Update on Clinical Management, Laboratory Diagnosis and Infection Control of Zika Virus Infection

19 February 2016

Ad Hoc Clinical Infection & Public Health Forum: An Update on Zika Virus

Centre for Health Protection ICB / Hospital Authority IDCTC

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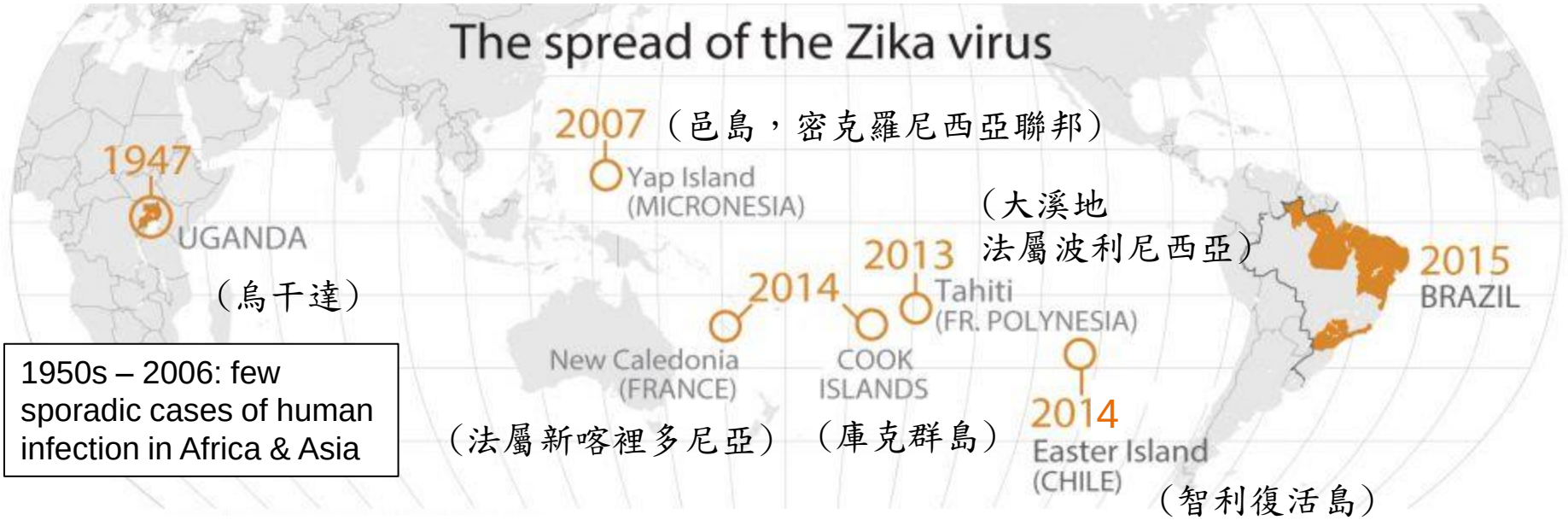
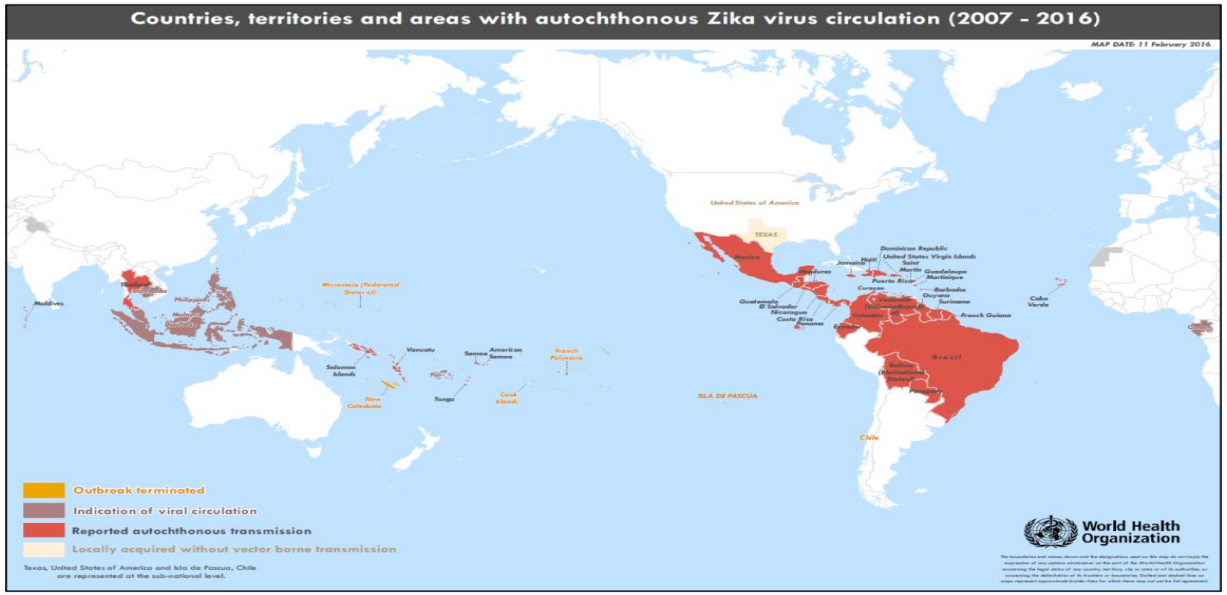
WHO: Public Health Emergency of International Concern (1 February 2016)

