South & Southeast Asia

Pathogen Genomics Prioritization & Implementation Workshop

September 9-13, 2024
Bangkok, Thailand

WORKSHOP PARTNERS

Arbovirus case study

Pathogen Prioritization Workshop Bangkok
9th- 13th September 2024

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WORKSHOP PARTNERS

Stillbirths linked to rare disease in Brazil raises fear of Zika re-run

Health ministry sounds alarm over spread of Oropouche after four cases of microcephaly found in newborns of mothers infected with virus

Verity Bowman *and* Maeve Cullinan, GLOBAL HEALTH SECURITY REPORTER

25 July 2024 • 12:37pm

2024 Chandipura virus outbreak is the largest in 20 years: WHO

This year's Chandipura virus outbreak is the largest in the past twenty years, with a 33% case fatality rate.

France, rest of Europe could see more local dengue outbreaks as world warms

Warmer rainy season triggers dengue spike across Indonesia

The Health Ministry recorded some 35,500 dengue cases nationwide from the beginning of January to March 18, with 290 deaths.

Nina A. Loasana
The Jakarta Post
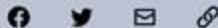
The Rising Concern of Malaria and Mosquito-Borne Diseases in the US

Jul 18, 2023
Benjamin Hottel, PhD, BCE

2 cases of rare mosquito-borne disease EEE detected in Alabama, including 1 death

Only one other U.S. case has been reported this year, according to the CDC.

By Mary Kekatos
August 23, 2023, 7:23 AM

Rare cases of mosquito- and tick-borne diseases, including Powassan and dengue, crop up across the U.S.

High temperatures may extend the tick and mosquito season into the fall, experts say.

Increasing threat of arboviruses to human health

- The pandemic potential of arboviruses, particularly in our region is increasing
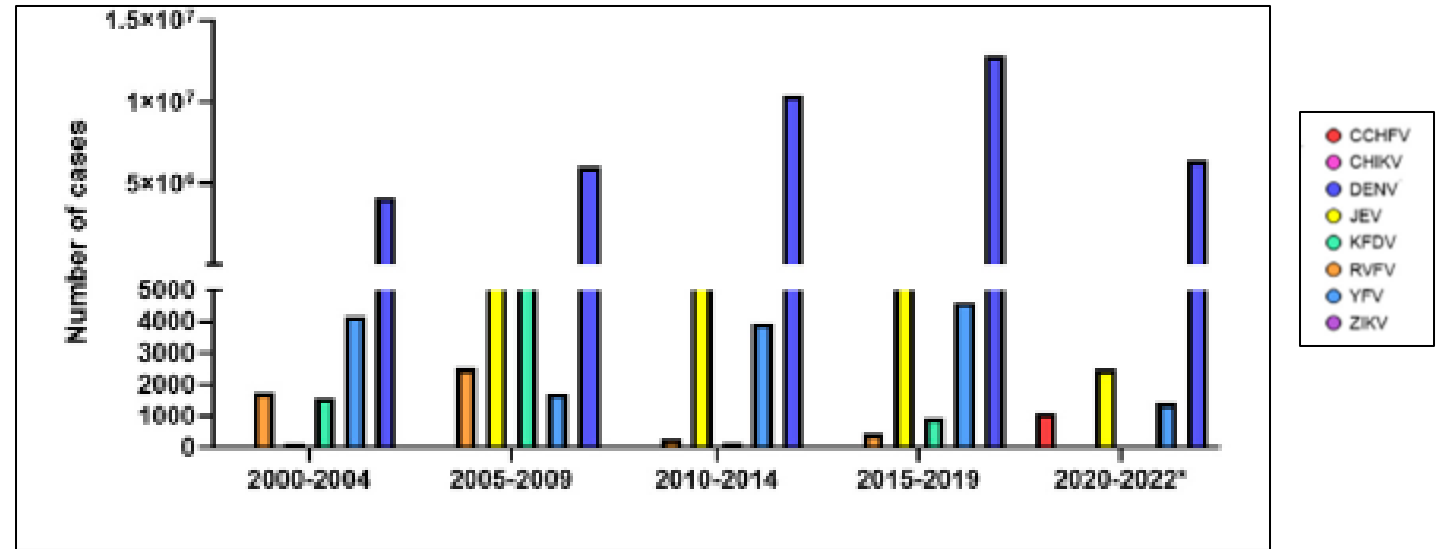
Rose-Nunes D et al 2023 Frontiers in Drug Discovery

Increasing threat of arboviruses to human health

- The pandemic potential of arboviruses, particularly in our region is increasing

- Driven by urbanization, climate change, human movement and adaptation of mosquitos are increasing the.

- Genomics can have a critical role in arbovirus surveillance

Table 2 | Non-climate drivers of the transmission and spread of vector-borne diseases

Globalization and environment	
Driver	Effect
Deforestation, mining and dams	Change vector and non-human host habitats
Ecosystem degradation/change	Changes vector and non-human host habitats
International travel and trade	Spreads pathogen and vector
Urbanization	Provides an ideal habitat for A. aegypti
Population displacement	Spreads pathogen to new locations or puts immunologically susceptible populations in contact with the vector and pathogen

Sociodemographic factors	
Driver	Effect
Population demographic composition	Children, the elderly and pregnant women may have elevated vulnerability
Level of economic development	Quality of housing (including presence of air conditioning) affects exposure to vectors
Baseline incidence of disease	Vulnerability to climate change may be highest at the margins of current endemic areas
Population health status	Low level of population health increases vulnerability
Humanitarian crises	

Rose-Nunes D et al 2023 Frontiers in Drug Discovery

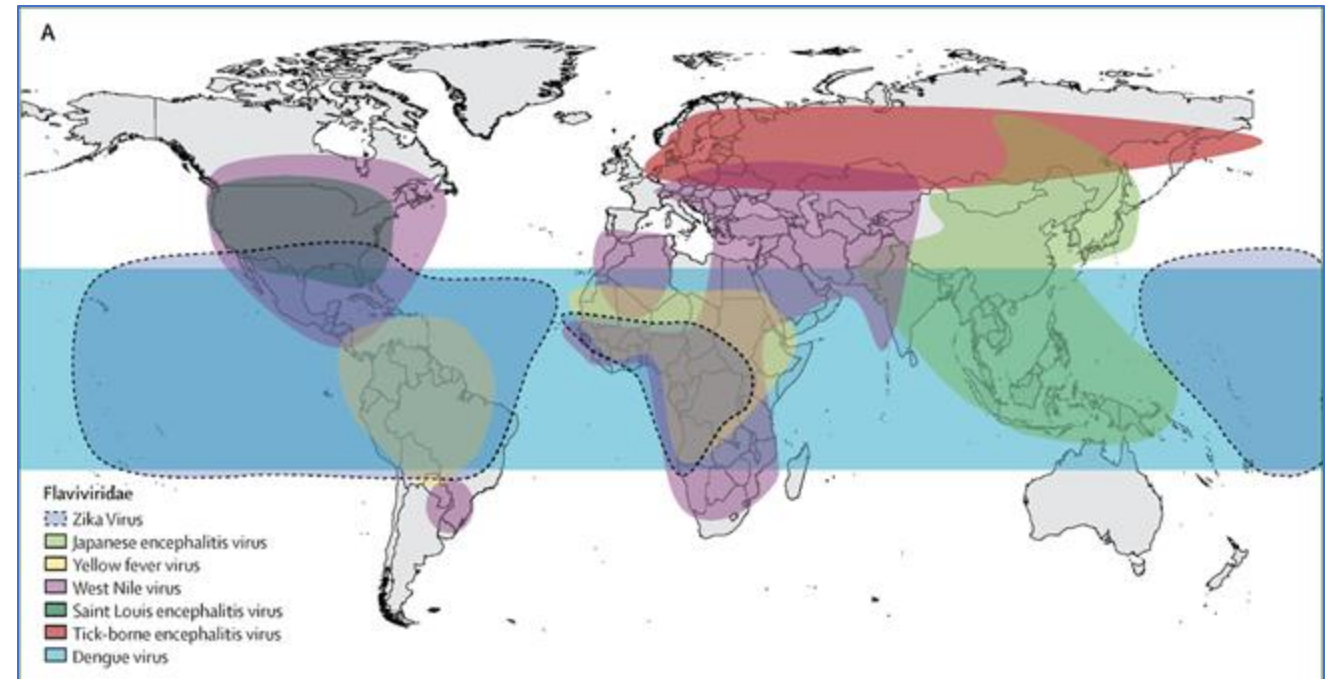*Rocklov J et al, Nature Immunology, 2020*

Arboviruses of public health significance

- Belong to three main viral families,
  - Flaviviruses
  - Alphaviruses
  - Bunyaviruses

- These viruses have different geographical distributions.

