Clinical, Diagnostics and Therapeutics in Arbovirus Infections

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Arboviruses

ALPHAVIRUSES

Human Disease
Ross River virus*
Barmah Forest virus
Sindbis virus, Chikungunya

FLAVIVIRUSES

Human disease
MVE, Kunjin/West Nile virus, dengue
Japanese encephalitis
Kokobera, Yellow Fever, Zika, TBE

BUNYAVIRUSES

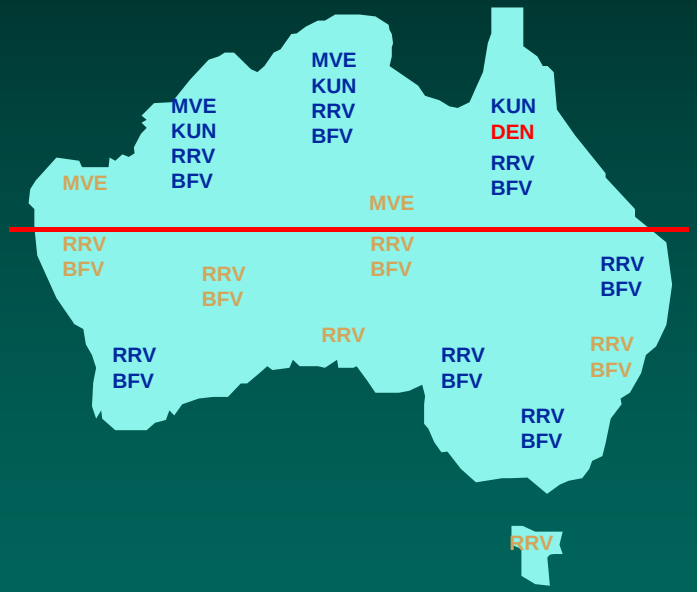
Human Disease
Gan Gan, Hantavirus
Trubanaman, Rift Valley Fever,
Crimean-Congo haemorrhagic fever

****Australian***

Arbovirus Notifications in Australia, 2013/14

	NSW	NT	Qld	SA	Vic	WA	TOTAL
RRV	509	434	1,845	111	161	1,485	4,569
BFV	254	129	1115	20	25	257	1,803
Dengue*	211	69	461	82	414	531	2,021
MVE	0	0	0	0	0	0	0
CHIK^	22	2	8	5	20	37	94
Yellow F	0	0	0	0	0	0	0

*mostly imported cases (404 local Queensland cases)
^all imported cases



National Arbovirus and Malaria Advisory Committee

Clinical aspects of arboviruses

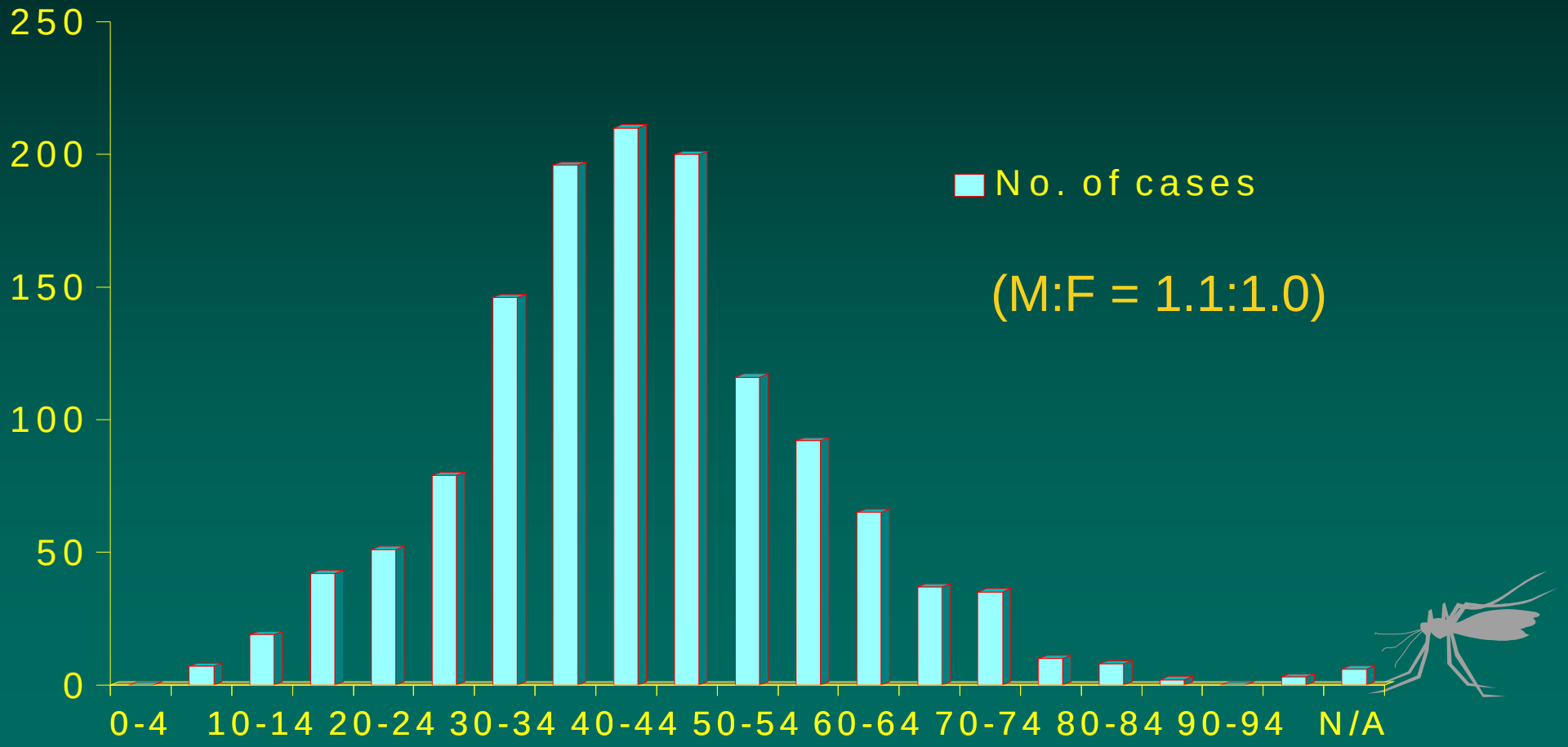
- Incubation period of 3 days to 2 weeks for most
- Most arbovirus infections are asymptomatic
- Ranges from mild febrile illness to severe encephalitis
- Acute symptom duration from 3-10 days
- Categorised into neuroinvasive and non-neuroinvasive

