



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



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

MVE **KUN** MVE **KUN RRV KUN** DEN **BFV RRV RRV BFV** MVE **BFV** MVE RRV **RRV RRV BFV** BFV **RRV RRV RRV BFV BFV RRV BFV**



National Arbovirus and Malaria Advisory Committee

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



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 nonneuroinvasive





Illnesses due to Arboviruses

Non-neurological:

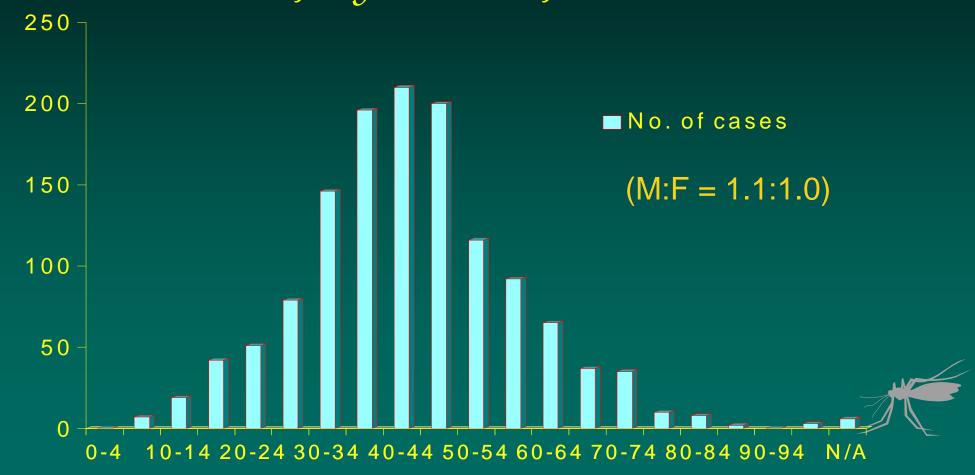
- Polyarthralgic illness: Ross River, Barmah Forest, Kunjin/West Nile virus, Kokobera, Gan Gan, Trubanaman, Chikungunya, Dengue
- Fever and rash: Ross River, Barmah Forest, Dengue, Zika
- Febrile illness: Dengue, MVE, Kunjin, JE
- Haemorrhagic fever: Dengue, CCHF, Rift Valley Fever, Hantavirus,
 Yellow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

Neurological

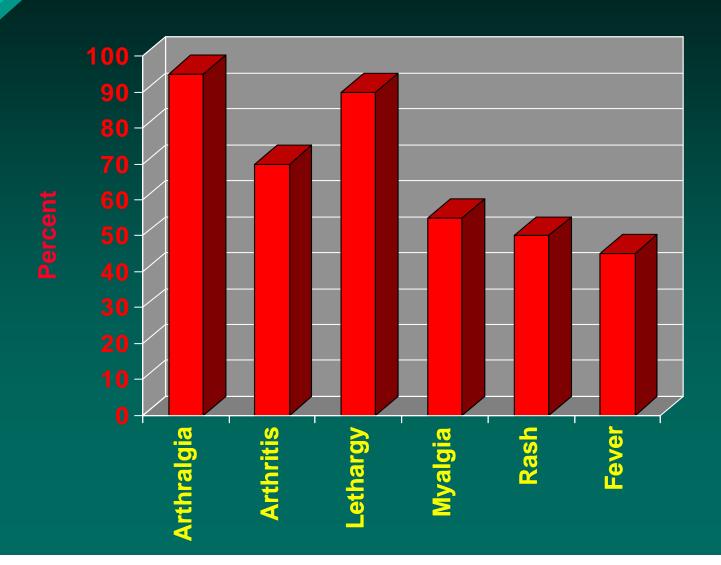
- Meningitis/Encephalitis/Acute flaccid paralysis: MVE, Kunjin, JE,
 TBE, Rift Valley Fever
- Guillain-Barre Syndrome: Zika
- Congenital cerebral malformations: Zika
- Ocular: Rift Valley Fever