Global Spread of Flaviviruses

Flaviviridae
- Zika Virus
- Japanese encephalitis virus
- Yellow fever virus
- West Nile virus
- Saint Louis encephalitis virus
- Tick-borne encephalitis virus
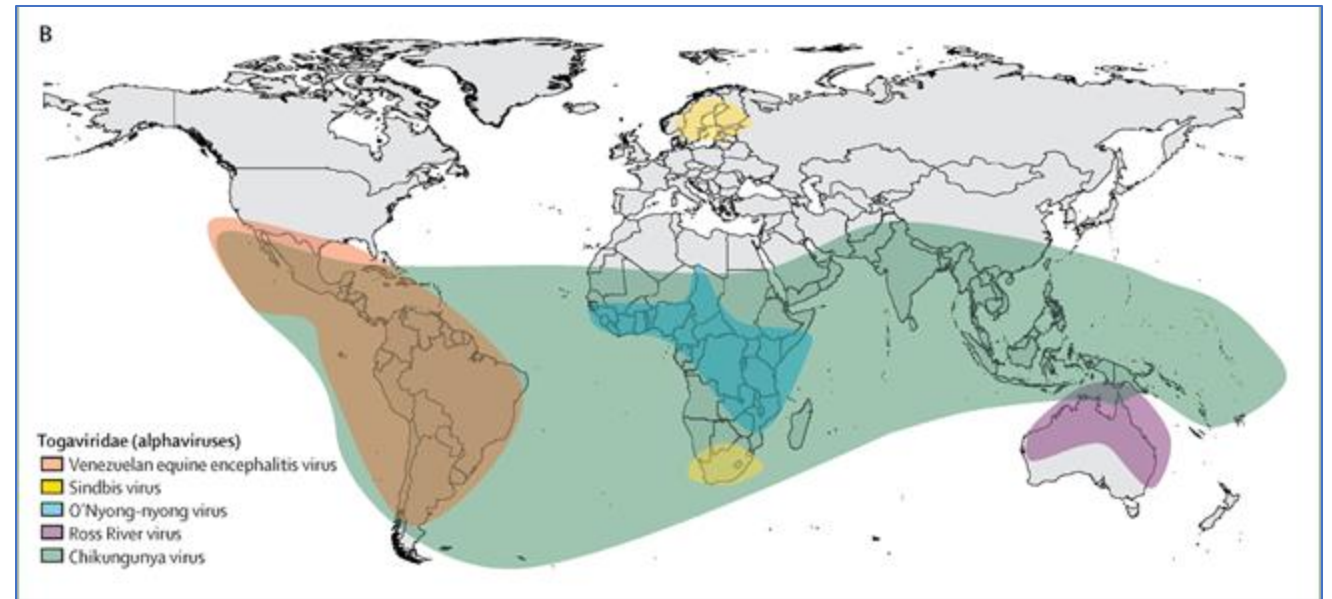- Dengue virus

Charlier, C et al 2017, Lancet Child & Adol

Arboviruses of public health significance

- Belong to three main viral families,
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  - Alphaviruses
  - Bunyaviruses

- These viruses have different geographical distributions.

Global Spread of Alphaviruses

Togaviridae (alphaviruses)
- Venezuelan equine encephalitis virus
- Sindbis virus
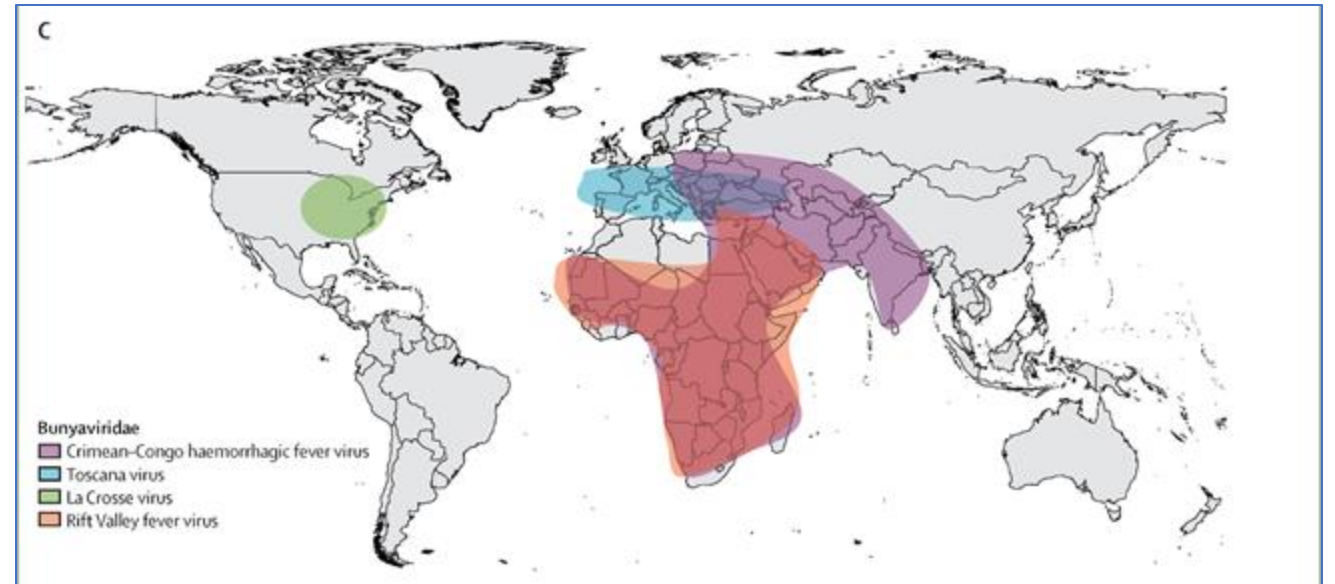- O'Nyong-nyong virus
- Ross River virus
- Chikungunya virus

Charlier, C et al 2017, Lancet Child & Adol

Arboviruses of public health significance

- Belong to three main viral families,
  - Flaviviruses
  - Alphaviruses
  - Bunyaviruses

- These viruses have different geographical distributions.

Global Spread of Bunyaviruses

Bunyaviridae
- Crimean–Congo haemorrhagic fever virus
- Toscana virus
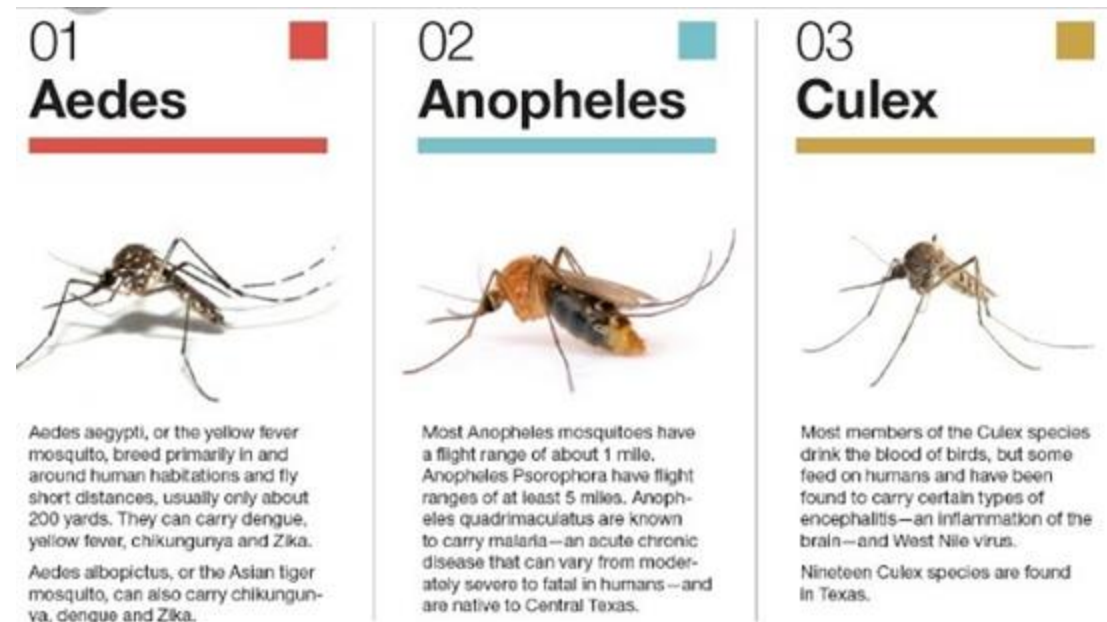- La Crosse virus
- Rift Valley fever virus

Charlier, C et al 2017, Lancet Child & Adol

Arboviruses of public health significance

- Belong to three main viral families,
  - Flaviviruses
  - Alphaviruses
  - Bunyaviruses
- These viruses have different geographical distributions.
- These viruses are spread by mosquito species.

01 **Aedes**

Aedes aegypti, or the yellow fever mosquito, breed primarily in and around human habitations and fly short distances, usually only about 200 yards. They can carry dengue, yellow fever, chikungunya and Zika.

Aedes albopictus, or the Asian tiger mosquito, can also carry chikungunya, dengue and Zika.

02 **Anopheles**

Most Anopheles mosquitoes have a flight range of about 1 mile. Anopheles Psorophora have flight ranges of at least 5 miles. Anopheles quadrimaculatus are known to carry malaria—an acute chronic disease that can vary from moderately severe to fatal in humans—and are native to Central Texas.

03 **Culex**

Most members of the Culex species drink the blood of birds, but some feed on humans and have been found to carry certain types of encephalitis—an inflammation of the brain—and West Nile virus.

Nineteen Culex species are found in Texas.

