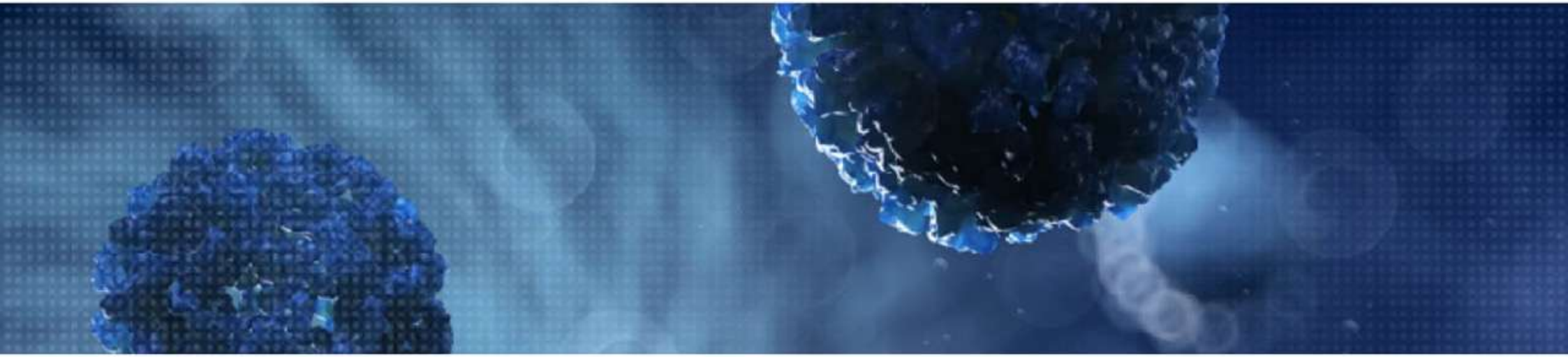
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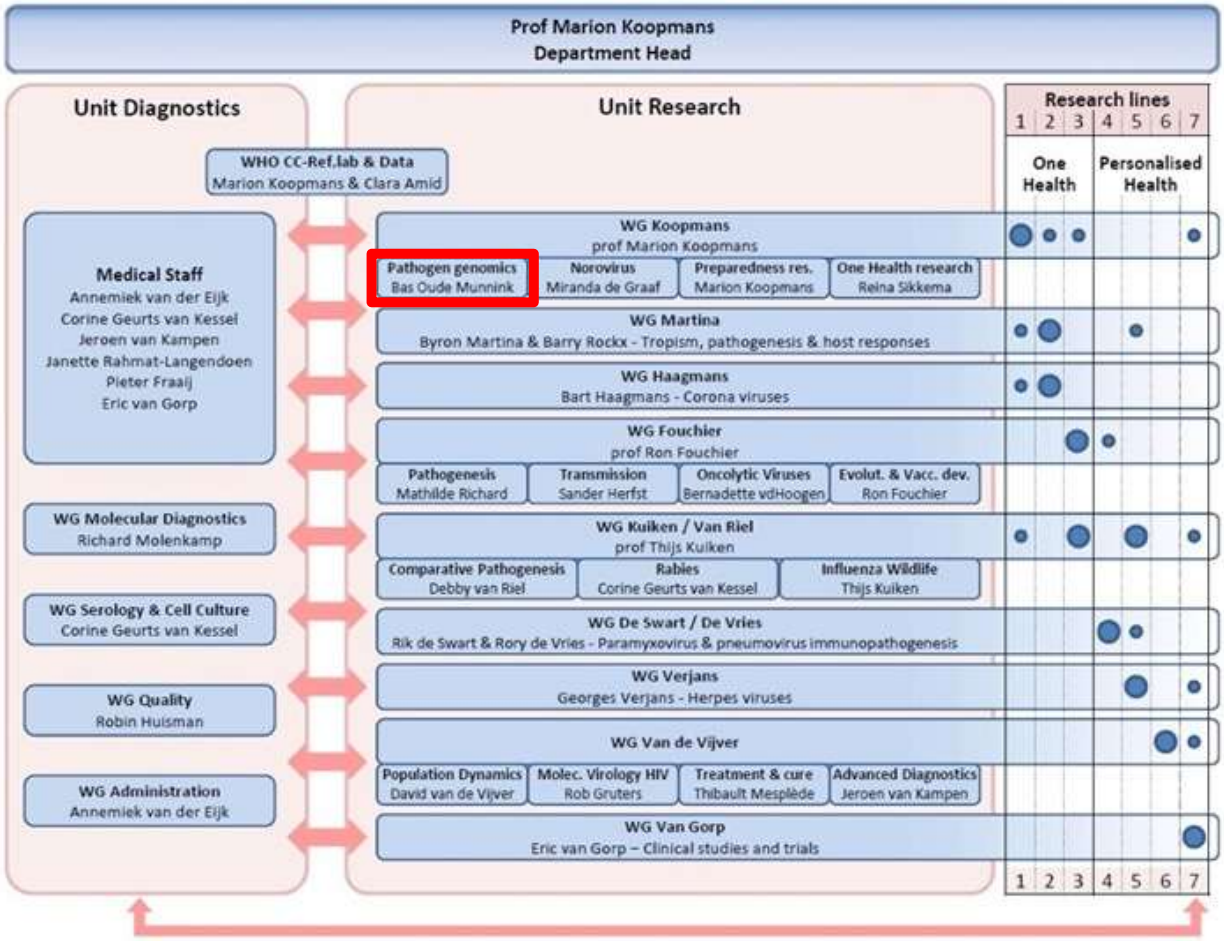
Viroscience lab
WHERE SKILLS MEET TO STUDY & PROTECT



Epidemiology and and genomic surveillance after the detection of a novel Mpox Clade Ib virus

Bas Oude Munnink (b.oudemunnink@erasmusmc.nl)

Viroscience – ErasmusMC







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University Medical Center Rotterdam

EMC – pathogen genomics group








- **Bas Oude Munnink**

- David Nieuwenhuijse
- Leonard Schule
- Hayley Cassidy
- Marjan Boter
- Susanne van der Boom
- Anne van der Linden
- Luc van Zon
- Emmanuelle Munger
- Nathalie Worp
- Shreya Gurung






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Genomic surveillance

- Virus discovery
  - Unexplained illnesses in humans
  - Unexplained illnesses in animals

- Virus typing
  - Outbreak investigations
  - Tracing origins of viruses

- Virus (virome) characterisation
  - Genotyping of the virus
  - Virome determinations in sewage

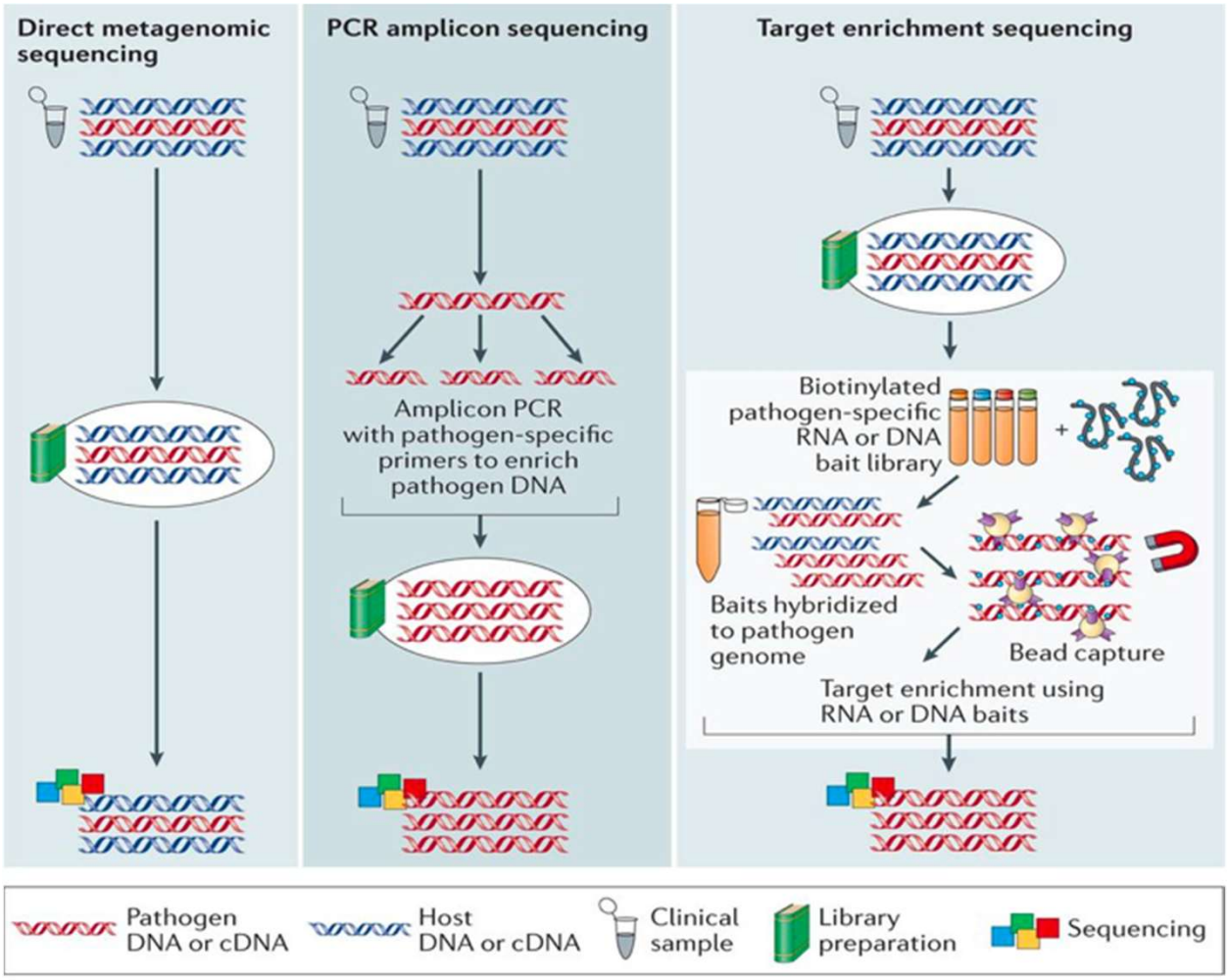
Genomic surveillance

- Virus discovery
  - Unexplained illnesses in humans
  - Unexplained illnesses in animals

- **Virus typing**
  - **Outbreak investigations**
  - **Tracing origins of viruses**

- Virus (virome) characterisation
  - Genotyping of the virus
  - Virome determinations in sewage

Sequencing methods



Houldcroft et al, Nature, 2017

Nature Reviews | Microbiology

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Nanopore sequencing



- Small
- Fast
- Relatively cheap
- Fieldable
- Produce data in real time
- High error rate / lower throughput



NextSeq 1000 & 2000



NovaSeq 6000 Series



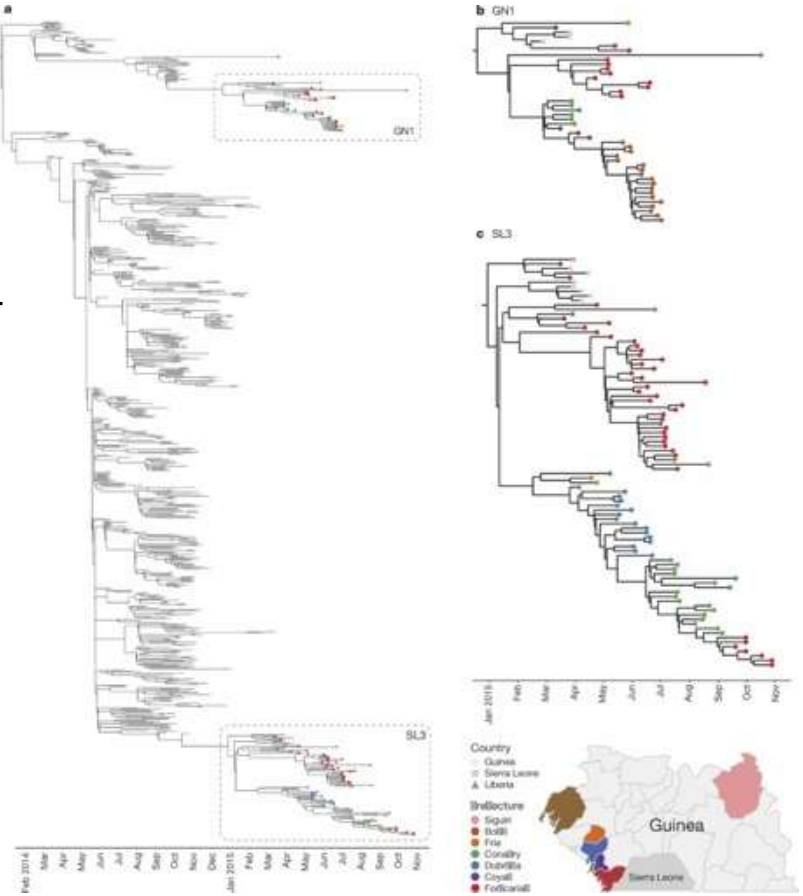
NovaSeq X Series

- High throughput
- Several applications (virocap)
- Paired-end sequencing
- High accuracy
- Expensive / short reads

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Nanopore sequencing for emerging viruses

- Ebola virus outbreak in West Africa in 2013-2016
- Results were generated within 24 hours after receiving an Ebola positive sample
- Showcasing real-time genomic surveillance



Letter | Published: 03 February 2016

Real-time, portable genome sequencing for Ebola surveillance

Joshua Quick, Nicholas J. Loman ✉, Sophie Duraffour, Jared T. Simpson, Ettore Severi, Lauren Cowley, Joseph Akoi Bore, Raymond Koundouno, Gytis Dudas, Amy Mikhail, Nobila Ouédraogo, Babak Afrough, Amadou Bah, Jonathan H. J. Baum, Beate Becker-Ziaja, Jan Peter Boettcher, Mar Cabeza-Cabrerizo, Álvaro Camino-Sánchez, Lisa L. Carter, Juliane Doerrbecker, Theresa Enkirch, Isabel García- Dorival, Nicole Hetzelt, Julia Hinzmann, ... Miles W. Carroll + Show authors

Nature **530**, 228–232 (2016) | Cite this article

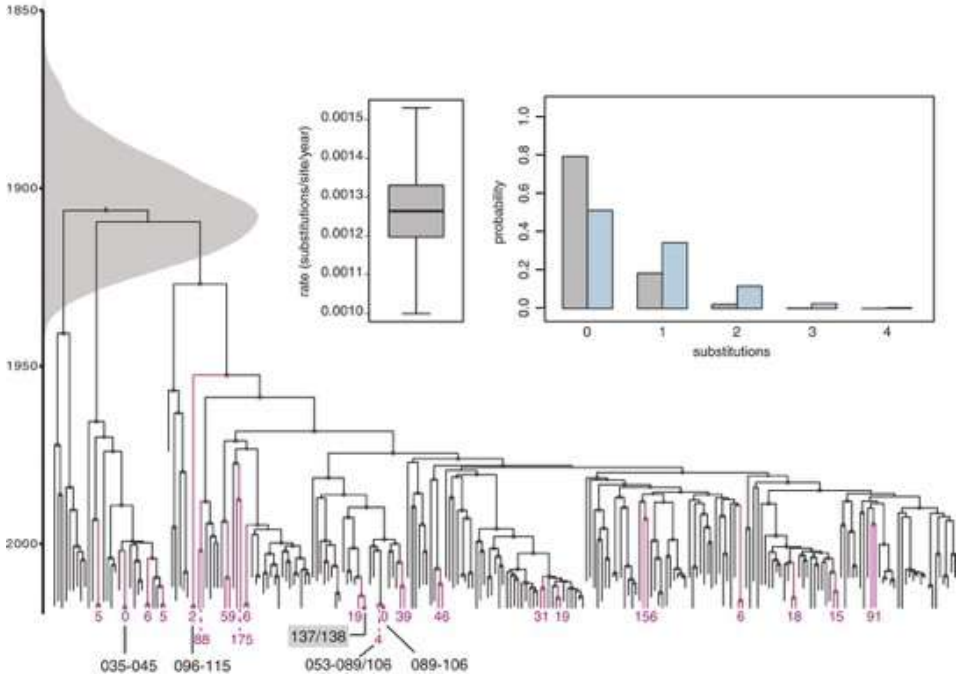
67k Accesses | **1359** Citations | **796** Altmetric | Metrics

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Nanopore sequencing for emerging viruses (2)

- Surge in Lassa virus cases in Nigeria from 102 cases in 2017 to 1,495 cases in 2018

- Urgent questions:
  - Is there an outbreak of a novel strain with increased transmission and human to human transmission?



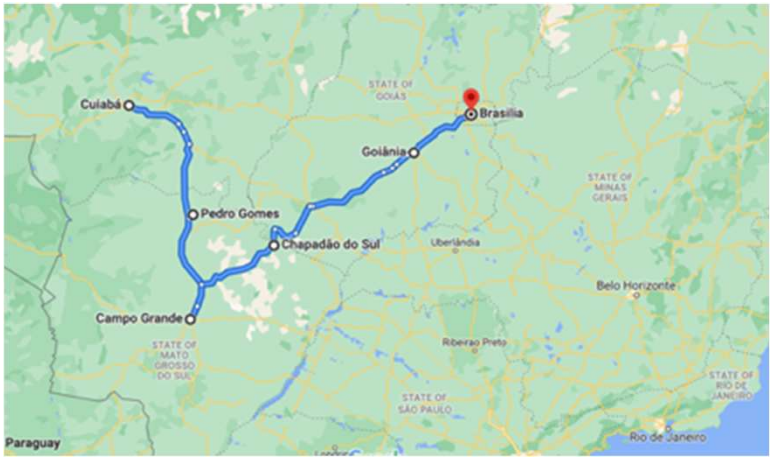
Metagenomic sequencing at the epicenter of the Nigeria 2018 Lassa fever outbreak

L. E. KAFETZOPOULOU, S. T. PULLAN, P. LEMEY, M. A. SUCHARD, D. U. EHICHIOYA, M. PAHLMANN, A. THIELEBEIN, J. HINZMANN, L. OESTEREICH, [...], AND S. DURAFFOUR +55 authors Authors Info & Affiliations

SCIENCE • 4 Jan 2019 • Vol 363, Issue 6422 • pp. 74-77 • DOI: 10.1126/science.aau9343

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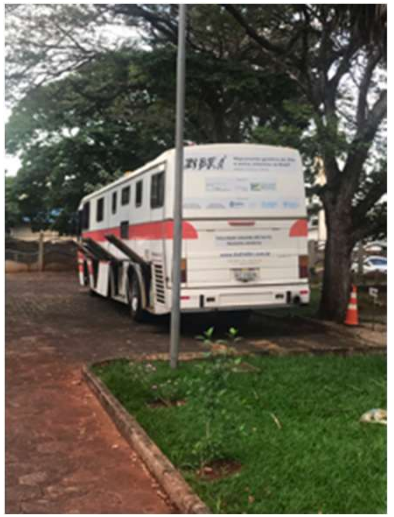
Amplicon-based arbovirus sequencing in Brazil











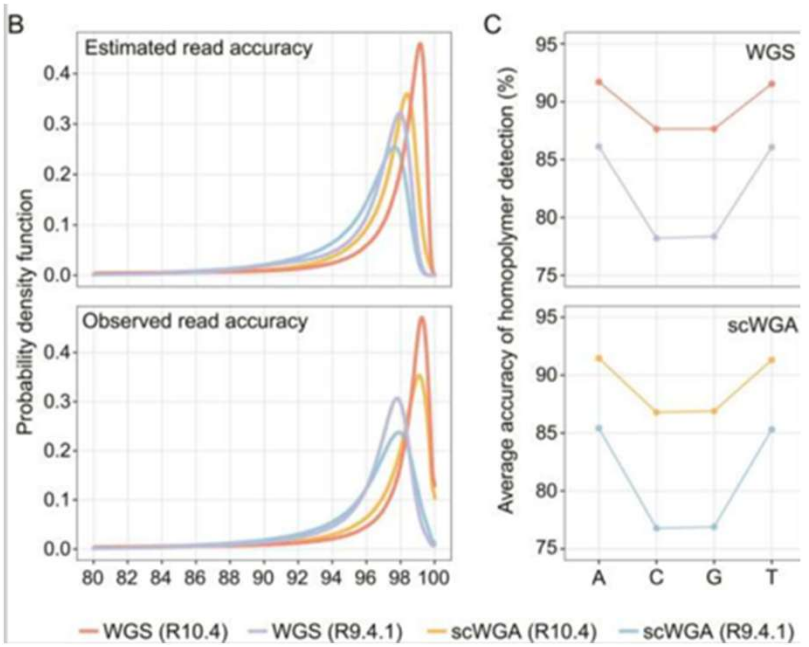
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Nanopore sequence quality improved

- Improvements in flow-cells
- Improvements in base-calling software
- Improvements in throughput
- Improvements in stability







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SARS-CoV-2 pandemic

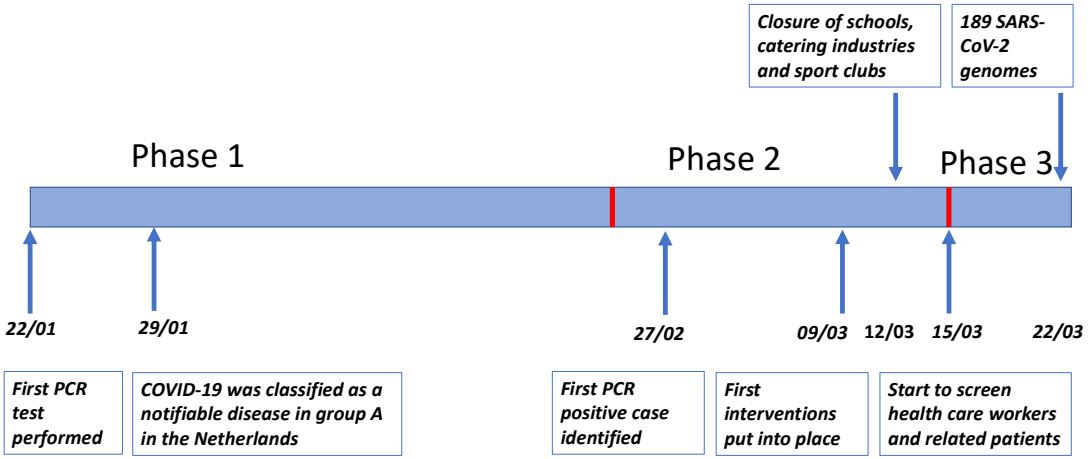
The first SARS-CoV-2 infection in the Netherlands was confirmed on February 27th 2020 and an additional case one day later. The genomes of these first two positive samples were generated and analyzed by the February 29th.



