WET LAB DAY 1: INTRODUCTION TO MPOX GENOMICS

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CENTRE FOR PATHOGEN GENOMICS

World Health Organization
Western Pacific Region

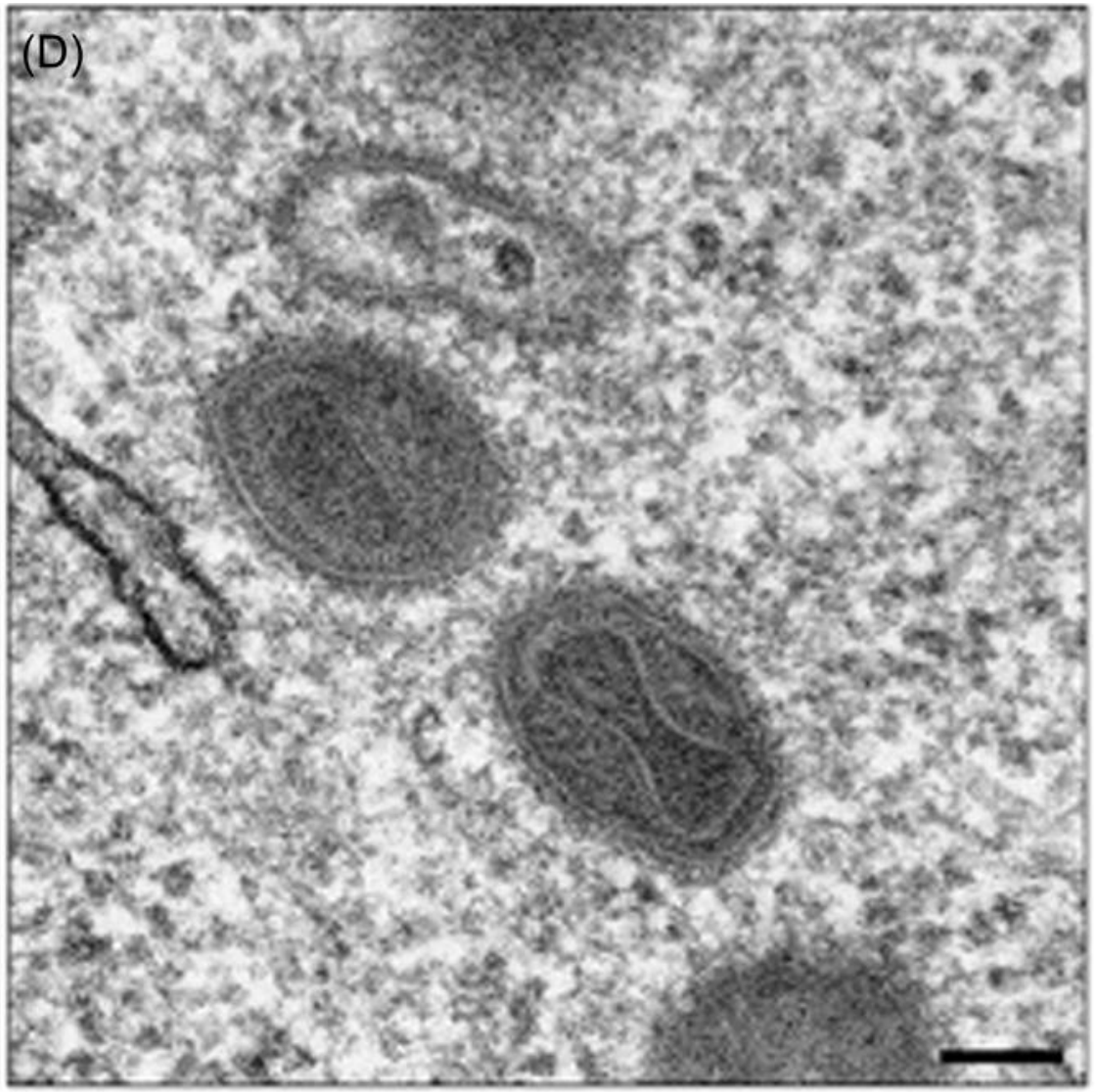
IPSN International Pathogen Surveillance Network
WHO hosted network