Illnesses due to Arboviruses

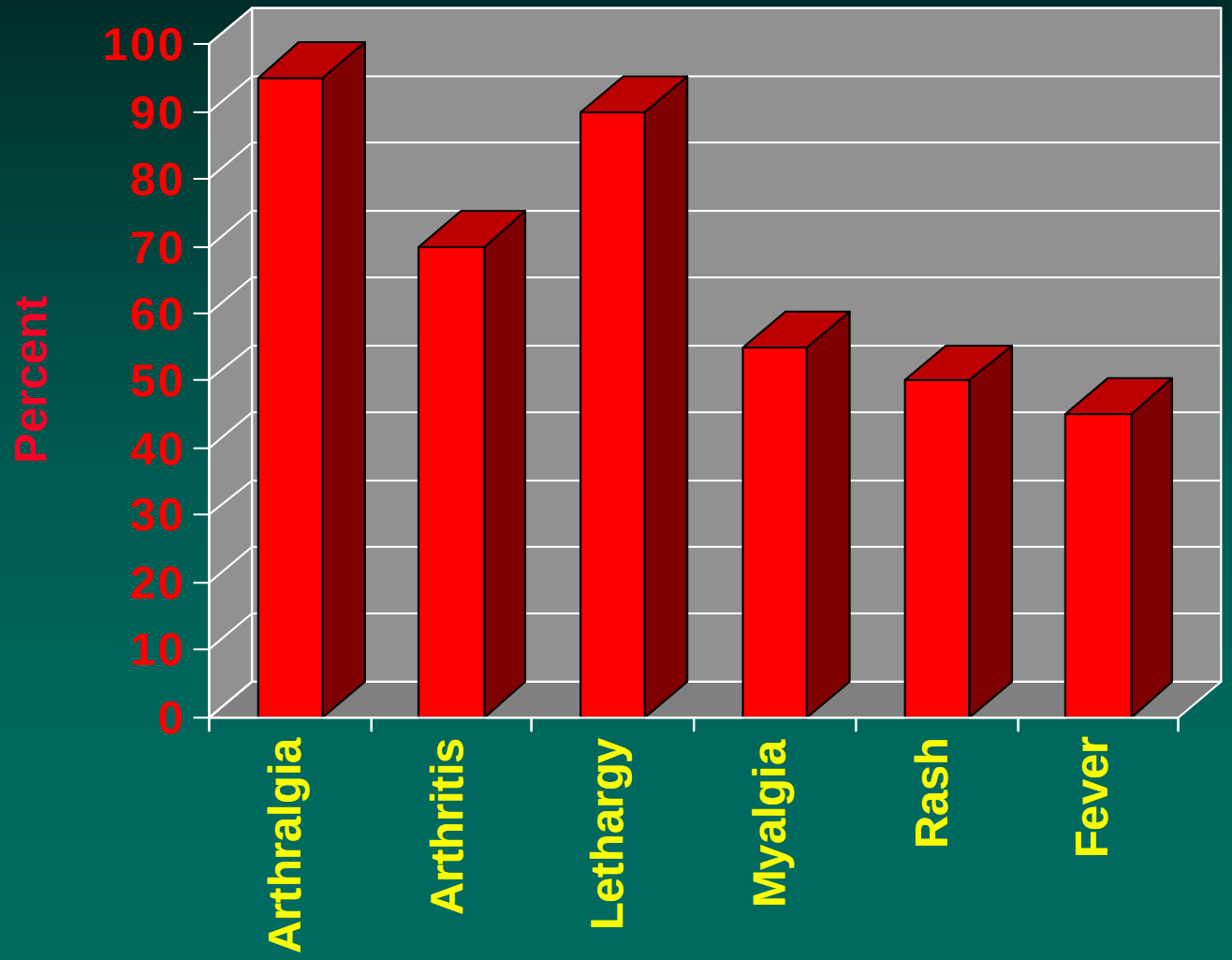
- **Non-neurological:**
  - Polyarthralgic illness: **Ross River , Barmah Forest, Kunjin/West Nile virus, Kokobera, Gan Gan, Trubanaman, Chikungunya, Dengue**
  - Fever and rash: **Ross River, Barmah Forest, Dengue, Zika**
  - Febrile illness: **Dengue, MVE, Kunjin, JE**
  - Haemorrhagic fever: **Dengue, CCHF, Rift Valley Fever, Hantavirus, Yellow Fever**

- **Neurological**
  - Meningitis/Encephalitis/Acute flaccid paralysis: **MVE, Kunjin, JE, TBE, Rift Valley Fever**
  - **Guillain-Barre Syndrome: Zika**
  - **Congenital cerebral malformations: Zika**
  - **Ocular: Rift Valley Fever**

Age distribution of cases of Ross River virus disease, south-west of WA, July 1995 - June 1996



Ross River Virus Infection



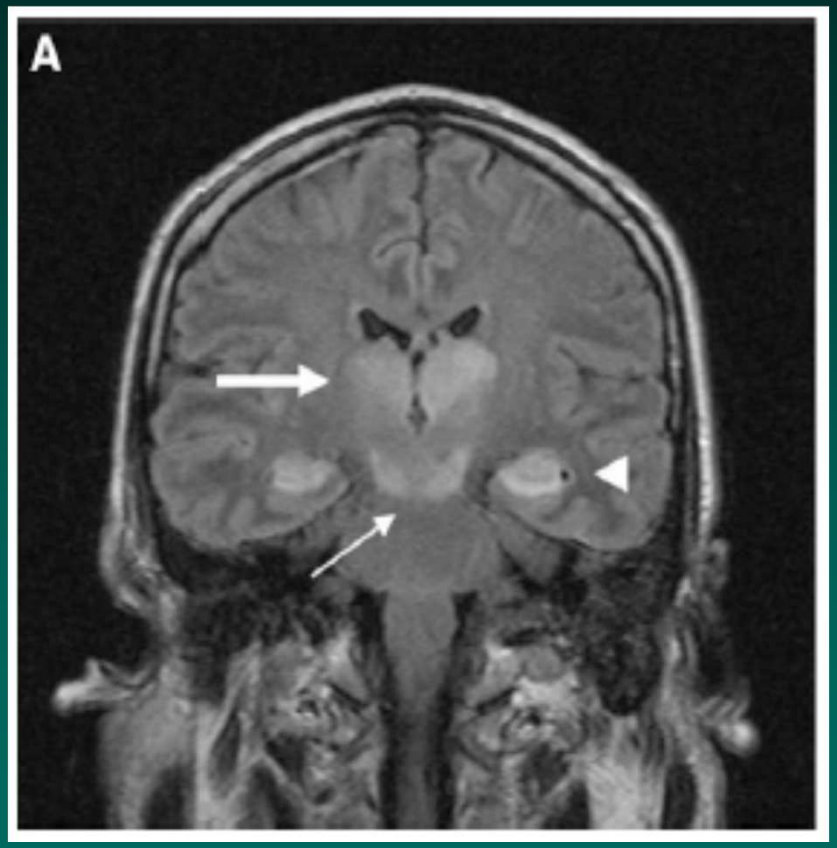
Comparison of disease due to Barmah Forest virus with Ross River virus