Arboviruses of public health significance

- Belong to three main viral families,
  - Flaviviruses
  - Alphaviruses
  - Bunyaviruses
- These viruses have different geographical distributions.
- These viruses are spread by mosquito species.
- Arboviruses of importance to Asia

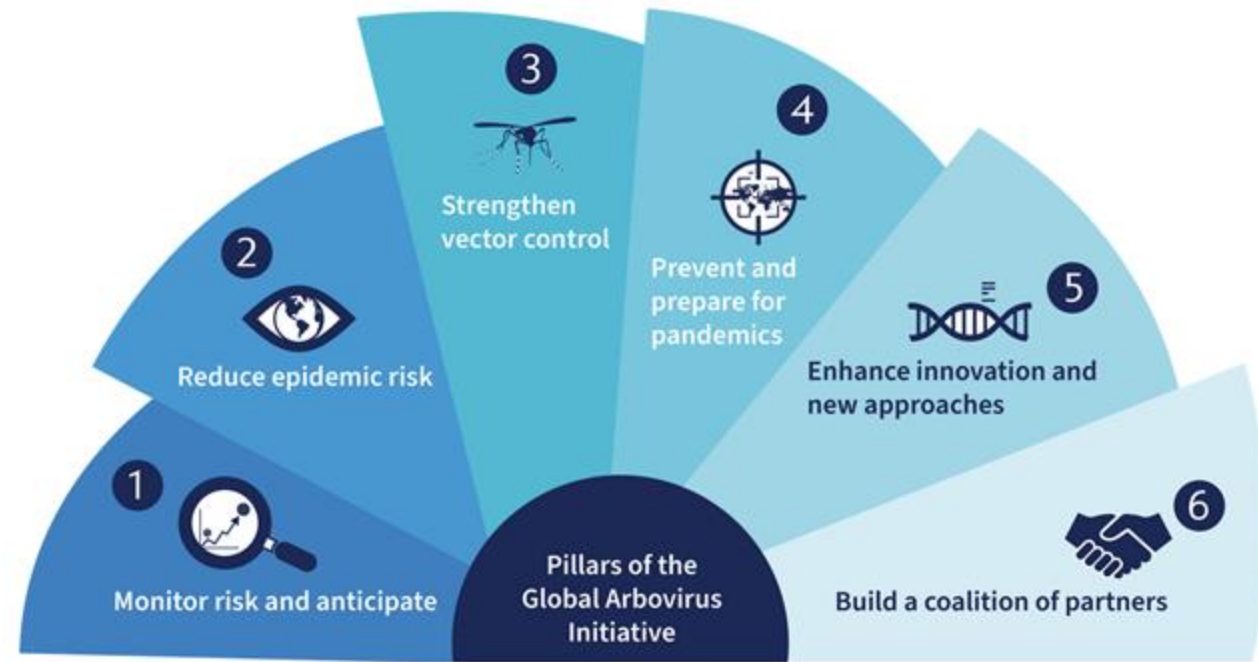|	
Dengue virus	Zika virus
Chikunguny a virus	Japanese encephalitis
West Nile virus	Arbovirus X

Many more important arboviruses

•Over 500 arboviruses have been described

•WHO has initially focused transmission via *Aedes spp.* vectors

•*Dengue, Zika, Yellow fever, Chikungunya*

Virus Family	Viral Genus	Virus		Vector Species
Bunyaviridae	Orthobunyavirus	California serogroup viruses		Mosquito (Aedes sp.)
	Phlebovirus	Rift Valley Fever virus		Mosquito (various)
	Phlebovirus	Toscana virus		Sandfly (Phelbotomus sp.)
	Phlebovirus	Phlebotomus fever virus		Sandfly (phelbotomus)
	Phlebovirus	Sandfly Fever Naples virus		Sandfly (phelbotomus)
	Phlebovirus	Sandfly Fever Sicilian virus		Sandfly (phelbotomus)
	Phlebovirus	Heartland virus		Tick (A. americanum)
	Phlebovirus	Severe fever with thrombocytopenia syndrome virus		Tick (H. longicornis)
	Nairovirus	Crimean Hemorrhagic Fever virus		Tick (Hyalomma sp.)
Flaviviridae	Flavivirus	Dengue Virus		Mosquito (Aedes sp.)
	Flavivirus	Zika virus		Mosquito (Aedes sp.)
	Flavivirus	Yellow fever virus		Mosquito (Aedes sp.)
	Flavivirus	West Nile Virus		Mosquito (Culex sp.)
	Flavivirus	St. Louis Encephalitis virus		Mosquito (Culex sp.)
	Flavivirus	Japanese encephalitis virus		Mosquito (Culex sp.)
	Flavivirus	Murray Valley encephalitis virus		Mosquito (Culex sp.)
	Flavivirus	Usutu		Mosquito (various)
	Flavivirus	Omsk Hemorrhagic fever virus		Tick (dermacentor)
	Flavivirus	Kyasanur Forest Diesease virus		Tick (Haemaphysalis sp.)
	Flavivirus	Tick-borne encephalitis virus		Tick (Ixodes and Haemaphysalis sp.)
	Flavivirus	Powassan virus		Tick (Ixodes sp.)
Orthomyxoviridae	Thogotovirus	Bourbon virus		Tick (A. americanum)
Reoviridae	Coltivirus	Colorado tick fever		Tick (dermacentor)
Rhabdoviridae	Vesiculovirus	Vesicular Stomatitis (New Jersey) virus		Sandflies (Lutz. Sp)	Mosquitos (various)
	Vesiculovirus	Chandipura		Sandlfy (Phlebotomus Sp.)
Togaviridae	Alphavirus	Barmah Forest Virus		Mosquito (Aedes and Culex sp.)
	Alphavirus	Chikungunya virus		Mosquito (Aedes sp.)
	Alphavirus	Venezuelan equine encephalitis virus		Mosquito (Culex sp.)
	Alphavirus	Sindbis virus		Mosquito (Culex sp.)
	Alphavirus	Equine encephalitis virus		Mosquito (Culex sp.)
	Alphavirus	Mayaro virus		Mosquito (Haemagogus sp.)

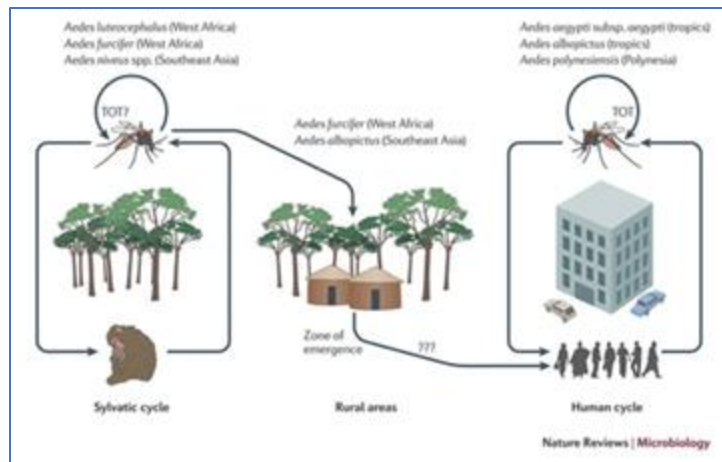# Public health management of arboviruses

- Understand host/virus/vector transmission drivers

- Improve diagnostics and surveillance

- Increase entomology capacity and surveillance

- Strengthen communication and risk detection between countries

- Use genomics to monitor arboviral genotypes

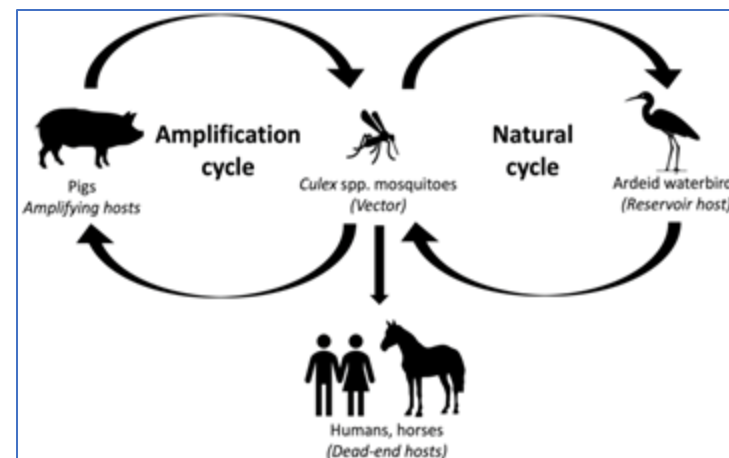- Build and strengthen partnerships and collaboration

3 Strengthen vector control

4 Prevent and prepare for pandemics

2 Reduce epidemic risk

5 Enhance innovation and new approaches

1 Monitor risk and anticipate

Pillars of the Global Arbovirus Initiative

6 Build a coalition of partners

One Health approach

- Environmental surveillance of both vectors and animals is an important consideration

- Particularly monitoring amplifying hosts/sylvatic cycles

ZIKA transmission cycle

DENV transmission cycle

JEV transmission cycle

McGuinness, S.L., *et al. Curr Infect Dis Rep* **25**, 111–122 (2023). https://doi.org/10.1007/s11908-023-00804-w