CHP: statutory notification of Zika virus (5 February 2016)



1950s – 2006: few sporadic cases of human infection in Africa & Asia

April 1947: isolation of ZIKV from a febrile sentinel rhesus macaque（恒河猴）in the Zika Forest of Uganda



~40 countries with locally acquired cases (12 Feb 2016)

Duffy MR et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med 2009;360:2536-43.
WHO: Zika situation report – 12 February 2016. http://who.int/emergencies/zika-virus/situation-report/12-february-2016/en/

Flaviviridae

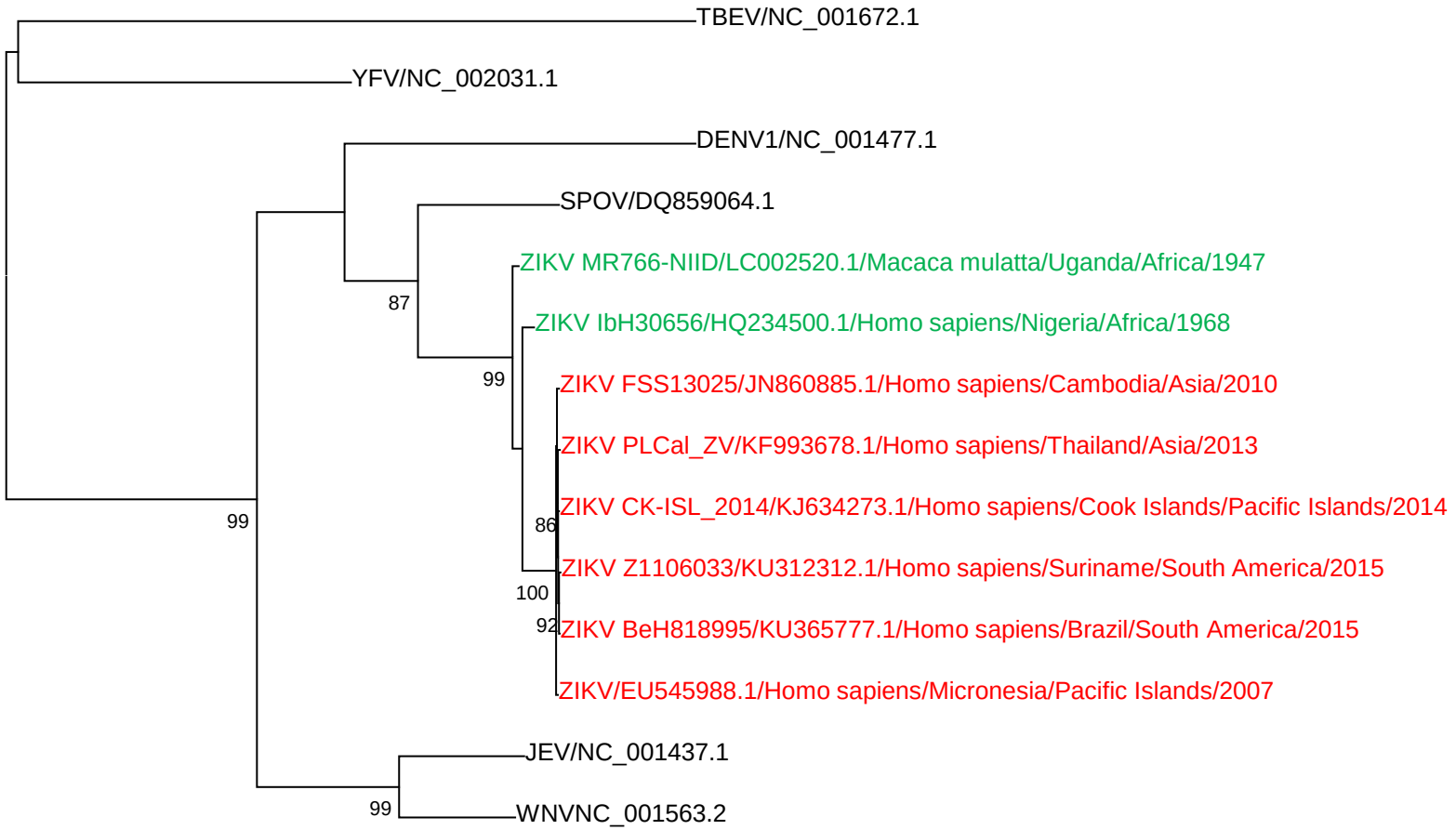
73 flaviviruses:

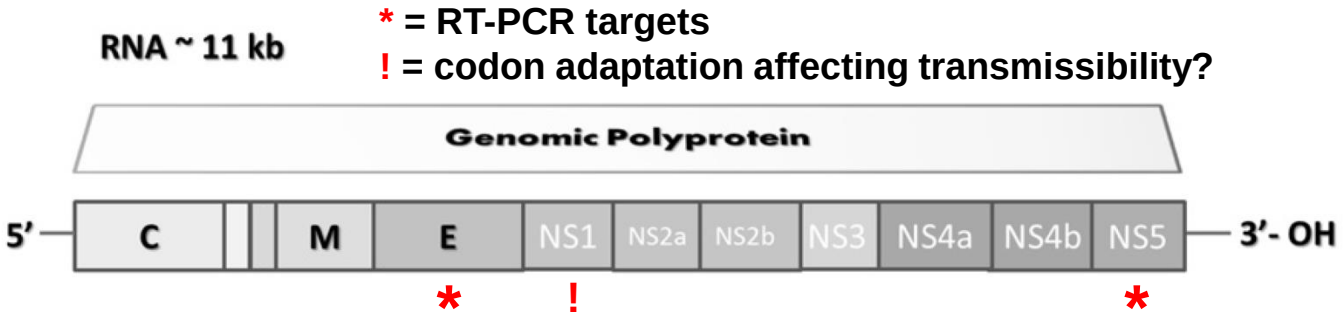
- **34 mosquito-borne (22 human pathogens)**
- **17 tick-borne (13 human pathogens)**
- **22 zoonotic**

Common or important human pathogens:

1. **Dengue** virus
2. **Japanese encephalitis** virus
3. **West Nile** virus
4. **Yellow fever** virus
5. **Zika virus**
6. (Spondweni virus, Tick-borne encephalitis virus in Russia, St. Louis encephalitis virus, Murray Valley encephalitis virus)

Phylogenetic tree of selected ZIKV strains with partial nucleotide sequences of E gene





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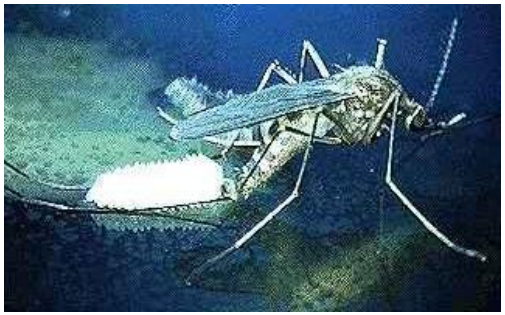
Mosquito-borne infectious diseases reported in Hong Kong

Aedes albopictus (白紋伊蚊)

Dengue (登革熱)
Chikungunya (基孔肯雅)

Anopheles sp. (瘧蚊)
Malaria (瘧疾)



Culex tritaeniorhynchus (三帶喙庫蚊)
Japanese encephalitis (日本腦炎)