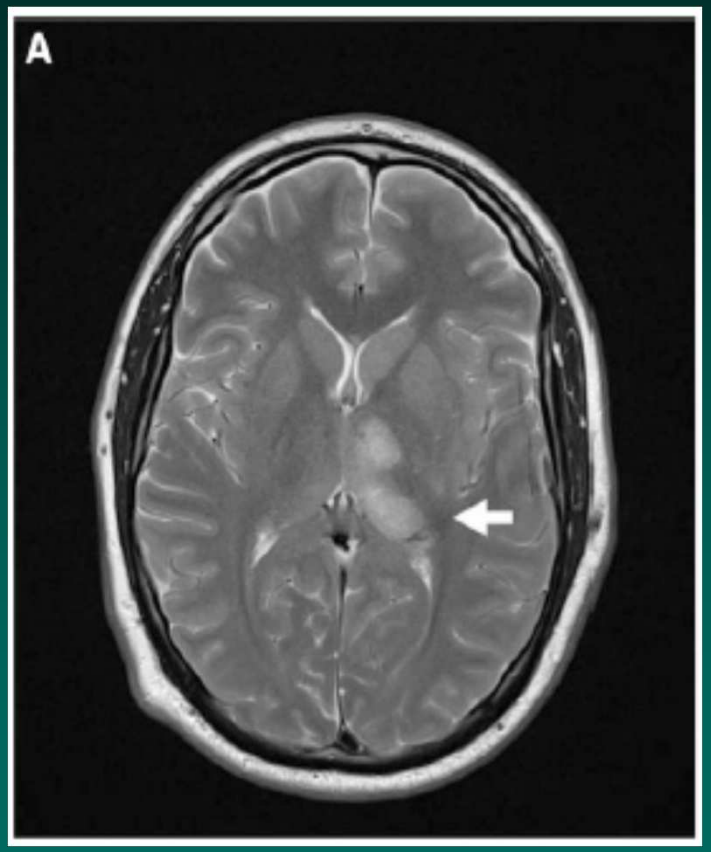
	BFV Disease	RRV Disease
Rash : Any type	52 - 100%	40 - 60%
: Maculopapular	90%	100%
: Vesicular	10%	Uncommon
Joint swelling/stiffness	30%	61 - 80%
Joint pain : Any	70 - 86%	83 - 98%
: ≥ 1 month	40%	80 - 98%
: ≥ 6 months	≥ 10%	57%
Myalgia	70 - 80%	43 - 67%
Fatigue	80%	62 - 94%
Fever	50%	20 - 59%
Lymphadenopathy	7%	0.6 - 20%

Murray Valley Encephalitis Virus

- Presentation
  - Altered mental state, seizures, tremor, weakness, paralysis
- Blood tests
  - Raised CRP, LFTs, neutrophils, platelets
- EMG
  - Diffuse slow wave pattern
- Radiology
  - Early CT head often normal
  - MRI:
    - thalamic signal worse prognosis
    - thalamic + brainstem, basal ganglia, cerebellum or cortex involvement devastating outcome

Murray Valley Encephalitis Virus





Dengue Virus

Primary infection

- Short incubation of < 9 days
  - fever, retro-orbital headache, myalgias, nausea
  - acute illness lasts 3-7 days, convalescence may last weeks

Dengue haemorrhagic fever

- Due to infection with a second serotype
  - following classical dengue but when fever breaks develop haemorrhages, circulatory collapse, thrombocytopenia
  - if severe vascular leak develop dengue shock syndrome

Reported clinical symptoms among confirmed Zika cases

Symptoms	N (n=31)	%
Macular or papular rash	28	90%
Subjective fever	20	65%
Arthralgia	20	65%
Conjunctivitis	17	55%
Myalgia	15	48%
Headache	14	45%
Retro-orbital pain	12	39%
Edema	6	19%
Vomiting	3	10%

Yap Island, 2007

Duffy M. N Engl J Med 2009

Clinical features: Zika c.f. dengue and chikungunya

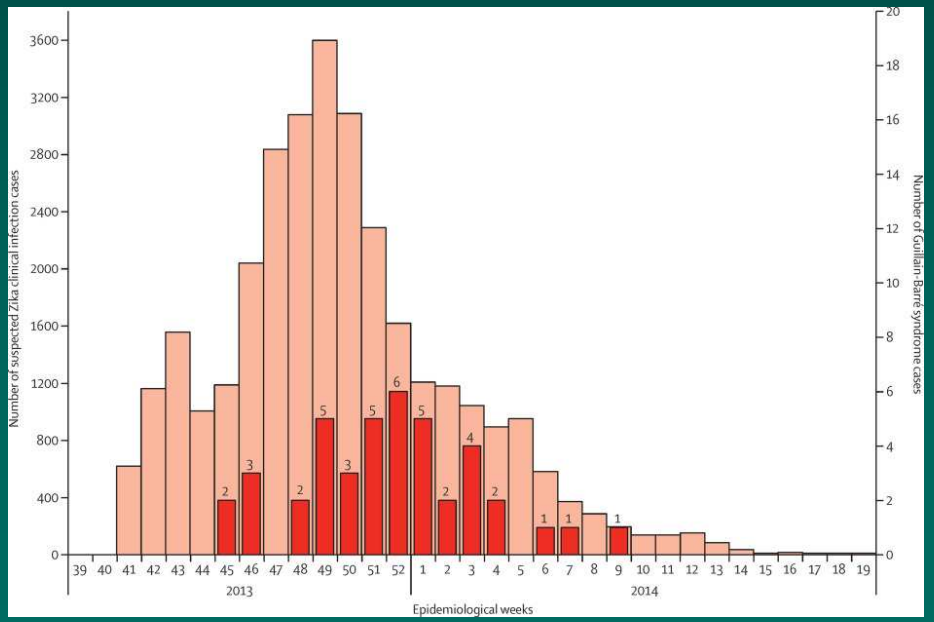
Features	Zika	Dengue	Chikungunya
Fever	++	+++	+++
Rash	+++	+	++
Conjunctivitis	++	-	-
Arthralgia	++	+	+++
Myalgia	+	++	+
Headache	+	++	++
Hemorrhage	-	++	-
Shock	-	+	-

Rabe, Ingrid MBChB, MMed "Zika Virus- What Clinicians Need to Know?" (presentation, Clinician Outreach and Communication Activity (COCA) Call, Atlanta, GA, January 26 2016)

Reported Zika neurological sequelae

- Guillain-Barré syndrome (GBS)
- Acute myelitis
- Meningoencephalitis
- Acute disseminated encephalomyelitis (ADEM)
- Sensory polyneuropathy
- Uveitis

French Polynesian outbreak
(Lancet 2016;387:1531)



Zika and pregnancy outcomes

(Congenital Zika Syndrome)

- Birth defects reported in 6% of maternal infections
  - No difference if maternal Sx (JAMA 2017;317:59)
- Miscarriage or stillbirth
- Microcephaly
  - esp 1st trimester infection
- Intracranial calcifications
- Absent or poorly developed brain structures
- Eye defects
  - esp 1st trimester infection
  - retinal scarring, optic nerve hypoplasia
- Hearing deficits (Brazil: 6% incidence)
- Limb contractures (arthrogryposis in 20%)
  - Due to brain stem, spinal cord abnormalities
- IUGR

Zika effects on human neurons

- Zika has tropism for:
  - neural stem cells
    - apoptosis (cell death)
  - radial glial cells
    - straddle base to surface of brain to provide scaffold used to populate cortex
- Induces increased centrosomes in foetal brain cells
  - associated with failure of cell division and microcephaly
- Result in:
  - insufficient neurons producing microcephaly
  - congenital cortical malformation (scaffolding defect)

Arbovirus Testing

- Combination of:
  - direct detection
  - serological methods
- Laboratory methods need to be considered together with other information:
  - vaccination and travel history
  - date of onset of symptoms
  - other arboviruses known to circulate in the geographic area

Direct detection methods

- NAAT:
  - blood (whole blood vs serum), urine, vaginal fluid, semen
  - arboviruses often detectable in serum for only the first few days of illness prior to appearance of antibody
- Antigen detection:
  - NS1 Ag for DENV
- Culture:
  - requires insect cell lines for 3-4 days then passaged into a mammalian cell line for another 2-3 days for a CPE
  - Previously suckling mouse brain or intrathoracic mosquito inoculation