Wet Lab schedule DAY 1

MONDAY	ACTIVITY	PRESENTER
8:45 – 9.00	Registration	Lisa
9.00 – 9.15	Overview of the Doherty Institute/CPG/VIDRL/MDU	Lisa
9.15 – 9:45	Welcome and introductions	
9:45 –10.00	Training overview	Jean
10.00– 10.30	LAB: Pipetting exercise	Louise
10.30 – 11.00	Morning tea	
11.00 – 11.30	**LECTURE: INTRODUCTION TO MPXV GENOMICS AND VIDRL**	**Jean**
11.30 – 12.30	LECTURE: Tiled amplicon MPXV/viral theory	Jean
12.30 – 13.30	Lunch	
13.30 – 15.30	LAB: Tiled amplicon PCR	Louise
15.30 – 16:00	Afternoon tea	
16:00 – 16.30	LECTURE: Introduction to ONT sequencing viruses	Louise
16.30 – 17:00	Group discussion: Opportunity for Q&A and further discussion	Nicole

MPXV

MPXV has a large genome (~200 kb)



Transmission electron micrograph provided by Dr. Jason Roberts (VIDRL)

Multiple intracellular mature MPXV particles can be seen with clearly defined dumbbell-shaped core and striated palisade layer between inner and outer membranes.

Scale bar = 100 nm

Lim CK, Roberts J, Moso M, et al. Mpox diagnostics: review of current and emerging technologies. J Med Virol. 2023;95:e28429. doi:10.1002/jmv.28429

NGS and Public Health

Understand the virus circulating in the population

Confirmation of the virus

Understand origin of infection

Source of community outbreaks

Evaluating Diagnostics/vaccines/therapies

MPXV Publications - VIDRL

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REVIEW

JOURNAL OF MEDICAL VIROLOGY WILEY

Mpox diagnostics: Review of current and emerging technologies

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Abstract

Mpox is a zoonotic disease caused by monkeypox virus (MPXV) from the *Orthopoxvirus* genus. Unprecedented transmission events have led to more than 70 000 cases reported worldwide by October 2022. The change in mpox epidemiology has raised concerns of its ability to establish endemicity beyond its traditional geographical locations. In this review, we discuss the current understanding of mpox virology and viral dynamics that are relevant to mpox diagnostics. A synopsis of the traditional and emerging laboratory technologies useful for MPXV detection and in guiding "elimination" strategies is outlined in this review. Importantly, development in MPXV genomics has rapidly advanced our understanding of the role of viral evolution and adaptation in the current outbreak.

KEYWORDS
diagnostics, genomics, monkeypox, mpox, orthopoxvirus, serology

ELSEVIER

The Lancet Microbe
Available online 7 October 2024, 100999
In Press, Corrected Proof ⓘ What's this?

Comment

Mpox genomics in outbreak control: challenges and limitations

Kathryn Edenborough [a b #], Ammar Aziz [a b #], Nicola Sexton-Oates [a b], Ivana Savic [a b], Eike Steinig [a b], Brendan Quinn [c], Mihaela Ivan [c], Alicia Arnott [a b], Leon Caly [a b ✉], Chuan Kok Lim [a b ✉]

JOURNAL OF MEDICAL VIROLOGY

RESEARCH ARTICLE | Open Access

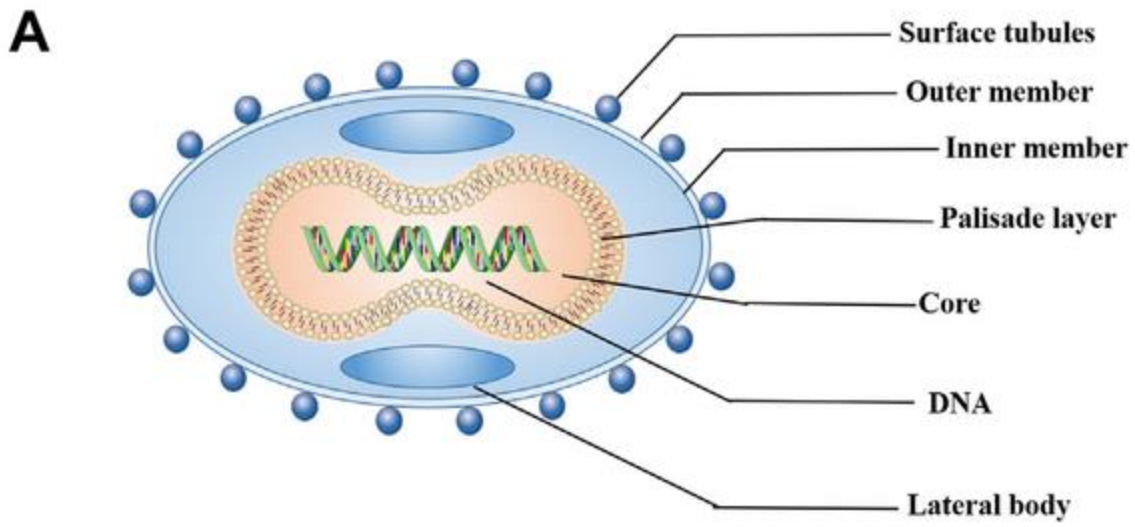
Intra- and interhost genomic diversity of monkeypox virus