Vasilakis, N., Cardosa, J., Hanley, K. *et al. Nat Rev Microbiol* **9**, 532–541 (2011). https://doi.org/10.1038/nrmicro2595
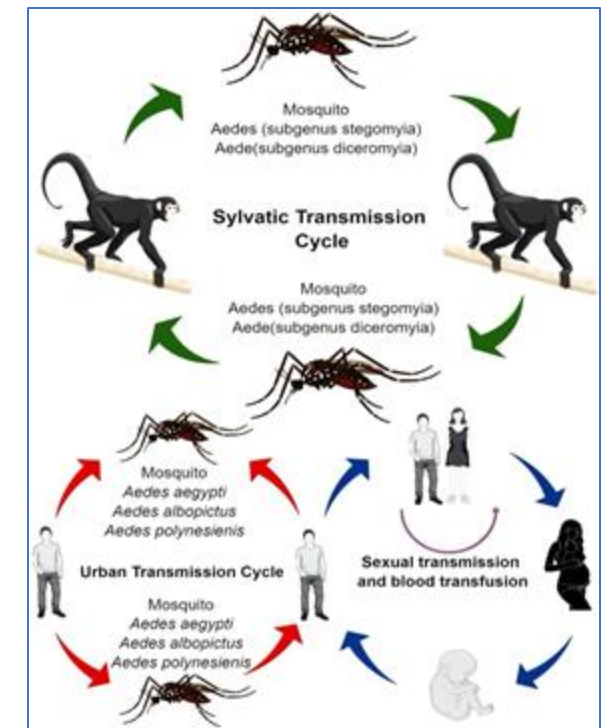
Public health action

It is widely recognized that no strategy alone can fully address the problem. However, some intervention tools have helped reduce the disease burden.

- Vector control, including insecticide treatment and environmental management
  - residual spraying to reduce mosquito populations

- Personal protection

- Clinical diagnosis and management, laboratory-based surveillance
  - To guide access to clinical services

- Vaccination
  - Vaccines are available to protect against Japanese encephalitis and yellow fever, and the first dengue vaccine licensed in 2015

Use cases for arboviral genomic surveillance

- Essential for early warning of virus outbreaks

- Understanding epidemic potential of endemic and newly introduced virus strain

- Early introduction of new strains into naive populations

- Detection of emergence of more virulent lineages and virus lineages resistant to interventions such as vaccines, antiviral drugs, and various vector control interventions (eg, *Wolbachia*-spp-infected mosquito strategies)

- Evaluation of intervention effectiveness

- Characterisation of the transmission network between vertebrates, including humans, and vector species

- Detection of genomic variants that could affect molecular assays.

Pathogen Genomics Utility
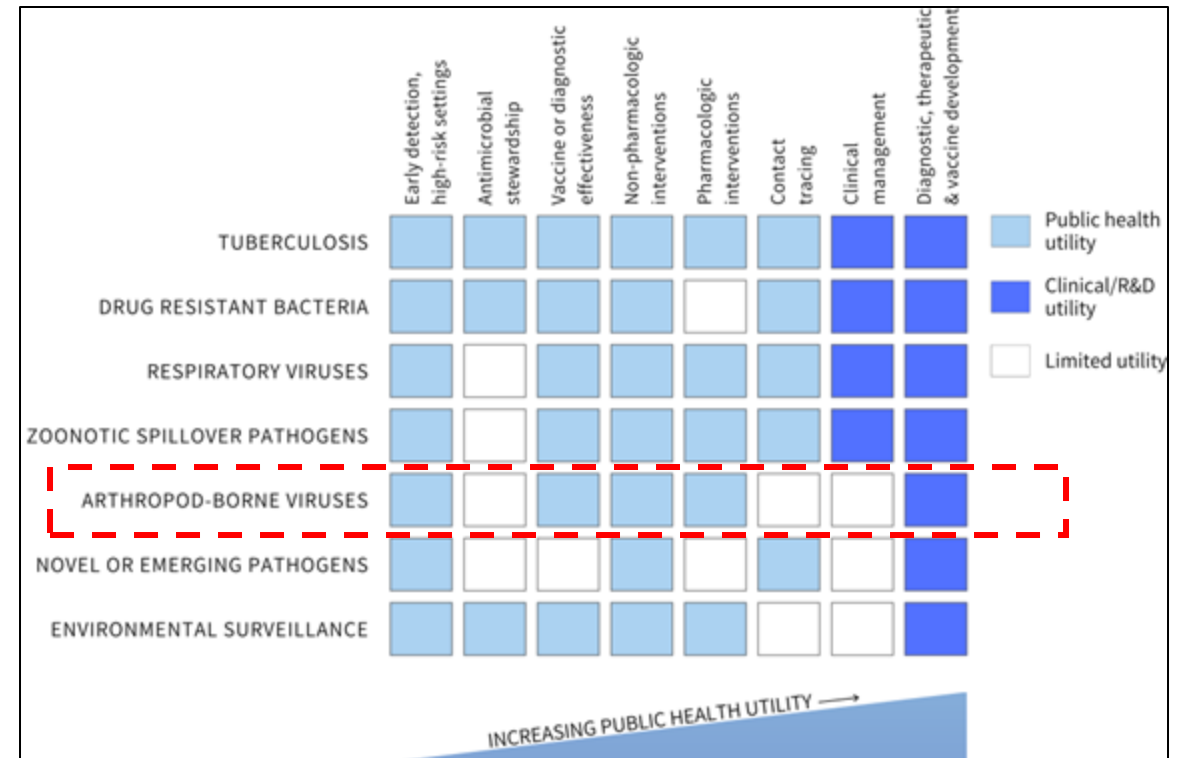
Challenges to arboviral genomic surveillance
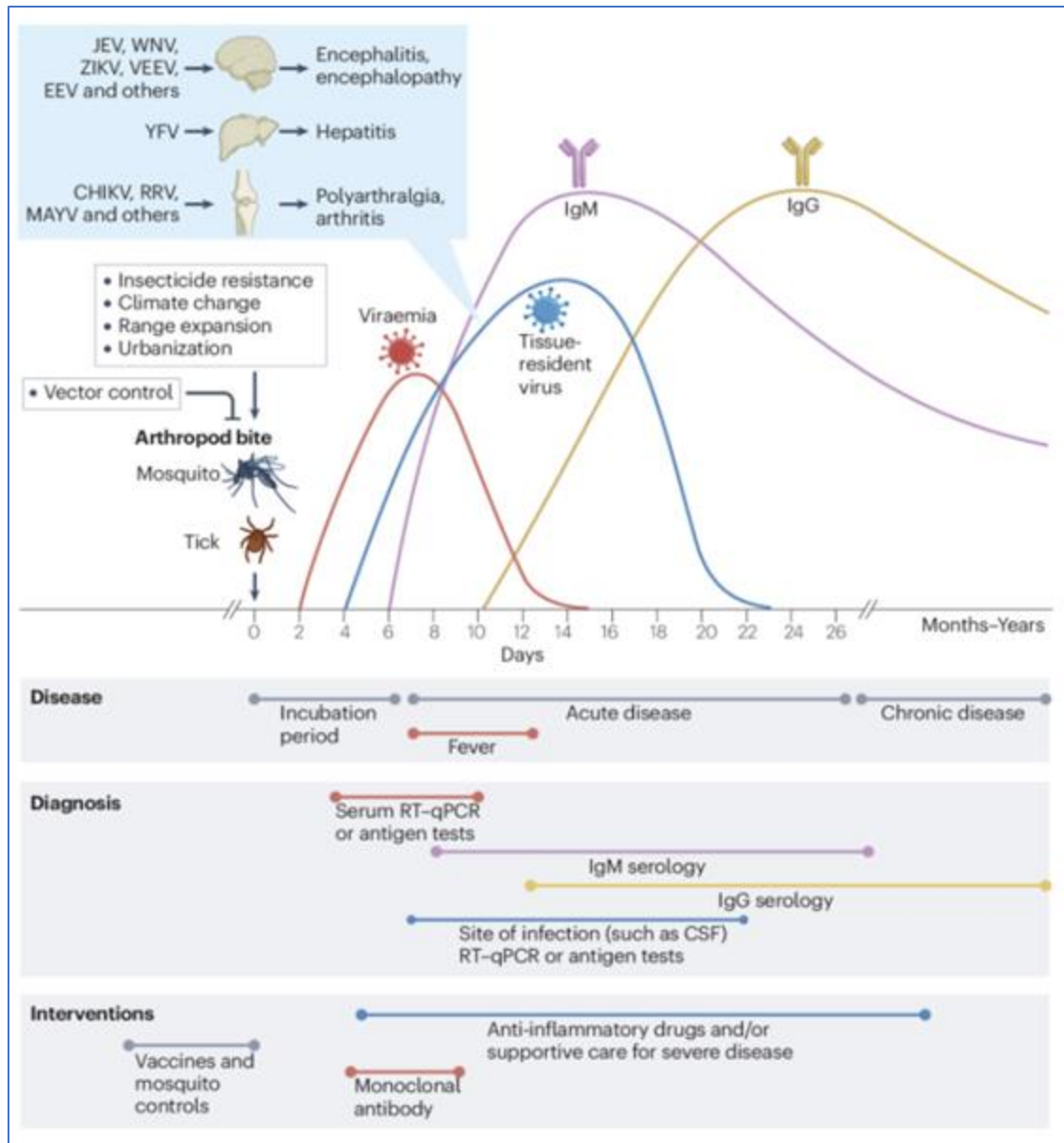
- Viremia is short lived

- Viremia peaks prior to acute symptoms

- Low level of symptomatic disease

- High diagnostic reliance on serological testing (which can be highly cross-reactive)

Integrated Surveillance of Arboviruses

Integrated Dengue Surveillance

Genomics use case
Zika genomics in Singapore

Zika case study - Singapore

- In 2016 (from Aug 27 to Nov 30), an outbreak of Zika virus infection (with 455 cases) were confirmed in Singapore.