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 Joint pain : Any : ≥ 1 month : ≥ 6 months	30% 70 - 86% 40% ≥ 10%	61 - 80% 83 - 98% 80 - 98% 57%
Myalgia	70 - 80%	43 - 67%
Fatigue	80%	62 - 94%
Fever	50%	20 - 59%
Lymphadenopathy	7%	0.6 - 20%



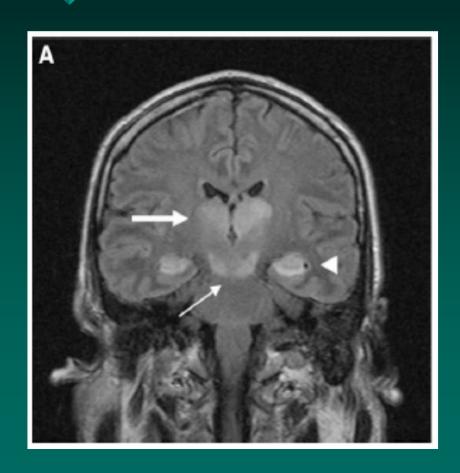


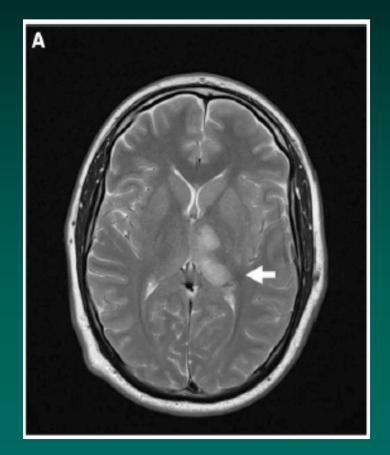
Murray Valley Encephalitis Virus

- 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









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%





Clinical features: Zika c.f. dengue and chikungunya

Features	Zika	Dengue	Chikungunya
Fever	++	+++	+++
Rash	+++	+	++
Conjunctivitis	++	-	-
Arthralgia	++	+	+++
Myalgia	+	++	+
Headache	+	++	++
Hemorrhage	-	++	-
Shock	-1	+	-

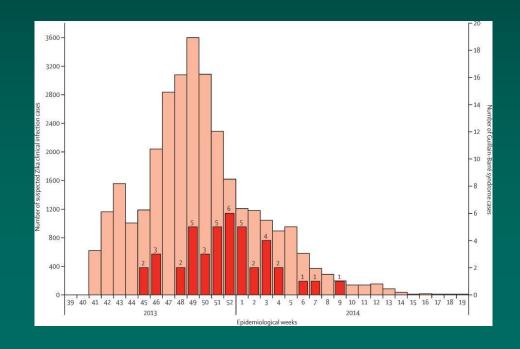
Rabe, Ingrid MBChB, MMed
"Zika Virus- What Clinicians
Need to Know?" (presentation,
Clinician Outreach and
Communication Application Action (Call, Atlanta, GA) January 26
2016)



