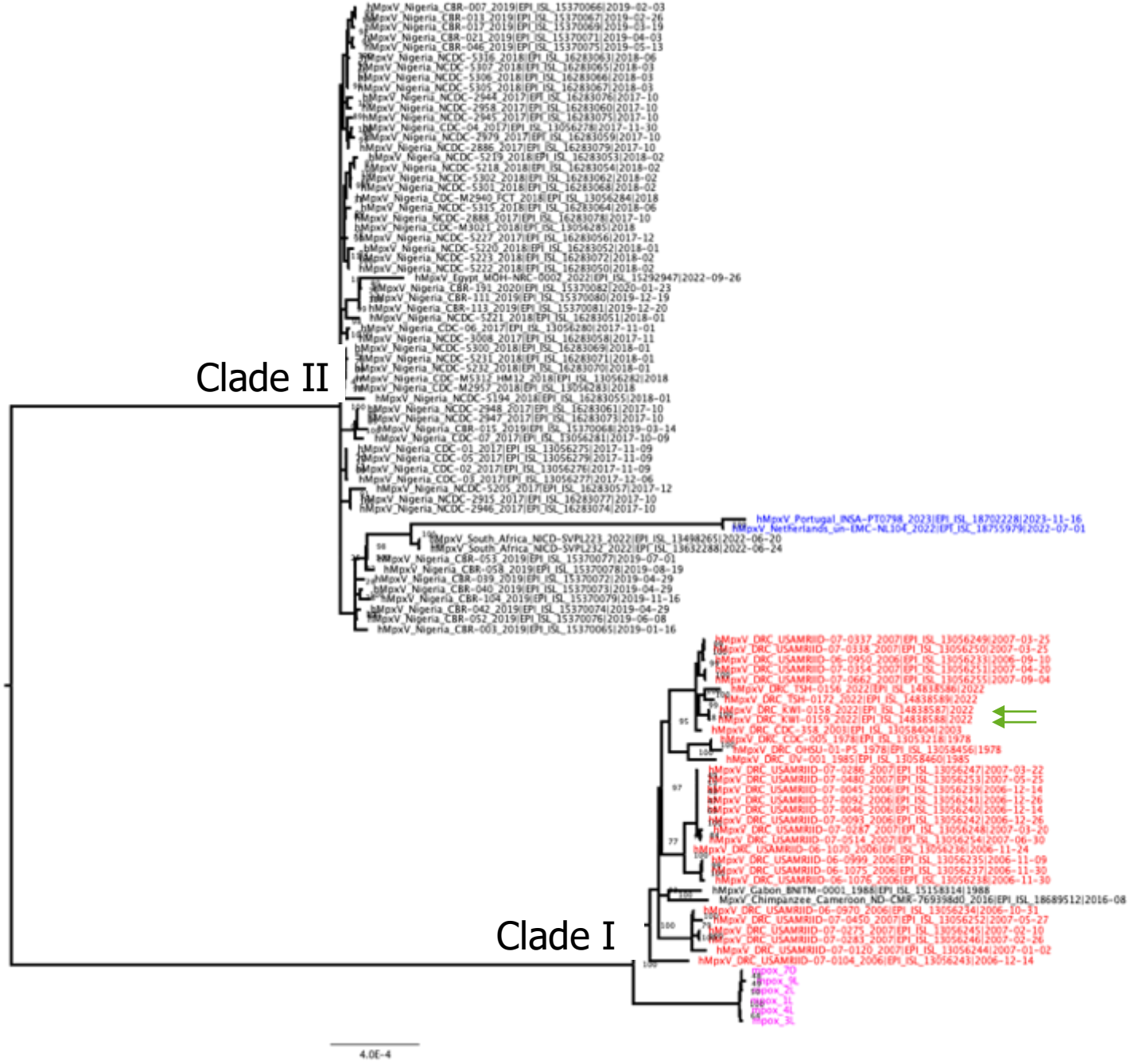






New data generated

- Sequences are at the root of the clade 1 viruses (n=6)
- Arrow indicate sequences which cluster with the 2023 Kinshasa sequences
- Several SNPs were observed within the sequenced strains
- OPG032 is deleted, similar to what has been found in all Clade II viruses