Not from one recent source, e.g. unlikely connected

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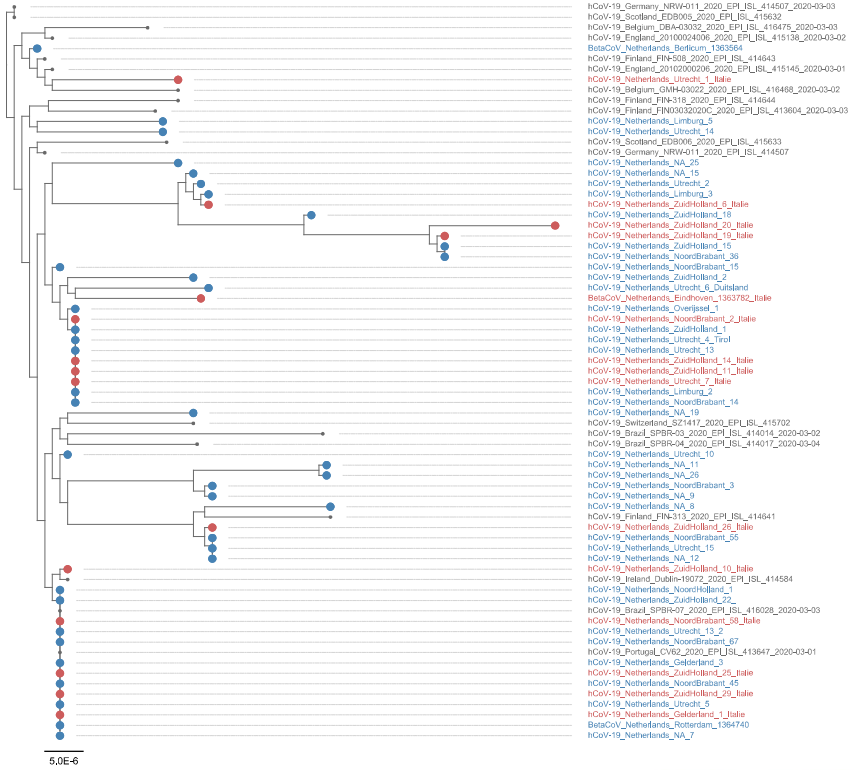
SARS-CoV-2 pandemic



nature medicine
LETTERS
https://doi.org/10.1038/s41591-020-0997-y
Check for updates

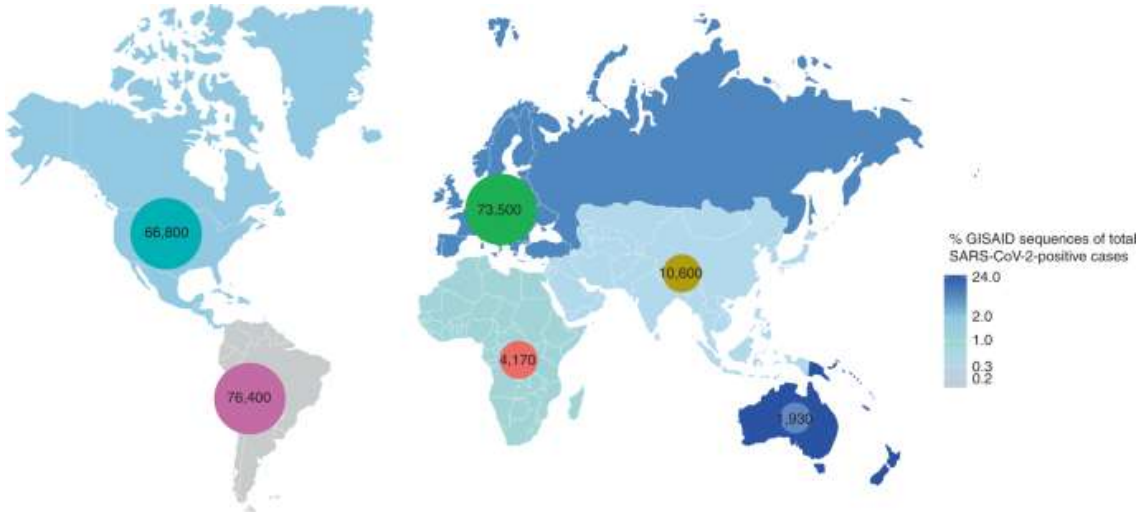
Rapid SARS-CoV-2 whole-genome sequencing and analysis for informed public health decision-making in the Netherlands

Bas B. Oude Munnink[1], David F. Nieuwenhuijse[1], Mart Stein[2], Áine O'Toole[3], Manon Haverkate[2], Madelief Mollers[2], Sandra K. Kamga[2], Claudia Schapendonk[1], Mark Pronk[1], Pascal Lexmond[1], Anne van der Linden[1], Theo Bestebroer[1], Irina Chestakova[1], Ronald J. Overmars[1], Stefan van Nieuwkoop[1], Richard Molenkamp[1], Annemiek A. van der Eijk[1], Corine GeurtsvanKessel[1], Harry Vennema[2], Adam Meijer[2], Andrew Rambaut[3], Jaap van Dissel[2], Reina S. Sikkema[1], Aura Timen[2,33], Marion Koopmans[1,33] ✉ and The Dutch-Covid-19 response team*

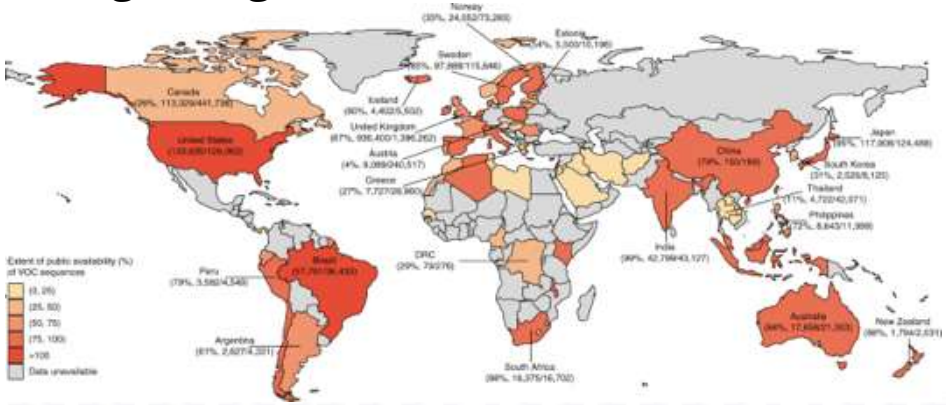


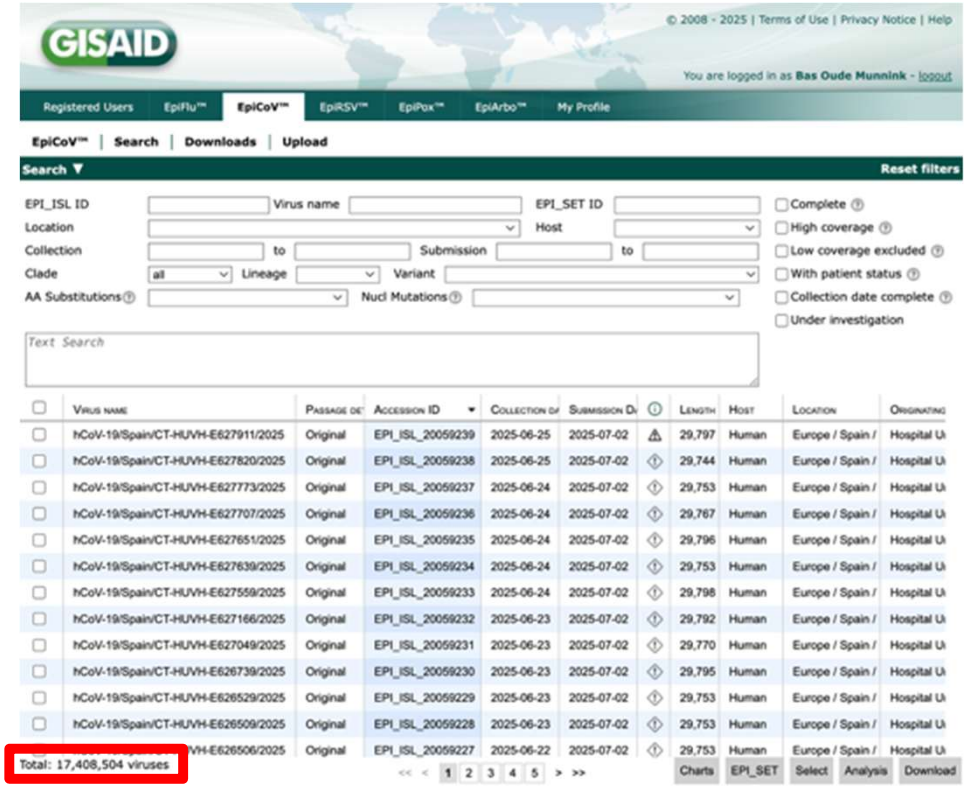
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Enormous amounts of sequences were generated



Beginning of 2021





66% of all viral entries in GenBank are SARS-CoV-2

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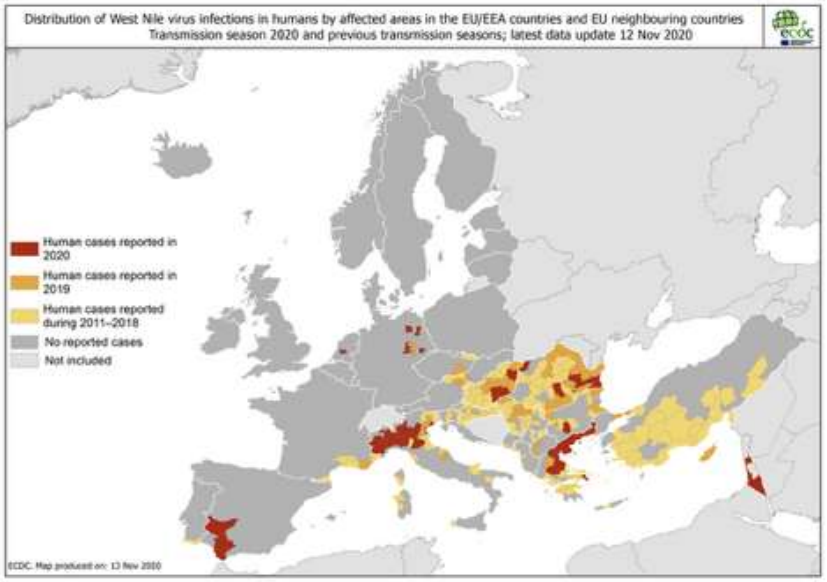
Where can genomic surveillance be used for?

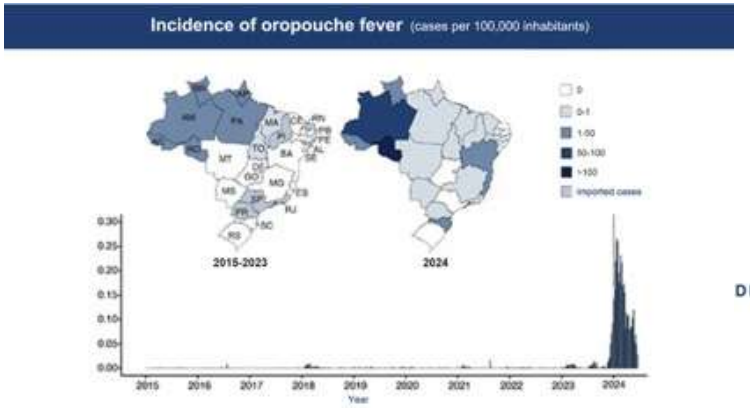
1) Molecular epidemiology of different viruses/variants
2) Local, regional and national support of source- and contact tracing and potentially internationally
3) Improved detection of genetically linked clusters
4) Surveillance in case of changes in disease presentation / epidemiological presentation
5) Early detection of novel genetic variants/deletion events that could influence diagnostic/surveillance capabilities
6) Monitoring of genetic variation related to potential vaccine responses

Epidemiology and genomic sequencing performed at EMC

- Emering arboviruses
  - Usutu virus
  - West Nile virus
  - Oropouche virus
  - Zika virus
  - Sindbis virus

- Emering other viruses
  - Influenza A virus
  - Measles virus
  - Mpox virus



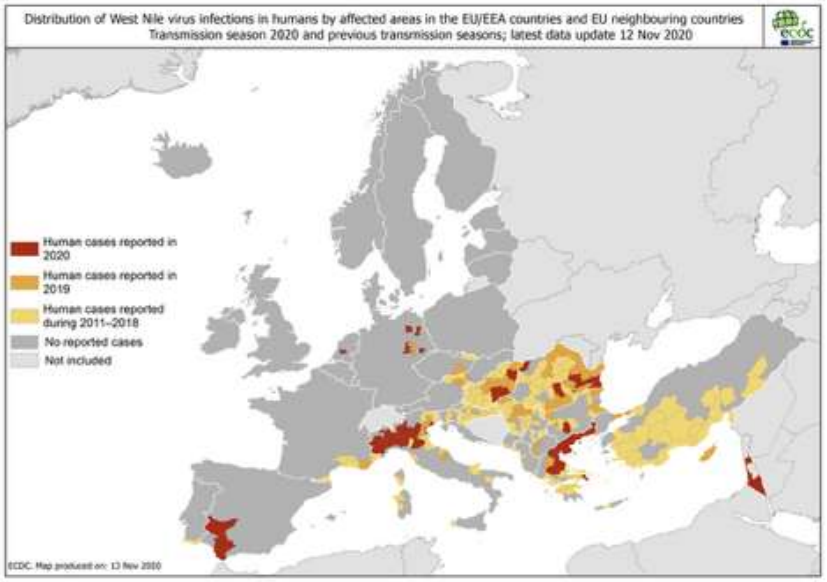


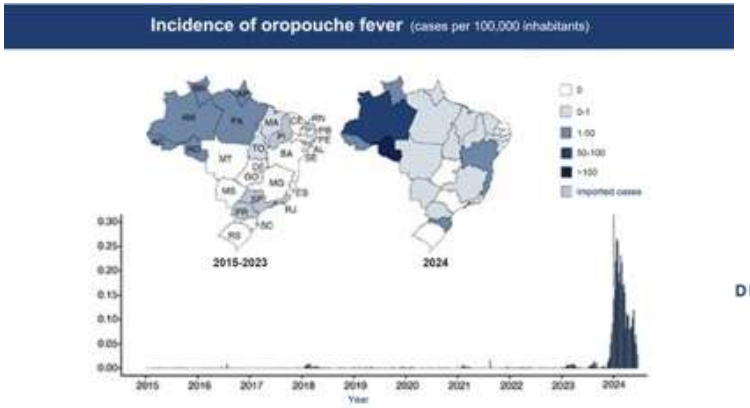
DEPARTMENT OF Erasmus MC University Medical Center Rotterdam

Epidemiology and genomic sequencing performed at EMC

- Emering arboviruses
  - Usutu virus
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  - Sindbis virus

- Emering other viruses
  - Influenza A virus
  - Measles virus
  - **Mpox virus**





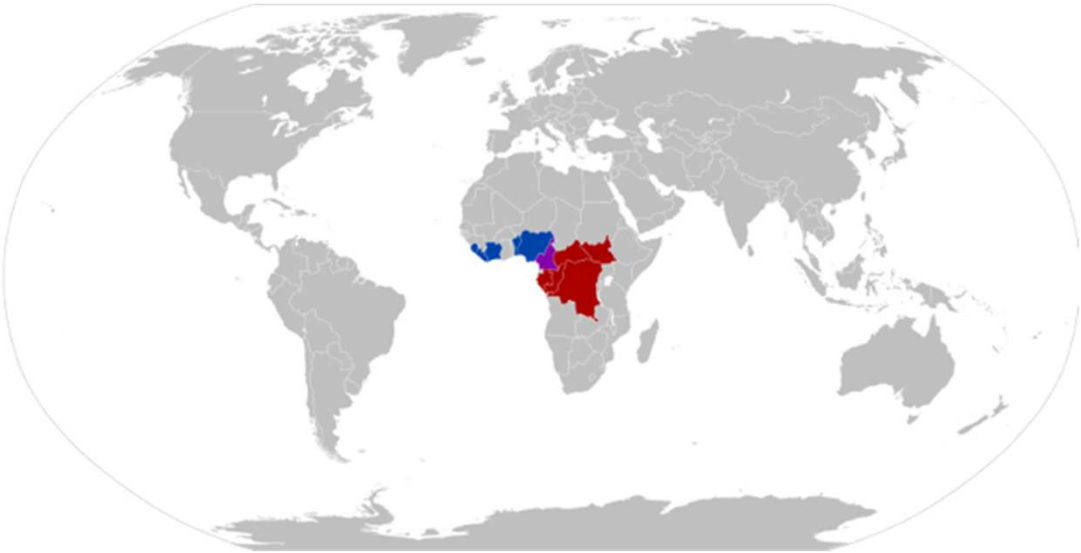
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Mpox historically

- 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.



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 Ojo, 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, A Akinpelu BSc, A Ahmad 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

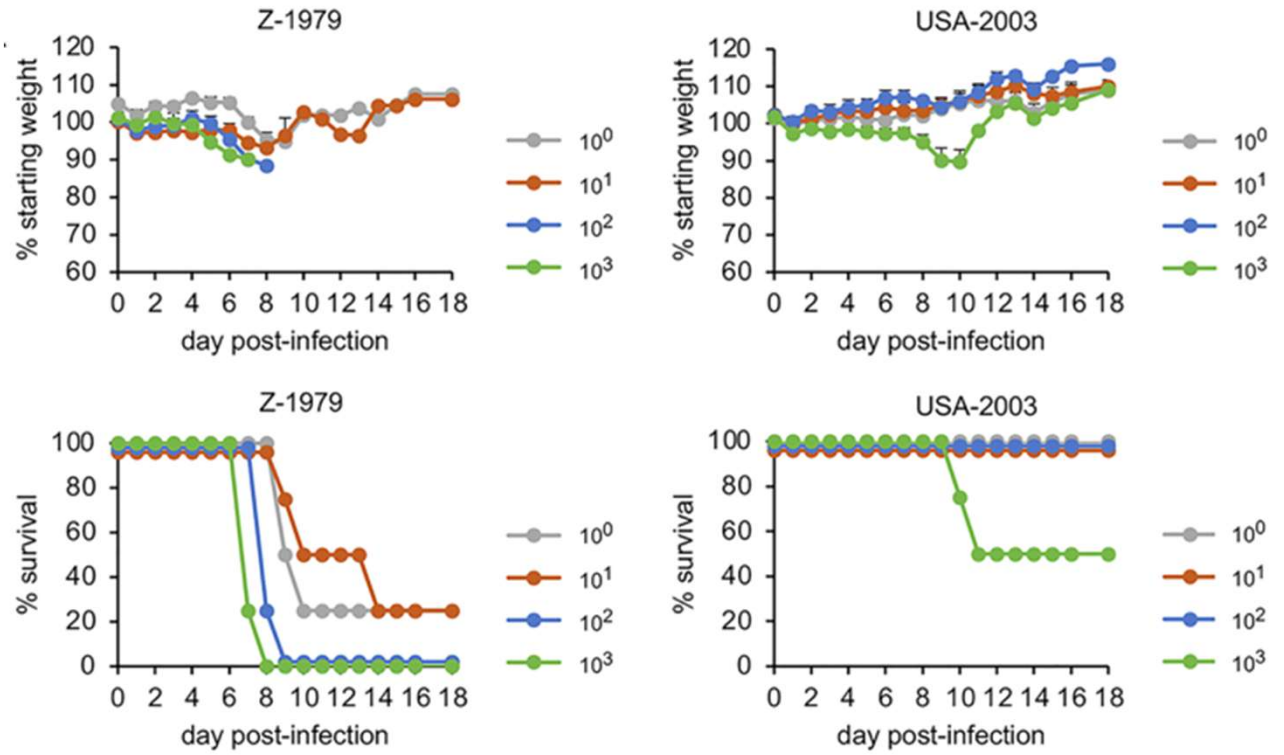
Two cases of monkeypox imported to the United Kingdom, September 2018

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Mpox clades

- **Clade I (Congo Basin strain)** is a zoonotic form of the virus, and responsible for a more severe disease presentation (~5-10% mortality) with pox present all over the body, and only limited human-human transmission is observed. **Clade II (West African strain)** is causing less severe disease (99% survival)

- Clade I MPXV (Z-1979) Is up to 1,000 Times More Virulent Than Clade IIa MPXV (USA-2003) in CAST Mice.