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



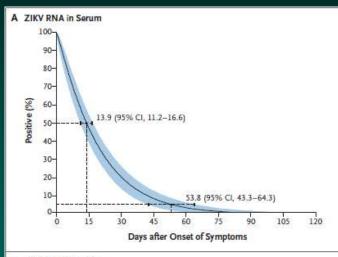


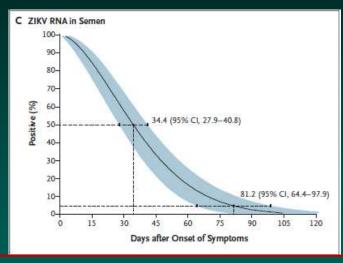
Detection of Zika Virus by specimen

- 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



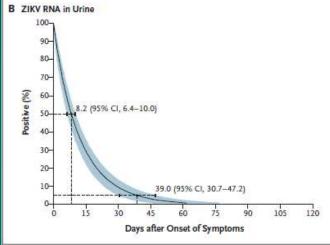
ZKV detection by PCR

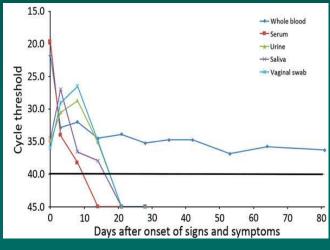




* 150 Zika cases

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



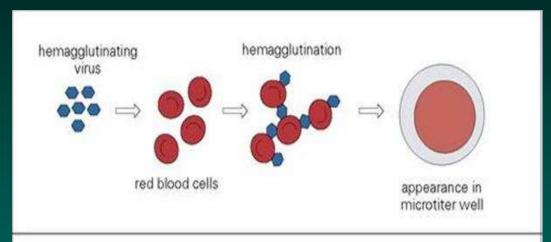
Arbovirus serological assays

- 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.

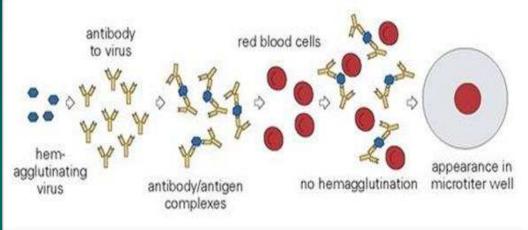


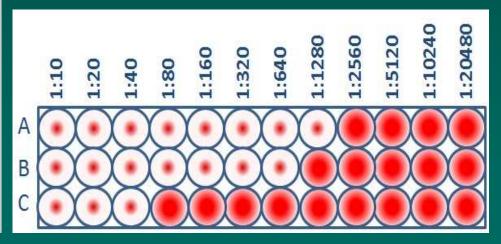


Haemagglutination inhibition



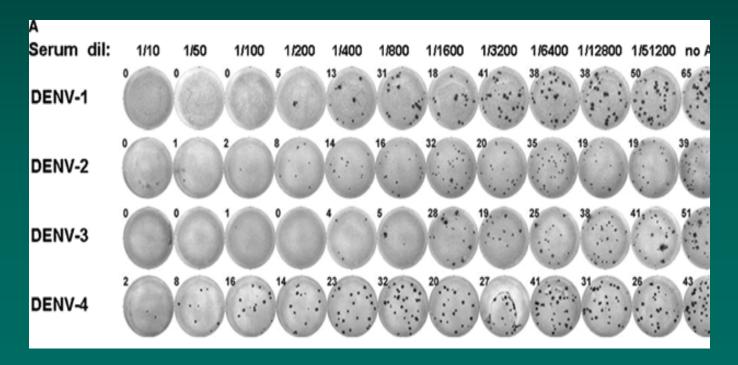
- Arboviruses naturally agglutinate goose RBCs, some mammalian RBCs
- May need to use virus-specific antigen





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 virusspecific 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