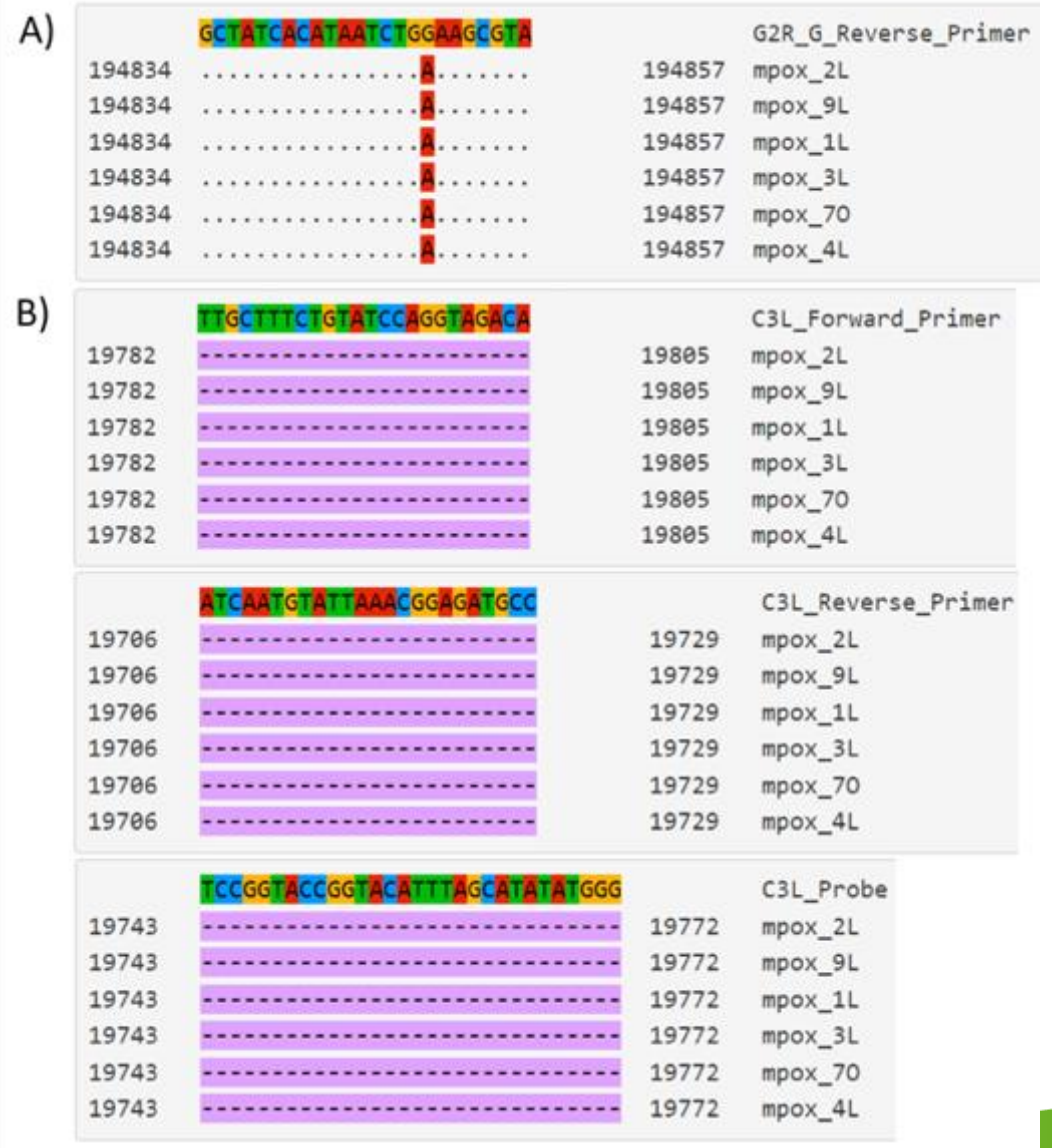


Diagnostic primer performance

- OPG032/D14L is the target recommended by the CDC to differentially diagnose Clade I versus Clade II mpox virus --> **Not working anymore**

CDC recommended primers

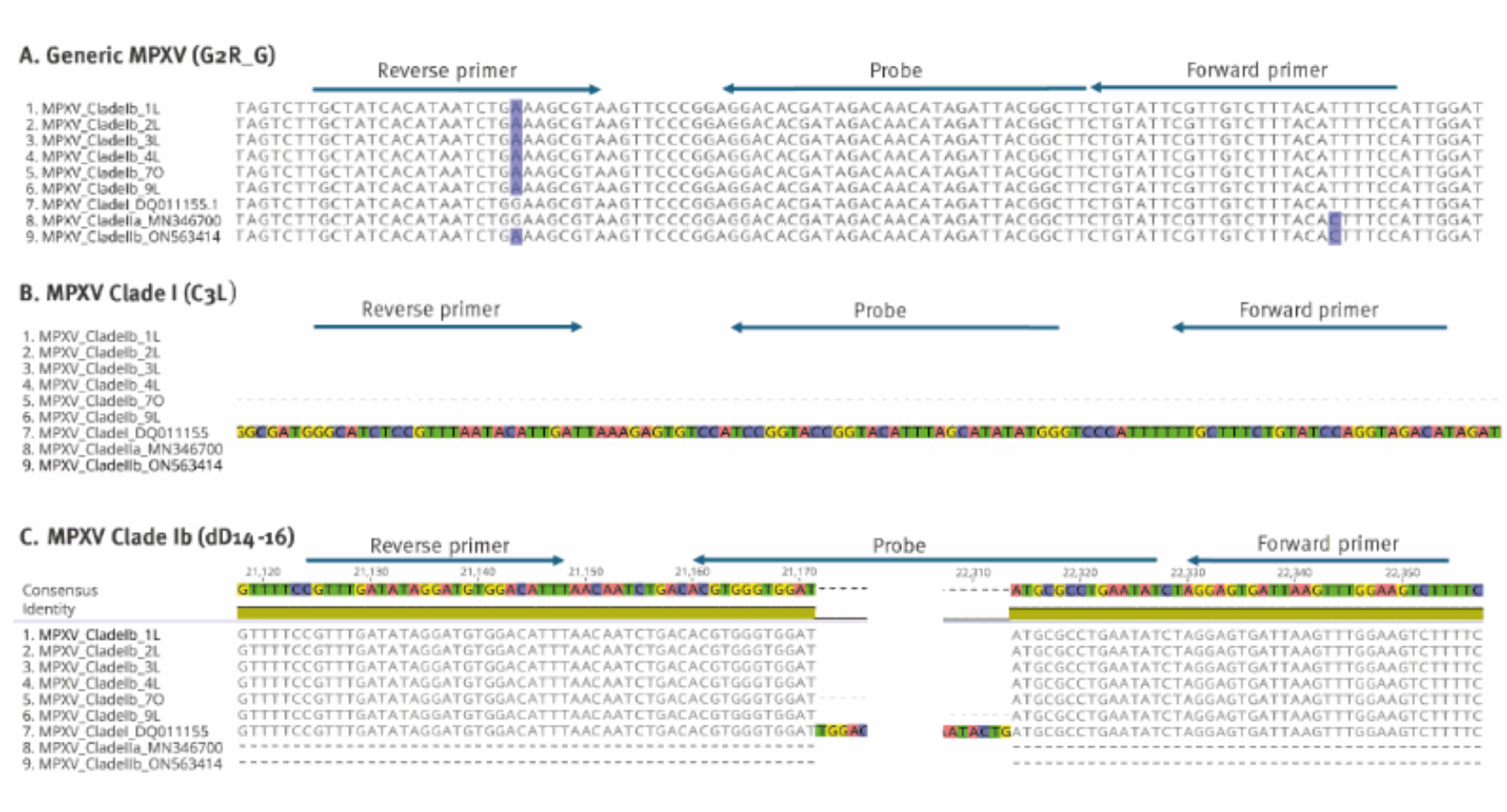
	Primers/Probe	Sequence (5' > 3')	Length
Generic	G2R_G Forward Primer	GGAAAATGTAAAGACAACGAATACAG	26
	G2R_G Reverse Primer	GCTATCACATAATCTGGAAGCGTA	24
	G2R_G Probe	FAM-AAGCCGTAATCTATGTTGTCTATCGTGTCC-BHQ1	30
Clade I	C3L Forward Primer	TGTCTACCTGGATACAGAAAGCAA	26
	C3L Reverse Primer	GGCATCTCCGTTTAATACATTGAT	24
	C3L Probe	FAM-CCCATATATGCTAAATGTACCGGTACCGGA-BHQ1	30