Mosquito species which are potential vectors for the transmission of ZIKV

Mosquito species	Findings
Genus *Aedes*	
Ae. aegypti	Considered as the primary vector for ZIKV transmission; natural infection reported. *In vitro*: susceptible to ZIKV infection with a short extrinsic incubation period of 5 days.
Ae. africanus	Natural infection: first mosquito species from which ZIKV was isolated from.
Ae. albopictus	Natural infection: *Ae. albopictus* reported (Gabon). *In vitro*: can be infected by and transmit >20 different arboviruses including ZIKV.
Ae. apicoargenteus	Natural infection reported.
Ae. dalzieli	Natural infection reported; phylogenetic analysis shows evidence to support its role as an important vector for ZIKV transmission in West Africa.
Ae. furcifer	Natural infection reported.
Ae. hensilli	Susceptible to ZIKV infection *in vitro*; the most abundant mosquito species on Yap Island (but no virus isolation was made from field-collected mosquitoes).
Ae. hirsutus	Natural infection reported.
Ae. luteocephalus	Natural infection reported; exhibited potential to transmit ZIKV *in vitro*.
Ae. metallicus	Natural infection reported.
Ae. opok	Natural infection reported.
Ae. taylori	Natural infection reported.
Ae. unilineatus	Natural infection reported; susceptible to ZIKV infection *in vitro*.
Ae. vittatus	Natural infection reported; exhibited potential to transmit ZIKV *in vitro*.
Genus *Anopheles*	
An. coustani	Natural infection reported.
An. gambiae s.l.	Natural infection reported.
Genus *Culex*	
Cx. perfuscus	Natural infection reported.
Genus *Mansonia*	
Ma. uniformis	Natural infection reported.

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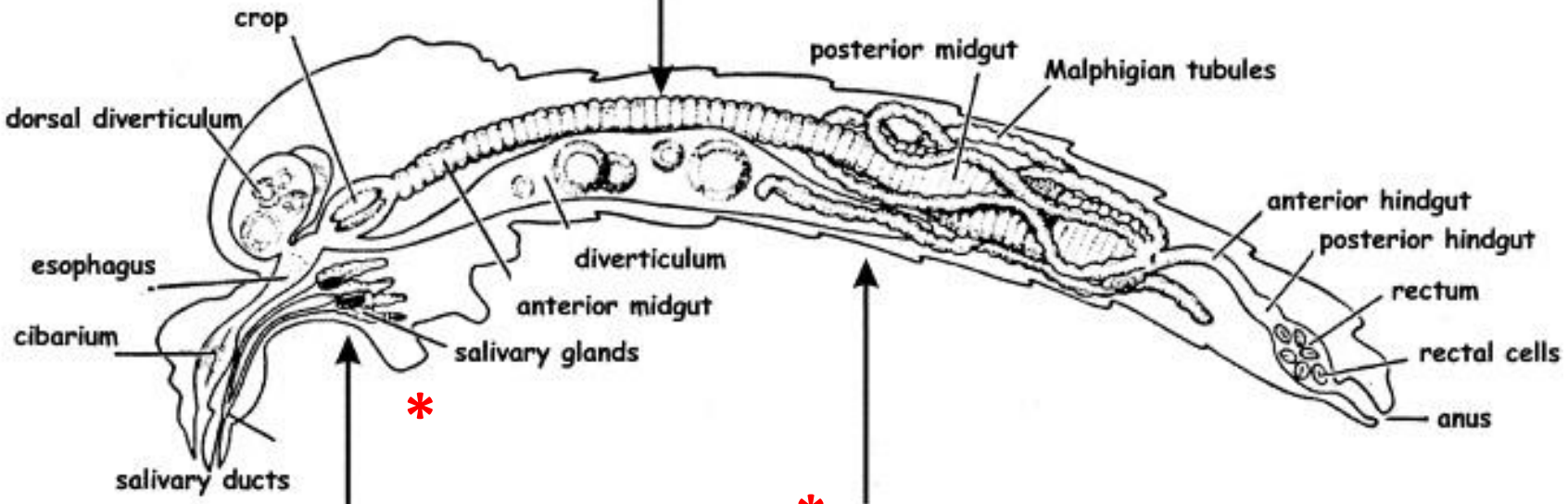
Midgut Infection Barrier