- Zika virus was detected in the blood samples of 97 (60%) patients and the urine samples of 157 (96%) patients
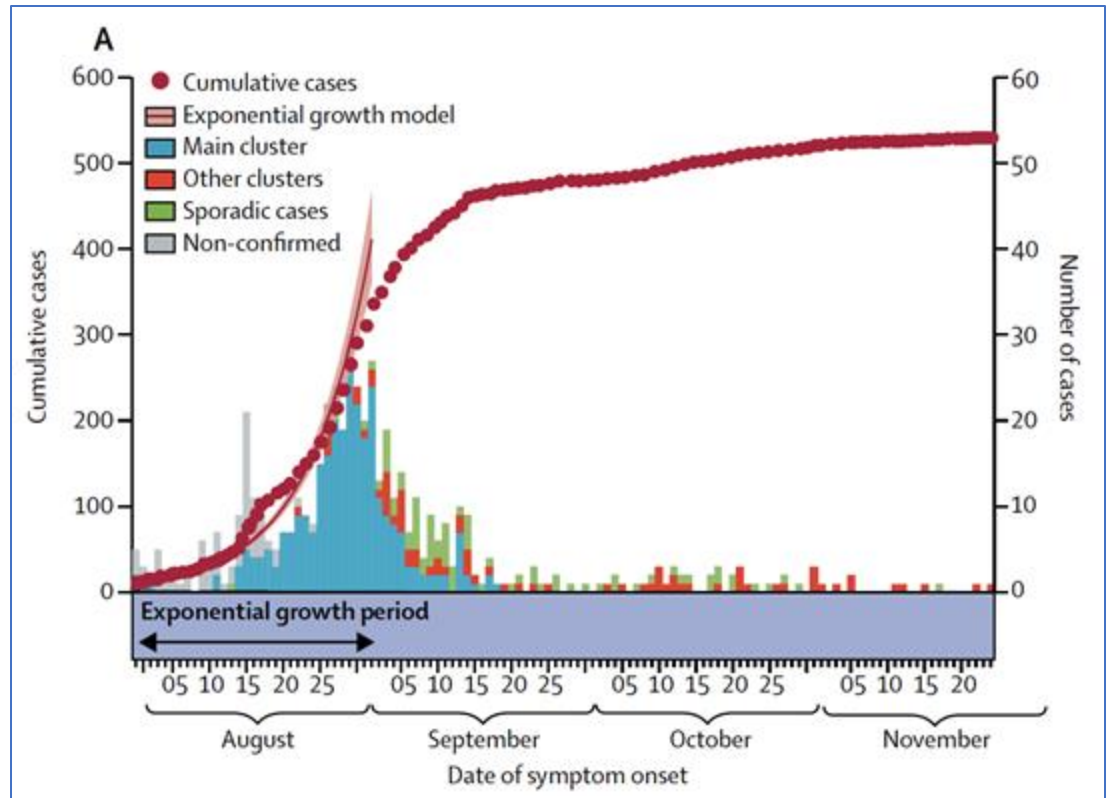
Singapore Zika Study Group, 2017, Lancet ID

Zika case study - Singapore

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- 15 clusters of cases, with the largest cluster traced back to workers from a construction site.

Zika cases
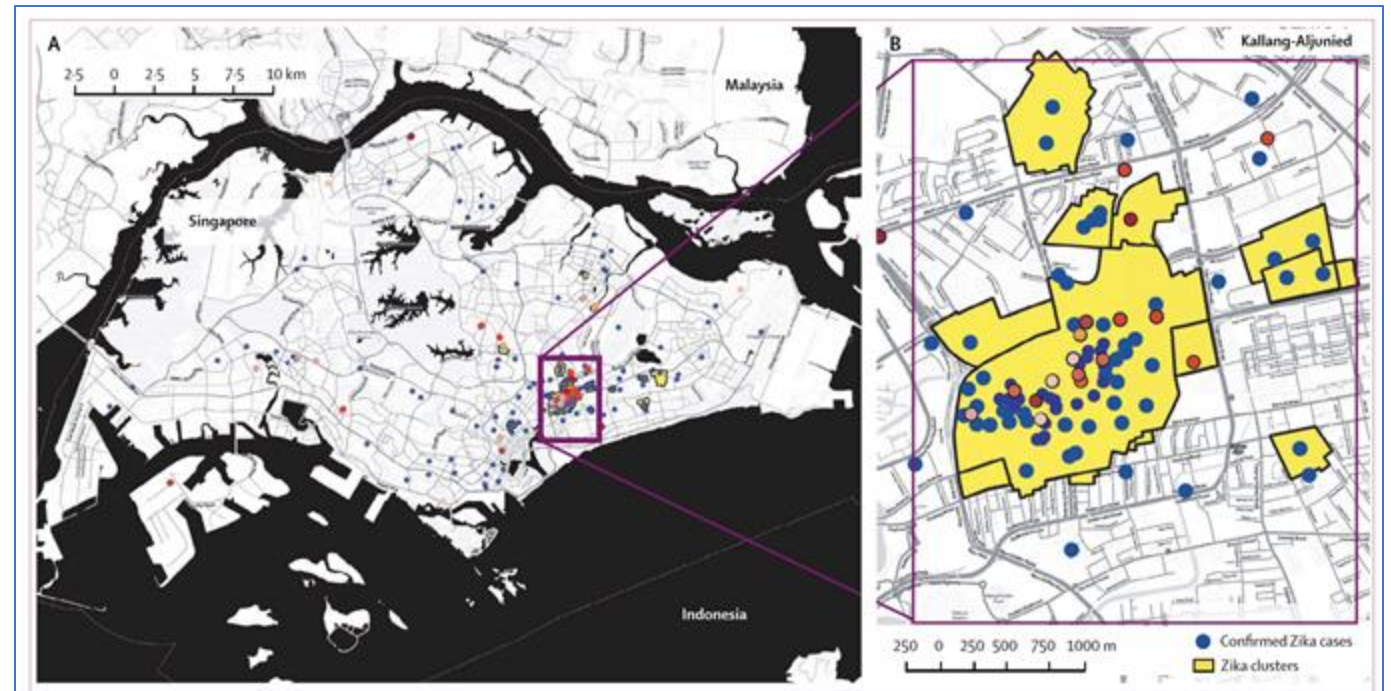
Singapore Zika Study Group, 2017, Lancet ID

Zika case study - Singapore

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- 15 clusters of cases, with the largest cluster traced back to workers from a construction site.

- 12 of the cluster neighborhoods had a high percentage of Ades Aegypti breeding sites. (2% PCR positive rate)

Ades aegypti breeding sites

Singapore Zika Study Group, 2017, Lancet ID

Captured mosquitoes were pooled for Zika virus screening; nine abdomen pools (**2%**) were positive for Zika virus.

Zika case study - Singapore

- Zika virus was sequenced from positive urine or serum clinical samples and mosquito samples.

- Sequenced using a full genome sequencing via a hybridization enrichment method. Using Illumina.

- 117 complete /nearly complete genomes (from 103 individual human samples and 14 mosquitoes).

Conclusions

- The lineage showed little diversity and was distinct from other Asian lineages.

- The estimated most recent common ancestor of the outbreak lineage was from May, 2016.

- Possibility of 3 separate introductions.