https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2024.29.32.2400486

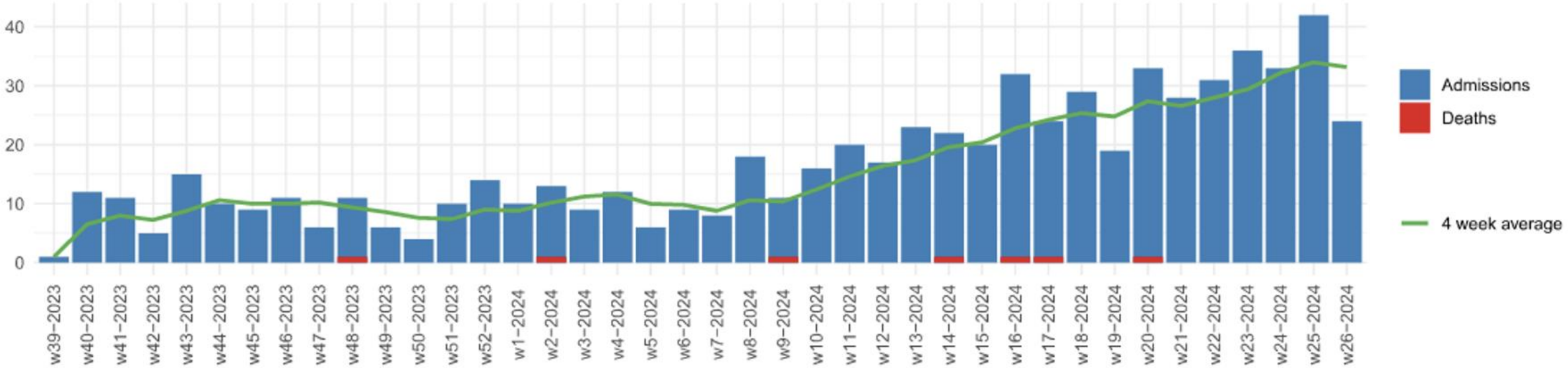
Development of new clade Ib specific RT-PCR

- We developed a novel real-time PCR assay (dD14-16) to detect the new MPXV Clade Ib.
- The assay can be performed alongside other TaqMan-based real-time targets to detect MPXV and distinguish between Clade I and Clade II.
- We designed a specific probe sequence to anneal directly upstream and downstream of the deletion in Clade Ib



https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2024.29.32.2400486

Epidemiology in Kamituga



- Situation was worsening
- Mpox virus detection in new health zones
- Request for a new training mission

Request for a second on-site training mission

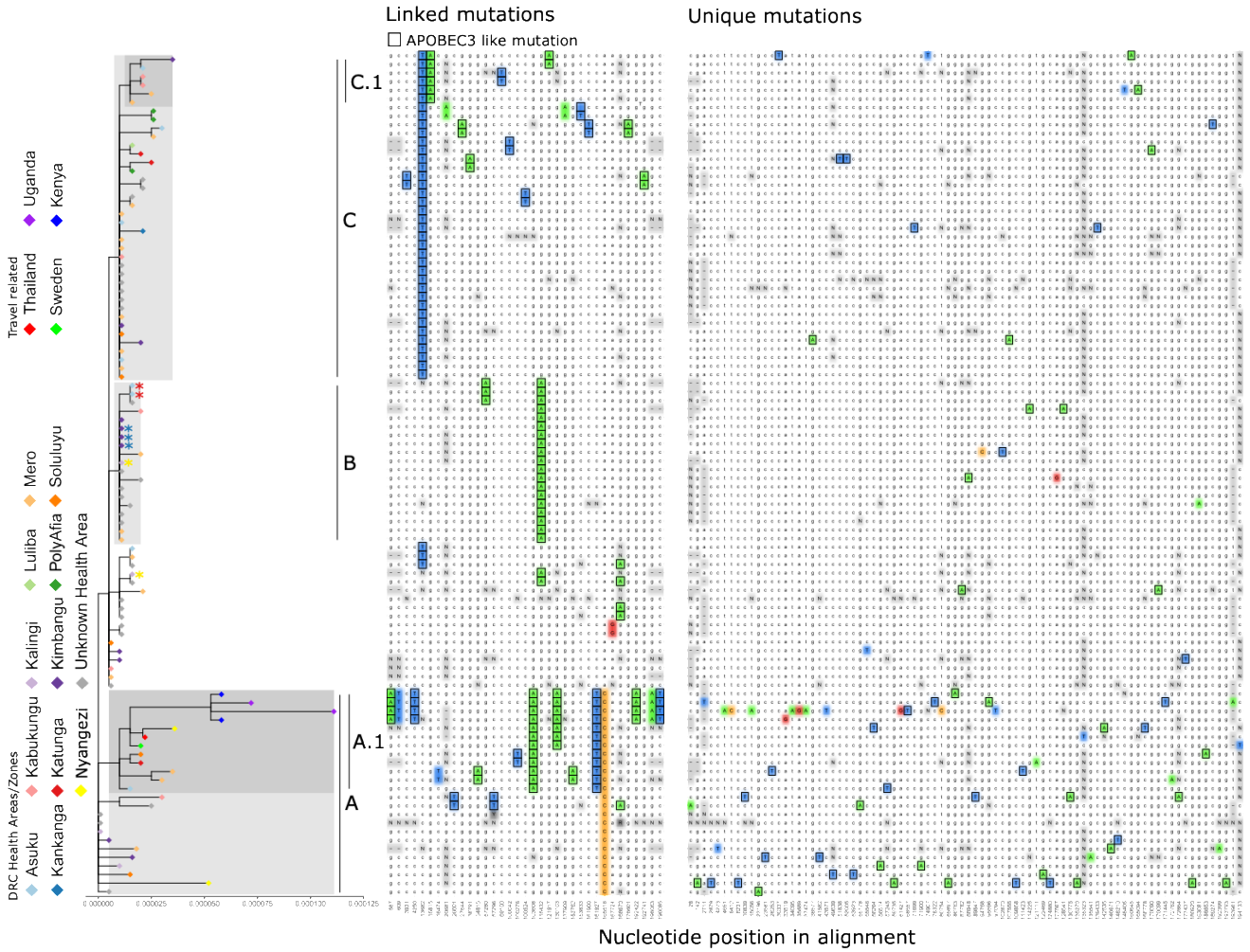


- Training in serology
- Training in diagnostics to detect clade Ib virus
- Second training in WGS (58 new complete genomes generated)
- Training in bioinformatics (challenging)
  - No Wifi
  - No programming knowledge