Mona L. Taouk ✉, Eike Steinig, George Taiaroa, Ivana Savic, Thomas Tran, Nasra Higgins, Stephanie Tran, Alvin Lee, Maxwell Braddick, Michael A. Moso, Eric P. F. Chow, Christopher K. Fairley ... See all authors

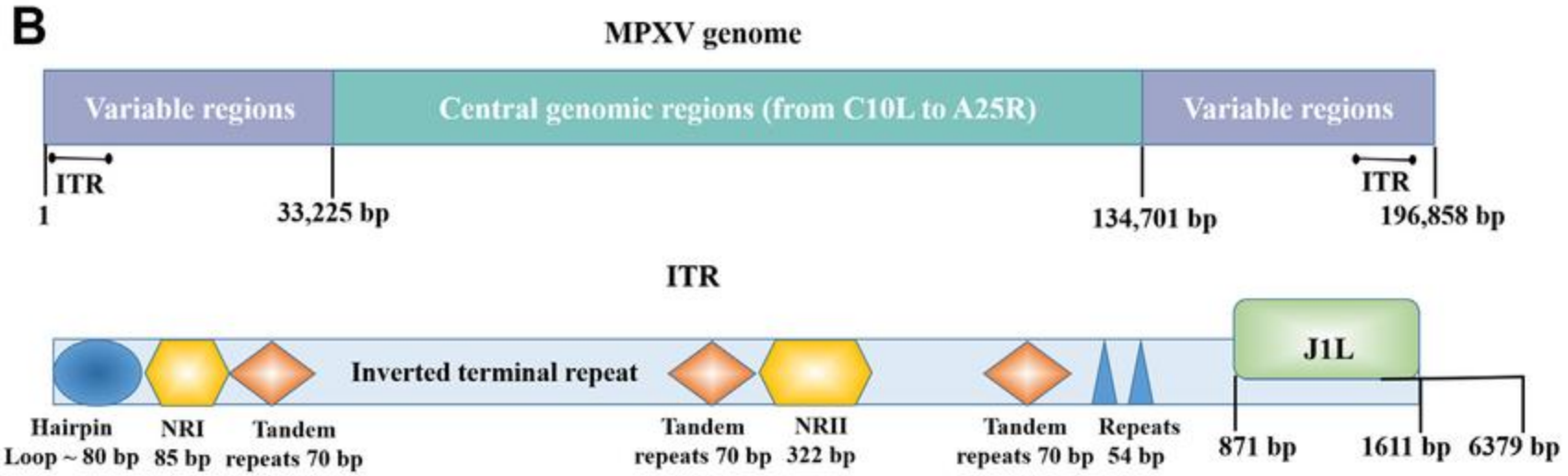
First published: 11 August 2023 | https://doi.org/10.1002/jmv.29029

Large double-stranded DNA virus

MPXV



MPXV has a large genome (~200 kb) with a covalently closed hairpin on both 5' and 3' ends with inverted tandem repeats (ITS).