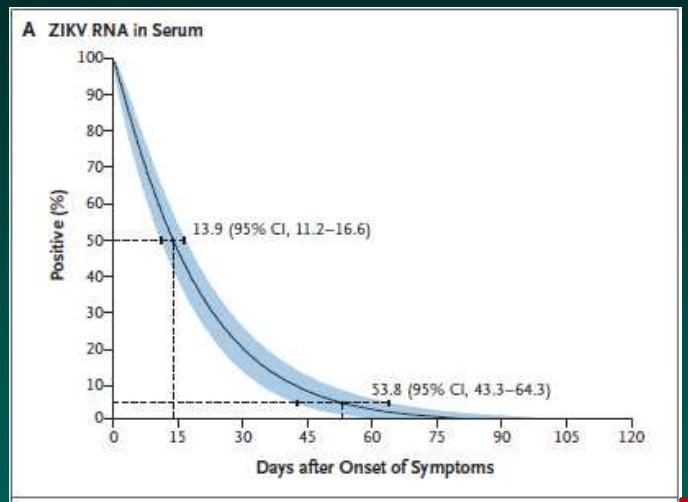
NAAT detection

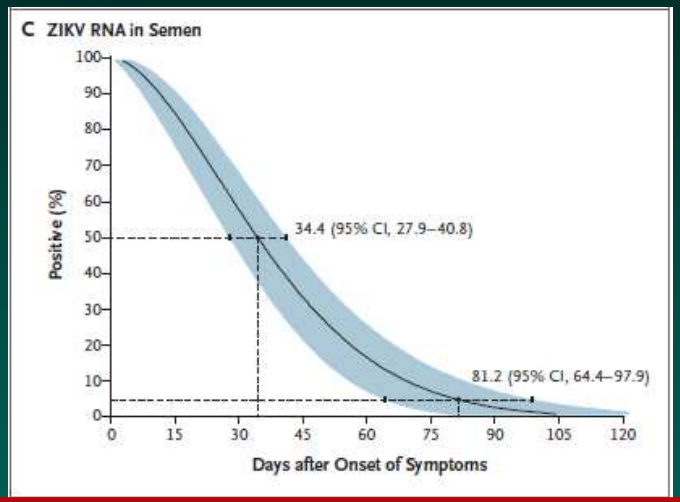
- Commercial assays available, many in-house assays
- Sensitivity peaks usually within 2-3 days in serum then declines rapidly through first week of illness
  - Can be serotype (DENV), lineage (CHIK, ZKV) variation in sensitivity
- Detection often dependent on body fluid
- Can also genotype, perform phylogenetic studies:
  - DENV 1-4
  - Zika virus Asian vs African lineage

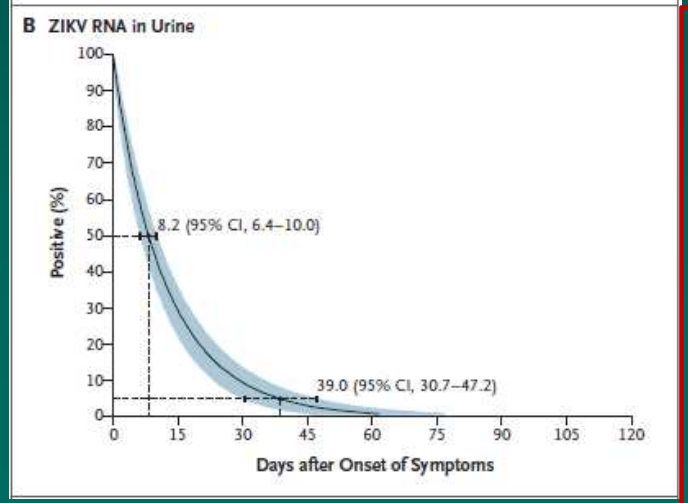
Detection of Zika Virus by specimen

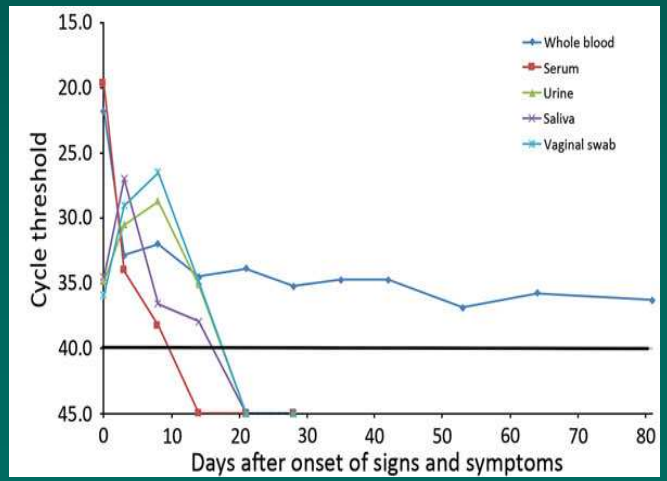
- Serum:
  - Up to 7 days
  - Case reports of prolonged viraemia in pregnancy ?due to foetal infection (NEJM 2016)
- Urine:
  - Up to 14 days
- Semen:
  - Detected by PCR up to 6 months after symptom onset, cleared by 3 months in 95%
  - Zika cultured from semen up to 93 days
  - Testicular atrophy, infertility reported in mouse model (Nature 2016)
- Vaginal fluids
  - PCR detection up to 14 days,
- Breast milk
  - RNA has been detected in breast milk.
  - Zika virus cultured from breast milk in one report (Lancet 2016;387(10023):1051)
  - No reports of transmission through breastfeeding.
- Eye
  - Up to 1 week

ZKV detection by PCR









- 150 Zika cases

Paz-Bailey et al., NEJM 2017

- single Zika case
  - Alpha/Flavivurses known to agglutinate some animal RBCs
  - WNV known to adhere to human RBCs (Rios et al, CID)