- Increased vector control measures.
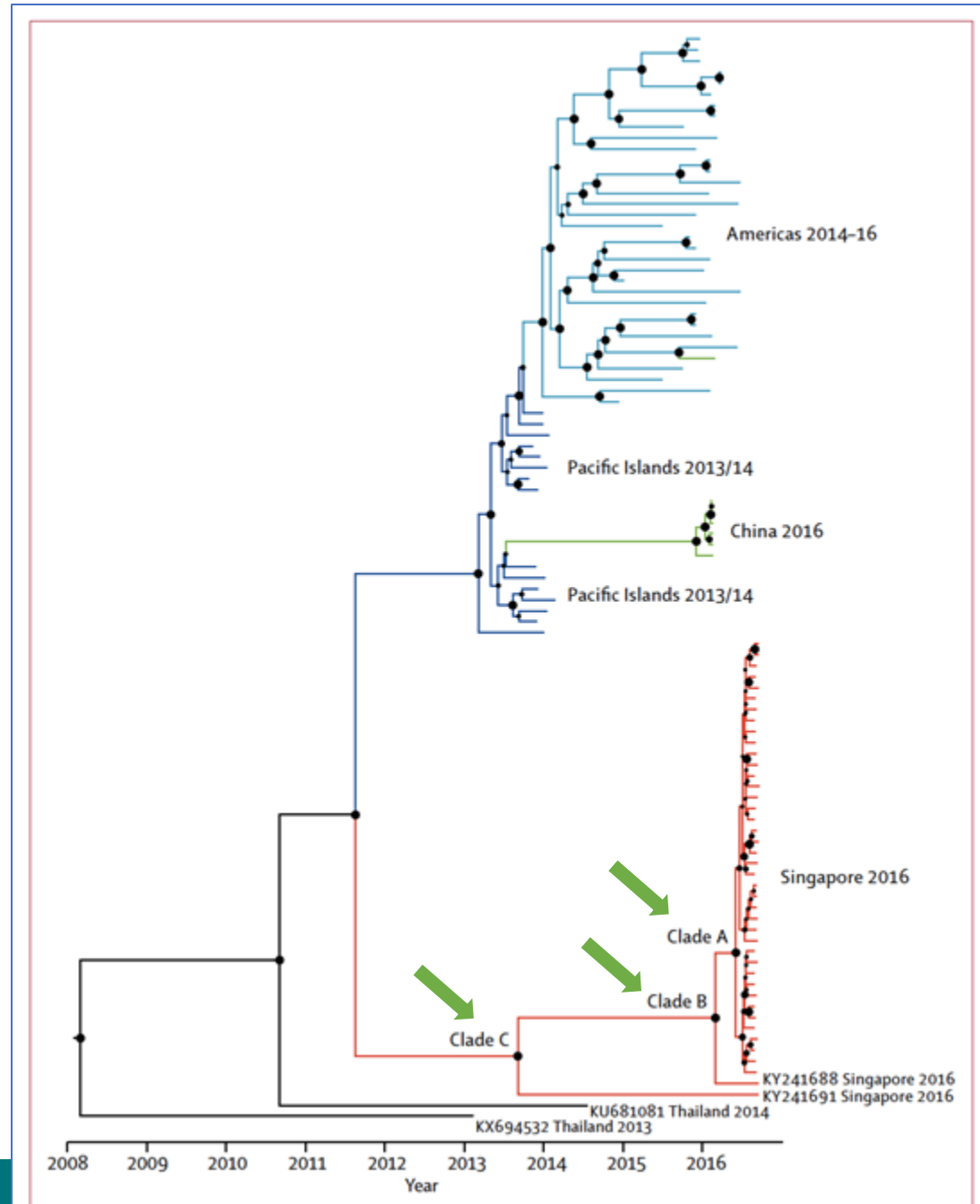
Figure 3: Temporal scale of the expansion of the Zika virus Asian genotype

Genomics use case
JEV Outbreak in Australia

Japanese encephalitis virus

- Most JEV infections are mild or asymptomatic

- Approximately 1 in 250 results in severe disease

- 68,000 cases of clinical disease annually and ~15,000 deaths

- Naturally infects wading birds

- Pigs amplifying hosts
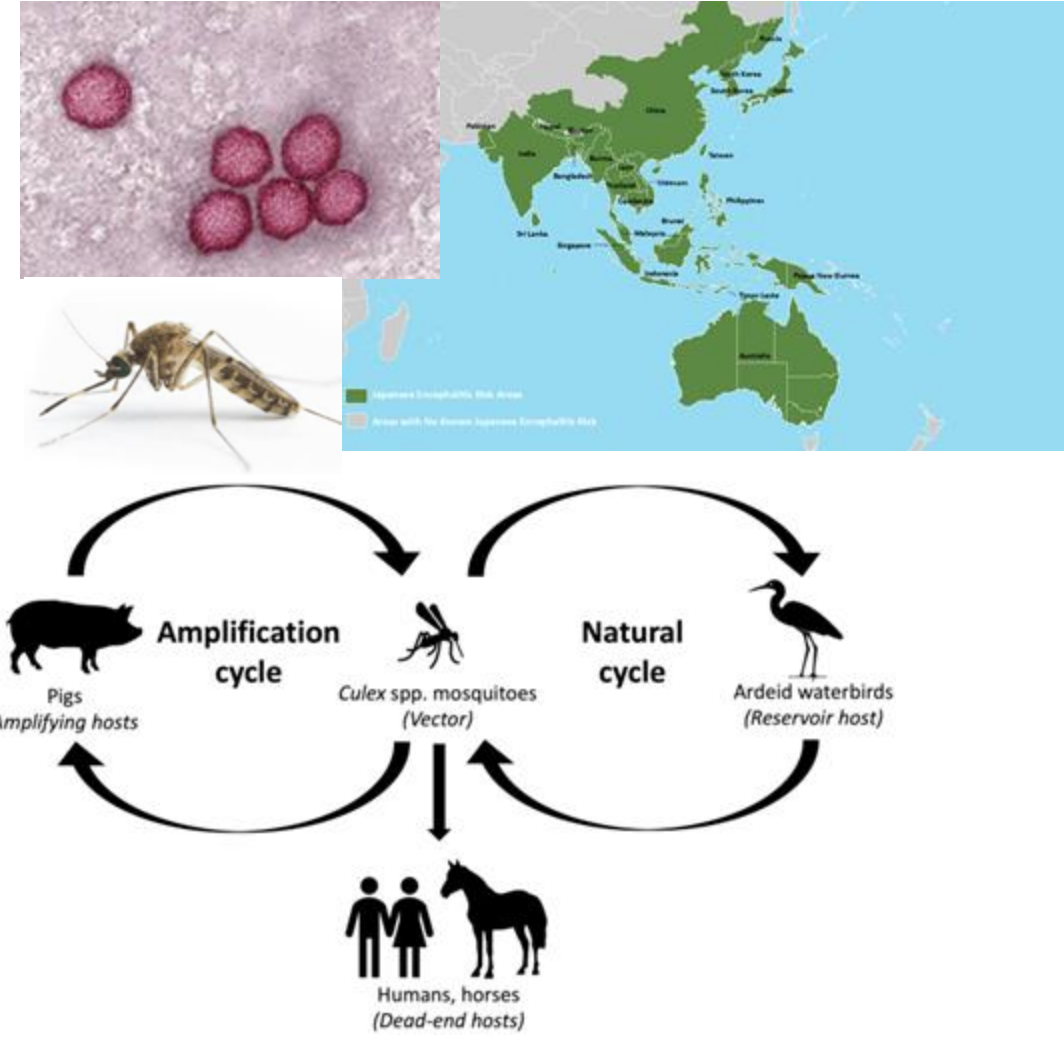
Phylogeography of JEV
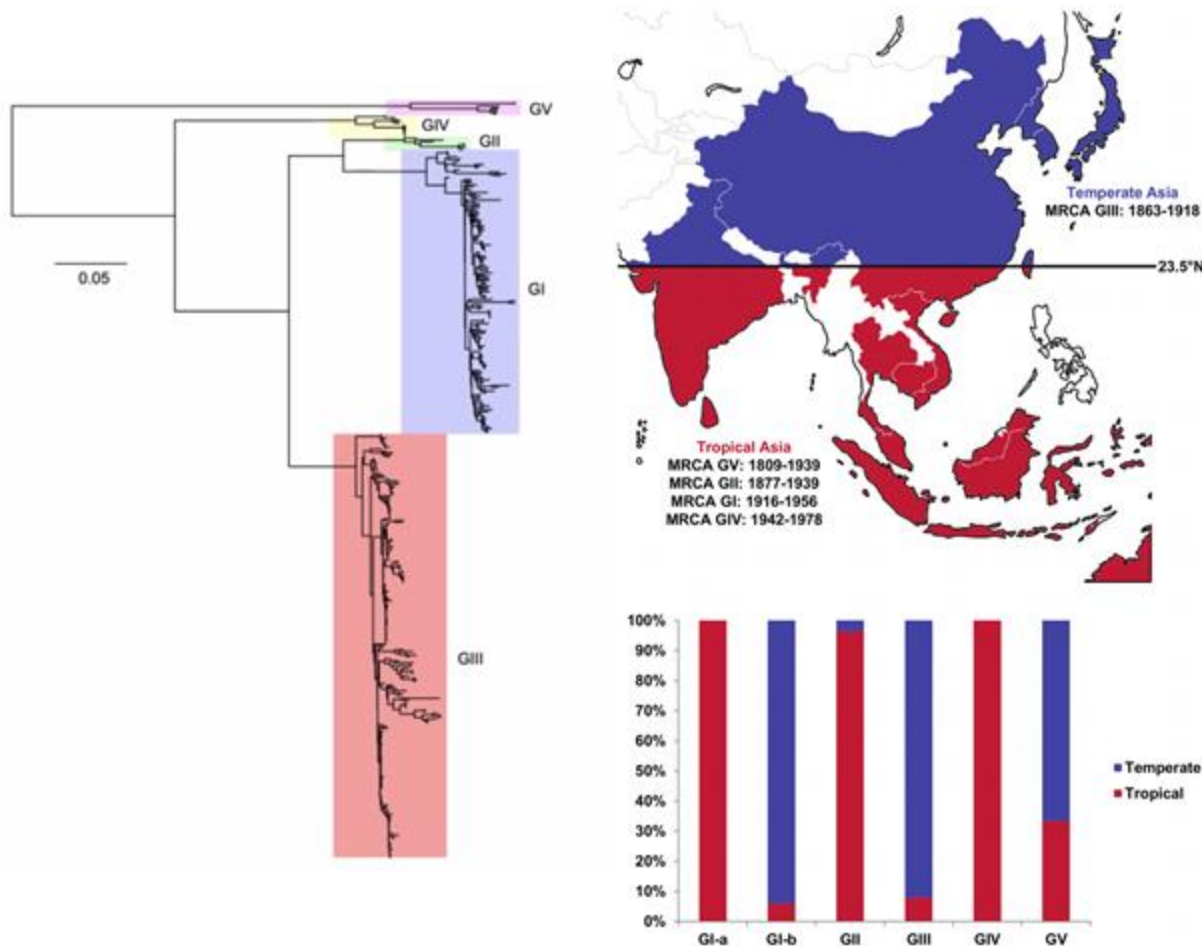
- Five distinct genotypes of JEV are recognised (I–V)