The land-scape of immune response to monkeypox virus - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Structure-and-genome-of-monkeypox-virus-MPXV-A-MPXV-contains-five-distinct_fig1_366652147 [accessed 10 Jun 2025]

MXPV Clades

The current emergency represents a concurrent outbreak involving multiple MPXV clades.

including the emergence of a novel lineage (Clade Ib), concentrated in the Democratic Republic of the Congo with ongoing community transmission

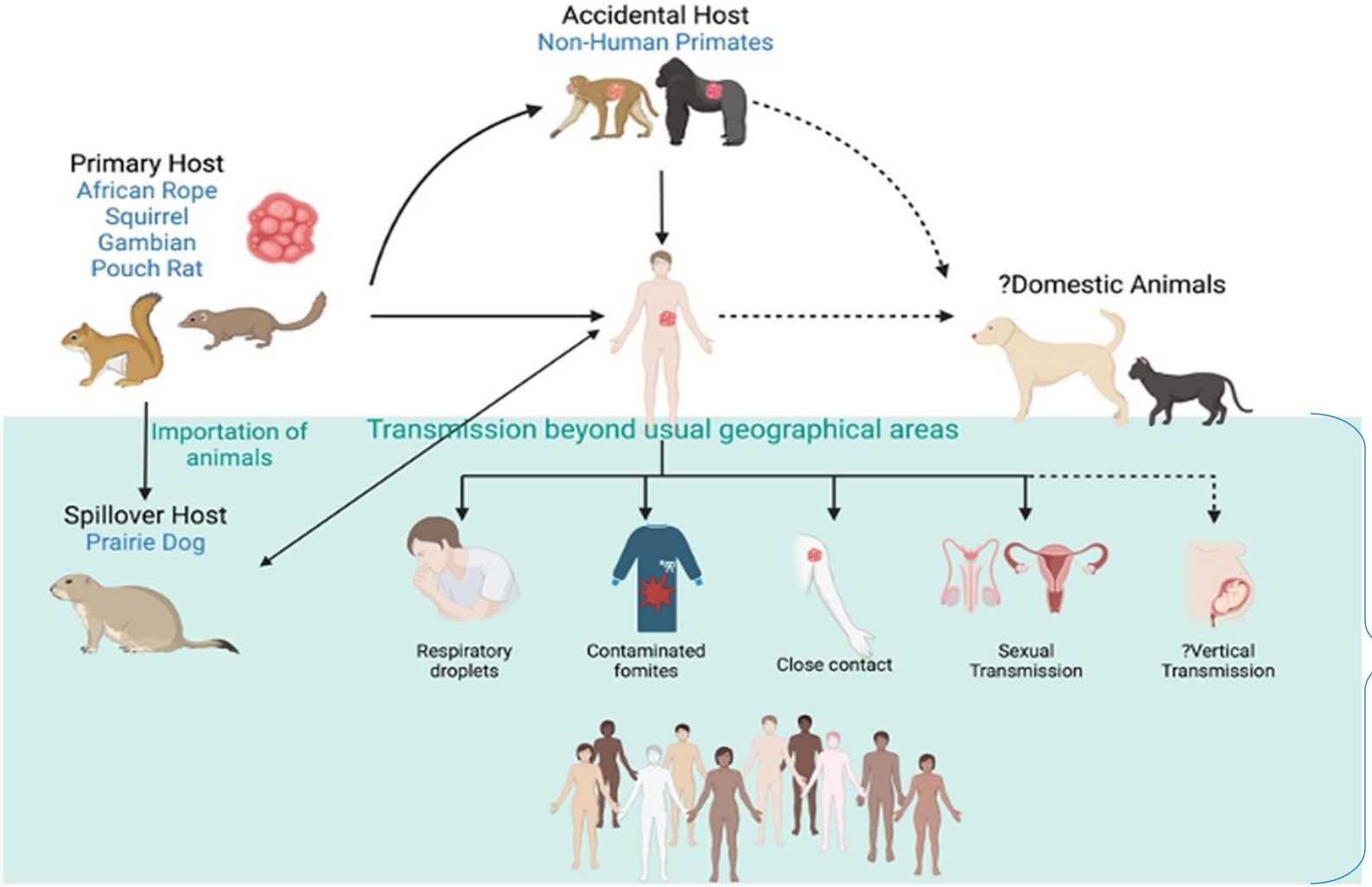
Two major clades, I and II, whose sub-clades (Ia, Ib, IIa, IIb) are circulating in distinct regions of Africa.

In 2022, cases of MPXV Clade IIb spread globally through

sustained human-to-human transmission within networks of men who have sex with men.

MXPV Transmission Routes & Hosts

Mpox is a viral zoonotic disease caused by monkeypox virus (MPXV).



Primary host: rodents endemic to the African continent

Spillover to humans or nonhuman primates through direct contact with lesions.