Outbreak of the Clade Ib virus is now widely spread



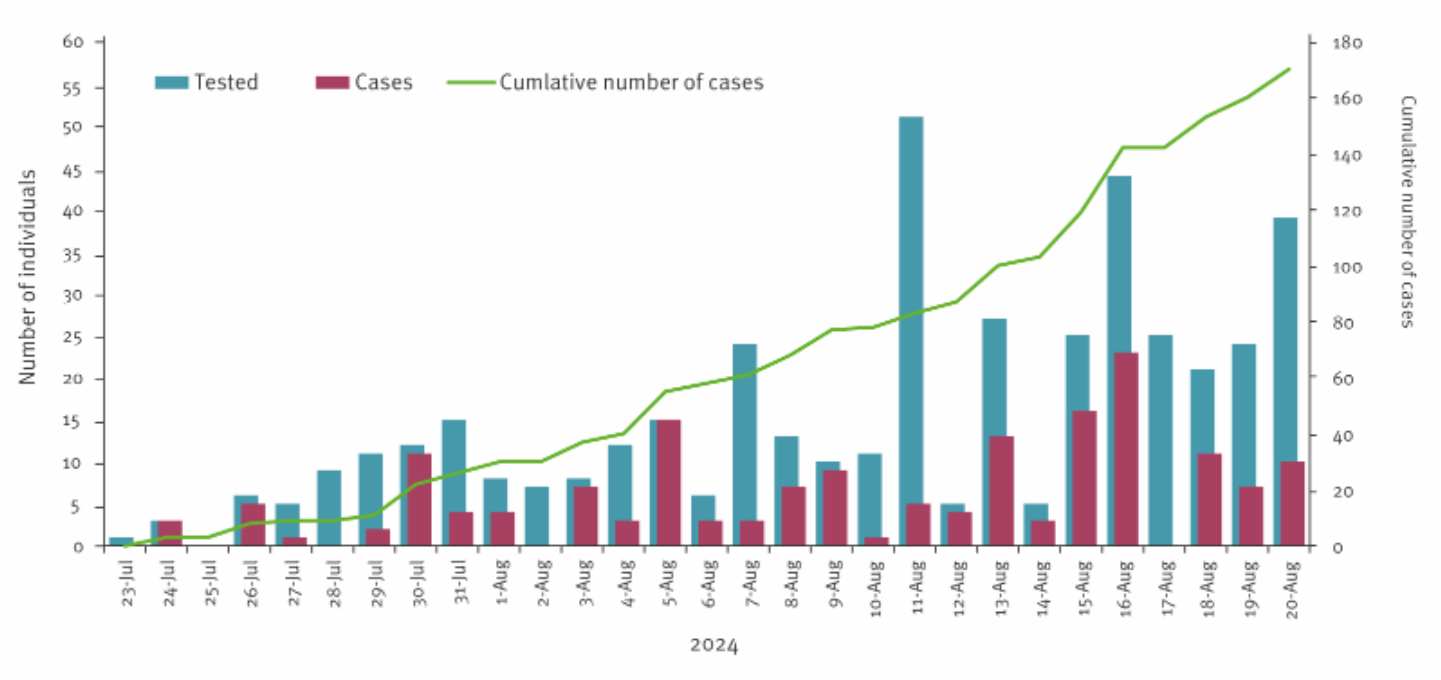
- 58 more complete WGS from the Clade Ib outbreak
- During this outbreak also 14 pregnant women were admitted among whom 8 aborted.

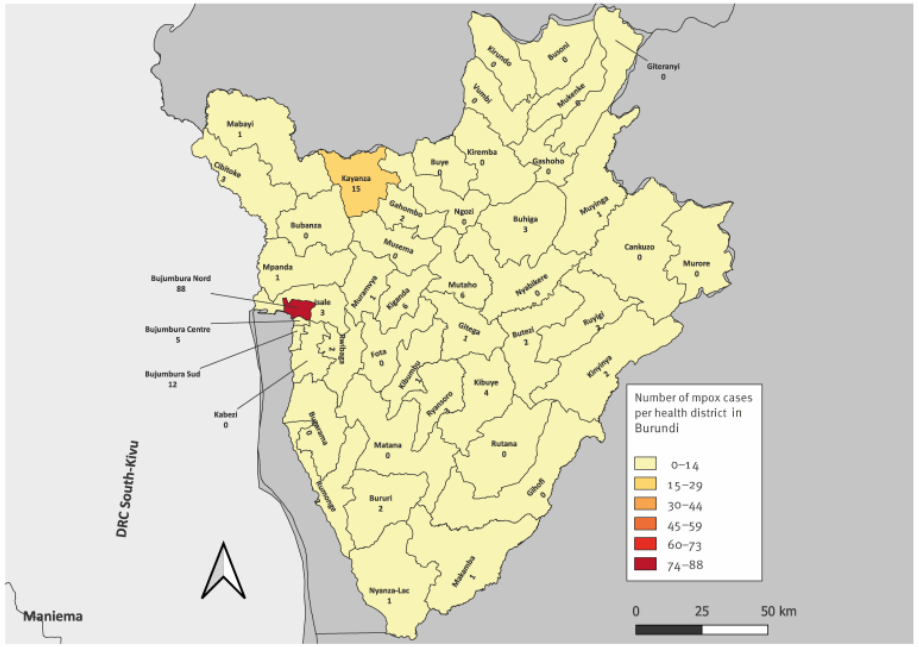


https://www.nature.com/articles/s41591-025-03582-1

Mpox Clade Ib is spreading to neighbouring countries

- Cases have been detected in Rwanda, Uganda and Burundi
- Mainly at the border region but later also throughout the country