Americo et al, PNAS, 2023

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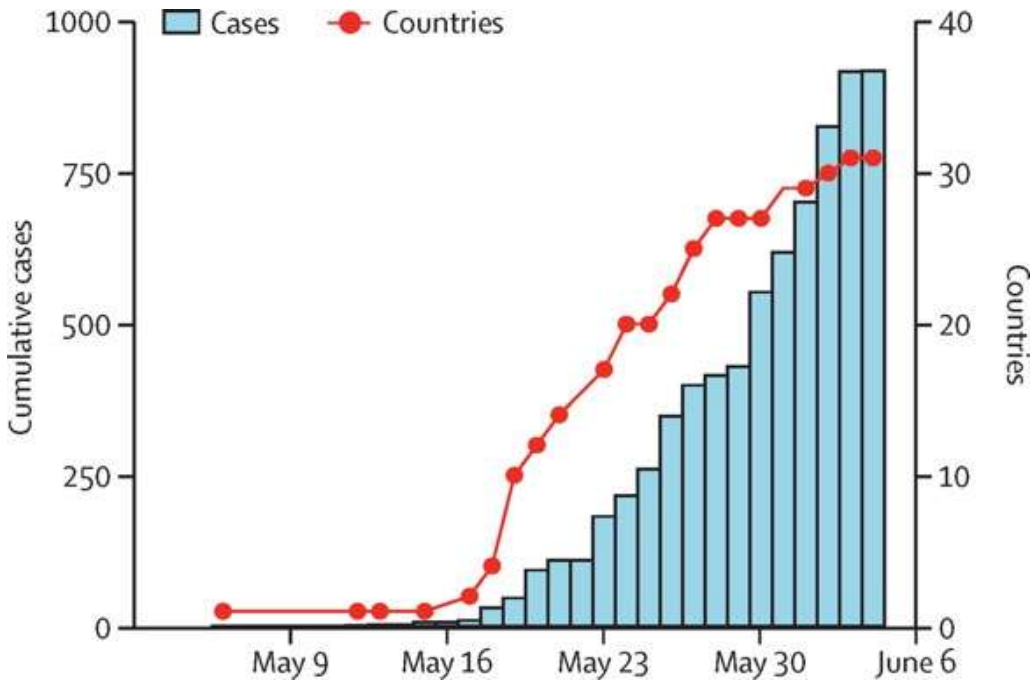
Differences between Clade I and Clade II

- **Clade I** caused higher numbers of severe illnesses than clade II, with up to 10% of people dying from it. Recent outbreaks have seen much lower death rates of about 1-3.3%.

- **Clade II** infections are less severe. More than 99.9% of people survive.

- **The D14L gene (OPG032 is deleted)** in the Clade II viruses which causes potential differences in virulence and transmissibility

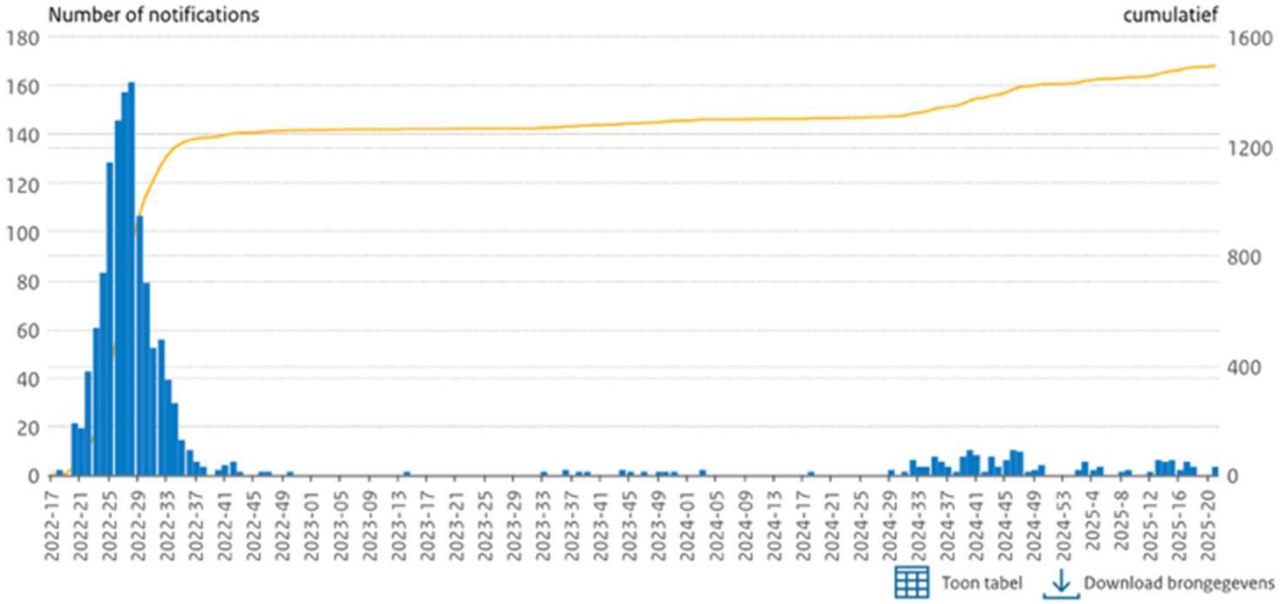
- No documented sustained human to transmission of both clades until 2022



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University Medical Center Rotterdam

Cases in the Netherlands

Reported cases of mpox in the Netherlands



First Monkeypox genome sequence from the Netherlands

MPXV Genome Reports



b.oudemunnink 3 May 2022

First Dutch genome sequence of Monkeypox virus

Bas Oude Munnink, Marjan Boter, Babette Weller, Richard Molenkamp, Janette Rahamat-Langendoen, Reina Sikkema, Marion Koopmans

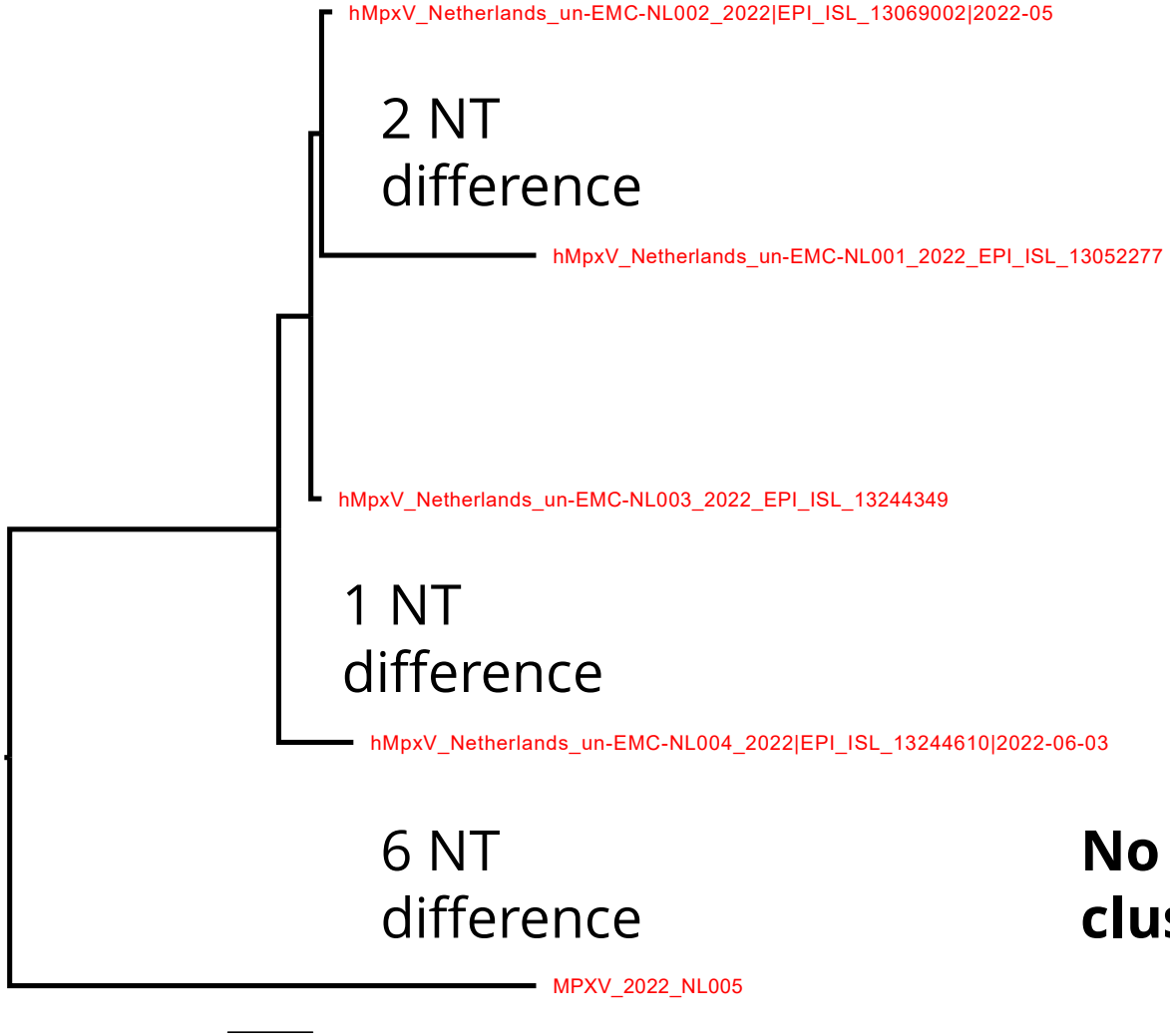
ErasmusMC, department of viroscience

Here we announce the first Dutch genome sequence of the monkeypox virus associated with the currently ongoing multi-country outbreak. The virus clusters with the previously obtained sequences and only differs on two positions compared to the other sequences.

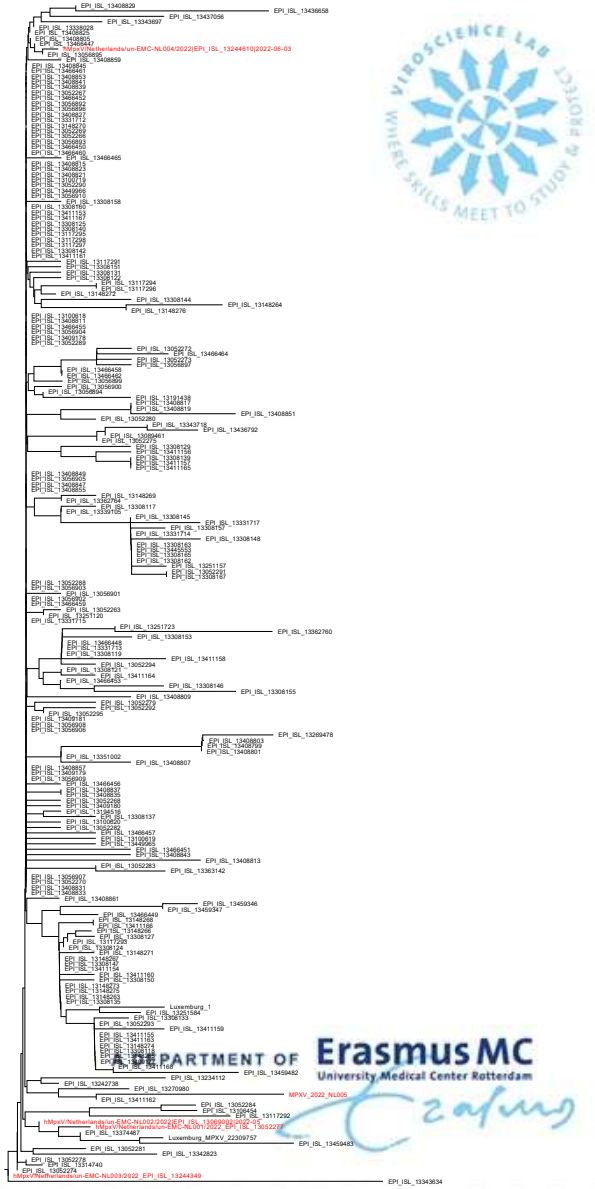


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First sequences from the Netherlands

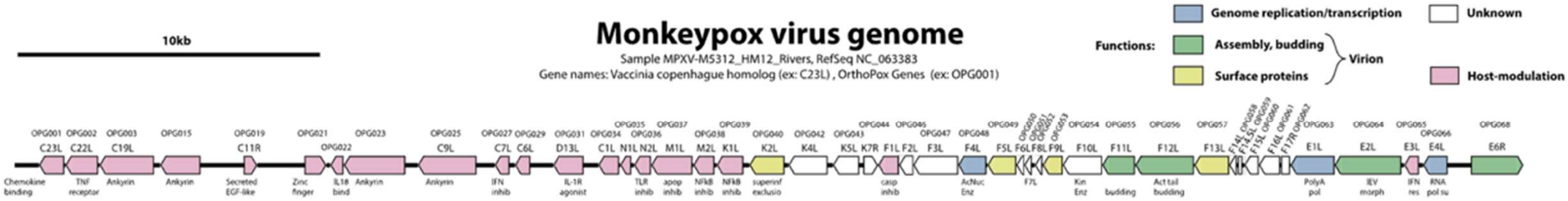


No geographical clustering yet



Mpox genomics

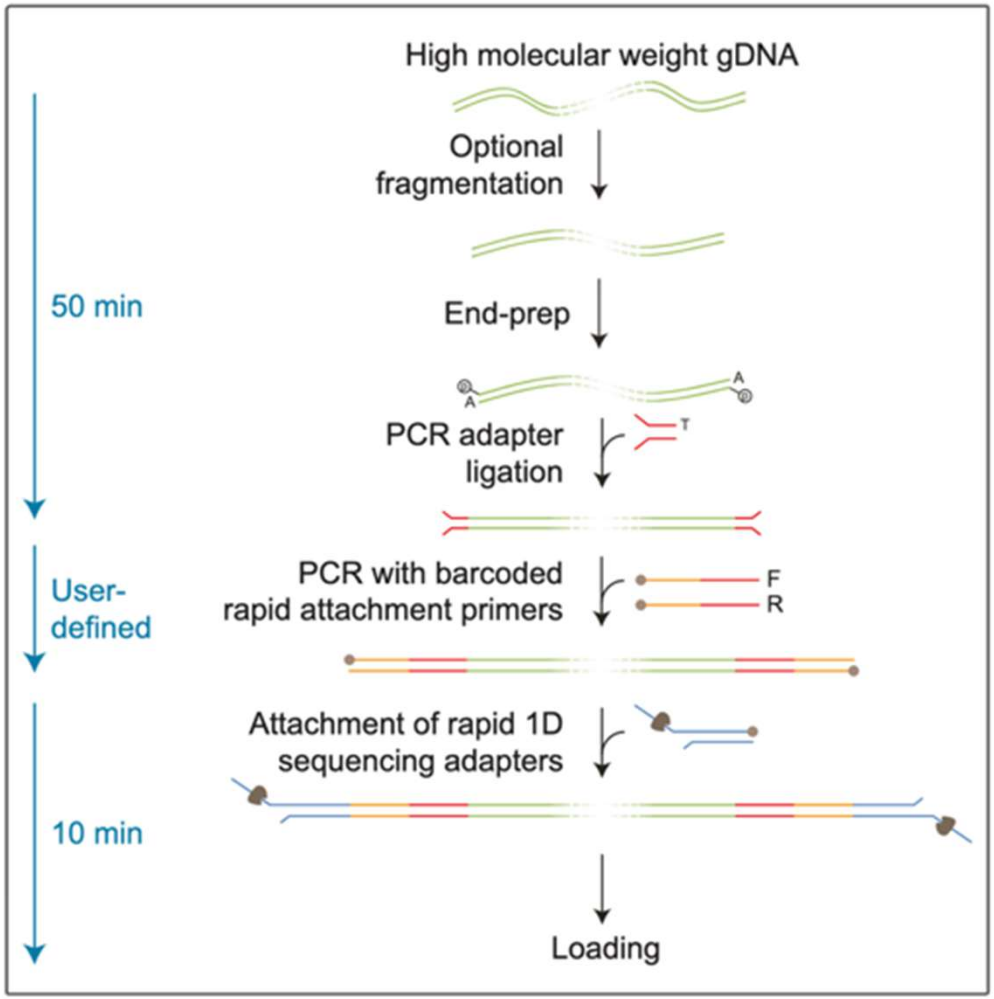
- Large genome of around 200kb encoding for around 200 different proteins
- Function of these proteins remain largely unknown
- Contains inverted terminal repeats
- Initial data suggests that the virus only changes 1-2 nucleotides per year
- Deletions and insertion are frequently observed



Metagenomic sequencing

Pretreatment:

- Centrifugation
- DNAse treatment
- Omnicleave treatment



Metagenomic sequencing
(DNase + centrifugation)
Work up to Ct value 20

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Amplicon-based sequencing

- If you know what to look for … → Amplicon based approach

PROTOCOL

Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples

Joshua Quick[1], Nathan D Grubaugh[2], Steven T Pullan[3], Ingra M Claro[4], Andrew D Smith[1], Karthik Gangavarapu[2], Glenn Oliveira[5], Refugio Robles-Sikisaka[2], Thomas F Rogers[2,6], Nathan A Beutler[2], Dennis R Burton[2], Lia Laura Lewis-Ximenez[7], Jaqueline Goes de Jesus[8], Marta Giovanetti[8,9], Sarah C Hill[10], Allison Black[11,12], Trevor Bedford[11], Miles W Carroll[3,13], Marcio Nunes[14], Luiz Carlos Alcantara Jr.[8], Ester C Sabino[4], Sally A Baylis[15], Nuno R Faria[10], Matthew Loose[16], Jared T Simpson[17], Oliver G Pybus[10], Kristian G Andersen[2,5] & Nicholas J Loman[1]

[1]Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Birmingham, UK. [2]The Scripps Research Institute, La Jolla, California, USA. [3]Public Health England, National Infection Service, Porton Down, Salisbury, UK. [4]Department of Infectious Disease and Institute of Tropical Medicine, University of São Paulo, São Paulo, Brazil. [5]Scripps Translational Science Institute, La Jolla, California, USA. [6]Massachusetts General Hospital, Boston, Massachusetts, USA. [7]Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil. [8]Fundação Oswaldo Cruz (FIOCRUZ), Salvador, Brazil. [9]University of Rome, Tor Vergata, Italy. [10]Department of Zoology, University of Oxford, Oxford, UK. [11]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA. [12]Department of Epidemiology, University of Washington, Seattle, Washington, USA. [13]University of Southampton, South General Hospital, Southampton, UK. [14]Instituto Evandro Chagas, Belem, Brazil. [15]Paul-Ehrlich-Institut, Langen, Germany. [16]DeepSeq, School of Life Sciences, University of Nottingham, Nottingham, UK. [17]OICR, Toronto, Canada. Correspondence should be addressed to N.J.L. (n.j.loman@bham.ac.uk).