The most recent human-to-human transmissions in the 2022 & 2024 outbreak

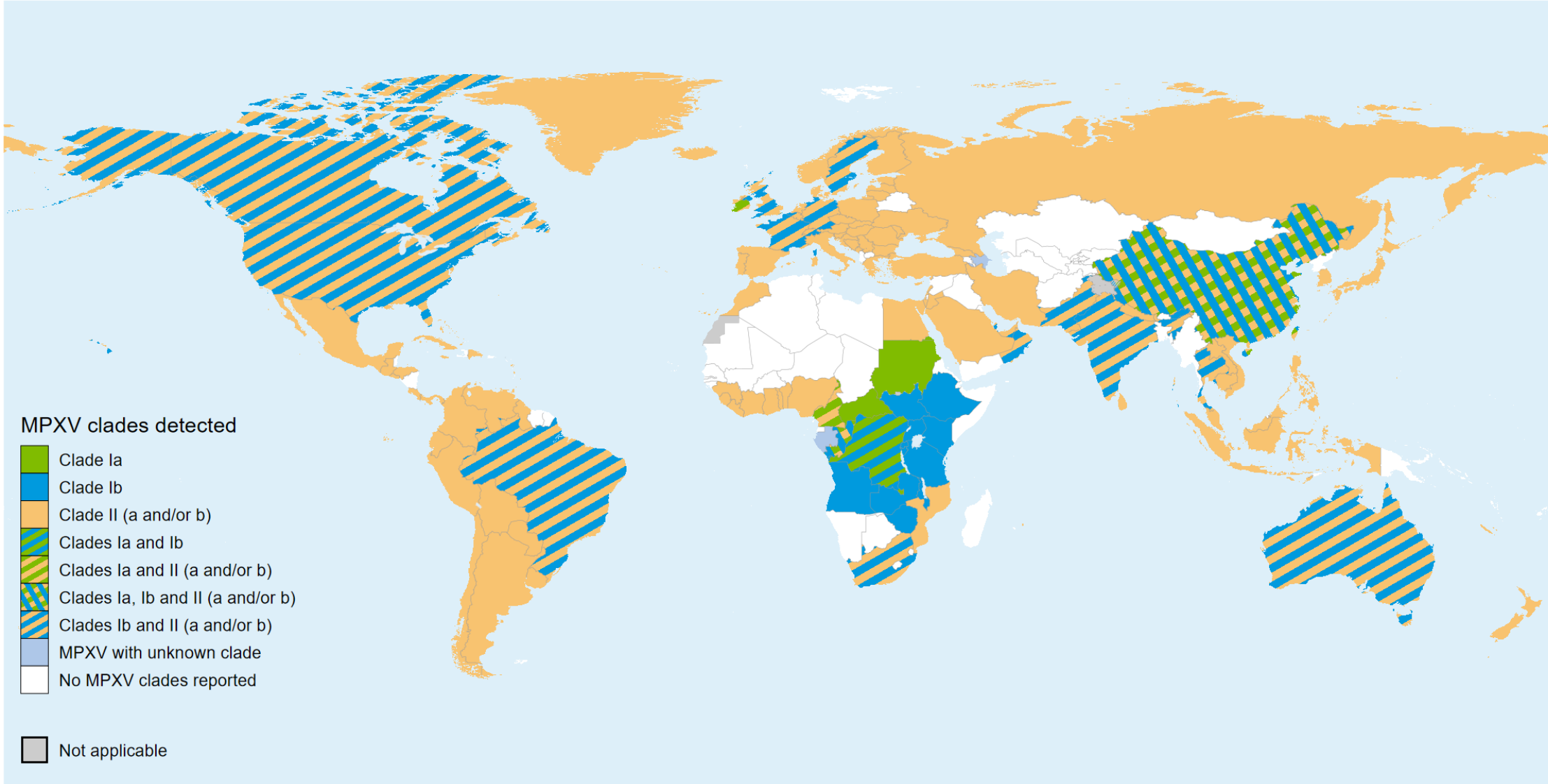
Journal of Medical Virology, Volume: 95, Issue: 1, First published: 26 December 2022, DOI: (10.1002/jmv.28429)