Murray et al., EID 2016

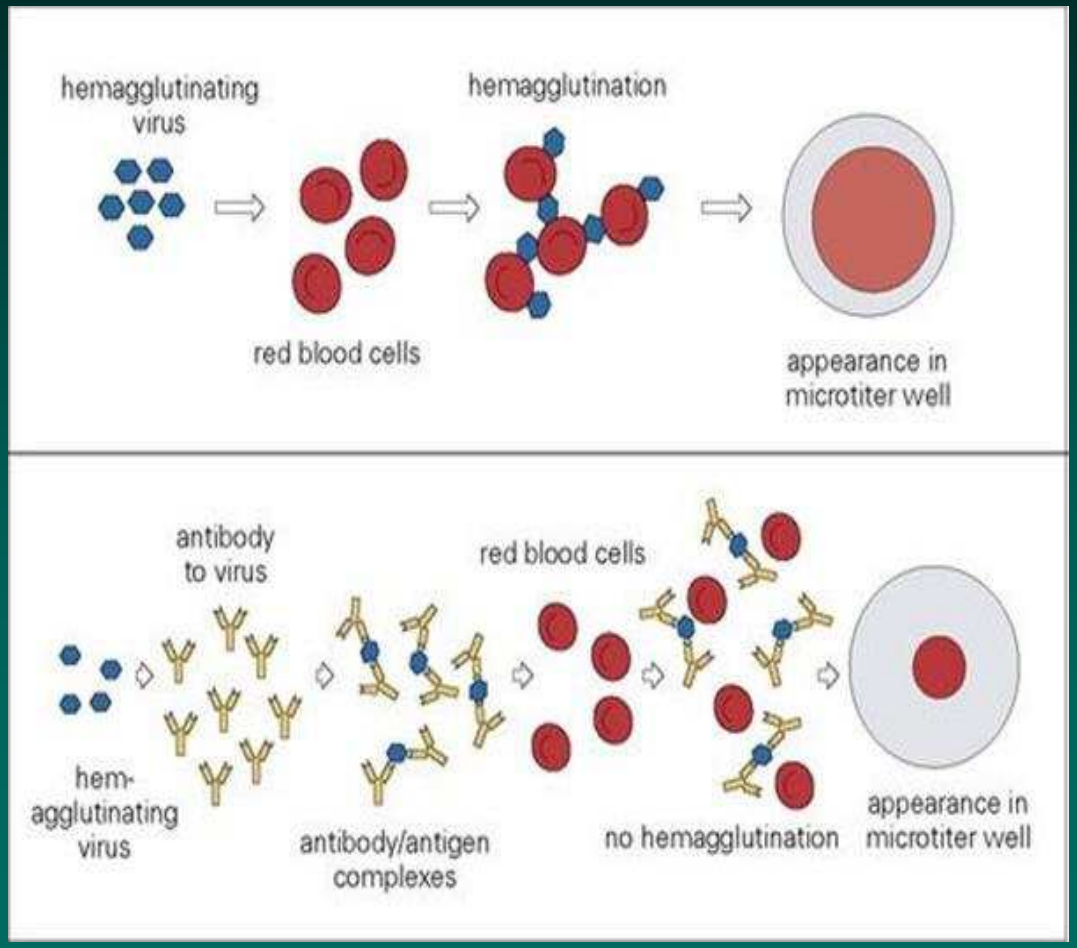
Arbovirus serological assays

- Serology
  - Serum, CSF

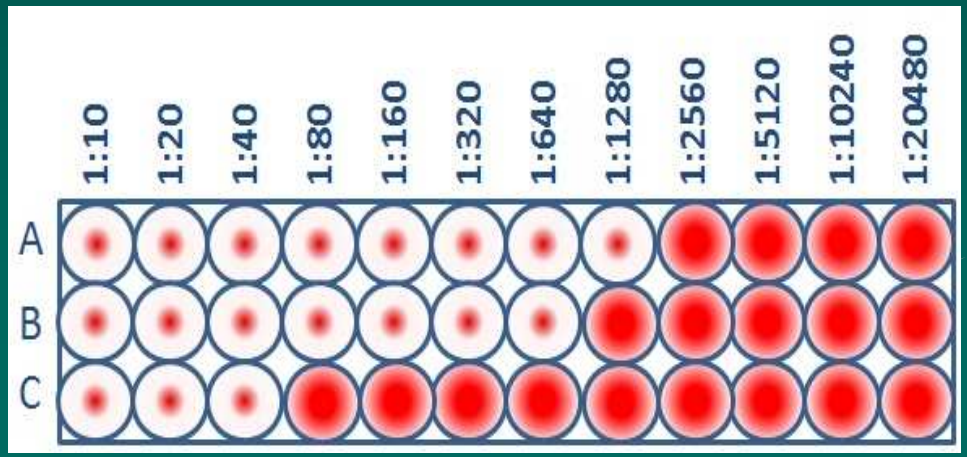
- Assays for the detection of IgM and IgG antibodies include:
  - enzyme immunoassay (EIA)
  - microsphere immunoassay (MIA)
  - haemagglutination inhibition assay (HI)
  - immunofluorescence assay (IFA).

- These assays provide a presumptive diagnosis
  - Need confirmatory testing in some circumstances, e.g. dengue IgM, NS1 Ag detection in a non-traveller
  - Confirmatory testing includes plaque reduction neutralization test (PRNT), other neutralisation platforms, monoclonal blocking EIAs.

Haemagglutination inhibition

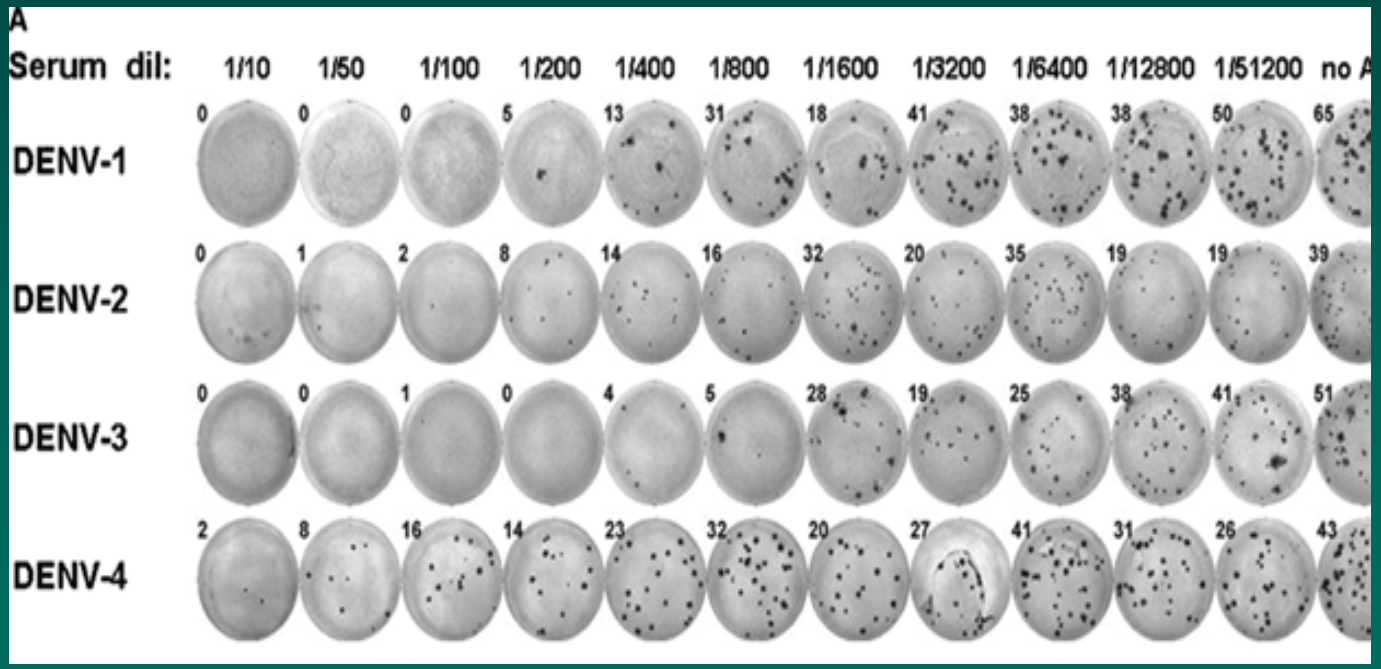


- Arboviruses naturally agglutinate goose RBCs, some mammalian RBCs
- May need to use virus-specific antigen



Plaque reduction neutralisation Titre (PRNT)

- Gold standard for arboviruses, other viruses, e.g. mumps
- Very laborious and difficult but measures virus-specific neutralising antibodies
- Must have a comparator virus



Interpreting arboviral laboratory serology results (1)

- Rise and fall of IgM antibodies:
  - IgM antibodies generally first detectable at 3-8 days after onset of illness and persist for 30-90 days

- Persistence of IgM antibodies:
  - Arboviral IgM antibodies may be detected in some patients months or years after their acute infection. e.g., up to 500 days for West Nile virus
  - virus-specific IgM antibodies in CSF, fourfold or greater rise in virus-specific antibody titres between acute- and convalescent-phase serum specimens more reliable.