1 => establish an infection in the midgut epithelium

2 => replicate in the midgut epithelium cells



Midgut Escape Barrier

3 => pass through the basal lamina

4 => replicate in other organs and tissues

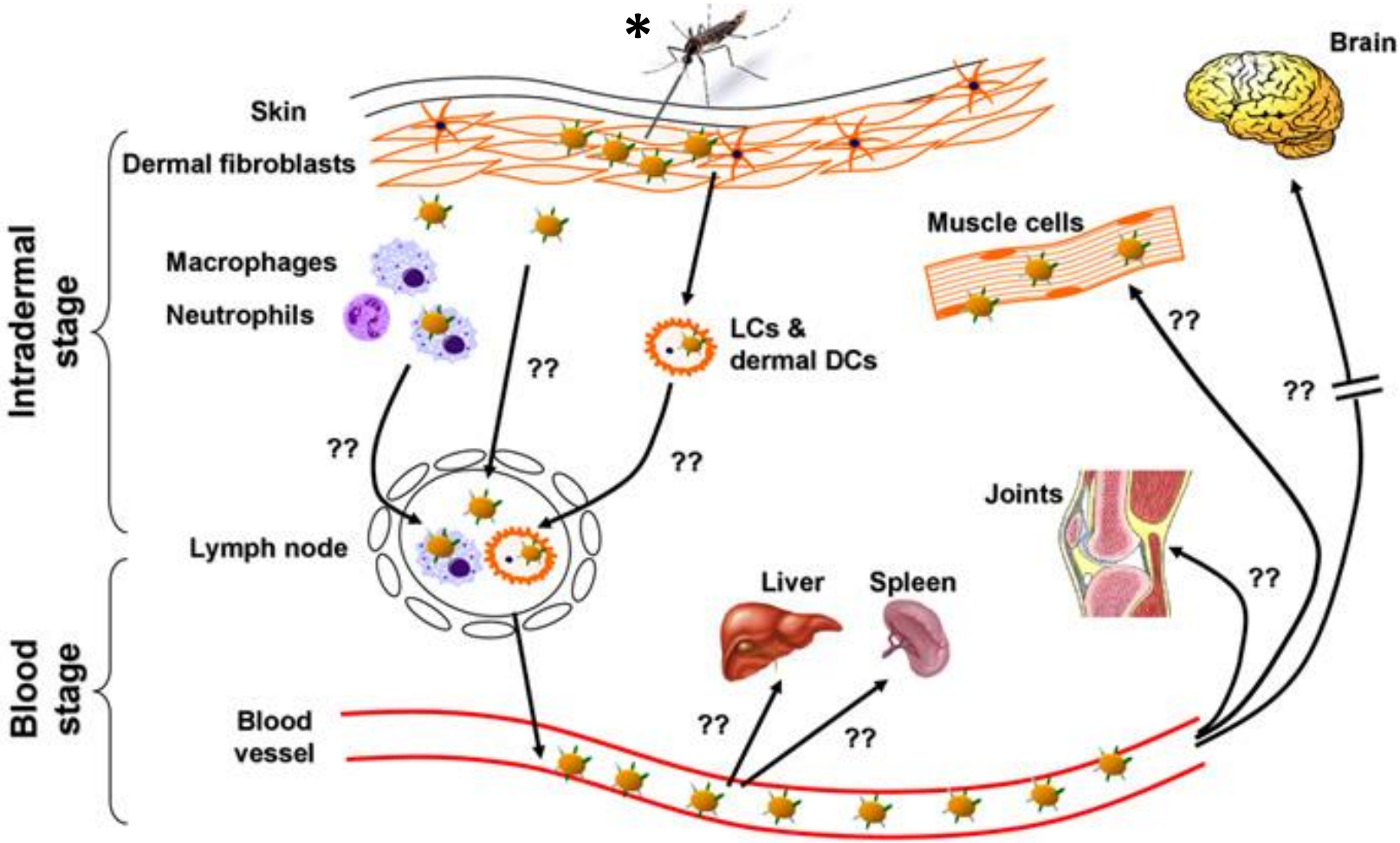
Transmission Barriers

5 => infect salivary glands

6 => escape into the lumen of the salivary gland

Extrinsic incubation period of ZIKV: 5 to 10 days

Wong PS et al. Aedes (Stegomyia) albopictus (Skuse): a potential vector of Zika virus in Singapore. PLoS Negl Trop Dis 2013;7:e2348.