MPXV clades detected globally

Includes imported cases; known distribution from 1 January 2022 to 08 Jun 2025

World Health Organization

Royal Melbourne Hospital



The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of WHO concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: World Health Organization
Map Production: WHO Health Emergencies Programme

https://worldhealthorg.shinyapps.io/mpx_global/

Mpox

MPXV clade	Areas/populations affected	Overall risk level[3]
Clade Ib	Predominantly affecting non-endemic areas for mpox in the Democratic Republic of the Congo and neighbouring countries	High
Clade Ia	Primarily affecting endemic areas for mpox within the Democratic Republic of the Congo	Moderate[4]
Clade II	Observed in Nigeria and endemic countries in West and Central Africa	Moderate
Clade IIb	Associated with the global mpox epidemic first documented in 2022	Moderate

https://worldhealthorg.shinyapps.io/mpx_global/

Mpox and Australia

Australian Government
Department of Health, Disability and Ageing

Australian Centre for Disease Control

Home

First mpox clade Ib confirmed in Australia

The first case of mpox clade Ib in Australia is a timely reminder to at-risk population groups to get vaccinated. The risk of community transmission from this case is low. However, the Australian Government is monitoring the situation.

16 May 2025 News

Sporadic cases in Australia for Clade II, mostly associated with travel.

Confirmed and characterized through and from genomic surveillance, particularly in Europe, North America and Asia.

MXPV and NGS
Mutations and outbreaks

MPXV exhibits a lower mutation rate than RNA viruses like SARS-CoV-2, However, it has been observed that sustained human-to-human transition of MPXV lineages exhibited elevated mutation rates that have been attributed to the activity of APOBEC3 enzymes.

The apolipoprotein B mRNA-editing catalytic polypeptide-like 3 (APOBEC3) enzyme is important for MPXV evolution and potential human adaptation.

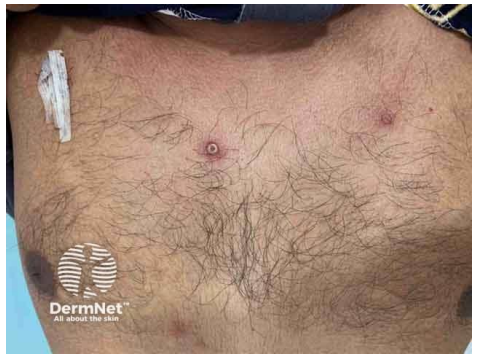
MXPV Genomics Summary
Clade I vs Clade II

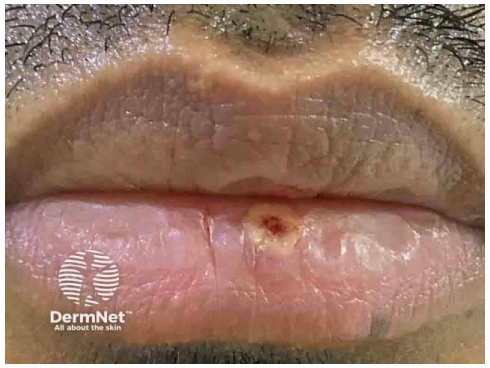
Feature	Characteristics
Genome size	Clade I: ~196 – 199 kb Clade II: ~196 – 211 kb
GC content	~33%
Number of protein-coding genes	~190 ORFs (variations due to clade-specific deletions or truncations)
Presence of ITRs	Both ends; ~6.5–17.5 kb each
Key clade makers for…	
Clade I	missing: E5R (homologue of VACV-Cop) truncated: K1R, homologues of VACV-Cop A47L and B11R
Clade Ib	missing: OPG032
Clade II	missing: D14L, D15L, D16L and D17L truncated: D4L, B14L and B15L
Clade IIa	missing: D2L additional: N3R, N2R, N1R and R1R (only in left ITR of Clade IIa)

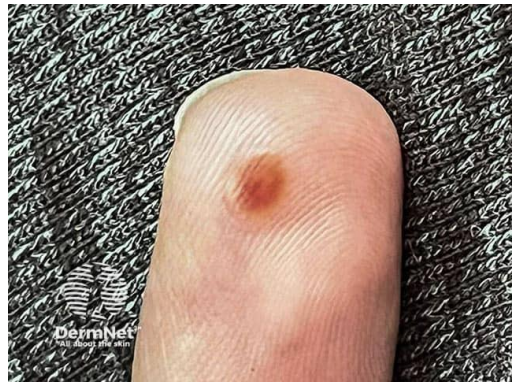
Mpox Symptoms
Victorian Department of Health

Symptoms of mpox may develop up to 21 days after contact with an infected person

Viral loads in skin lesion swabs are found to be generally higher than in oropharyngeal specimens.