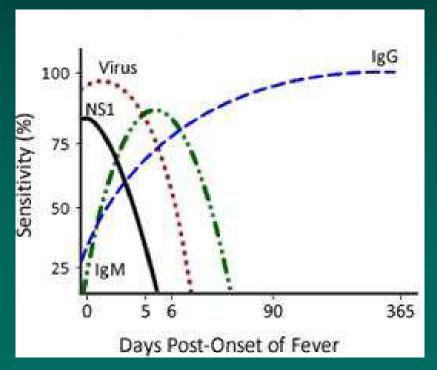


Serology profile depends on primary vs secondary infection

Primary DENV Infection

(%) Ainitisues 196 NS1 50 25 IgM 0 5 6 90 365 Days Post-Onset of Fever

Secondary DENV Infection



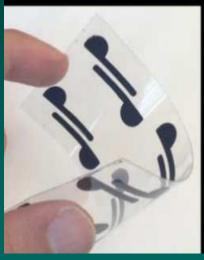


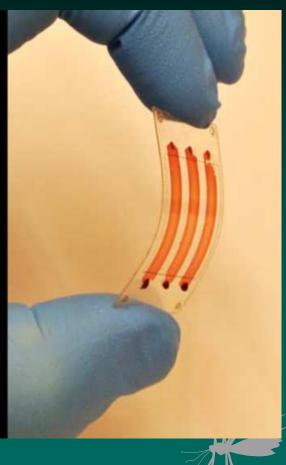


New testing modalities

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









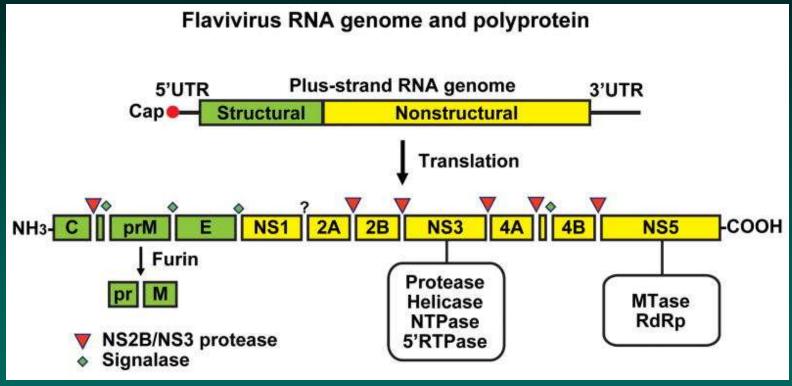
Arbovirus treatment

- 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)





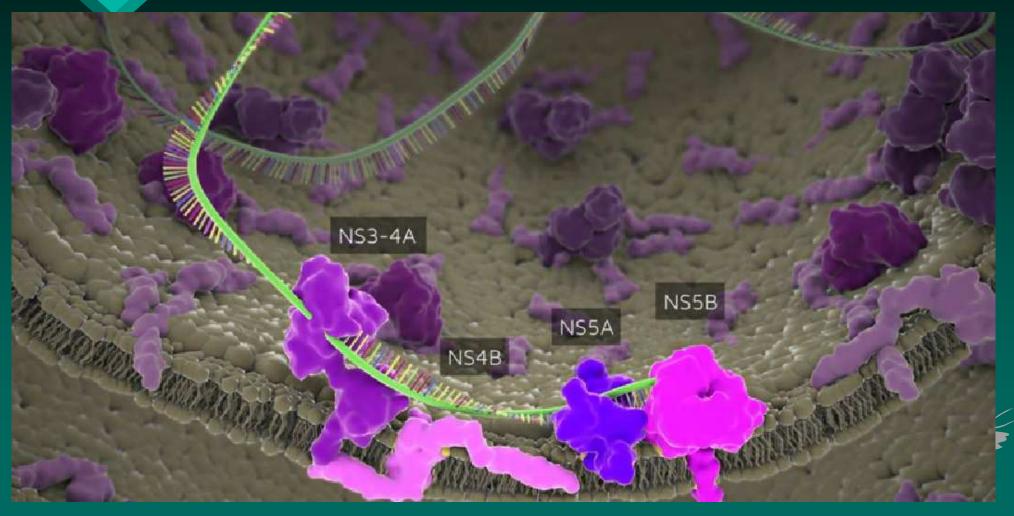
Flavivirus genome







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

Value of the second of the

- 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. Behnam¹*, Nikos Vasilakis³ and Christian D. Klein¹

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





Arbovirus (flavivirus) human vaccines

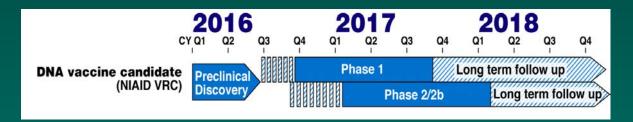
- Vaccines available in Australia
 - JEV: live and inactivated
 - Some animal model evidence for cross protective antibody response to MVEV
 - Yellow Fever: live 17D virus
 - Travel requirements, current shortage (African, Brazil outbreaks and supply problem)
 - WHO recommending 1/5 dose in outbreaks
- 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





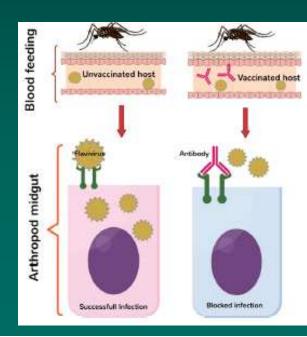
Novel vaccine strategies

- 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