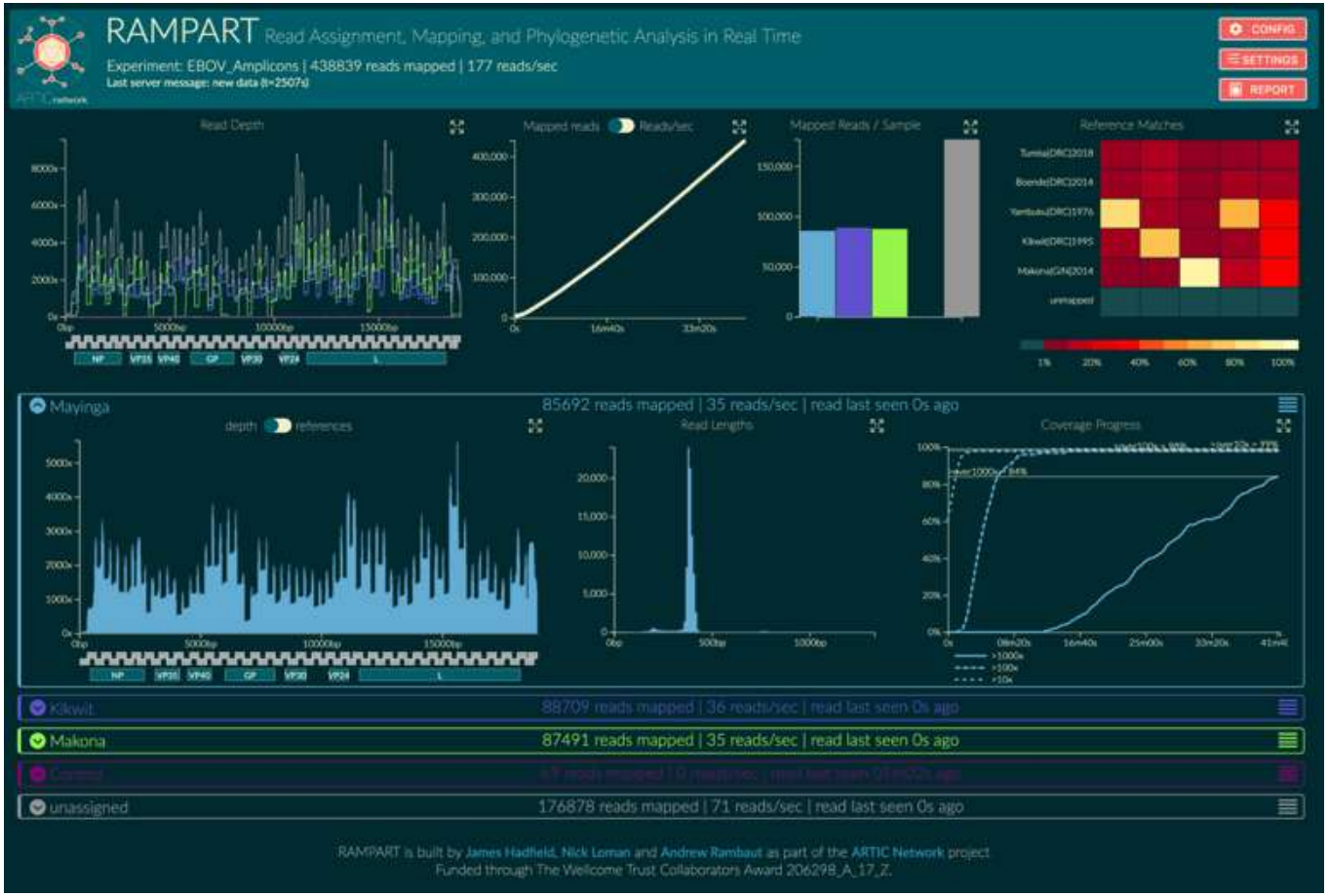


Amplicon sequencing Comparison:

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

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Bioinformatic analysis



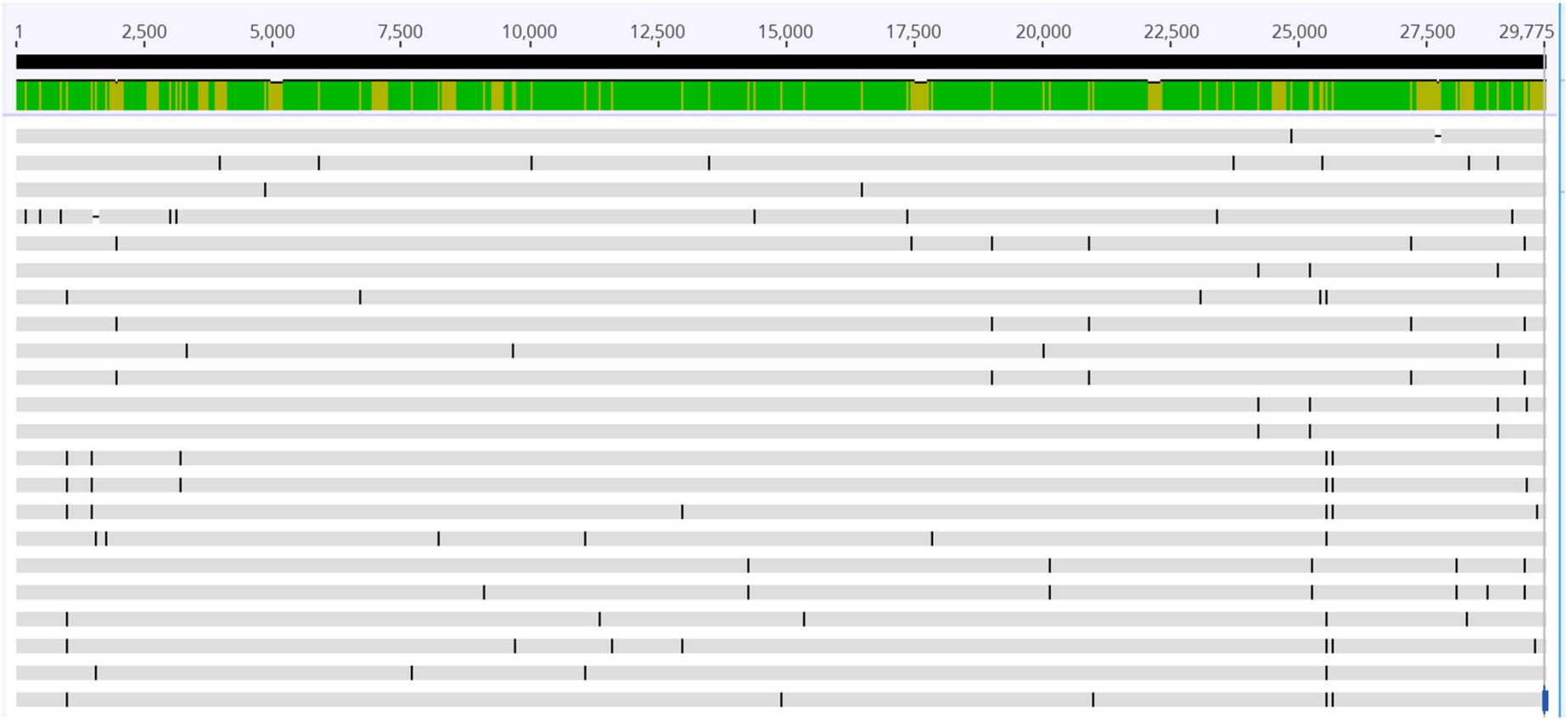
Custom script to automatically collect data from the machine and transfer to the server

Real-time automated data-analysis:

- Demultiplex
- Trim primers
- Align to reference
- Generate consensus sequence

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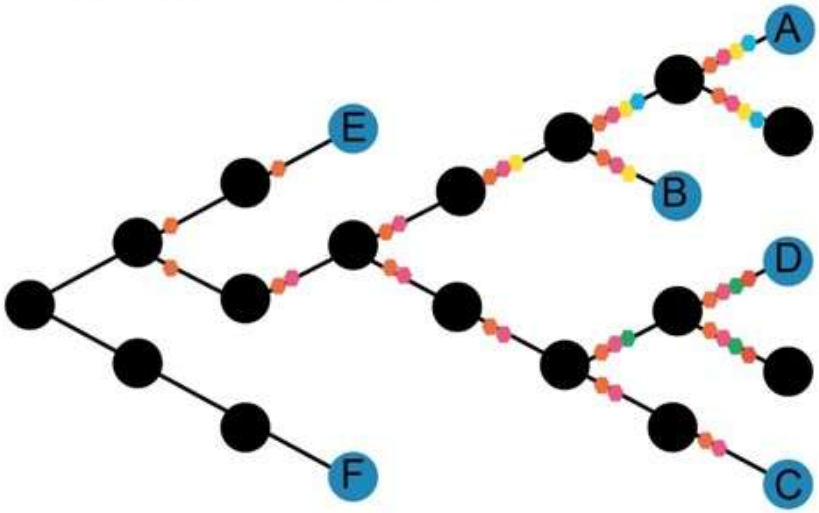
Multiple sequence alignment



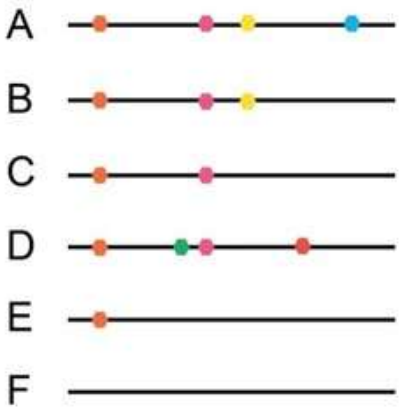
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Phylogeny

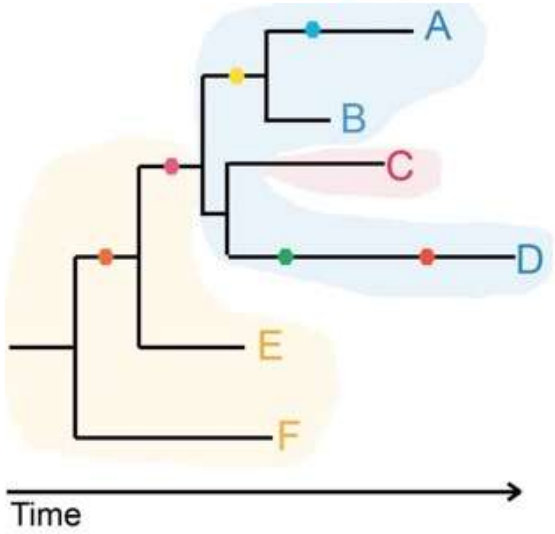
Transmission process



Sequences from samples

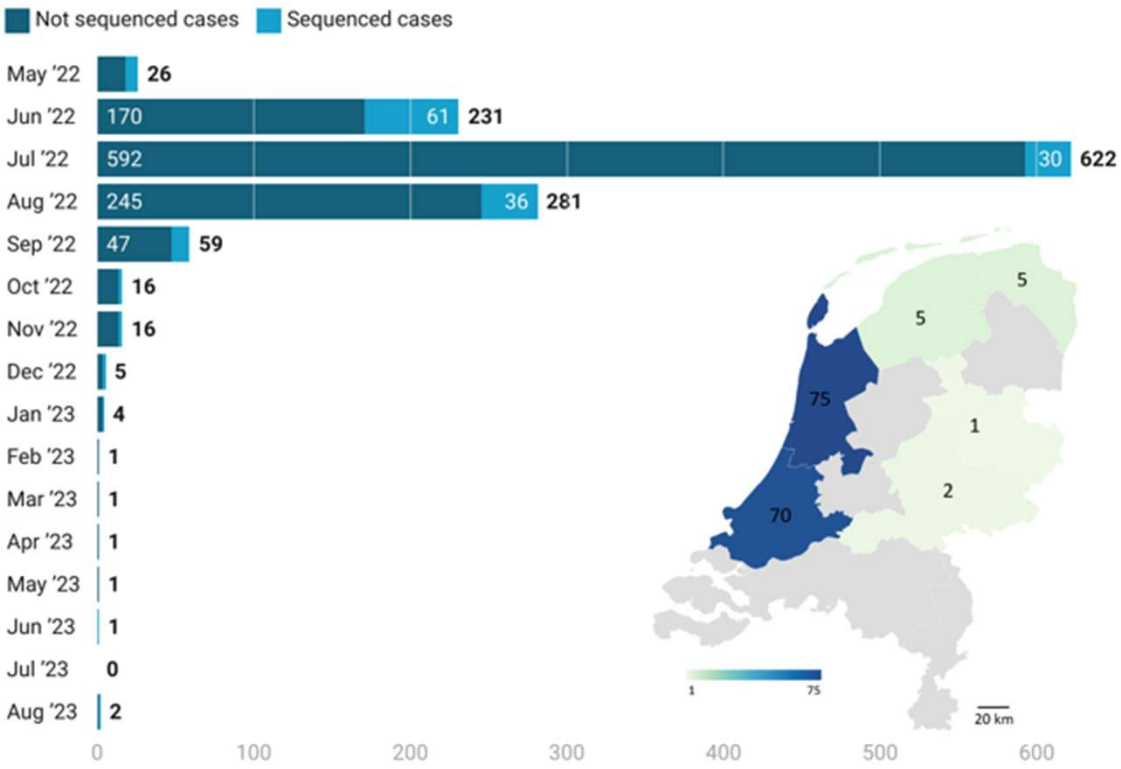


Reconstruction

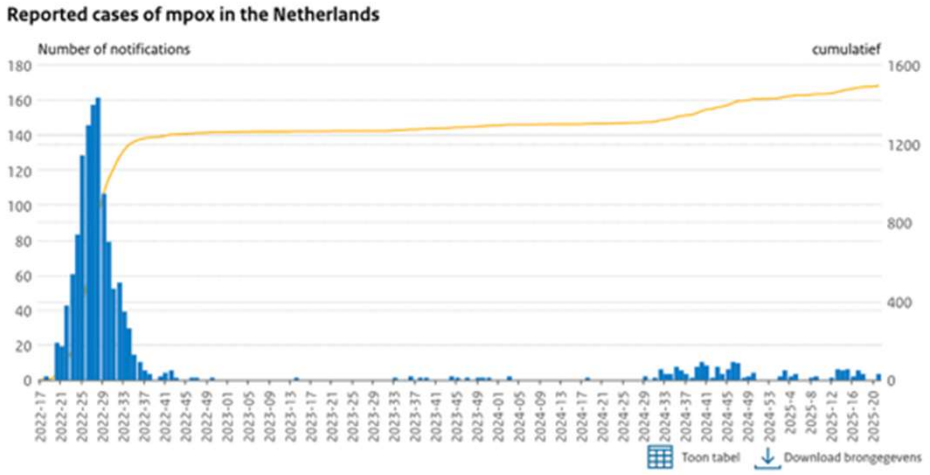


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The 2022 MXPV clade II outbreak



- Sustained human-to-human spread and sexual transmission in the MSM community
- Globally >93.000 cases have been reported with 177 reported deaths (as of 20-01-2024)
- At the moment in 2023 still limited spread of the virus in the Netherlands



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

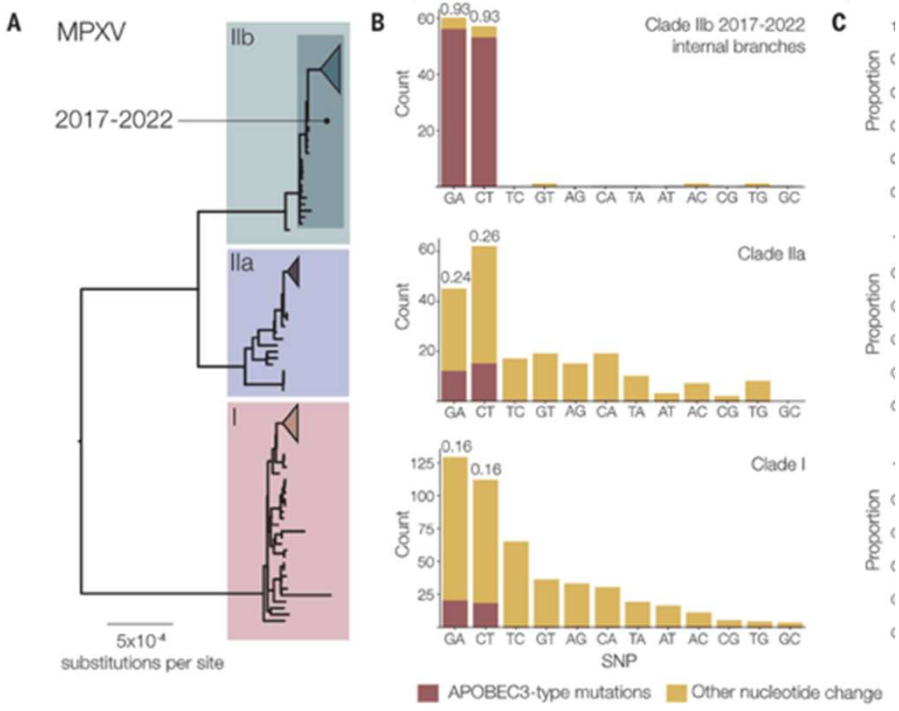
RESEARCH

MPOX

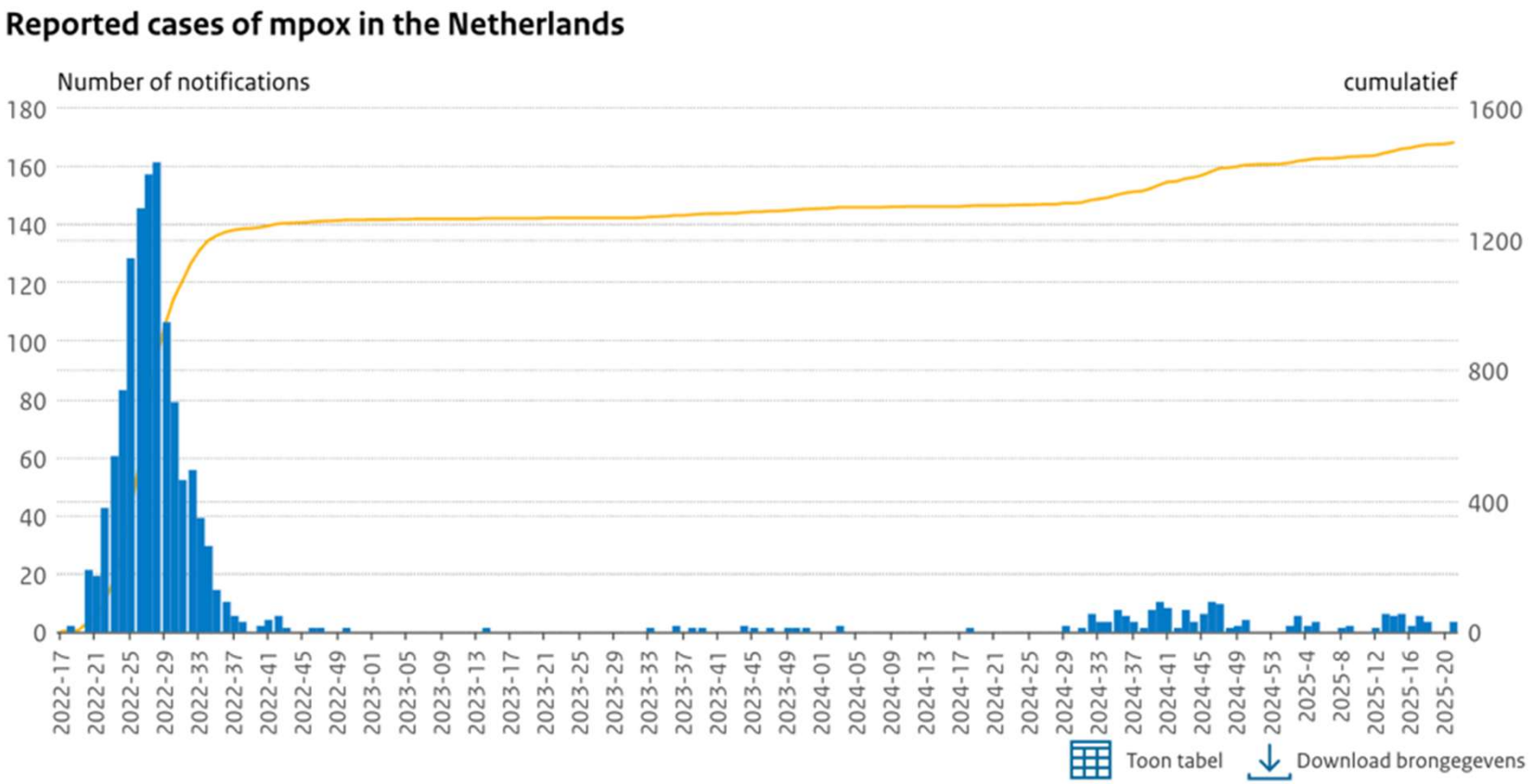
APOBEC3 deaminase editing in mpox virus as evidence for sustained human transmission since at least 2016

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[5], 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



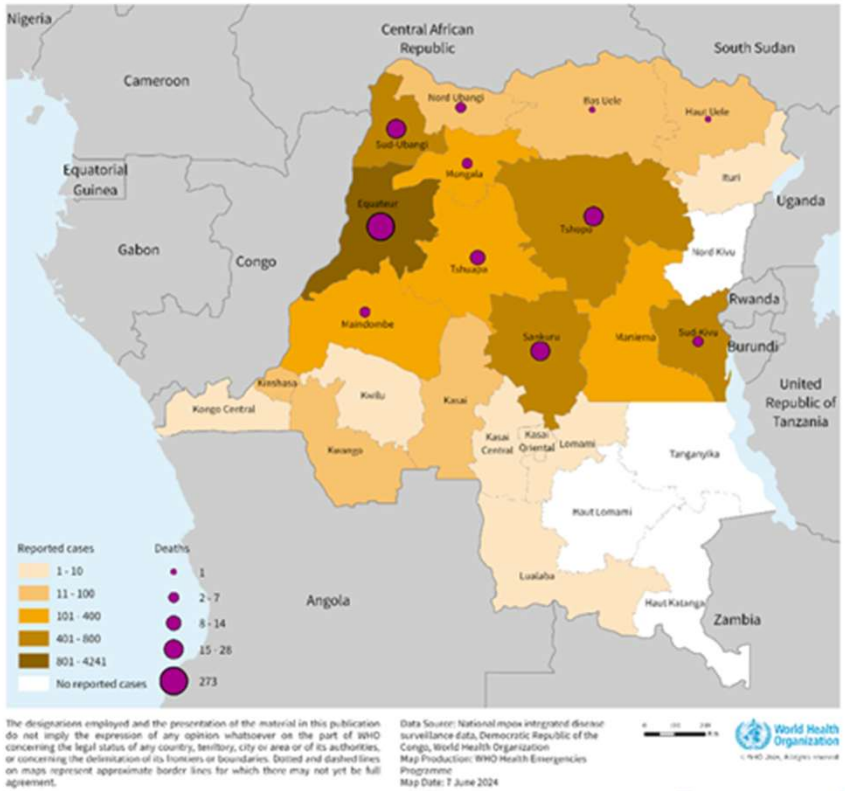
MXPV in the Netherlands



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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.