https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2024.29.42.2400666

Epidemiology in Burundi

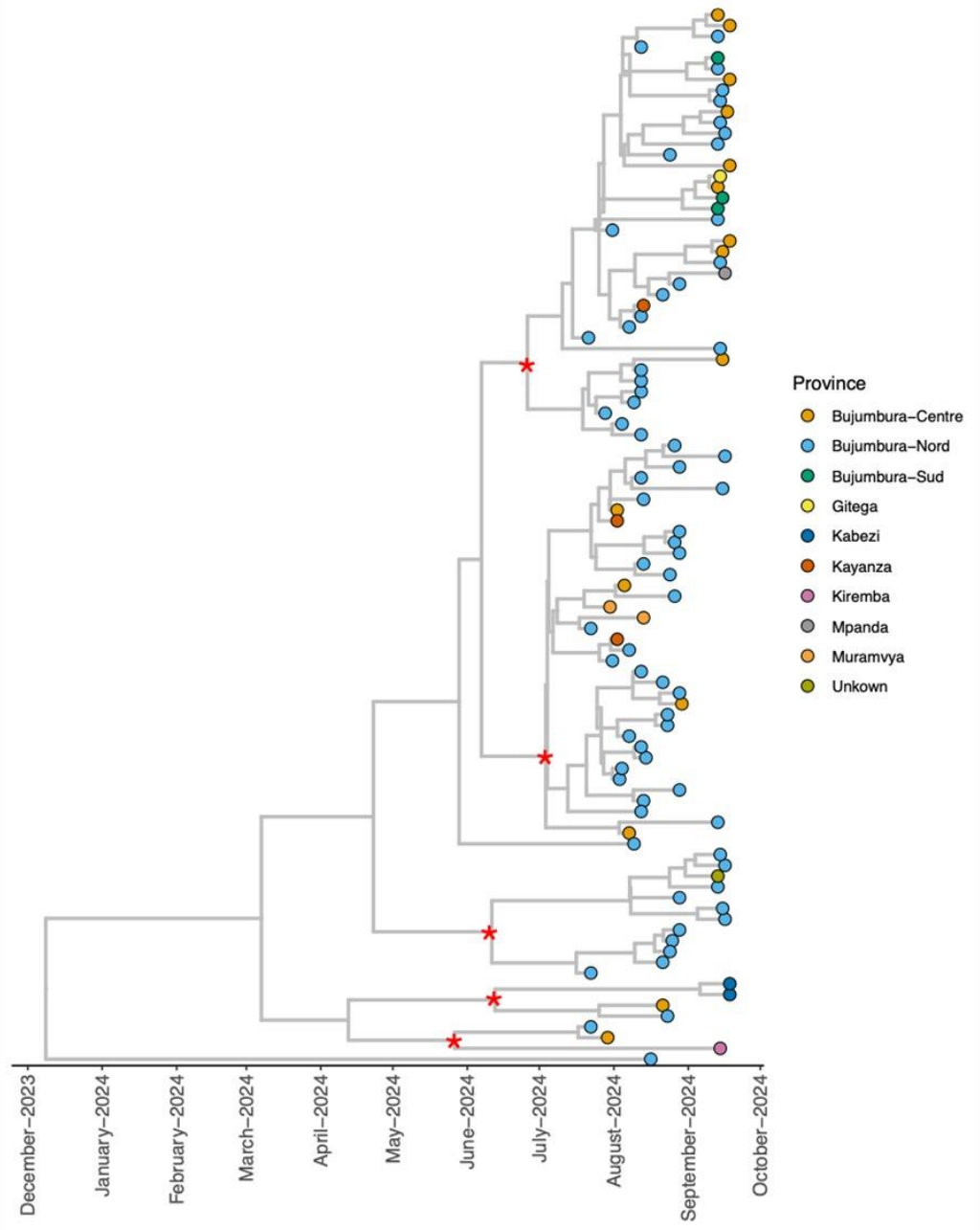
- 98 genomes were generated on site after the second training
- Several different introductions
- Sustained spread within the country
- Large proportion of ABOPEC-3 mutations indicating human-to-human transmission

https://www.nature.com/articles/s43856-025-01199-6



Epidemiology in Burundi

- Assuming the virus was detected fast after the introduction five different introduction events occurred leading to subsequent spread into the country
- After the initial detection of MPXV in Burundi the virus continued to spread with 3,359 detected cases on the 13th of February 2025.
- The epidemic is mainly concentrated in and around the highly densely populated city of Bujumbura, but infections have also been reported in other health zones in the country with cases in 45 out of 49 (91.8%) districts.



https://www.nature.com/articles/s43856-025-01199-6

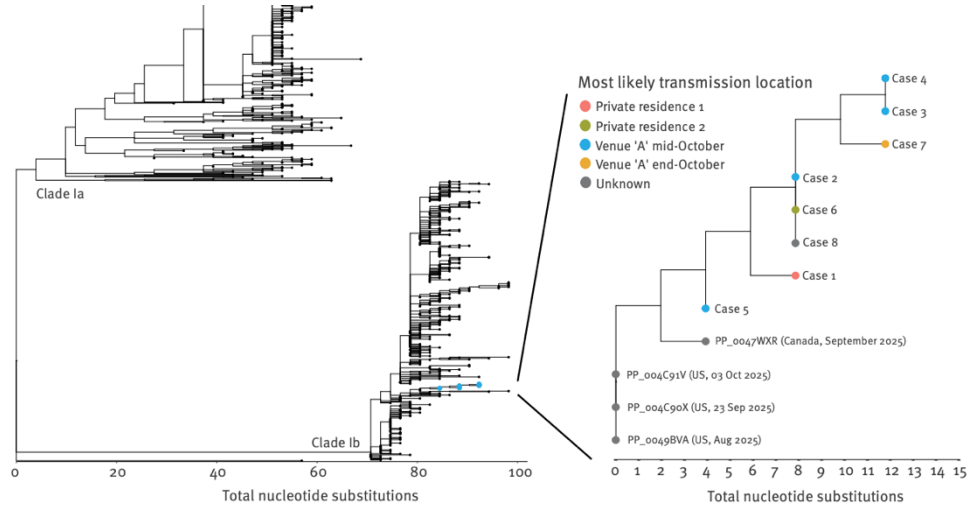
Mpox clade Ib is spreading globally

- Since half/end 2025 local transmission in Europe and the USA
- Co-circulation of Clade Ib and Clade IIb

RAPID COMMUNICATION

First detection and autochthonous transmission of monkeypox virus clade Ib in the Netherlands, October to November, 2025

Jelte Elsinga[1,2], Celine van de Ven[3], Anne de Vries[4], Hester Coppoolse[5], Mariska Petrignani[3], Brigitte van Cleef[5], Riemer van Markus[4], Nora Carpay[6], Catharina E van Ewijk[7], Sjoerd Rebers[1], Aldert Bart[8], Karin J von Eije[9], Brenda Westerhuis[5], Sylvia Bruisten[5], Leonard Schuele[9], Marjan Boter[9], Richard Molenkamp[9], Bregtje Lemkes[6], Suzanne Geerlings[6], Henry JC de Vries[2,10,11,12], Marion Koopmans[9], Marcel Jonges[1], Bas B Oude Munnink[9,**], Matthijs RA Welkers[1,2,**]