- Traditionally differentiated with E gene sequencing

- Genotypes I, II and III are the most prevalent and account for 98% (1935 to 2009)

- Vaccine is GIII

JEV in southeastern Australia

In March 2022, the first JEV outbreak in mainland Australia was identified

45 people have been infected with JEV in Australia, seven deaths

JEV detected in 80 piggeries from four states

JEV vaccination targeted to protect those at greatest risk of infection

NSW Case Detections
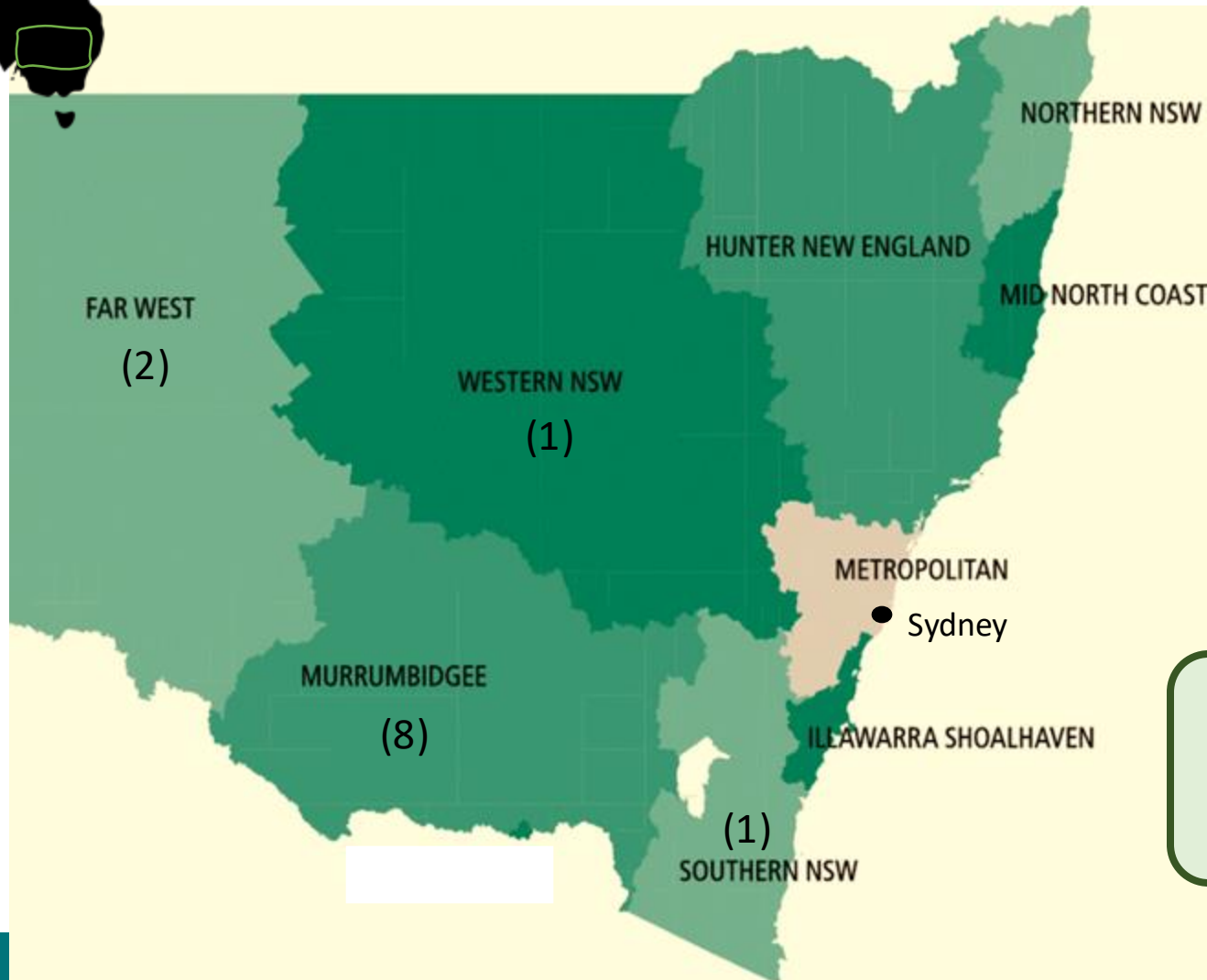
n = 12

Median age: 49.7 years

IQR 30.4 to 65.9 years

Range 10.1 to 73.7 years

Male:Female ratio 2.0

1 paediatric case – diagnosed by serology

First 3 months of outbreak:

1.5 JE cases per 1,000,000 population (NSW)

Prevalence of non-encephalitic disease unknown

Clinical utility of JEV genomics

Table 3. Diagnostic results for the twelve confirmed cases of Japanese encephalitis from New South Wales tested in our laboratory over the period 4 March 2022 to 31 May 2022.

Case #	CSF JEV IgM IF *	Serum JEV IgM IF *	Serum JEV IgG (Interval)	JEV RT-PCR	mNGS	Confirmed/Probable JE
1	detected	detected	>8× titre rise (7 days)	detected (brain tissue and CSF)	JEV sequence detected (brain tissue)	confirmed
2	not detected	not detected	not detected	detected (CSF)	-	confirmed
3	not detected	detected	4× titre rise (29 days)	not detected (CSF)	-	confirmed
4	-	detected	>4× titre rise (7 days)	-	-	confirmed
5	-	detected	falling IgG titres	-	-	confirmed
6	-	detected	4× titre rise (36 days)	-	-	confirmed
7	-	detected	>16× titre rise (9 days)	-	-	confirmed
8	-	detected	>4× titre rise (25 days)	-	-	confirmed
9	-	detected	>4× titre rise (19 days)	-	-	confirmed
10	-	detected	detected; no convalescent serum available	-	-	confirmed
11	-	detected	detected; no convalescent serum available	-	-	confirmed
12	-	detected	>8× titre rise (41 days)	-	-	confirmed

CSF, cerebrospinal fluid; IF, immunofluorescence; JE, Japanese encephalitis; JEV, Japanese encephalitis virus; mNGS, metagenomic next generation sequencing; RT-PCR, real-time polymerase chain reaction; * for all samples in which JEV IgM was detected by IF, MVEV and WNVKUNV IgM (and dengue, yellow fever and/or Zika IgM if appropriate travel history) was performed by IF and found to be negative.
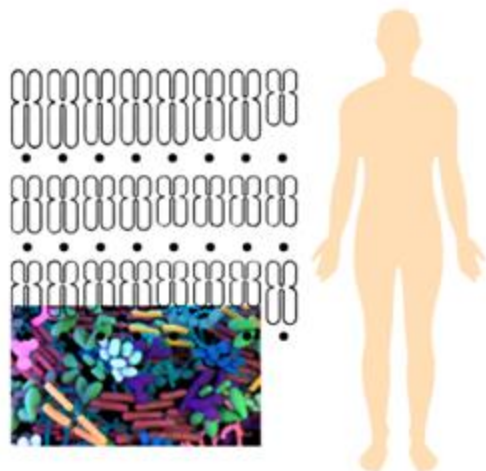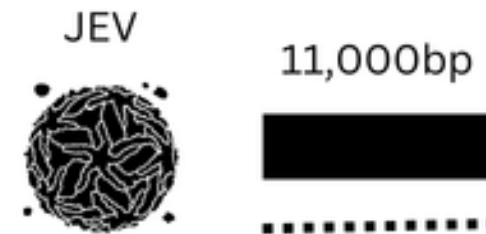
JEV in southeastern Australia