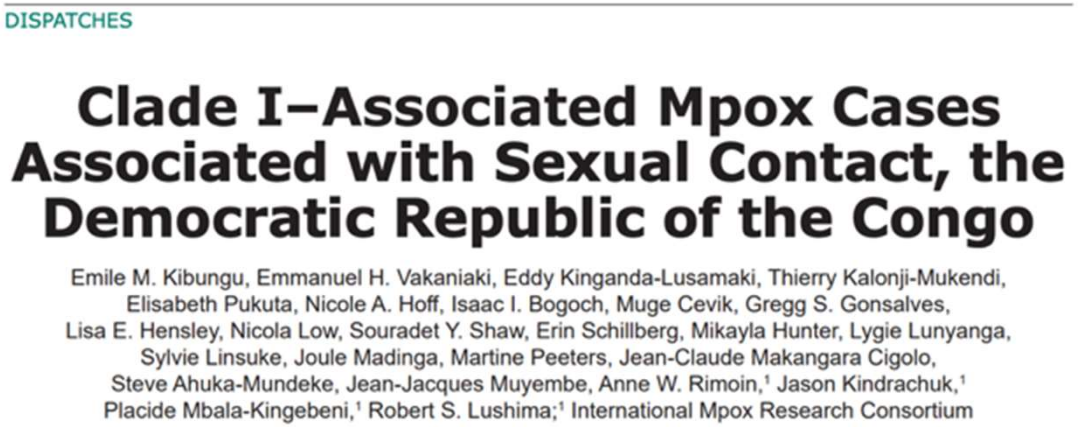
A DEPARTMENT OF Erasmus MC University Medical Center Rotterdam

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

Sexual transmission of Clade I MPXV

Mostly zoonotic infections occur with limited human-to-human transmission within households

*"A man from DRC in his late 20s (case-patient 1) reported having 2 sexual encounters with a man (suspected primary case-patient) in **Europe 1 week before returning to DRC.** The suspected primary case-patient frequently visited DRC"*



DISPATCHES

Clade I–Associated Mpox Cases Associated with Sexual Contact, the Democratic Republic of the Congo

Emile M. Kibungu, Emmanuel H. Vakaniaki, Eddy Kinganda-Lusamaki, Thierry Kalonji-Mukendi, Elisabeth Pukuta, Nicole A. Hoff, Isaac I. Bogoch, Muge Cevik, Gregg S. Gonsalves, Lisa E. Hensley, Nicola Low, Souradet Y. Shaw, Erin Schillberg, Mikayla Hunter, Lygie Lunyanga, Sylvie Linsuke, Joule Madinga, Martine Peeters, Jean-Claude Makangara Cigolo, Steve Ahuka-Mundeke, Jean-Jacques Muyembe, Anne W. Rimoin,[1] Jason Kindrachuk,[1] Placide Mbala-Kingebeni,[1] Robert S. Lushima;[1] International Mpox Research Consortium

*"Case-patient 1 reported having sexual contact with 9 partners (6 men and 3 women) after arriving in DRC. A prescreening of those contacts identified suspected mpox in 3 sexual contacts (2 men and 1 **woman**, 30–35 years of age) who had fever and lesions in the genital and perianal region."*

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Sequencing and analysis

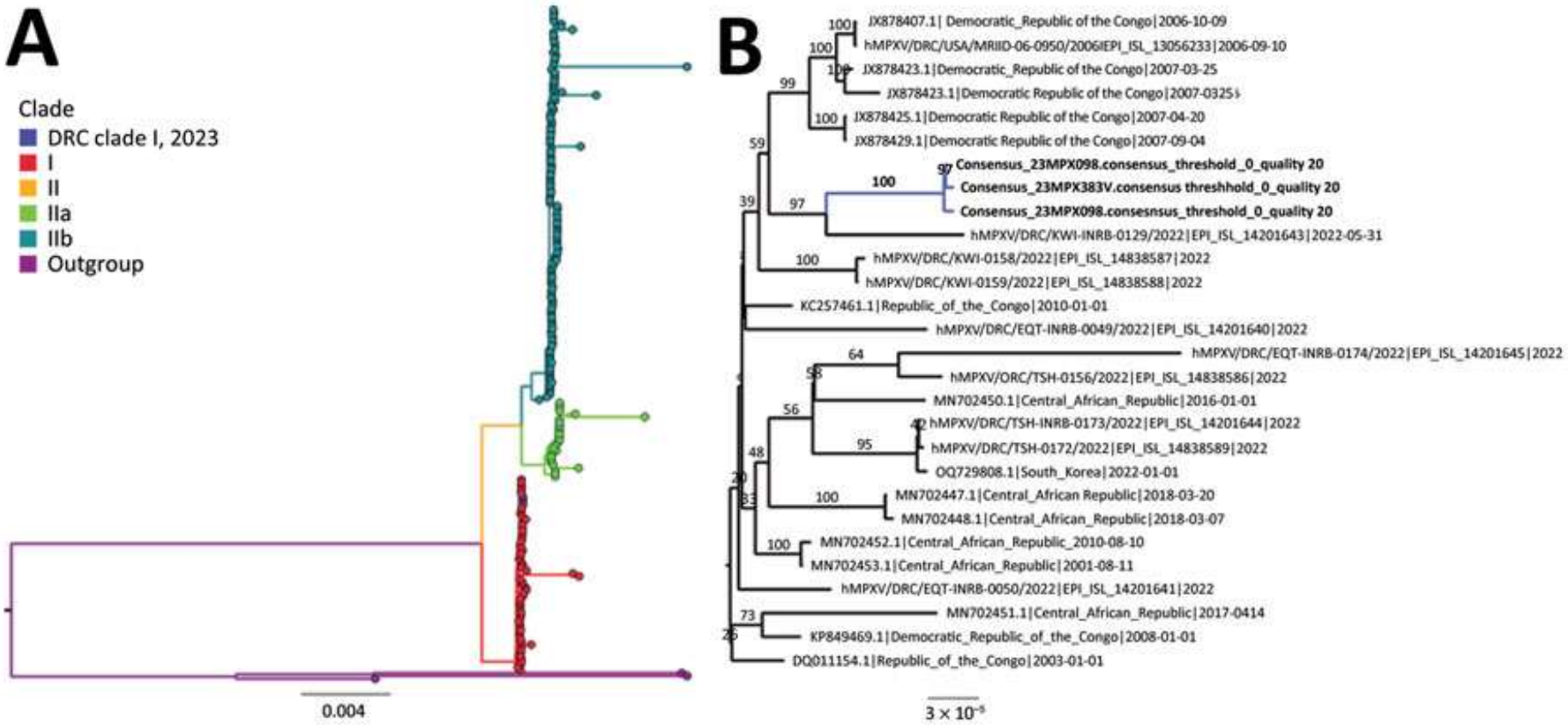


Figure 2. Phylogenetic analysis of MPXV sequences from a cluster of mpox cases described in Kwango Province, Democratic Republic of the Congo. A) MPXV global phylogeny showing that the Kwango Province outbreak cluster belongs to clade I MPXV. B) Phylogenetic analysis of MPXV genome sequences from the reported cases and clade I MPXV sequences from Central Africa. Note that 23MPX098 (or 23MPX0245V) and 23MPX099 (or 23MPX0245C) are vesicle and crust samples from case-patient 1.

Mpox in Congo (GREAT-LIFE)

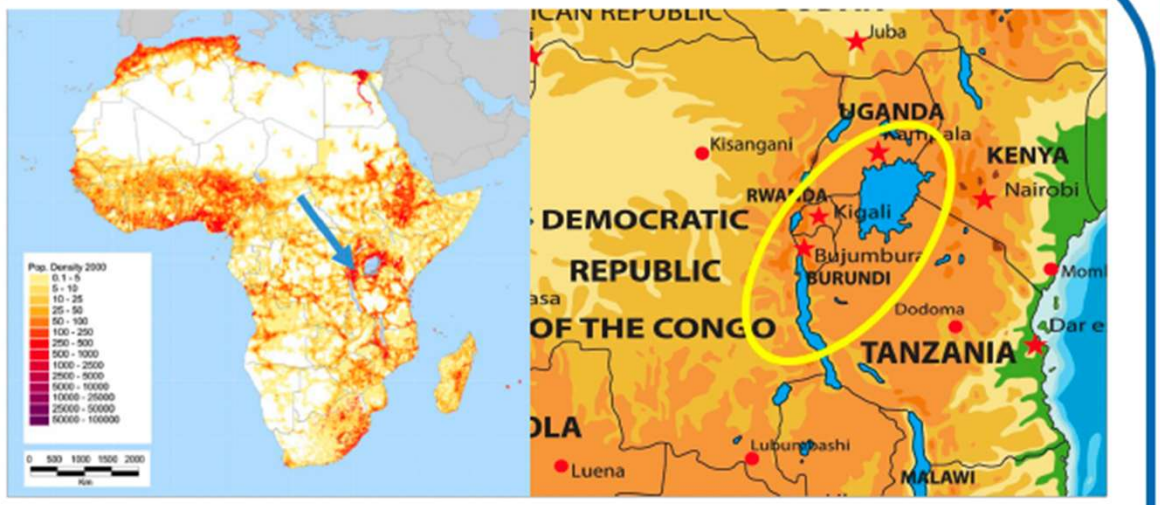


Figure 1. Area of the proposed research. The central part of the African Great Lakes Region, with high population density, high biodiversity and huge endemic and epidemic disease challenges, but yet limited surveillance.



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

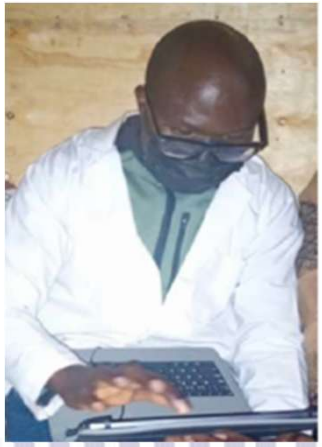
Epi data and contact tracing

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*

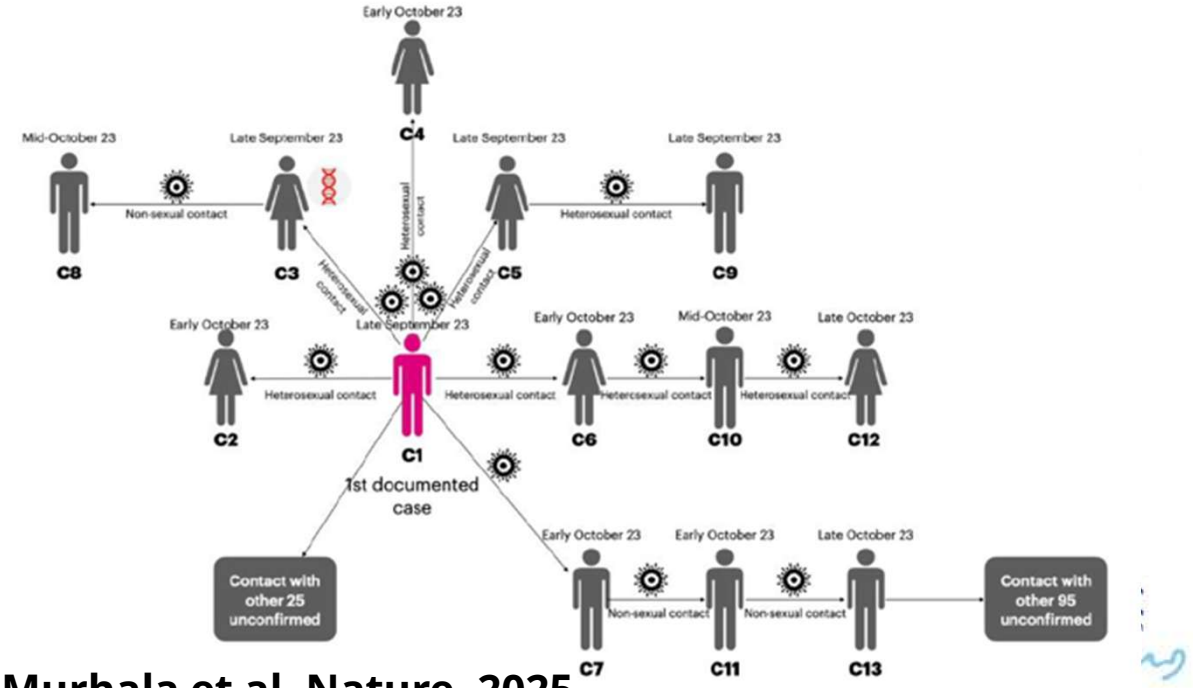
Leandre Murhula Masirika[1,2], Jean Claude Udahemuka[3], Pacifique Ndishimye[4,5], Gustavo Sganzerla Martinez[6,7], Patricia Kelvin[6,7], Maliyamungu Bubala Nadine[8], Bilembo Kitwanda Steeven[8], Franklin Kumbana Mweshi[8], Léandre Mutimbwa Mambo[9], Bas B. Oude Munnink[10], Justin Bengehya Mbiribindi[11], 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]

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	-



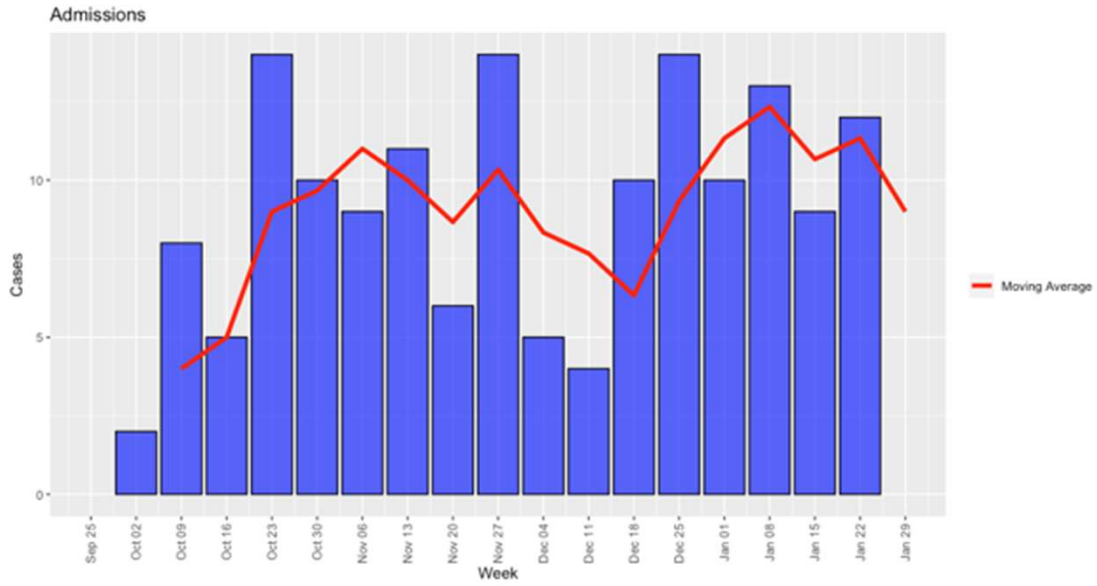




Murhala et al, Nature, 2025

Mpox outbreak in Kamituga

- 51 hospitalised individuals with 37 (73%) of 51 who were PCR diagnosed with mpox infection
- 2 patients died during the course of the study (4%)
- Structured, de-identified case report forms and interviews were conducted to determine the possible origins and modes of transmission of the Mpox outbreak



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Cohort characteristics

Table 1 – Characteristics of the cohort.

	Female	Male
N	26	25
Confirmed case/Negative case/Pending	21/1/4	16/1/8
Age (mean; median; Q1-Q3)	19.7; 20; 17-21	24.3; 23; 18.5-30.5
Sexual orientation		
Heterosexual	22	25
Homosexual	0	0
Bisexual	2	0
More than one sexual partner within the last 6 months (yes)	16	15
Profession/occupation		
Sex Worker[2]	19	5
Gold miner	0	5
Merchant	0	2
Farmer	2	2
Student	2	4
Other/none	3	7

- Mainly (female) sexworkers
- Heterosexual orientated
- Very young individuals
- Limited sexual conntact with >1 partner

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On site training in amplicon-based Nanopore sequencing







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Amplicon-based sequencing

- Amplicon based approach

- Using 2 different primer sets on 6 samples





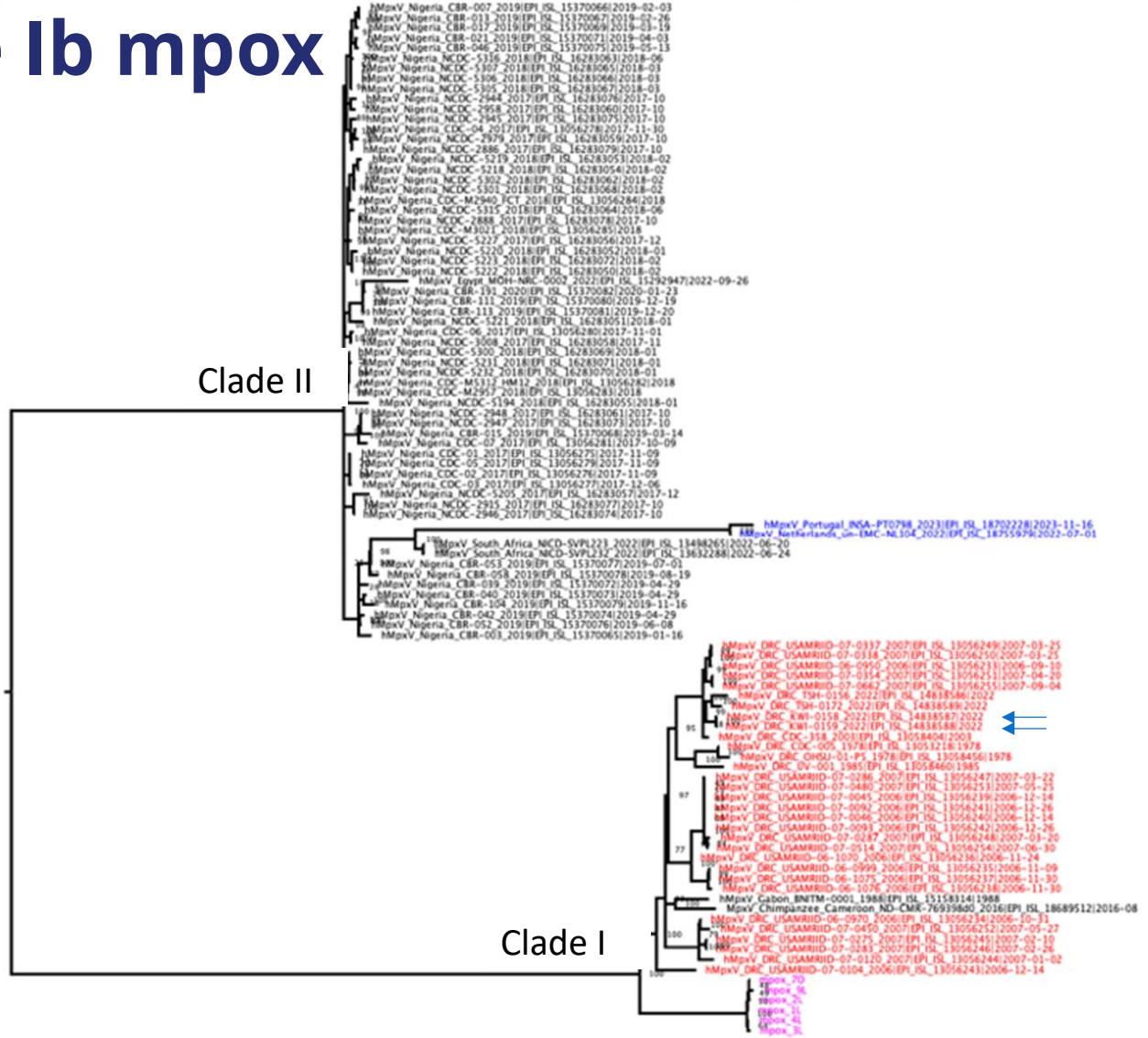


Amplicon sequencing Comparison:

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

Phylogeny –- new clade Ib mpox virus

- 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



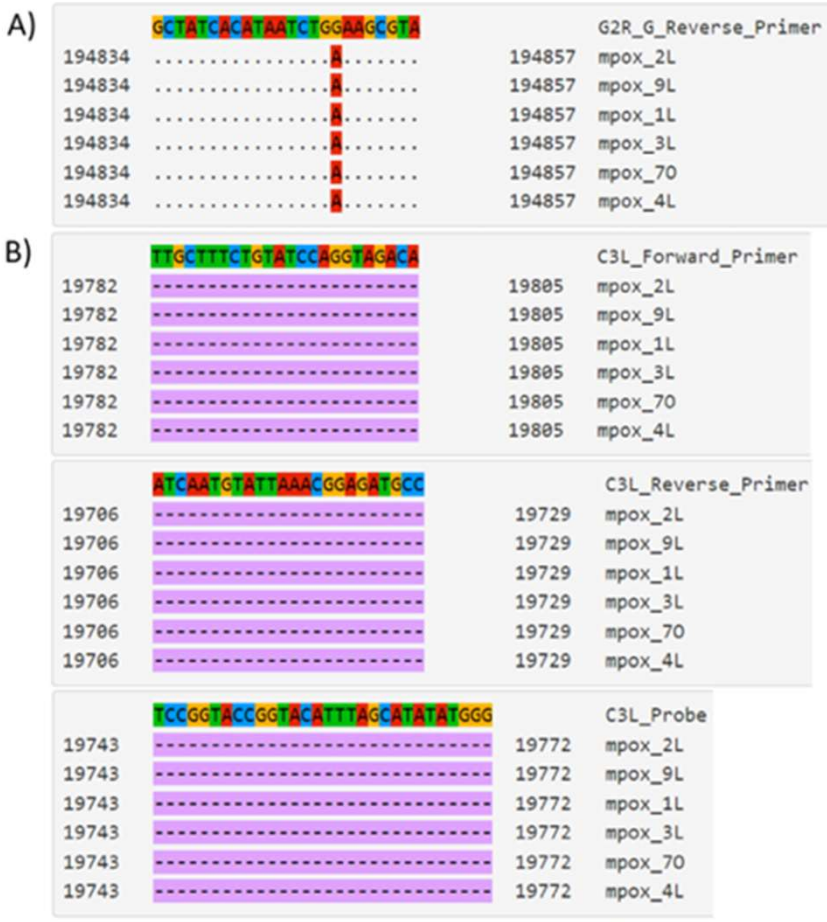
Murhula et al, Eurosurveillance, 2024

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



Diagnostic primer performance (2)

- Deletion validated by other groups after sharing the ”raw” data
- PCR for detection is still working
- Other screening assays not

DISPATCHES

Clade I–Associated Mpox Cases Associated with Sexual Contact, the Democratic Republic of the Congo

Emile M. Kibungu, Emmanuel H. Vakaniaki, Eddy Kinganda-Lusamaki, Thierry Kalonji-Mukendi, Elisabeth Pukuta, Nicole A. Hoff, Isaac I. Bogoch, Muge Cevik, Gregg S. Gonsalves, Lisa E. Hensley, Nicola Low, Souradet Y. Shaw, Erin Schillberg, Mikayla Hunter, Lygie Lunyanga, Sylvie Linsuke, Joule Madinga, Martine Peeters, Jean-Claude Makangara Cigolo, Steve Ahuka-Mundeke, Jean-Jacques Muyembe, Anne W. Rimoin,[1] Jason Kindrachuk,[1] Placide Mbala-Kingebeni,[1] Robert S. Lushima;[1] International Mpox Research Consortium

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Volume 30, Number 2—February 2024

Letter

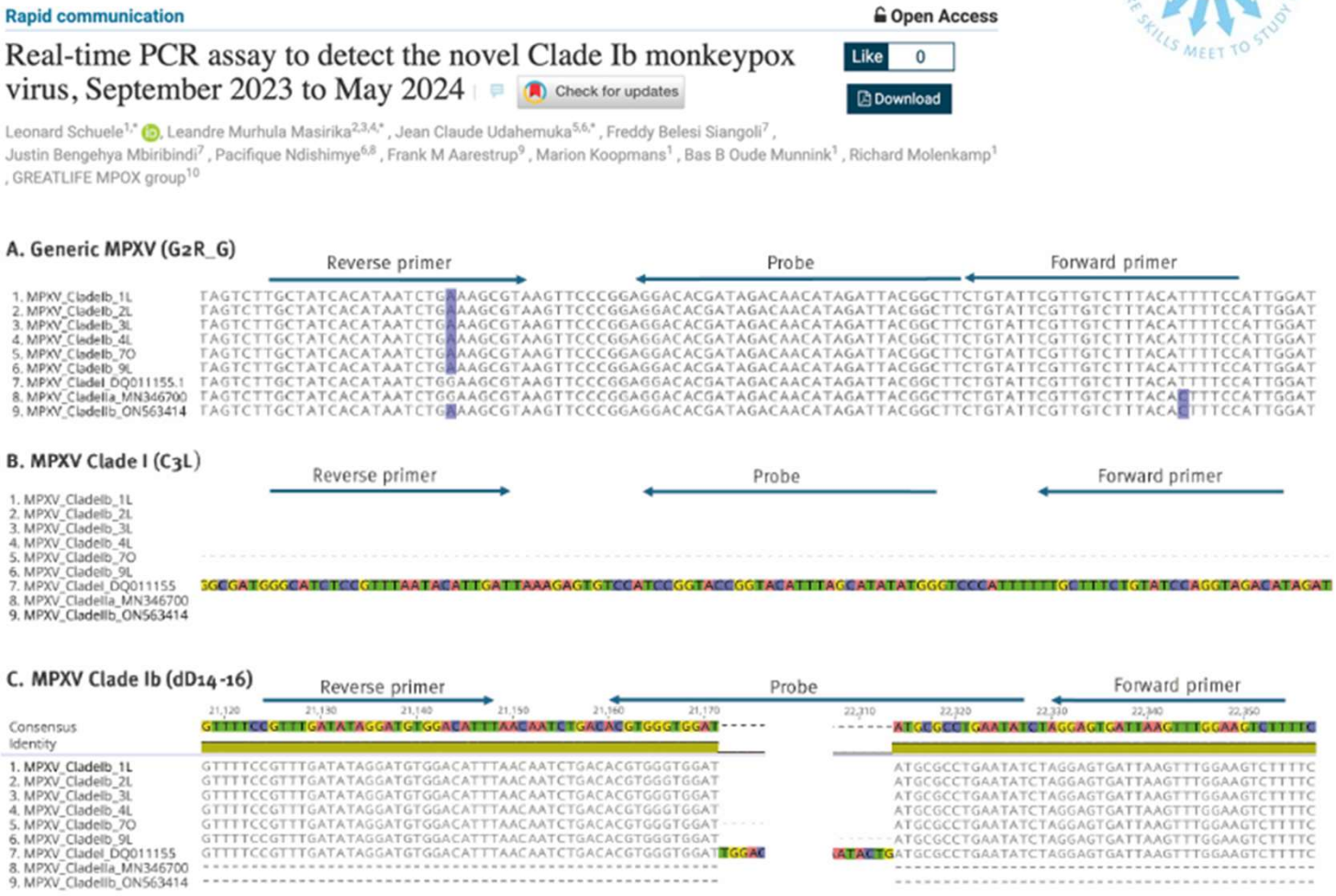
No Evidence for Clade I Monkeypox Virus Circulation, Belgium

Cite This Article

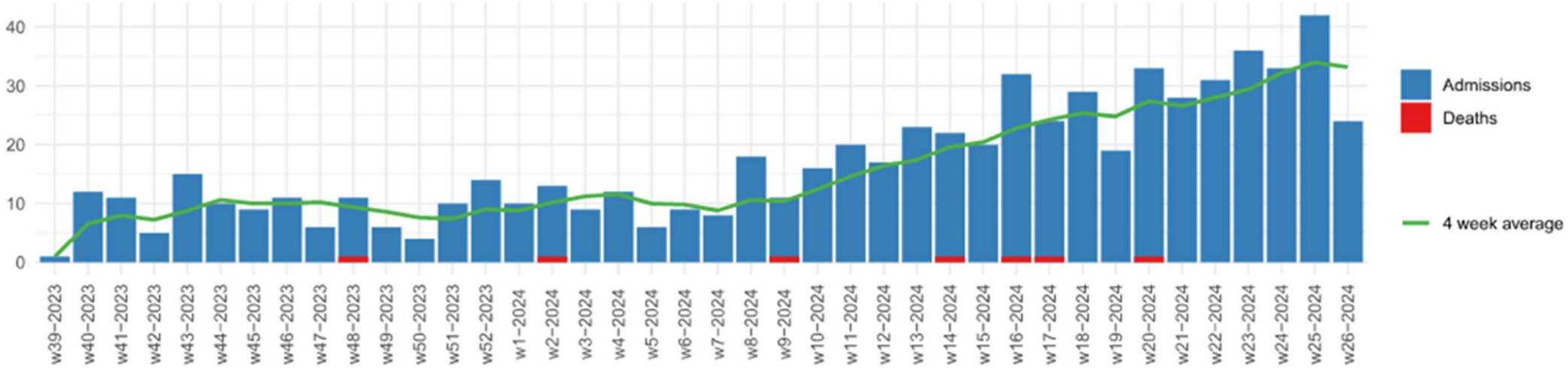
A DEPARTMENT OF Erasmus MC University Medical Center Rotterdam

Development of new Clade Ib specific 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 Ia, Clade Ib and Clade II.
- We designed a specific probe sequence to anneal directly upstream and downstream of the deletion in Clade Ib



Epidemiology in Kamituga



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

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Second training on site training session (2 weeks hands-on)



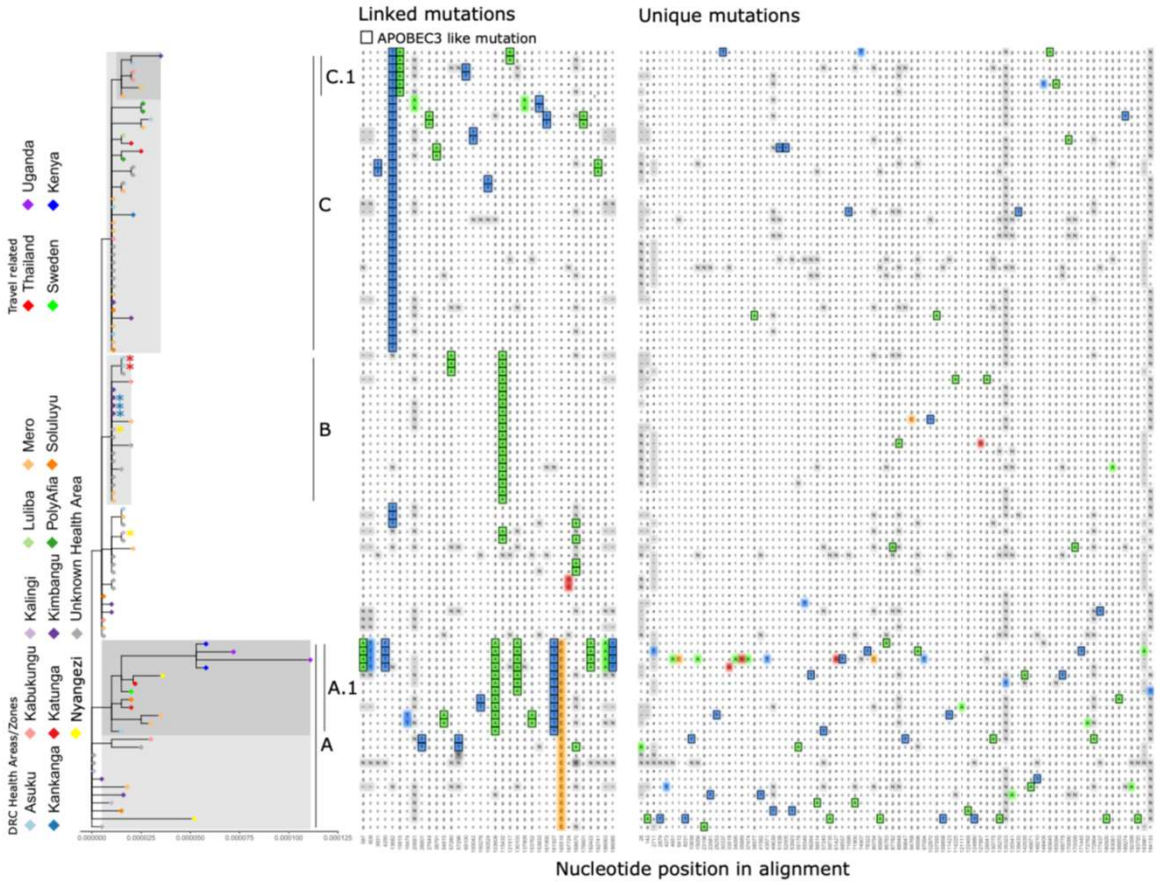
- Training in serology
- Training in diagnostics to detect clade Ib virus
- Second training in WGS (58 new complete genomes generated)
- Training in bioinformatics (challenging)
  - No Wifi
  - No programming knowledge





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Outbreak of the novel 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.
- In addition, MPXV DNA was detected in breast milk



Murhula et al, Nature Med, 2025

Observations

- 52.4% were in females and 47.6% in males. The majority (83.4%) were linked to professional sexual interactions.
- Seven deaths occurred, and three healthcare workers acquired mpox
- We observed an abundance of APOBEC3 mutations (28/35 linking mutations and 55/82 unique mutations) indicative of continuous human-to-human transmission.
- No clear link between sequence cluster, bar or health area was observed. These data suggest rapid spread mostly through sexual contact within densely populated areas

Table 1 | Epidemiological data of the ongoing mpox outbreak

Kamituga health areas	Population	Mpox cases	Relative frequency (%)	Sequenced samples (% total)	PSWs*	Bars
Kele Sidem	10,618	10	0.09		0	0
Asuku	11,002	73	0.66	8 (11.0%)	38	3
Bigombe	7,274	3	0.04		18	2
Isopo	5,403	1	0.02		0	0
Kabukungu	13,758	115	0.84	7 (6.1%)	152	10
Kalingi	12,888	28	0.22	4 (14.3%)	57	5
Kankanga	5,622	6	0.11	1 (16.7%)	0	0
Katunga	13,492	43	0.32	3 (7.0%)	7	1
Kele Katutu	11,234	3	0.03		21	0
Kibe	12,073	2	0.02		34	3
Kimbangu	16,132	105	0.65	11 (10.5%)	45	5
Luliba	2,870	11	0.38	1 (9.1%)	18	2
Mero	25,598	205	0.80	14 (6.8%)	161	13
Ngambwa	4,025	2	0.05		15	0
Poly Afia	12,105	13	0.11	3 (23.1%)	78	5
Poudriere	12,931	14	0.11		32	6
Soluluyu	10,921	36	0.33	4 (11.1%)	75	8
Total	**187,946**	**670**	**0.36**	**56 (8.4%)**	**751**	**63**

Description of number of inhabitants, bars, individuals employed as sex workers and notified mpox cases for the health areas in Kamituga that reported cases during the study period.
*Number of self-reported PSWs.

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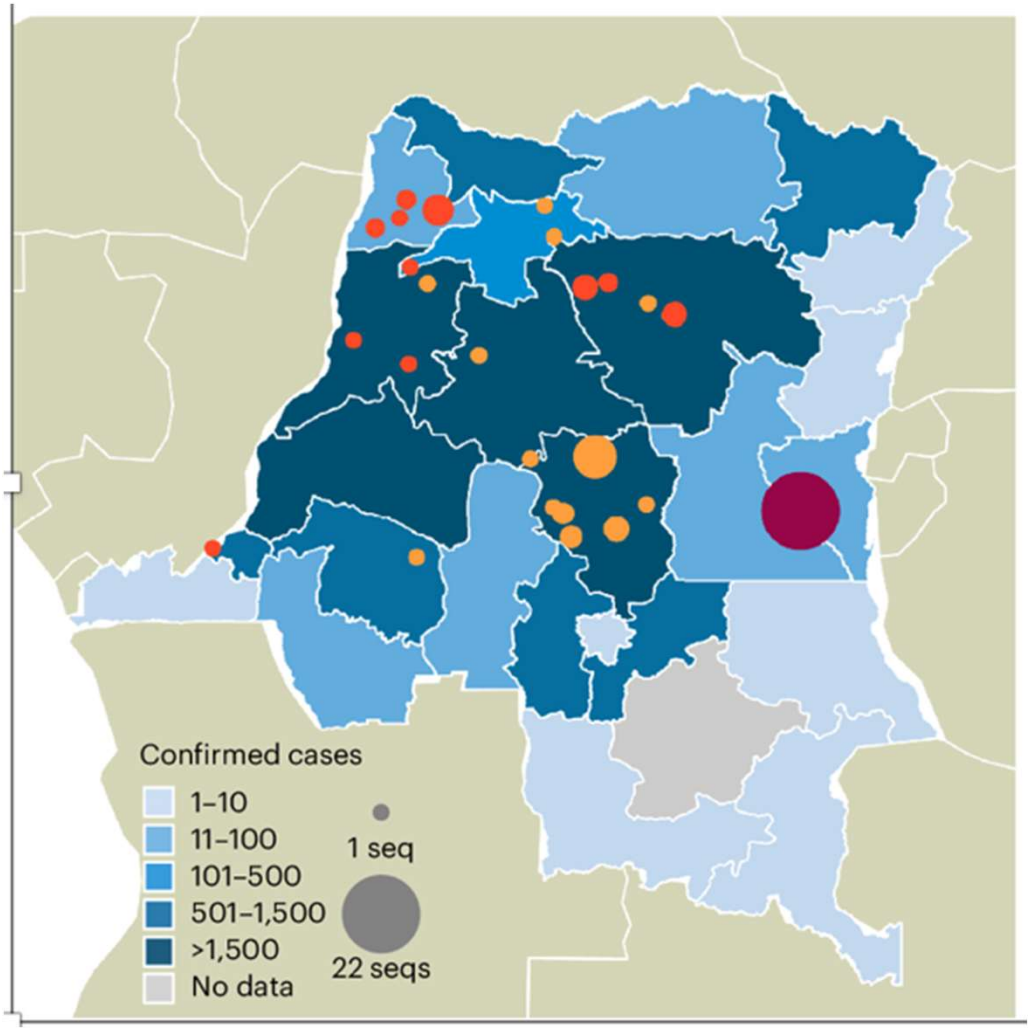
Pregnant women

- During this outbreak, also 14 pregnant women were admitted at Kamituga hospital among whom 8 aborted, supporting the potential for intrauterine transmission of MPXV in pregnant people as also previously suggested.

- Five women lost their pregnancies in the first trimester and three in the second trimester.
  - Three were housewives
  - Two were professional sex workers (PSWs)
  - One was a health worker
  - One was a trader
  - One reported no profession.

- In 2008 a MPXV Clade I case of fetal death after placental infection was reported which suggested risks of intrauterine infection emphasizing that clinicians should be aware of this potential complication.