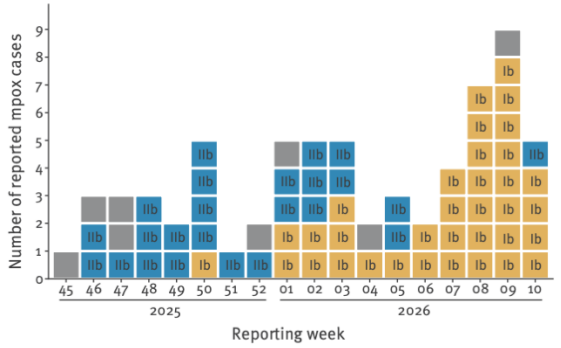
RAPID COMMUNICATION

Rapid spread of MPXV clade Ib with high genetic relatedness among men who have sex with men, Berlin, Germany, week 50 2025 up to week 10 2026

Alexander Bartel[1], Klaus Jansen[2], Ronja Boberg[1], Julia Bitzegeio[1], Annika Brinkmann[3], Livia Schrick[3], Raskit Lachmann[4], Daniel Sagebiel[1], Andreas Nitsche[3], Claudia Ruscher[1], Janine Michel[3]

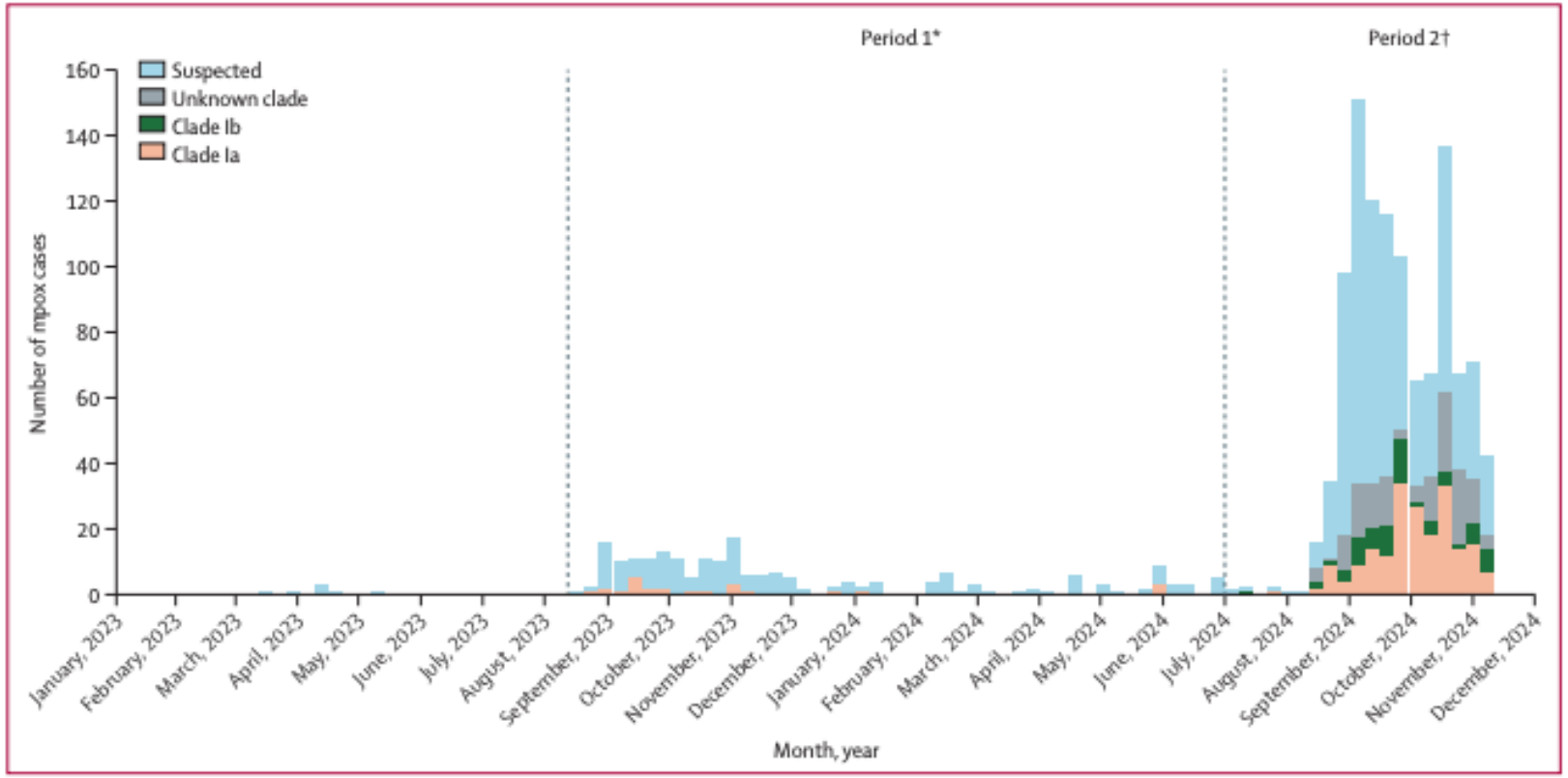
FIGURE 1

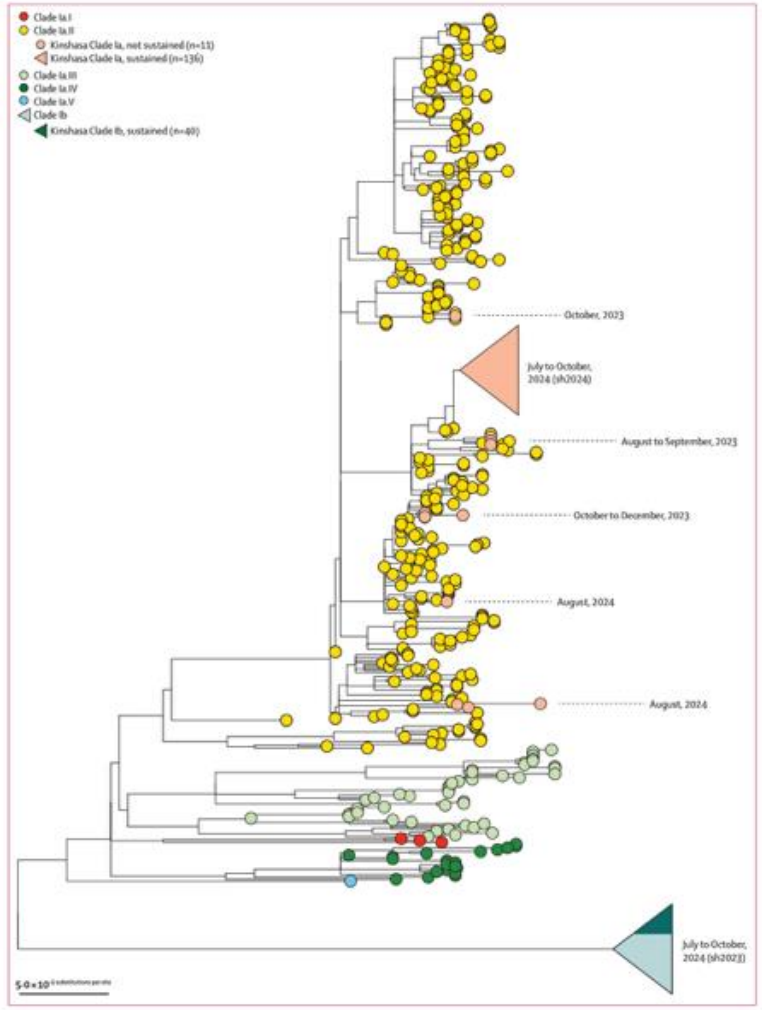
Epidemiological curve of all notified mpox cases by reporting week, Berlin, Germany, November 2025 (week 45)–March 2026 (week 10) (n = 67)



Virus clades of reported mpox cases are labelled and indicated by colour with blue for clade IIb (n = 24), yellow for clade Ib (n = 35) and grey unknown (n = 8). The epidemiological curve was created using ggsurveillance in R [21].