Genomics for JEV

Metagenomics
Cost: ~$1000
Time: 12 – 24hours

JEV amplicon tiling
Cost: ~$200
Time: 24 – 48hours

JEV

11,000bp

One health genomic surveillance

Not detected

Not collected

Vector surveillance

- *Culex annulirostris* primary vector for the 2022 JEV outbreak

- Widespread across mainland Australia

- Freshwater habitats including natural and constructed wetlands

- Opportunist feeding on a wide range of animals: birds, pigs, humans

- Can disperse up to 12km from larval habitats

- Demonstrated capacity to transmit various arboviruses including JEV (EIP 7-10 days post infection)
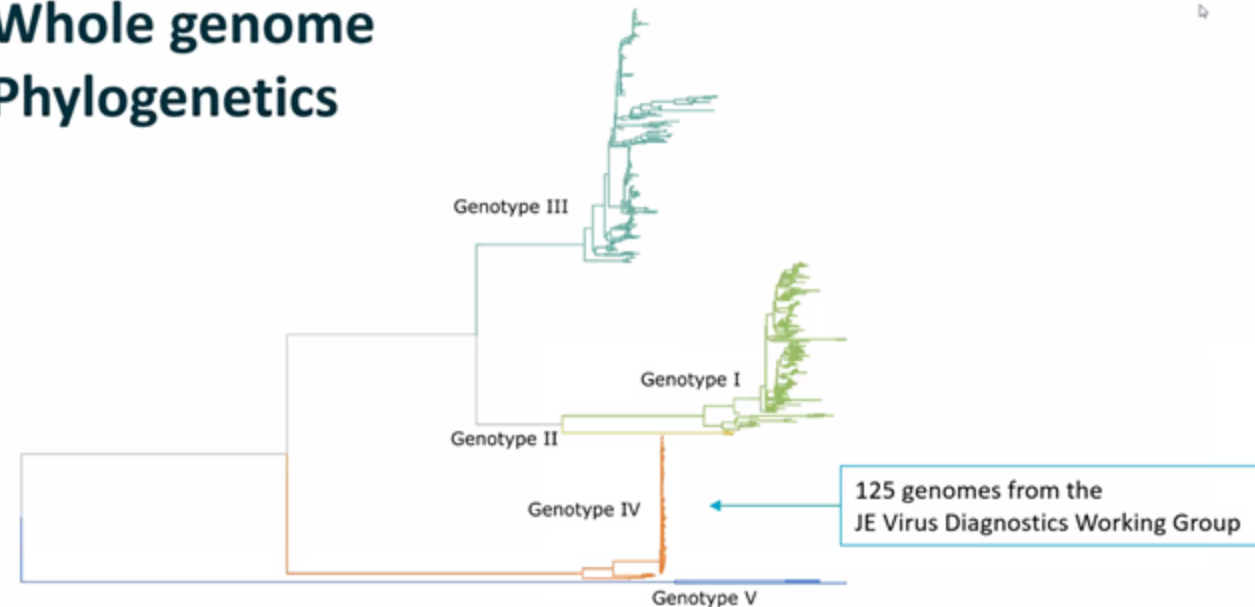
Slide courtesy: Annaelise Howard-Jones

Strengths of genomics for arboviral surveillance

- First detection of genotype IV in a temperate climate

- Genomic surveillance revealed circulation of genotype IV in Tiwi Islands in 2021

- One health surveillance key to clearly identifying the causal genotype

- Diagnostic challenges for rare and unusual arboviruses overcome using metagenomics

Whole genome Phylogenetics

Genotype III

Genotype I

Genotype II

Genotype IV

125 genomes from the JE Virus Diagnostics Working Group

Genotype V

Activity 1

"Using genomics for Arboviral outbreaks"

Group case studies

- Each scenario can be conducted at your current table as a team

- Each team address key questions that assist when implementing genomic surveillance during an arboviral outbreak

- Please outline on the slide templates/easel with butchers' paper below key drivers and barriers to conducting genomic surveillance in your scenario

- Results will be presented to the group during an open discussion

Capacity for genomics surveillance

| Low capacity	Moderate capacity	High capacity
• Multiple arboviruses usually **endemic** • High startup cost could mean minimal interest in genomics for arboviral control.	• Frequent seasonal outbreaks of arbovirus • Genomics could be used to strengthen arbovirus control activities • More stringent inclusion criteria for sequencing	• Frequent seasonal outbreaks of arbovirus • Genomics to aid preparedness, vector control and targeted vaccination • Replace partial genome sequencing

Case assignments

Moderate capacity

- Frequent seasonal outbreaks of arbovirus

- Genomics could be used to strengthen arbovirus control activities

- More stringent inclusion criteria for sequencing
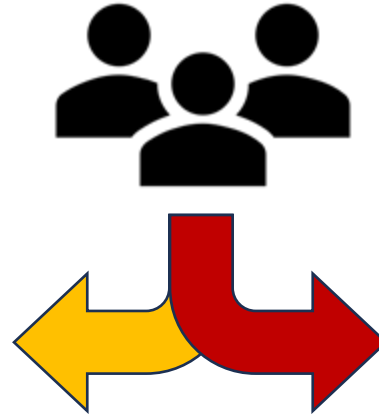
High capacity

- Low arboviral incidence

- Genomics to aid preparedness, vector control and targeted vaccination

- Replace partial genome sequencing

Assumptions for exercise

- In all scenarios, the assumption will be that the diagnostic/public health laboratory is fully stocked and can conduct molecular testing for a range of arboviruses

- Sampling, genomics capacity and genomic activities will be unique in each case.

Questions for considerations

- What are the likely uses of genomics in your scenario ?

- Outline potential sectors and surveillance partners to address the main aim of the scenario?

- What would be the most feasible and cost-effective sampling strategy?

- Does the scenario setting have sufficient capacity to address the main aim of genomics surveillance? If insufficient, what actions can be taken to strengthen in an outbreak scenario?

- Who are the target reporting stakeholders and how often should reports be generated?

- What are the key barriers to implementing genomic surveillance within existing surveillance systems for your pathogen?

Setting the scene: Group 1

Pathogen	Dengue
Aim	A new vaccine is about to be rolled out in a country with multiple circulating serotypes of Dengue. MOH is keen to use genomics along with other complimentary methods to assess the impact of vaccination on circulating dengue viruses in the country.
Case numbers	Hyper-endemic with high seasonal case numbers
Current genomics activities	• Convenience sampling of Dengue cases for E gene Sanger sequencing • Routine SARS-CoV-2 genomic surveillance • Other traditional surveillance methods such as PCR, serology assays for Dengue are available.
Sequencing platforms (status)	• Illumina Nextseq 2000 • Oxford Nanopore GridION (have access if required)

Setting the scene: Group 2

Pathogen	Zika virus
Aim	There has been a recent increase of microencephaly in newborns. In a private hospital, a case has come up PCR positive for Zika virus. Zika is not new to this Asian country, although not previously linked with microencephaly. MOH is keen to sequence the circulating strains to inform treatment and control response.
Case numbers	5 cases of microencephaly within two months
Current genomics activities	• Dengue sequencing capacity, but no routinely conducted • SARS-CoV-2 genomic surveillance • Other traditional surveillance methods such as PCR, serology assays for Zika are available.
Sequencing platforms (status)	• Illumina Nextseq 500 (workhorse) • Oxford Nanopore GridION (have access if required)

Setting the scene: Scenario 3

Pathogen	Japanese encephalitis virus (JEV
Aim	In a <u>non-endemic</u> country, there has been a case of encephalitis that is PCR confirmed to be JEV. The case has no history of travel. The MOH is keen to investigate with genomics the possible exposure event.
Case numbers	>50 RT-PCR confirmed cases
Current genomics activities	Genomic surveillance of Dengue virus only Samples of convenience used for dengue surveillance
Sequencing platforms	• Illumina iSeq • Oxford Nanopore GridION

Setting the scene: Scenario 4

Pathogen	Arbovirus X
Aim	An outbreak of severe febrile illness with neurological symptoms is occurring. Coincidental deaths of birds at the zoo have also been reported. Serum antibodies give weak signal to dengue, hence it is suspected the outbreak maybe caused by a flavivirus.
Case numbers	>100 cases
Current genomics activities	• Whole genome sequence of Dengue cases only • Other traditional surveillance methods such as PCR, serology assays are only available for dengue.
Sequencing platforms (status)	• Illumina Nextseq 500 (workhorse)

Template for team answers

- What are the likely uses of genomics in your scenario ?

- Outline potential sectors and surveillance partners to address the main aim of the scenario?

Template for team answers

- What would be the most feasible and cost-effective sampling strategy?

- Does the scenario setting has sufficient capacity to address the main aim of genomics surveillance? If insufficient what actions can be taken to strengthen in an outbreak scenario?

Template for team answers

- Who are the target reporting stakeholders and how often should reports be generated?

- What are the key barriers to implementing genomic surveillance within existing surveillance systems for your pathogen?

Activity 2

Pathogen Prioritization for Dengue

Pathogen prioritisation activity

Pathogen Prioritization for Dengue

Thank you!

WORKSHOP PARTNERS

Resources

- Sequencing protocols:
  - WNV (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10305458/)

  - Dengue (https://www.protocols.io/view/dengueseq-a-pan-serotype-whole-genome-amplicon-seq-kqdg39xxeg25/v3)

  - WNV, Dengue, Zika, YF, CHIK, Lassa (https://docs.google.com/spreadsheets/d/1zMfUv1IV5-Sy-AeOPhKHdoZMUxhD1R_RsB5gPswqx40/edit?pli=1&gid=0#gid=0)

  - Make your own primer scheme (https://www.protocols.io/view/primalseq-generation-of-tiled-virus-amplicons-for-n2bvjyqw5vk5/v)