Situation in the rest of the DRC

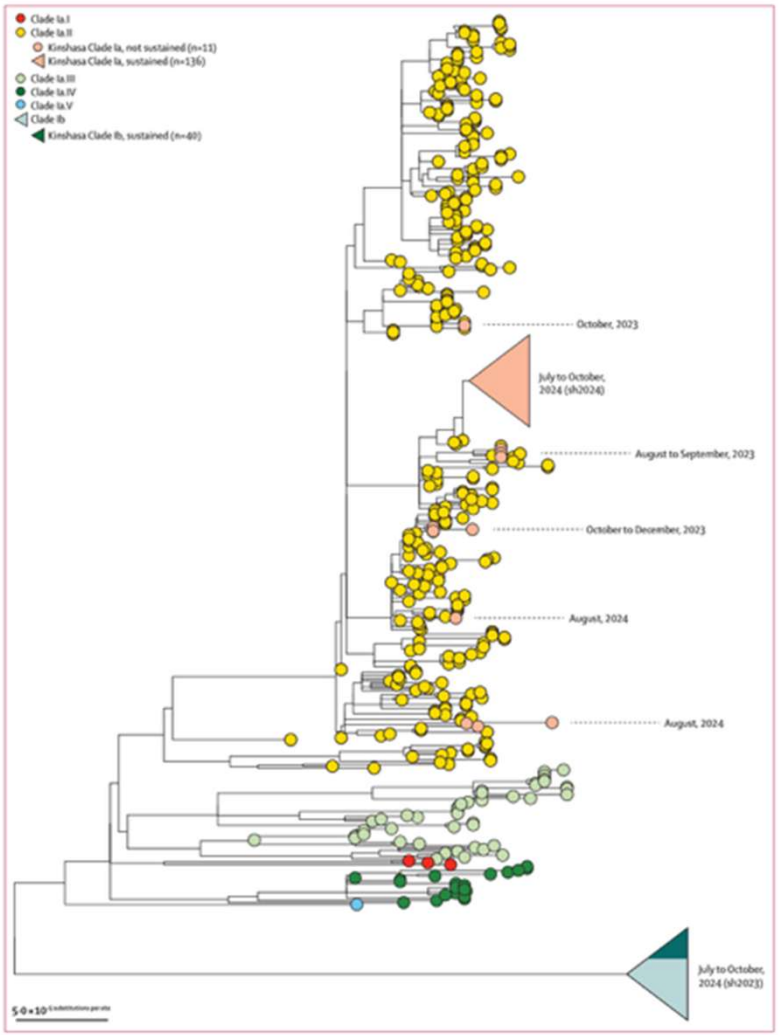


- Several outbreaks of MPXV are ongoing in DRC mainly by zoonotic transmission followed by limited human-to-human transmission
- Clade Ib (at the time) only detected in the provinces Nord and South-Kivu

Vakaniaki et al, Nature Medicine, 2024

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Epidemiology in the Kinshasa



- Recently also a cluster of mpox Clade Ib cases was detected in Kishasa, DRC
- However, also a cluster of cases with mpox Clade Ia infection with sustained human-to-human transmission was identified

https://virological.org/t/apobec3-deaminase-editing-supports-human-to-human-transmission-in-escalating-mpox-outbreaks-of-both-clade-ia-and-ib-in-kinshasa-democratic-republic-of-the-congo-july-september-2024/982



Epidemiology in the Kinshasa

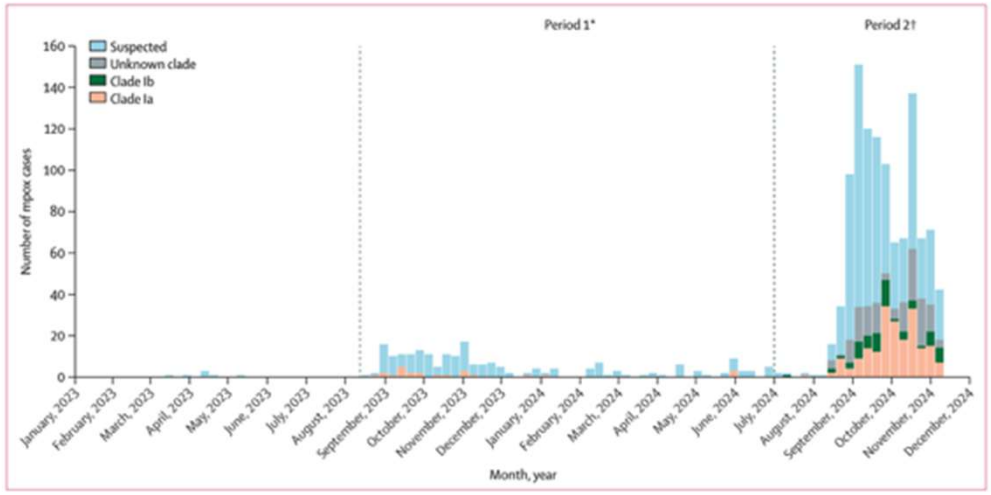


Figure 1: **Epidemiological curve of weekly reported, suspected, and PCR confirmed mpox cases (n=440) in Kinshasa from January, 2023, to October, 2024**
*Period 1 occurred from August, 2023, to June, 2024, with continuous low-grade clade Ia transmission. †Period 2 occurred from July to October, 2024, with established cocirculation of clades Ia and Ib.

Epidemiology and phylogenomic characterisation of two distinct mpox outbreaks in Kinshasa, DR Congo, involving a new subclade Ia lineage: a retrospective, observational study

Tony Wawina-Bokalanga, Sydney Merritt*, Eddy Kinganda-Lusamaki*, Daan Jansen*, Megan Halbrook, Áine O'Toole, Elisabeth Pukuta-Simbu, Emmanuel Hasivirwe Vakaniaki, Rilia Ola-Mpumbe, Papy Kwete-Mbokama, Prince Akil-Bandali, Cris Kacita, Ange Ponga-Museme, Nelson Mapenzi-Kashali, Adrienne Amuri-Aziza, Olivier Tshiani-Mbaya, Princesse Paku-Tshambu, Pedro H L F Dantas, Tessa De Block, Emmanuel Lokilo-Lofika, Chloé Muswamba-Kayembe, Jean-Claude Makangara-Cigolo, Gradi Luakanda-Ndelema, David J Kelvin, Catherine Pratt, Ahidjo Ayouba, Sofonias Tessema, Antonio Mauro Rezende, Lisa E Hensley, Eric Delaporte, Dieudonné Mwamba, Lorenzo Subissi, Laurens Liesenborghs, Nicole A Hoff, Martine Peeters, Nicola Low, Steve Ahuka-Mundeke, Jean-Jacques Muyembe-Tamfum, Anne W Rimoin†, Jason Kindrachuk†, Koen Vercauteren†, Andrew Rambaut†, Placide Mbala-Kingebeni†*

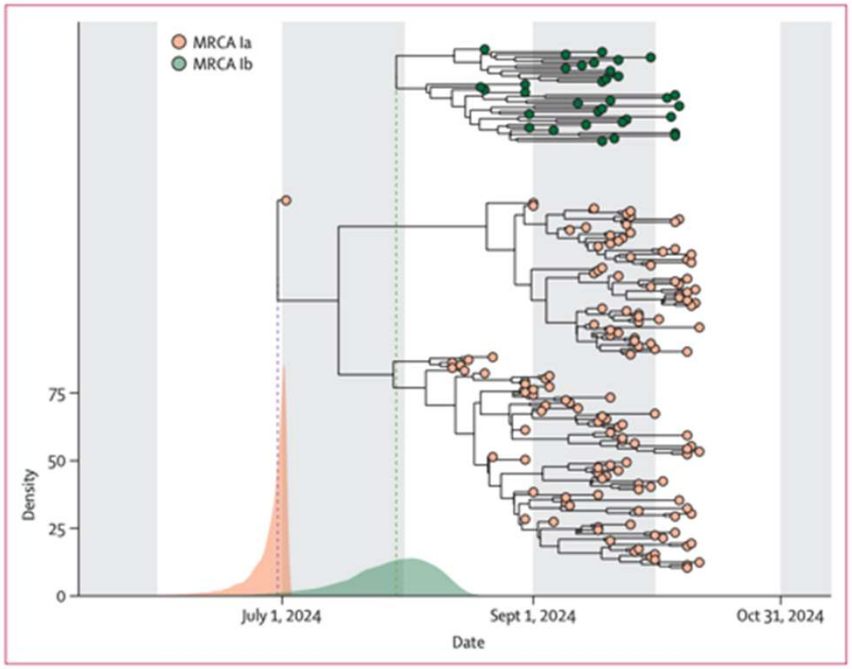
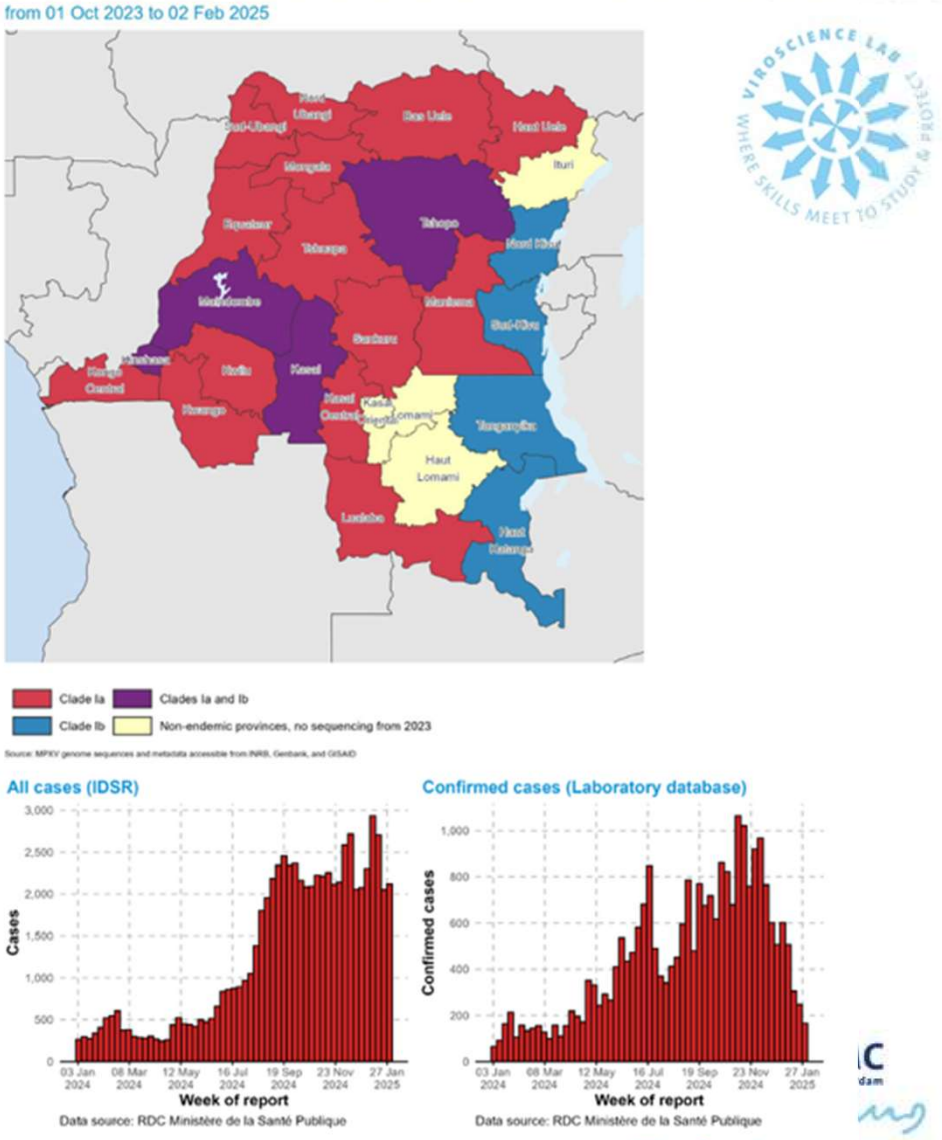


Figure 5: **Maximum clade credibility time trees estimated for Kinshasa subclade Ia and Ib outbreaks**
Based on the exponential growth model run in BEASTX, the timing of the emergence of subclade Ia in Kinshasa is slightly earlier (median date June 29, 2024 [95% highest probability density June 17, 2024 to July 2, 2024]) than that of Ib (median date June 29, 2024 [July 7, 2024, to Aug 15, 2024]), shown as the MRCA. MRCA=most recent common ancestor.

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Current situation in the Eastern African region

- Co-circulation of different clades of mpox virus
- More cases are suspected but laboratory confirmation is difficult (also since there is an armed conflict ongoing now)
- Clade Ib has also spread to Burundi (3359 cases) and Uganda (2479 cases) causing big outbreaks

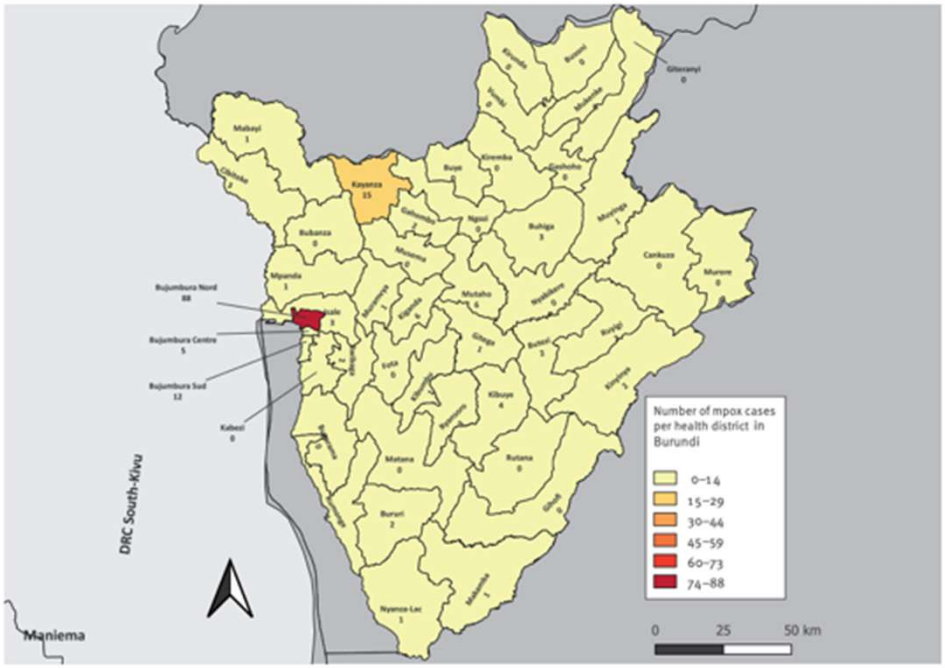


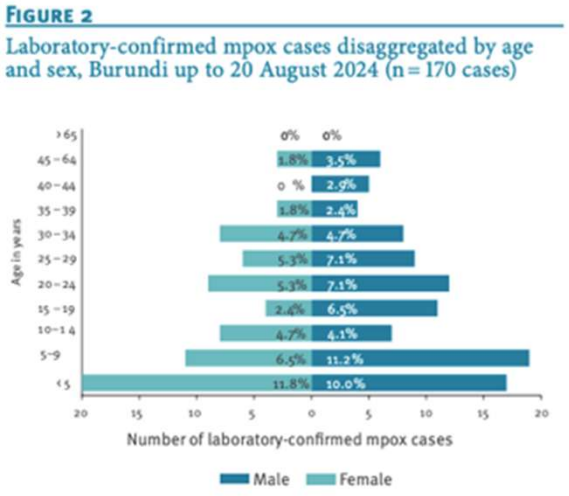
https://www.who.int/publications/m/item/multi-country-outbreak-of-mpox--external-situation-report--47---13-february-2025

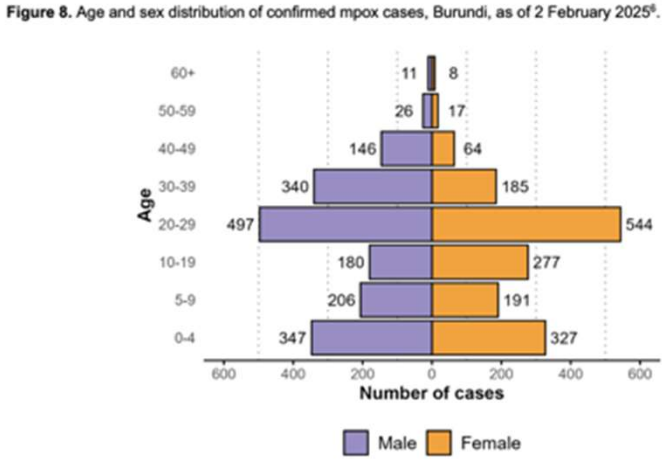
Epidemiology in Burundi

- Mainly in the East of the country
  - Also most densily populated
  - Close to South Kivu

- Intialy, mainly younger children affected







Nzoyikorera et al, Eurosurveillance 2024

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Phylogeny

- 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



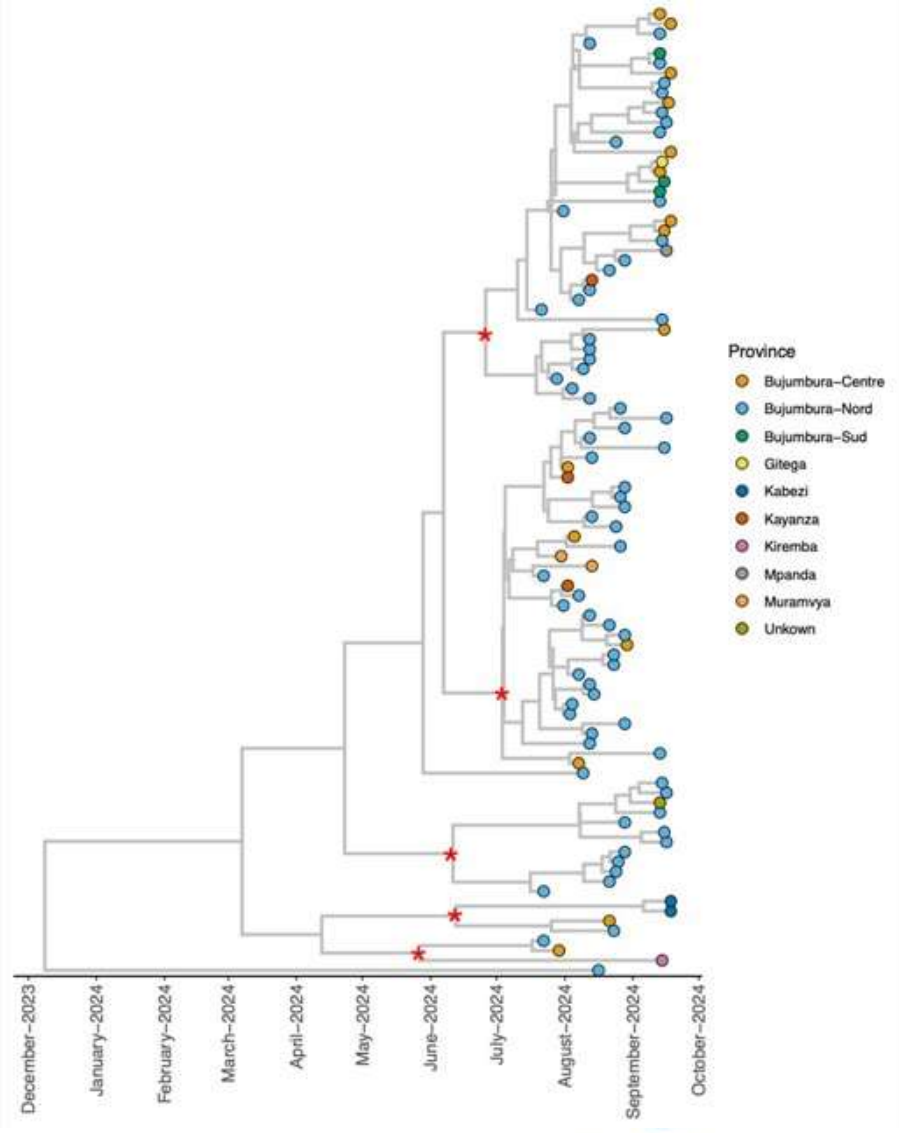
Nzoyikorera et al, unpublished data

Current situation in Burundi

- Assuming the virus was detected fast after the introduction several 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.