- ZIKV infects human **dermal fibroblasts, epidermal keratinocytes & immature dendritic cells.**
- Human cell receptors: mainly **AXL & Tyro3 +/- TIM-1** for ectodermal epithelial cells; under influence of **gonadotropin-releasing hormone affecting neuronal survival & migration**; **DC-SIGN** for macrophage/dendritic cells.
- Cell infection inhibited by **neutralizing antibody, type I & II interferons & autophagy inhibitors**.
- Highly **neurotropic** in suckling mice
- Persistence of flaviviruses

Hamel R et al. Biology of Zika Virus Infection in Human Skin Cells. J Virol 2015;89:8880-96.

Potential non-vector-borne transmission routes of ZIKV

Transmission routes	Findings
Blood transfusion	• 2.8% (42/1505) of asymptomatic blood donors in French Polynesia (21 November 2013 to 17 February 2014) tested positive for ZIKV by RT-PCR.
Semen	• ZIKV was isolated by RT-PCR and viral culture (Vero cells) from the semen of a patient from French Polynesia (Tahiti) who presented with haematospermia. • An American scientist acquired ZIKV infection in Senegal and developed prostatitis and haematospermia. After he returned to USA, his wife (never travelled to Africa and Asia) developed symptoms and serological evidence of ZIKV infection after having sexual intercourse with the index patient. • The viral load in semen (10^7 copies/ml) may be higher than that in the concomitant urine (10^3 copies/ml) and serum (undetectable) samples.
Perinatal / Transplacental	• At least 2 episodes of perinatal transmission reported in French Polynesia. • Preliminary epidemiological and virological evidence support transplacental transmission of ZIKV leading to microcephaly and other congenital anomalies.
Breastfeeding	• Breast milk samples of infected women inoculated on Vero cells were positive for viral RNA by RT-PCR, but replicative virus particles were not detected.
Saliva	• Viral RNA can be detected in saliva of infected patients, but it is unknown whether replicative virus particles can be detected.
Monkey bite	• Suspected transmission after monkey bite.
Others (reported for other flaviviruses)	• Mucocutaneous: DENV and WNV • Haemodialysis: WNV • Organ transplantation (HSCT and SOT): DENV and WNV

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Clinical features & complications of ZIKV infection

Table 1. Clinical Characteristics of 31 Patients with Confirmed Zika Virus Disease on Yap Island during the Period from April through July 2007.

Sign or Symptom	No. of Patients (%)
Macular or papular rash **(pruritic)**	28 (90) *
Fever* **(low-grade; 37.8-38.5°C)**	20 (65)
Arthritis or arthralgia	20 (65)
Nonpurulent conjunctivitis	17 (55)
Myalgia	15 (48)
Headache	14 (45)
Retro-orbital pain	12 (39)
Edema	6 (19)
Vomiting	3 (10)

- **Dengue-like illness** in returning travelers from affected areas: watch out for co-infection (& don't forget OTHER pathogens!)
- Incubation period: 3-14 days (?)
- Symptomatic rate: 18% (?); self-limiting 2-7 days

Duffy MR et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med 2009;360:2536-43.

Table 1
Comparison of symptoms for dengue fever, chikungunya, and Zika.
Clinique comparée de la dengue, du chikungunya et du Zika.

Symptoms	Dengue	Chikungunya	Zika
Fever	++++	+++	+++
Myalgia/arthralgia	+++	++++	++
Edema of extremities	0	0	++
Maculopapular rash	++	++	+++
Retro-orbital pain	++	+	++
Conjunctivitis	0	+	+++
Lymphadenopathies	++	++	+
Hepatomegaly	0	+++	0
Leukopenia/thrombopenia	+++	+++	0
Hemorrhage	+	0	0

Adapted from Halstead, et al. and from the Yap State Department of Health Services presentation.

Not pathognomonic!