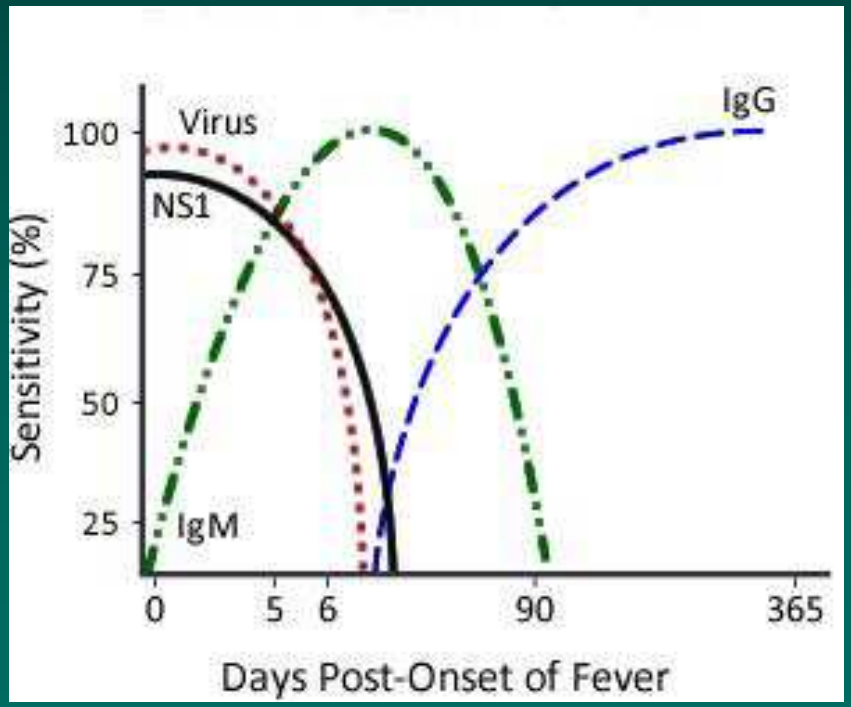
- Persistence of IgG and neutralizing antibodies:
  - Arboviral IgG and neutralizing antibodies can persist for many years.
  - The presence of these antibodies alone is only evidence of infection at some time

Interpreting arboviral laboratory serology results (2)

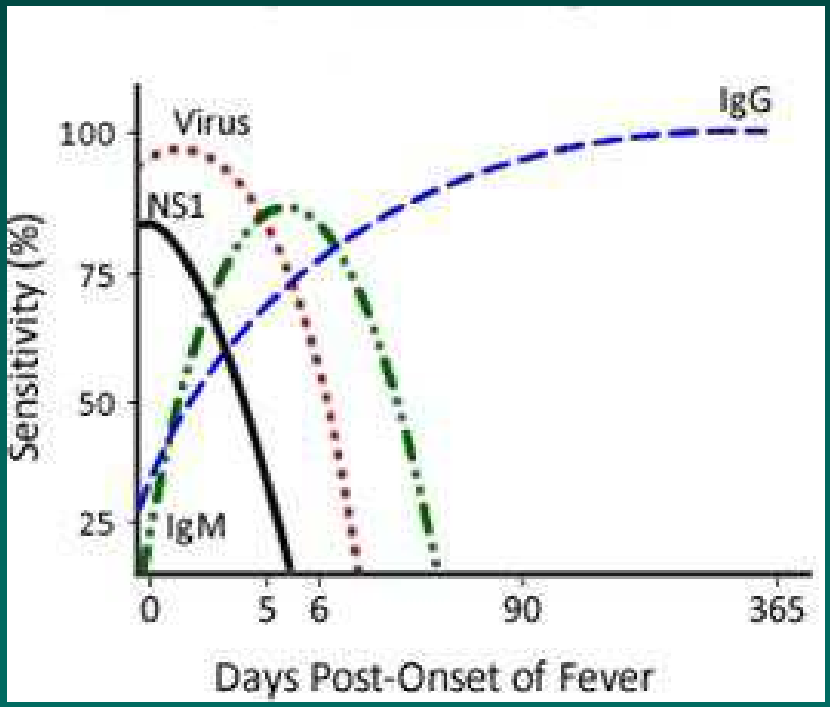
- Serologic cross-reactivity:
  - arboviruses from the same genus produce cross-reactive IgM and IgG antibodies.
  - Case report of Zika virus infection causing false positive dengue NS1 Ag
    - used SD Dengue Duo NS1 Ag device (Swiss Med Wkly 2016;146)
    - Platelia Dengue NS1 Ag kit not found to cross-react in 65 acute ZKV cases (EID 2016;22:1692)
- In regions where two or more closely-related arboviruses occur, problem of 'Original antigenic sin'
  - React to past flavivirus infection before reacting to current flavivirus infection
    - higher titre to past virus infection or false negative for recent infection
  - Problem with past infection or vaccination
    - Previous Dengue infection or YF vaccination and recent Zika
    - Recent KUNV/MVEV and past MVEV/KUNV