Other symptoms that can occur before or alongside the rash, include:

- Fever, chills and aches
- swollen lymph nodes exhaustion
- headache
- sore throat
- anal and rectal pain
- pain during urination

Image source: DermNet <https://dermnetnz.org/images/mpox-images

Mpox and other STIs
Victorian Department of Health

PHLN
Public Health Laboratory Network

Presence of other STI pathogens is also very common in the 2022 outbreak and detection of an alternative pathogen should not be used to exclude Mpox infection

Symptoms can resemble sexually transmitted infections (STIs) like herpes or syphilis as well as other diseases with a rash such as measles or chickenpox.

Mpox and other STIs
Victorian Department of Health

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

Are you eligible for the mpox vaccine?

- Sexually active gay, bisexual and other men who have sex with men and their partners
- Sexually active transgender and gender diverse people and their partners
- Sex workers, particularly those whose clients are at risk of mpox exposure
- People living with HIV if at risk of mpox exposure, and their partners

Specimen Handling Mpox Samples

Diagnostic testing for MPXV from primary specimens should be conducted in a Physical Containment 2 (PC2) laboratory with appropriate PPE.

Culture of MPXV should only be performed at specialised reference laboratories under PC level 3 or 4.

Standard laboratory precautions should be taken when processing samples from patients with suspected mpox. Vaccination of laboratory personnel handling these specimens is not recommended, in line with international guidance

Laboratory Case Definition Mpox

CENTRE FOR PATHOGEN GENOMICS
Doherty Institute
THE UNIVERSITY OF MELBOURNE
The Royal Melbourne Hospital
A joint venture between The University of Melbourne and The Royal Melbourne Hospital

PHLN
Public Health Laboratory Network

Test Method – Nucleic acid

One kit on Australian Register of Therapeutic Goods (ARTG):

HA TECH Monkeypox Virus PCR Diagnostic Kit is a real time PCR test acid

In-house and commercial assays typically target the F3L, G2R or TNF receptor genes

If Orthopoxvirus DNA is detected, MPXV-specific NAA assays should be performed to confirm the diagnosis

MXPV and Humans
Direct Testing

Multisite studies confirmed that polymerase chain reaction (PCR) testing of skin lesions has the highest yield (clinical sensitivity 91%–100%).

Direct sequencing of clinical samples containing MXPV possible

PCR amplicon

Hybridisation-probe capture

Metagenomics sequencing

Sequencing Approaches Mpox

EXAMPLE	SEQUENCING APPROACH
Clinical samples with low viral load (Ct > 28)	Amplicon-based WGS OR Target capture
Detection of structural variants, ITR variation	Amplicon-based WGS or Metagenomic sequencing with long-read technology
Accurate SNP calling and clade typing	Amplicon-based WGS
Validation of amplicon dropout or suspected deletions	Metagenomic sequencing or Target capture
High-throughput routine surveillance	Amplicon-based WGS
Field deployment with minimal infrastructure	Amplicon-based WGS with long-read technology
Wastewater or environmental samples	Metagenomic sequencing or Target capture with short amplicons Possibly: amplicon-based sequencing with short amplicons
Resource-limited labs with basic capacity	Amplicon-based WGS

Viral Genome Sequencing
Mpox

Genome sequencing from clinical samples with sufficient viral load can assist in determining MPXV **clades, subclades and lineages.**

In addition, sequencing may help identify or rule out transmission chains, provide contextualisation for resolving new international incursions or local circulation

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Public Health Laboratory Network

Public Health Laboratory Network laboratory case definition documents for nationally notifiable diseases within Australia.

NGS at VIDRL

NGS provides **enhanced sensitivity** and increased diagnostic yield when compared to qPCR in addition to whole genome sequencing.

SARS: VOC and VOI monitoring case surge investigation

High and low throughput NGS solutions serves a critical role in genomic surveillance

Collaboration between scientists and policy makers enables evidence based public health policies both at the local and country level.

THANK YOU

centre-pathogen@unimelb.edu.au

CENTRE FOR PATHOGEN GENOMICS

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