S. Ioos et al. / Médecine et maladies infectieuses 44 (2014) 302–307

Clinical features & complications of ZIKV infection

Clinical features	Comments
Asymptomatic	>80% of infected human are asymptomatic.
Zika fever	
Systemic	Fever, chills, rigors, malaise, headache, retro-orbital pain, anorexia, sore throat, lymphadenopathy (cervical, submandibular, axillary, and/or inguinal), hypotension, and conjunctivitis.
Neurological / Ophthalmological	Guillain-Barre syndrome, encephalitis, meningoencephalitis, paraesthesia, photophobia, vertigo, hypertensive iridocyclitis, auditory (bilateral dull and metallic hearing), facial paralysis, and myelitis.
Musculoskeletal	Myalgia, arthralgia with periarticular oedema (wrists, knees, ankles, and small joints of the hands and feet).
Gastrointestinal	Nausea, vomiting, diarrhoea, constipation, abdominal pain, and jaundice.
Genitourinary	Haematuria, prostatitis (perineal pain and mild dysuria), and haematospermia.
Dermatological and mucocutaneous	Diffuse maculopapular rash, pruritis, aphthous ulcer, and gingival bleeding.
Haematological	Leucopenia, neutropenia, lymphopenia or activated lymphocytes, monocytosis, thrombocytopenia, and elevated erythrocyte sedimentation rate; and immune-thrombocytopenic purpura.
Biochemical	Elevated serum lactate dehydrogenase, aspartate aminotransferase, γ-glutamyl transferase, C-reactive protein, fibrinogen, and ferritin levels.
Death	At least 3 deaths: • An adult male with systemic lupus erythematosus on corticosteroids, rheumatoid arthritis, and alcoholism. • A 16-year-old female who died in 1 month after symptom onset. • A neonate with microcephaly, foetal anasarca, and polyhydramnios who died within the first five minutes of birth.

- **Sickle cell anemia**

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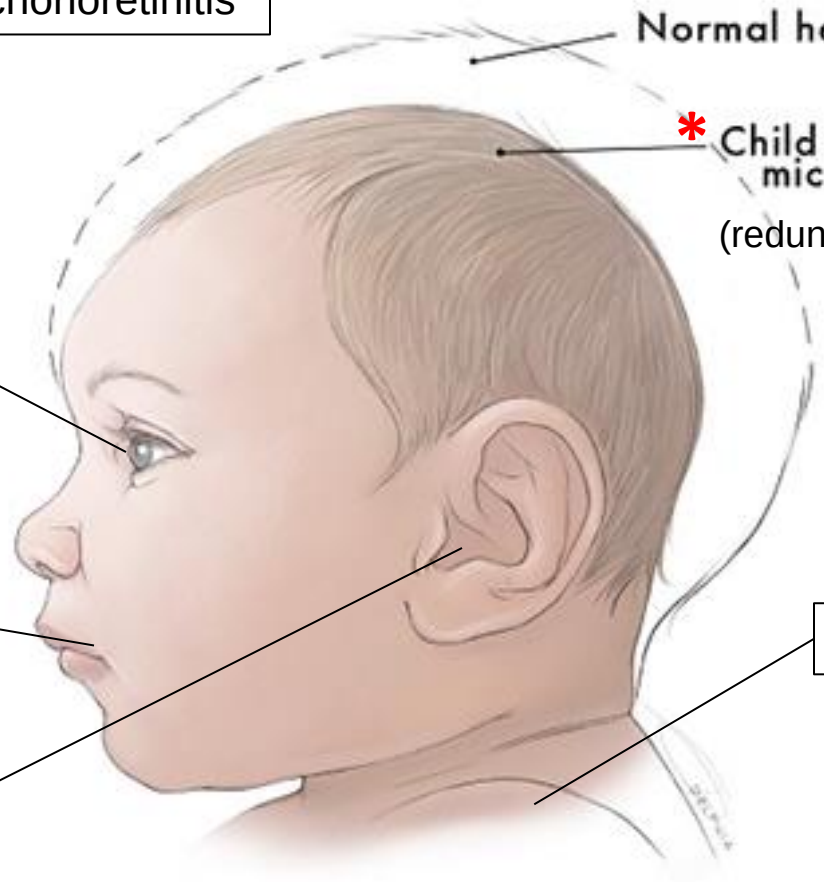
Suspected congenital ZIKV syndrome

General: LBW, ↓foetal movement, polyhydramnios, miscarriage; ?rash, ?hepatosplenomegaly, ?chorioretinitis



* Ophthalmological defects

Absence of swallowing (USG)