Serology profile depends on primary vs secondary infection

Primary DENV Infection

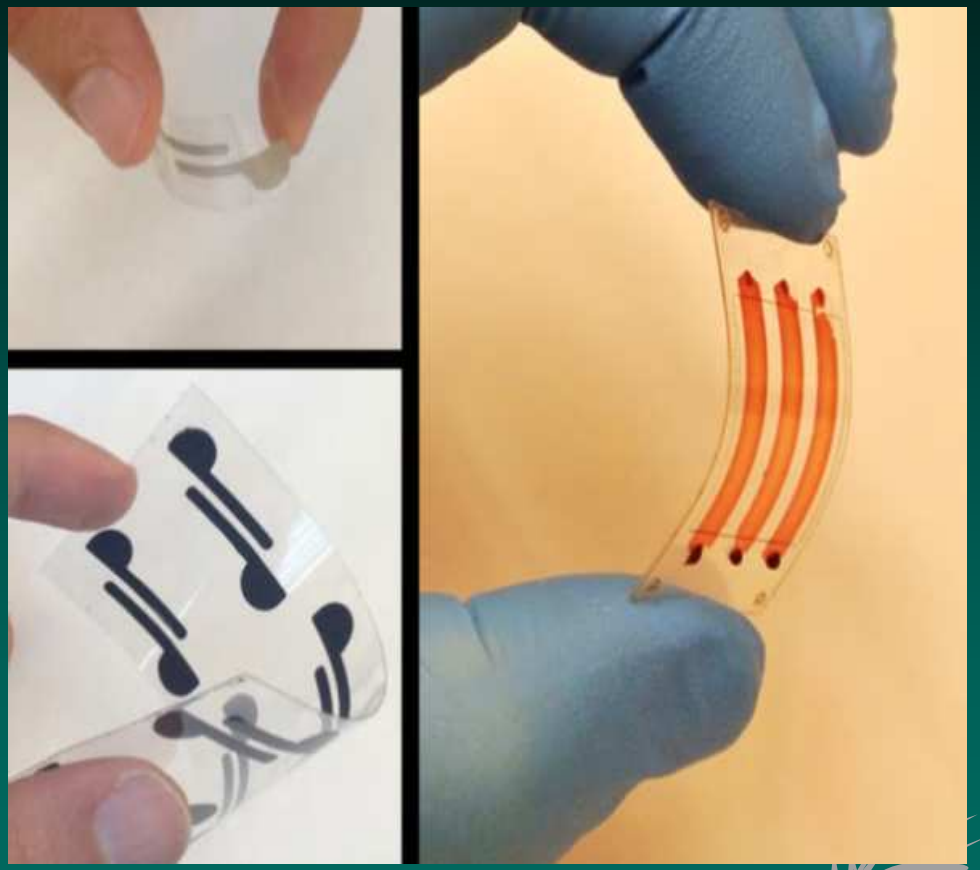


Secondary DENV Infection



New testing modalities

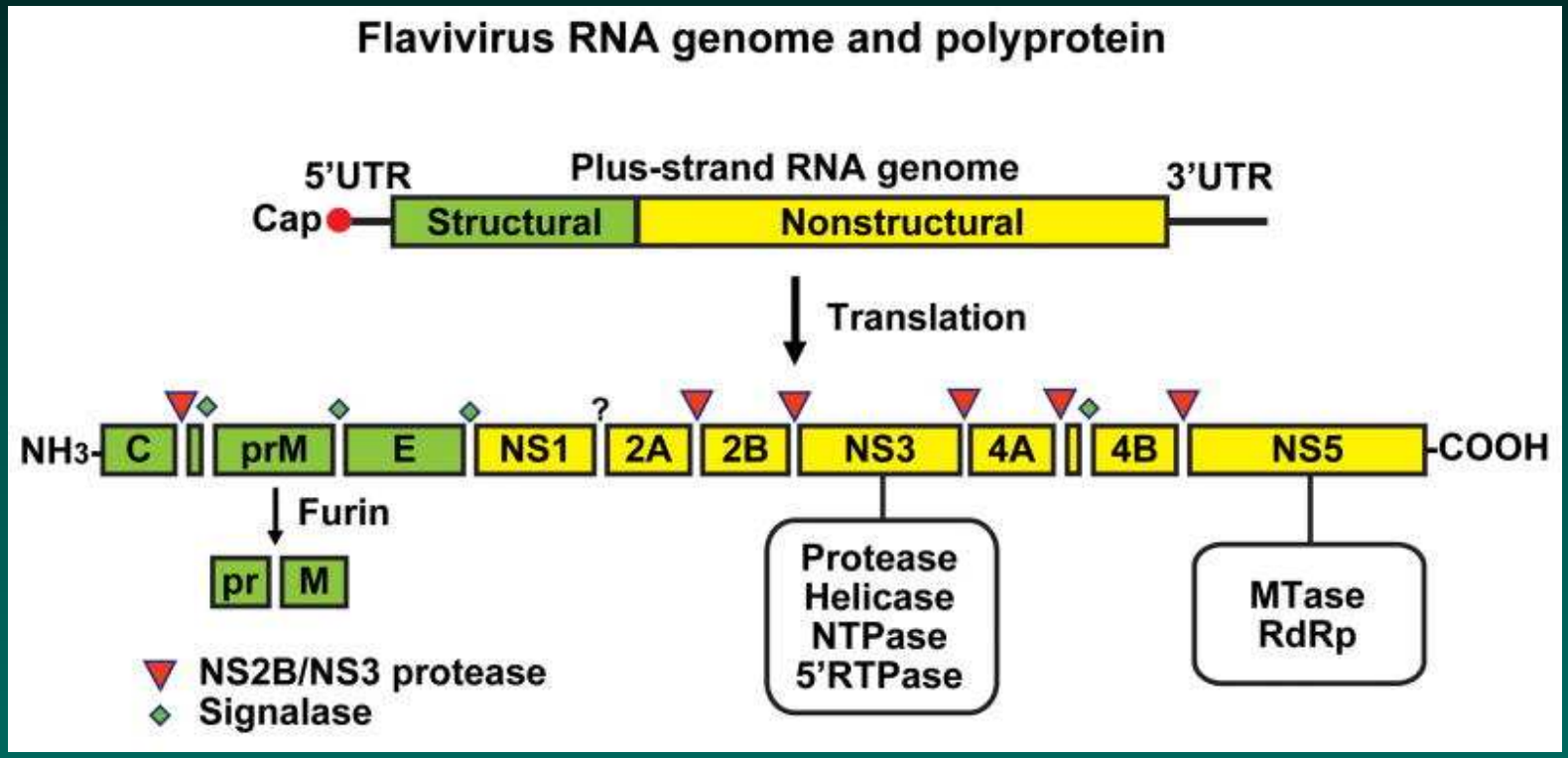
- Microfluidics advances:
  - Can test saliva, blood, urine
  - Little skill required
  - Cheap, rapid TAT
  - Suitable as POCT
  - Being adapted to ZIKV detection