Nzoyikorera et al, revisions stage

Case fatality rate is lower than anticipated before

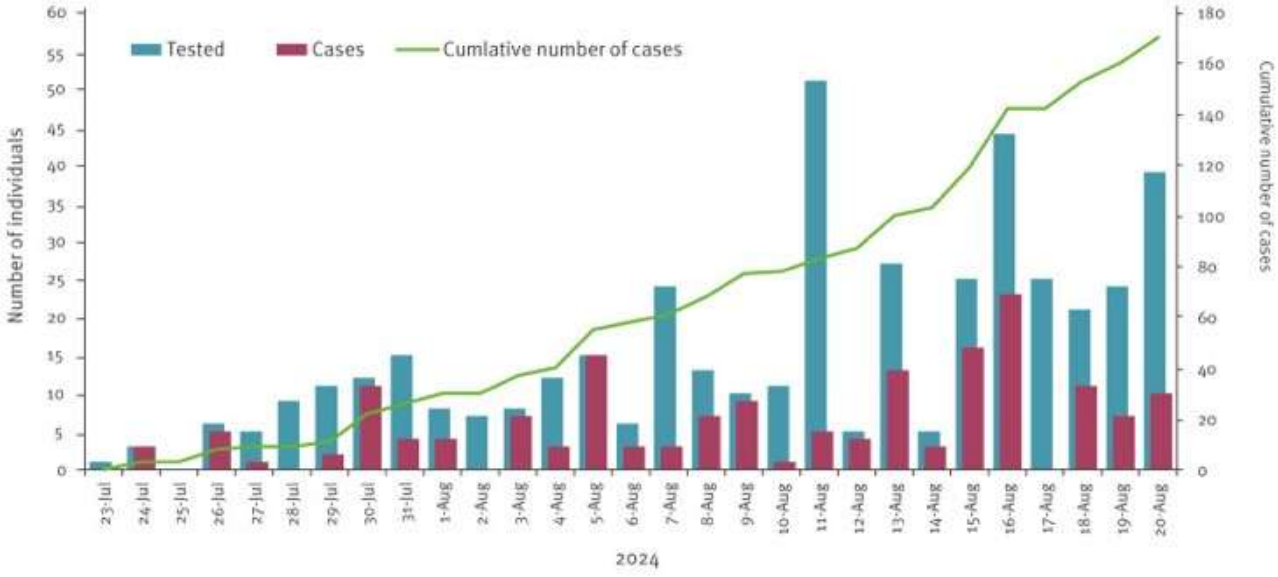
- Published estimates of the case fatality rate (CFR) for clade Ib are 1%, 1.4% and 0.5%
- These studies were carried out in individuals hospitalized so may overestimate the overall CFR.
- Syndromic surveillance data suggest an overall CFR of 0.3% in South and North Kivu
- Mild cases are more likely to be missed which may result in overestimation of the CFR.
- Furthermore, one mpox death has so far been confirmed in Burundi amongst over 2600 cases, and six deaths have been confirmed in Uganda amongst over 1000 cases.
- These data suggest that the CFR for clade Ib is lower than that of clade Ia and closer to that of clade IIb.

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https://cdn.who.int/media/docs/default-source/documents/epp/tag-ve/mpxv_clade_ib_risk_evaluation.pdf?sfvrsn=f33f1522_3&download=true

Suspected cases which were not tested / differential diagnosis

- Diagnostic capicity is limited and most of the times done by eye
- 30 samples suspected of MPXV were used for differential diagnosis:
  - 20 samples were positive for varicella zoster virus (chickenpox)
  - 4 samples were positive for herpes simplex virus 1 or 2



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Nzoyikorera et al, Eurosurveillance, 2024

Potential role of co-infections (proof of concept)

- The presence of microorganisms and their potential role in co- and secondary infections in mpox lesions is poorly studied
- Pox lesions could be more prone to bacterial colonization and infection depending on the clinical context, microbial burden and host immune response
- Such co-infections could contribute to a higher morbidity or delayed recovery
- 20 samples were sequenced using metagenomic sequencing (at the EMC) in an attempt to detect both bacteria and viruses

Bacterial content (proof of concept)

- *Enterococcus faecalis* (3 samples), *Shewanella seohaensis* (2 samples), Klebsiella sp. (2 samples)

- **A total of 45 different antimicrobial resistance genes (ARGs) were identified in the 10 samples (between 2 to 17)**, with a breadth of coverage above 90%, depth of coverage above 3.0x and at least half the depth of the species detected in the corresponding sample.

- The antimicrobial treatment provided to the patients involved in this study was in all cases ceftriaxone, cloxacillin, and metronidazole and among our patients we would only expect ceftriaxone or cloxacillin to be effective against the bacteria identified in patients 7, 9 and 10.

Sample	Bacteria	ARGs
1	*Enterococcus faecalis, Shewanella seohaensis*	blaOXA-436*, blaCARB-2, catA2*
4	Shewanella spp. (possibly seohaensis) / Pseudomonas spp. (possibly sediminis)	blaOXA-436*, blaCARB-2, catA2*, sul1*, tet(M)*
8	Klebsiella spp. (possibly michiganensis or oxytoca)	aph(3')-Ia*, blaOXY-1-4*, OqxB*
9	Gardnerella spp. (possibly piotii)	aph(3')-Ia, OqxB*
5	*Enterococcus faecalis, Staphylococcus sciuri, Macrococcus caseolyticus*	aac(6')-aph(2'')*, ant(6)-Ia, mecA, erm(B)*, sal(A)*, fexA, tet(K)*, tet(S)*, dfrE
10	*Staphylococcus aureus* (main), *Streptococcus dysgalactiae* and *Streptococcus pyogenes*	blaZ*, erm(C), tet(M), tet(K), dfrG
7	*Enterococcus faecalis, Staphylococcus haemolyticus*	aac(6')-aph(2''), ant(6)-Ia*, aph(3')-III, erm(B), erm(C), lsa(A)*, cat*, cat(pC221)*, tet(M), tet(K)*, dfrG
17	*Elizabethkingia meningoseptica*	blaGOB-17, blaB-12
14	*Klebsiella pneumoniae* and Serratia sp.	aph(3')-Ia, aac(6')-Ib-cr*, aac(3)-IIa, blaSRT-2*, blaOXA-1*, blaTEM-1C, blaZ_78*, blaCTX-M-15, fosA6*, mef(A)_2*, catB3*, catA1*, qnrS1, OqxB*, sul1*, sul2*, dfrA15
12	*Elizabethkingia meningoseptica*	aac(6')-Ij*, blaOXA-420, blaGOB-17, blaB-12, tet(39)

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Schuele et al, Micrbiol Spectr. 2025

Viral co-infections

# ID	Health zone	Gender	Age	Ct MPXV	MPXV coverage (> 95%)	Viral detections	Bacteria linked to SSTI
1	Mwenga	Male	20	25.9	98.5%	MPXV, TTV	**Yes**
2	Nyangezi	Male	22	16.7	100%	MPXV, TTV	
4	Miti Murhesa	Female	18	ND	-	TTV	**Yes**
5	Miti Murhesa	Female	30	14.3	100%	MPXV, TTV	**Yes**
6	Kadutu	Male	28	16.3	100%	MPXV, TTV	
7	Nyangezi	Female	8	17.0	100%	MPXV, TTV	**Yes**
8	Miti Muresha	Male	15	11.1	100%	MPXV, TTV	**Yes**
9	Miti Murhesa	Female	6	22.5	100%	MPXV, TTV, **VZV** (Ct 31.9), HPV-175, HBoV-1	**Yes**
10	Miti Murhesa	Female	11	ND	-	TTV, **VZV** (Ct 29.5)	**Yes**
11	Kamituga	Female	19	17.4	99.9%	TTV	
12	Kamituga	Female	25	17.7	99.8%	TTV	**Yes**
13	Kamituga	Female	22	16.6	99.8%	TTV	
14	Kamituga	Male	39	32.8	-	TTV	**Yes**
15	Kamituga	Male	35	16.6	99.9%	TTV	
16	Kamituga	Female	20	15.8	100.0%	TTV	
17	Kamituga	Male	40	27.2	98.5%	TTV	**Yes**
18	Kamituga	Male	28	16.5	100.0%	TTV	
19	Kamituga	Female	23	17.5	99.9%	TTV	
20	Kamituga	Male	27	14.9	100.0%	TTV, **HSV2** (Ct 27.0)	

In line with the findings from Burundi where differential diagnosis found that 20 of the 30 mpox suspected but negative tested samples tested positive for VZV and 4 for HSV-1,2

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Schuele et al, Micrbiol Spectr. 2025 **Nzoyikorera et al, Eurosurveillance 2024**

Second training on site training session (2 weeks hands-on)



- Training in serology (results pending)
- 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





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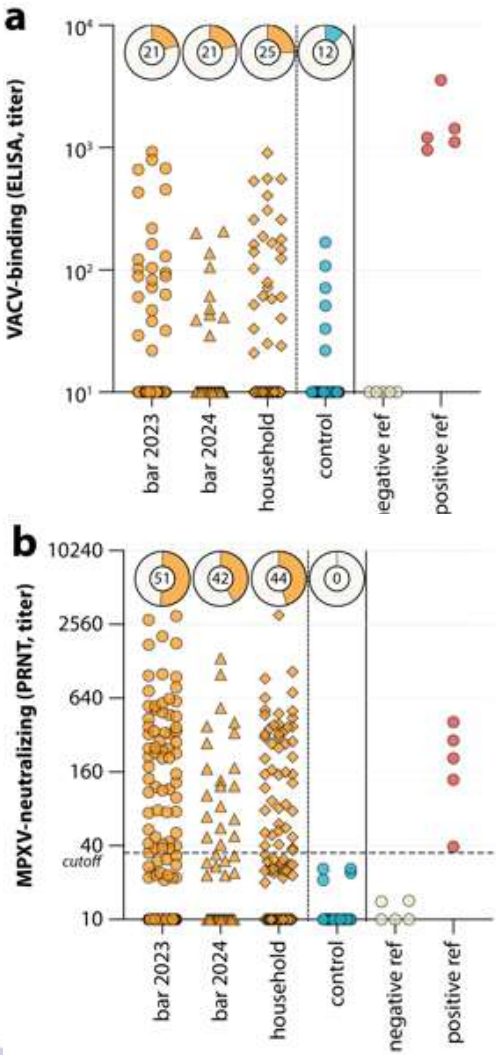
Cohort description

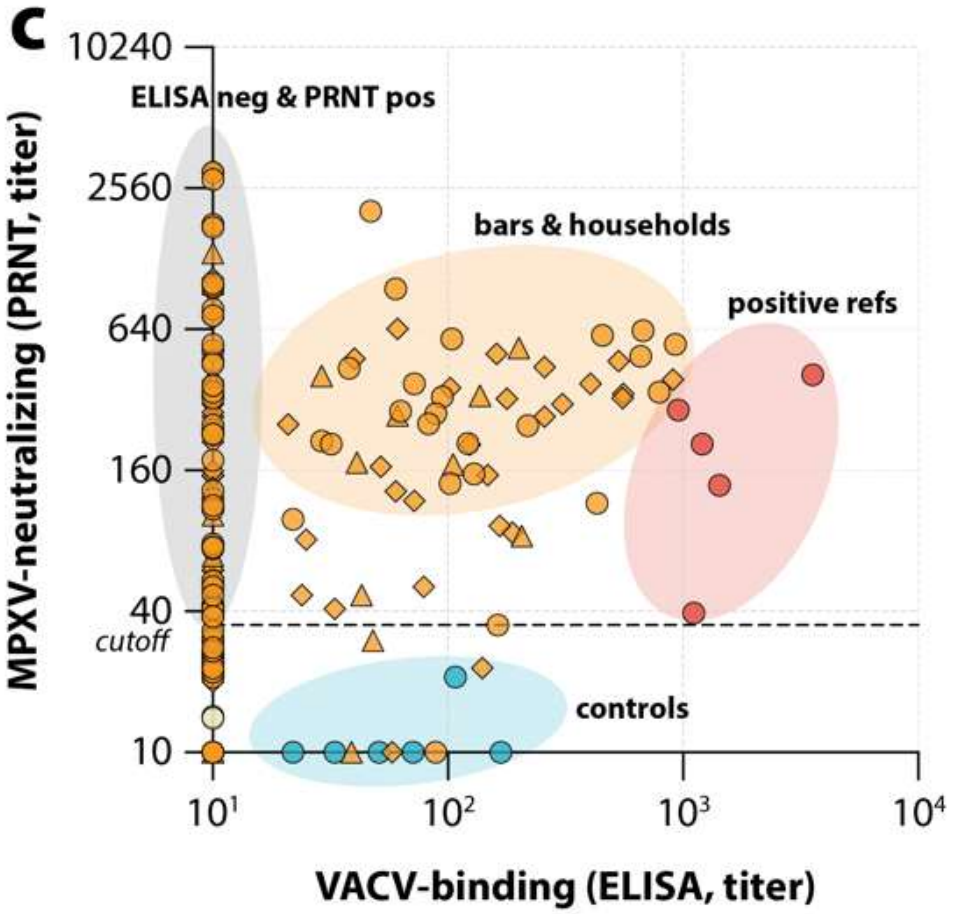
	Sera n	Female n (%)	Male n (%)	Age (median, range)	HIV+ n (%)
Potentially exposed	276	202 (73)	74 (27)	22 (1-74)	21 (8)
Bar (Nov/Dec 2023)	120	96 (80)	24 (20)	23 (17-48)	7 (6)
Bar (May 2024)	48	29 (60)	19 (40)	22 (15-74)	10 (21)
Household	108	77 (71)	31 (29)	20 (1-48)	4 (4)
Pre-outbreak	51	29 (57)	22 (43)	44 (15-87)	6 (12)

- Bar cohorts: samples collected in Nov/Dec 2023 and in May 2024 after identificiation of index cases in September 2023
- Household cohort: samples collected between September 2023 and May 2024
- Index cases not included

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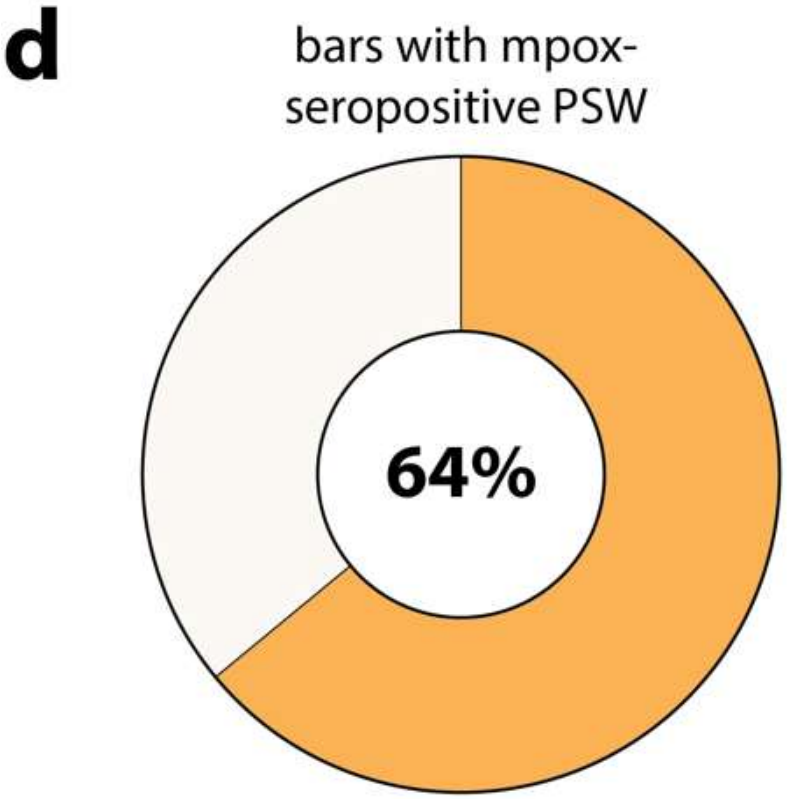
ELISA in the field versus PRNT

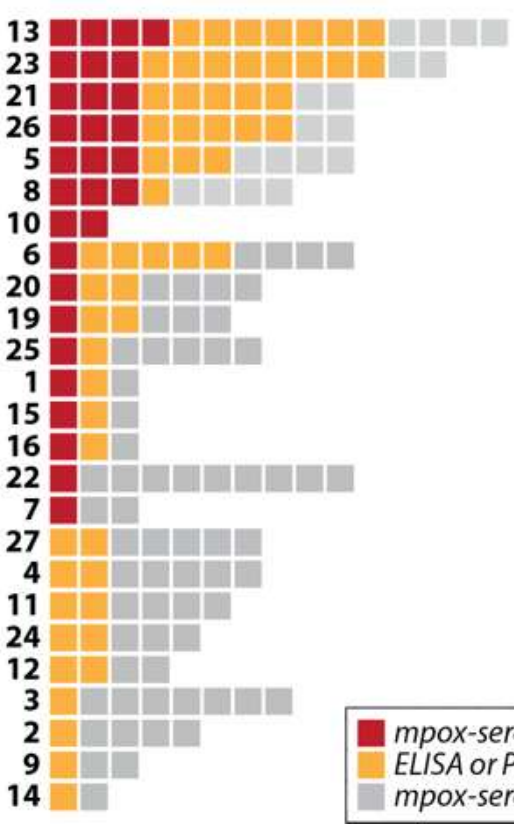




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Mpox seropositivity among professional sex workers

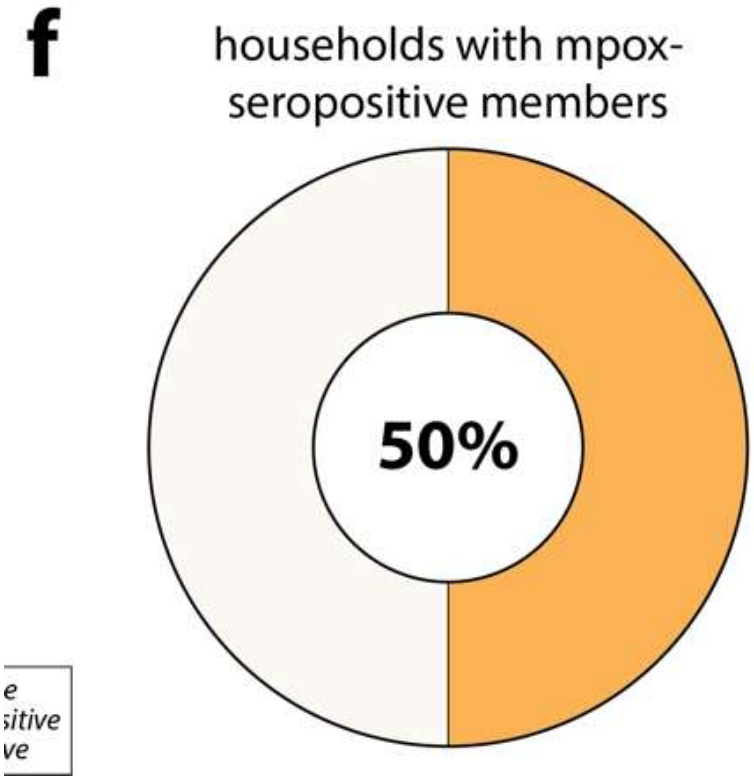


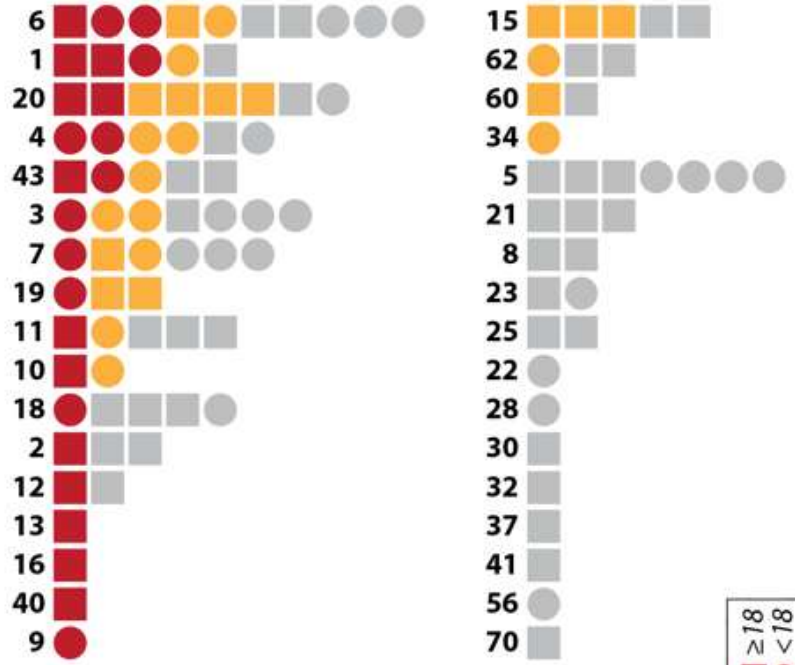


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Non-sexual close-contact transmission





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Discussion on serology

- Good concordance of data from the VACV ELISA but suboptimal sensitivity **which can be performed locally**
- About 1 in 5 high-risk individuals had MPXV-specific antibodies
- Professional sex workers played an important role in the spread of clade Ib MPXV
- Evidence for non-sexual close-contact transmission within half of all tested households with an index case

Discussion

- 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

- CFR / disease severity is difficult to assess due also due to potential coinfections with HIV, VZV, HSV, bacteria etc

- Comparing genomes is can be complex since the assembly is very complicated
  - Sharing raw data is essential

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

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sequencing infectious diseases – African Great Lakes Region
GREAT-LIFE

JUA KIVU
MPOX OUTBREAK RESEARCH

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