Sexual transmission of Clade Ia has also been described in the DRC

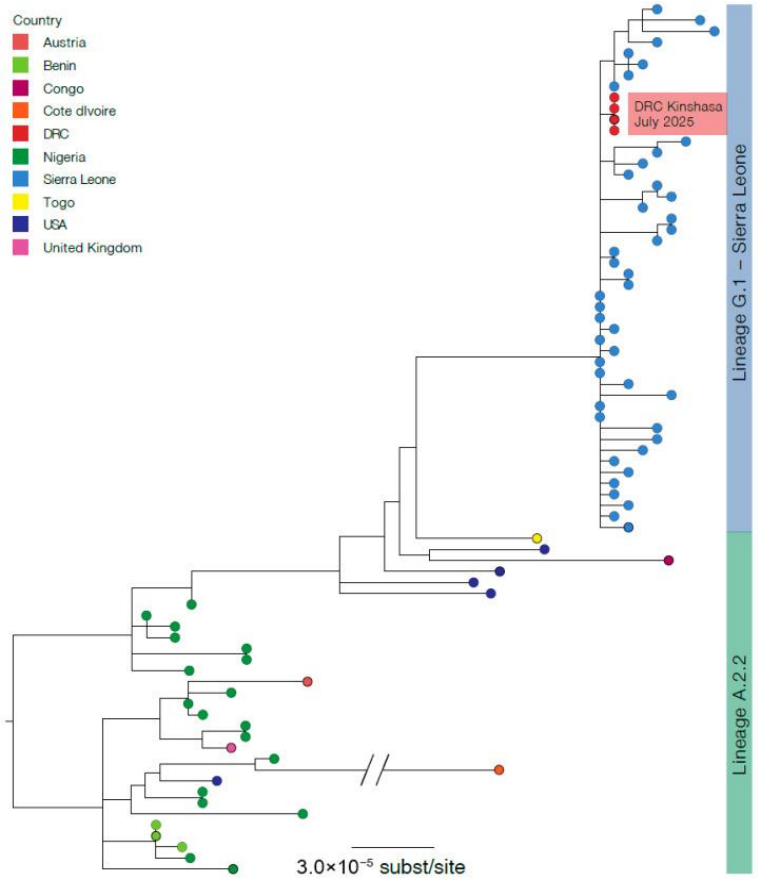


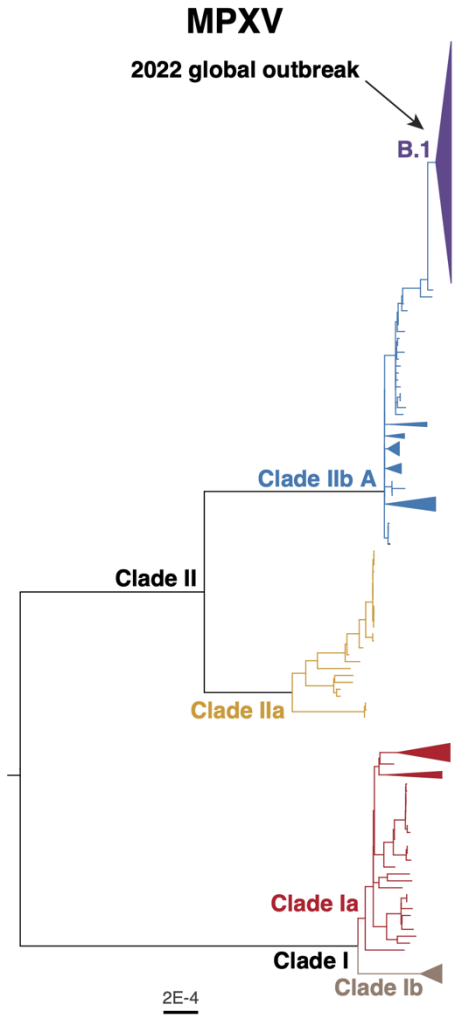


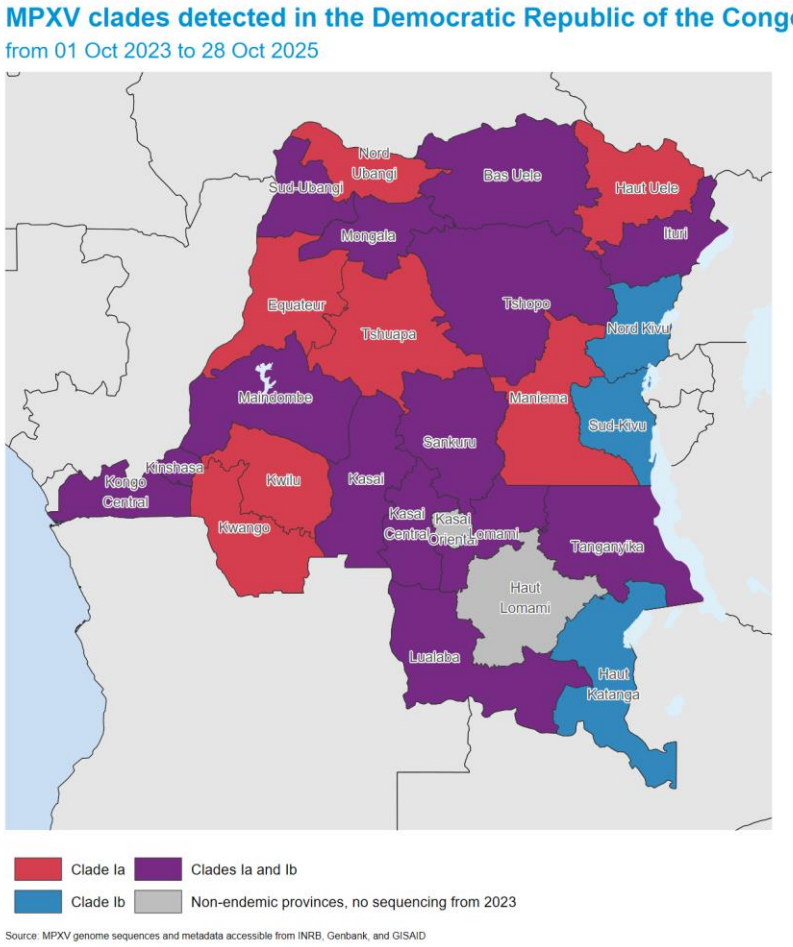
https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(25)00294-6/abstract

Current situation in the Eastern African region

- Several clades (Ia, Ib, IIa and IIb) are co-circulating







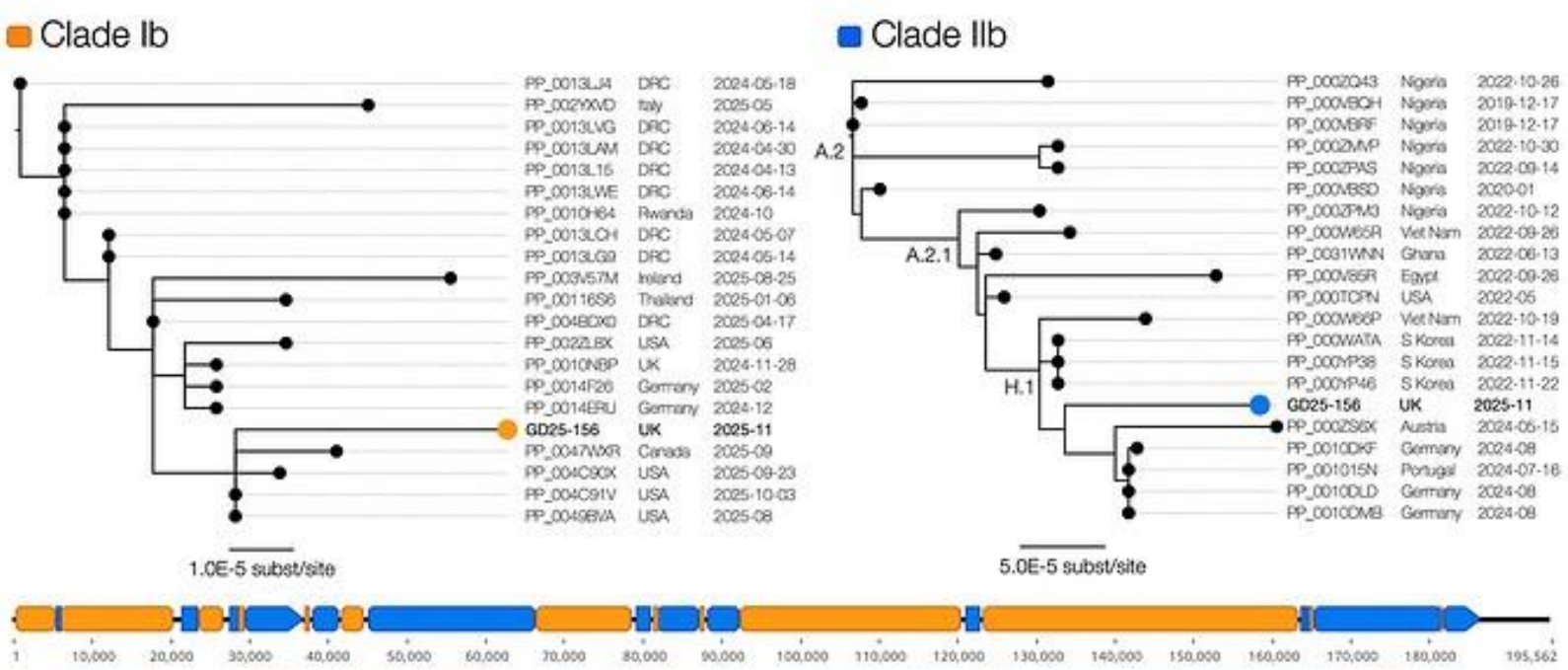
https://pmc.ncbi.nlm.nih.gov/articles/PMC12846488/

https://worldhealthorg.shinyapps.io/mpx_global/#sec-afr

Recombinant clade Ib/IIb has been detected

To confirm this unusual finding, amplicon sequencing was repeated on:

- A technical amplification and sequencing repeat from the original extract
- A fresh extract from the same primary sample
- A second, independent swab collected from the patient at the same time
- A VERO E6 cultured isolate derived from the initial swab



https://virological.org/t/inter-clade-recombinant-mpox-virus-detected-in-england-in-a-traveller-recently-returned-from-asia/1015

In summary

- Several zoonotic mpox introductions/outbreaks are ongoing in DRC mostly driven by sexual contact but also via household transmissions / close contacts

- Diagnostic PCR recommended by the US CDC cannot be used to discriminated between clade I(b) and clade IIb mpox viruses.
  - New PCR developed and validated in field settings to diagnose clade mpox clade Ib

- Why is MXPV now widely circulating?
  - Smallpox vaccination has stopped?
  - More close interaction with the reservoir host?

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