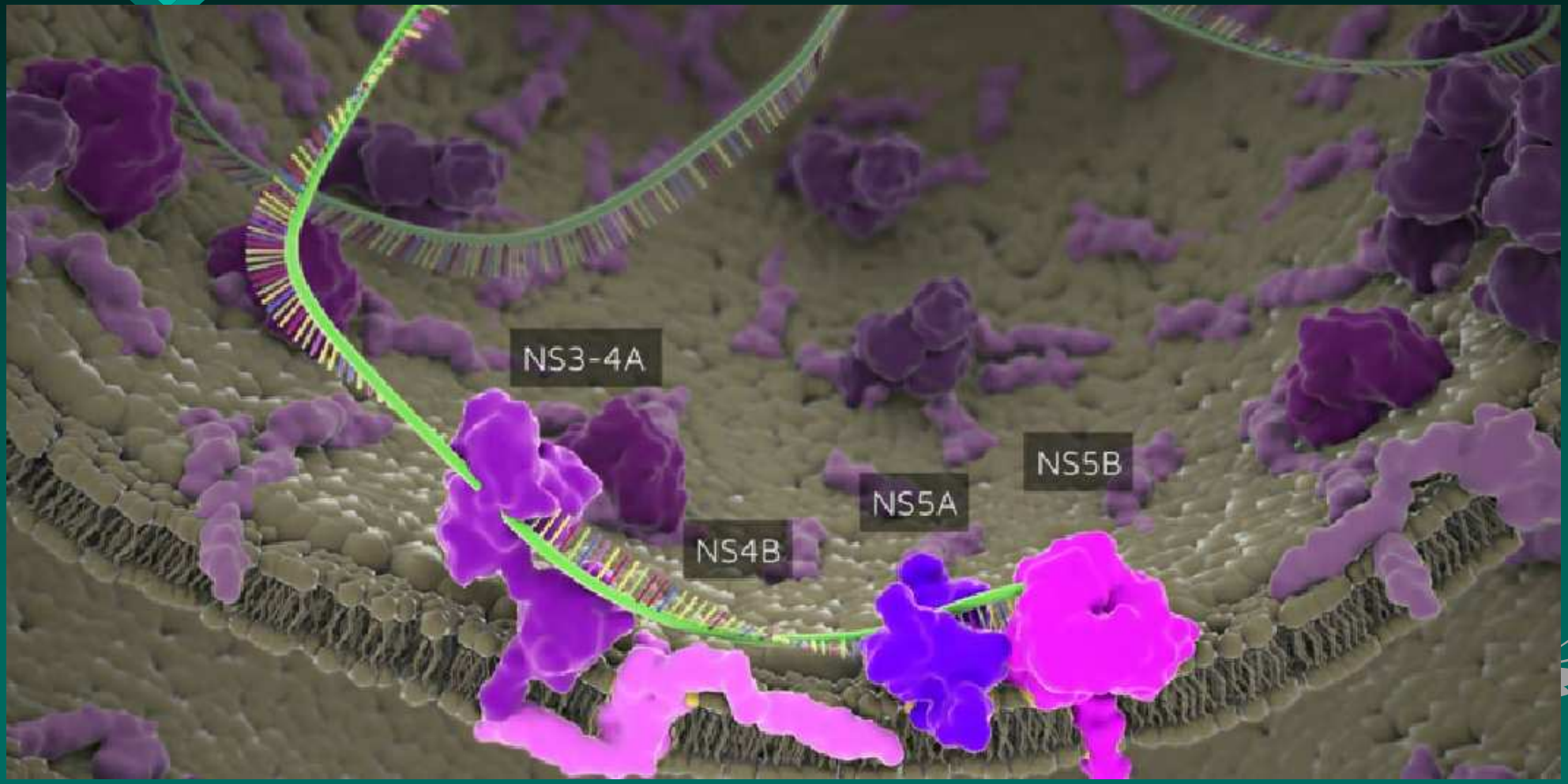
Arbovirus treatment

- No specific antivirals available
- Symptomatic treatment
  - Rest
  - Drink fluids to prevent dehydration
  - Paracetamol to reduce fever and pain
    - Avoid NSAIDs and aspirin in DNV infection
- Experimental therapies for flaviviruses
  - Neutralising antibody
    - Ab to envelope, NS1 flavivirus proteins
    - Zika antibody therapy protected foetus in mouse model
  - Repurposing existing compounds
  - Antiviral development (hepatitis C in Flaviviridae family)

Flavivirus genome



Flavivirus replication



Existing drugs

- Nitazoxanide
  - Antiparasitic drug for Giardia
  - Active against JEV, DENV-2 and YFV in cell culture
  - Active against JEV in mouse model
- Bromocriptine
  - Dopamine agonist
  - Active against DENV 1-4, TBEV in focus reduction assays
- Ivermectin
  - Broad-spectrum antiparasitic drug
  - Active against DENV 1-4, ZIKV

Broad-spectrum agents for flaviviral infections: dengue, Zika and beyond

Veaceslav Boldescu[1,2], Mira A. M. Behnam[1]*, Nikos Vasilakis[3] and Christian D. Klein[1]*

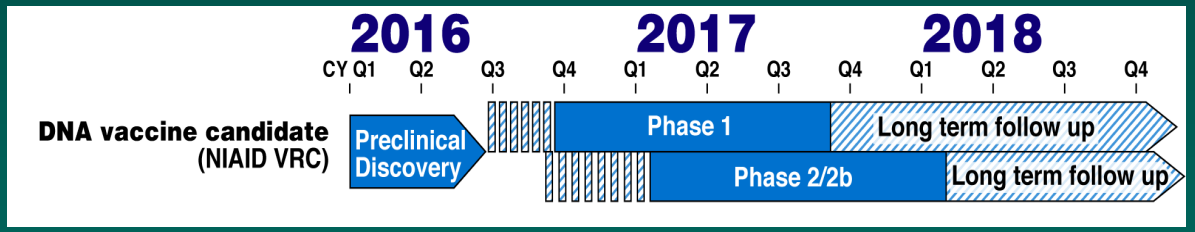
- Nature Reviews / Drug Discovery
  - online 5 May 2017 doi:10.1038/nrd.2017.33

Arbovirus (flavivirus) human vaccines

- Vaccines available in Australia
  - JEV: live and inactivated
    - Some animal model evidence for cross protective antibody response to MVEV
  - Yellow Fever: live 17D virus
    - Travel requirements, current shortage (African, Brazil outbreaks and supply problem)
    - WHO recommending 1/5 dose in outbreaks
- Vaccines available overseas
  - Dengue: live attenuated tetravalent
    - Hindered by concerns of immune enhancement
    - Dengvaxia®
  - TBE: inactivated
    - Can access through travel clinics in Aust via SAS

Zika vaccines

- 40 vaccine candidates:
  - 5 entering phase 1 studies by Feb 2017
    - Inactivated, mRNA, DNA vaccines
  - 2-3 years for registration, expected 2020



Rubella: The last virus to cause an epidemic of congenital defects

WHO agenda for arbovirus vaccines

- Area 1 (Dengue):
  - development of second generation dengue vaccines
  - dengue diagnostic algorithms post dengue vaccine introduction
- Area 2 (Zika):
  - analyse Zika vaccines, diagnostics and therapeutics
  - define strategic priorities
- Area 3 (Yellow fever):
  - Yellow fever vaccine fractional dose agenda
- Area 4 (Arboviruses in general):
  - advance the arboviral vaccine development agenda

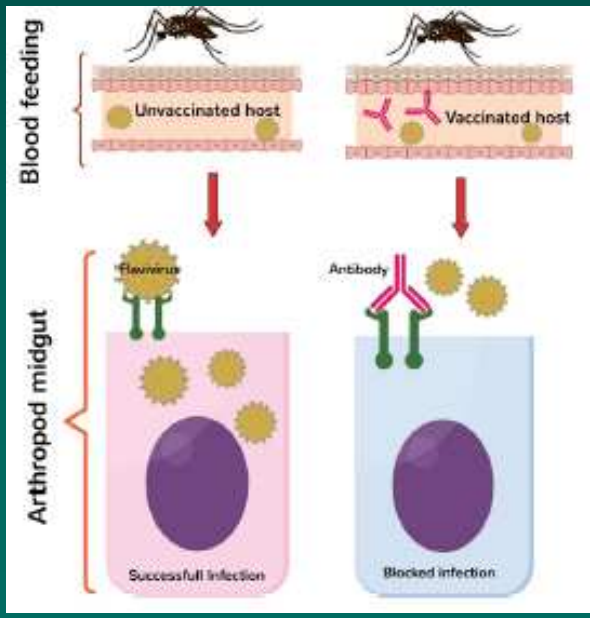
Novel vaccine strategies

- Vaccines in animals/development/experimental
  - Killed RRV (Wressnigg et al 2015 Clin Vacc Immunol)
  - CHIK (Metz et al 2013 PLoS Negl Trop Dis)
  - WNV DNA equine vaccine
  - MVE mouse model (Hall et al 1996, J Gen Virol)
  - Rift Velley Fever

- Baculovirus vaccines
  - Insect virus non-pathogenic in humans
  - Express other enveloped virus glycoproteins correctly and at high level

- Transmission blocking vaccines
  - Stop mosquito infection rather than human infection

Parasites & Vectors 2016



Summary

- Clinical
  - Non-neurological
    - Fever,
    - Fever and rash
    - Fever, rash arthralgia
  - Neurological
    - Encephalitis
    - Congenital cerebral malformation
- Laboratory diagnosis of arboviruses complicated by:
  - High rate of asymptomatic cases
  - Serological cross-reactivity
  - Brief early viraemia in many arbovirus infections limiting direct detection
- Treatment
  - Symptomatic at present, novel inhibitors in the future