?Hearing

Arthrogryposis

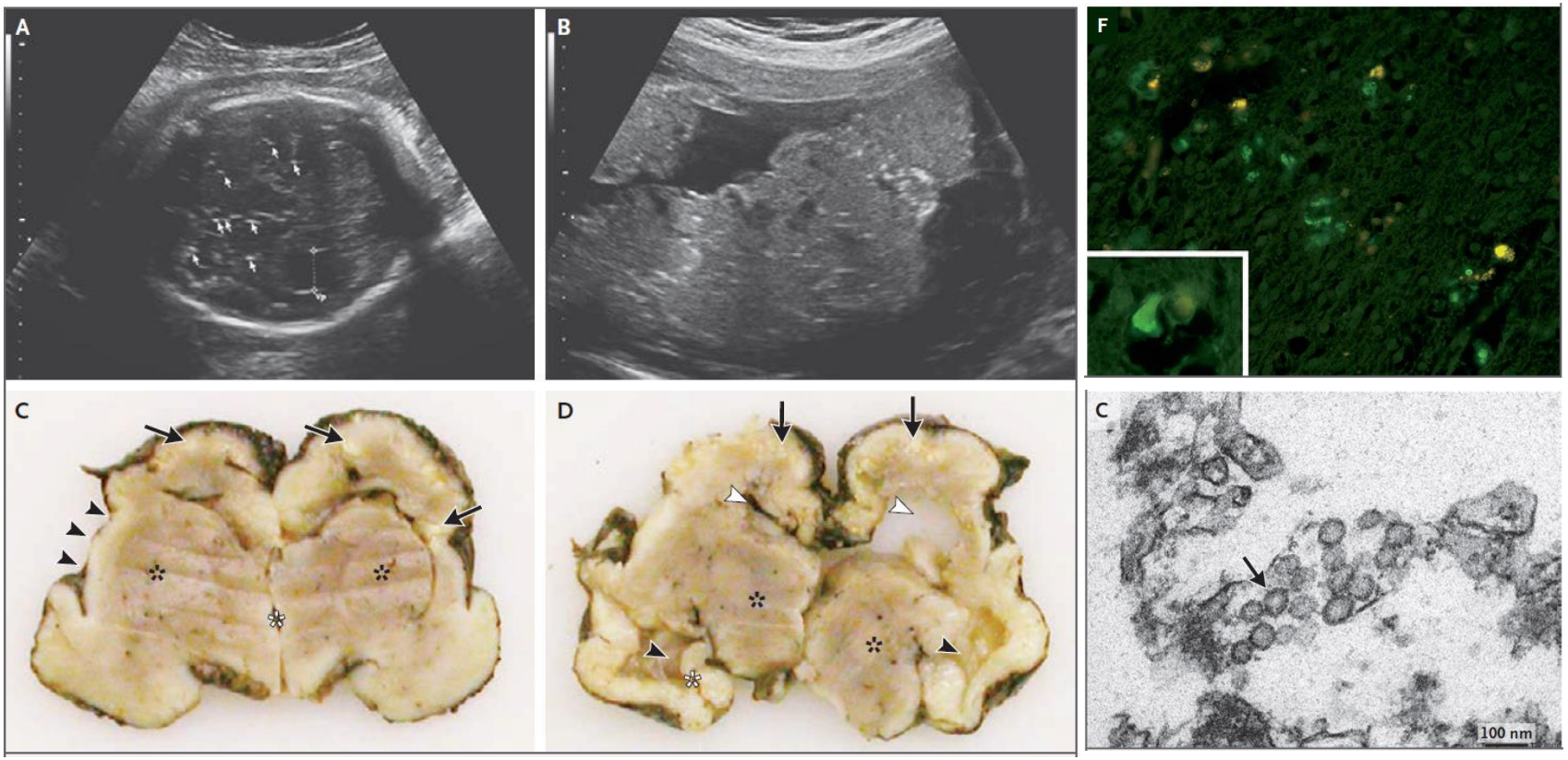
Head circumference <31.5-32 cm at birth; or ≥2 SD below the mean for sex and gestational age at birth

Suspected congenital ZIKV syndrome

USG: Intracranial calcifications & dilated lateral ventricle

USG: Placental calcifications

IF: intracytoplasmic reaction



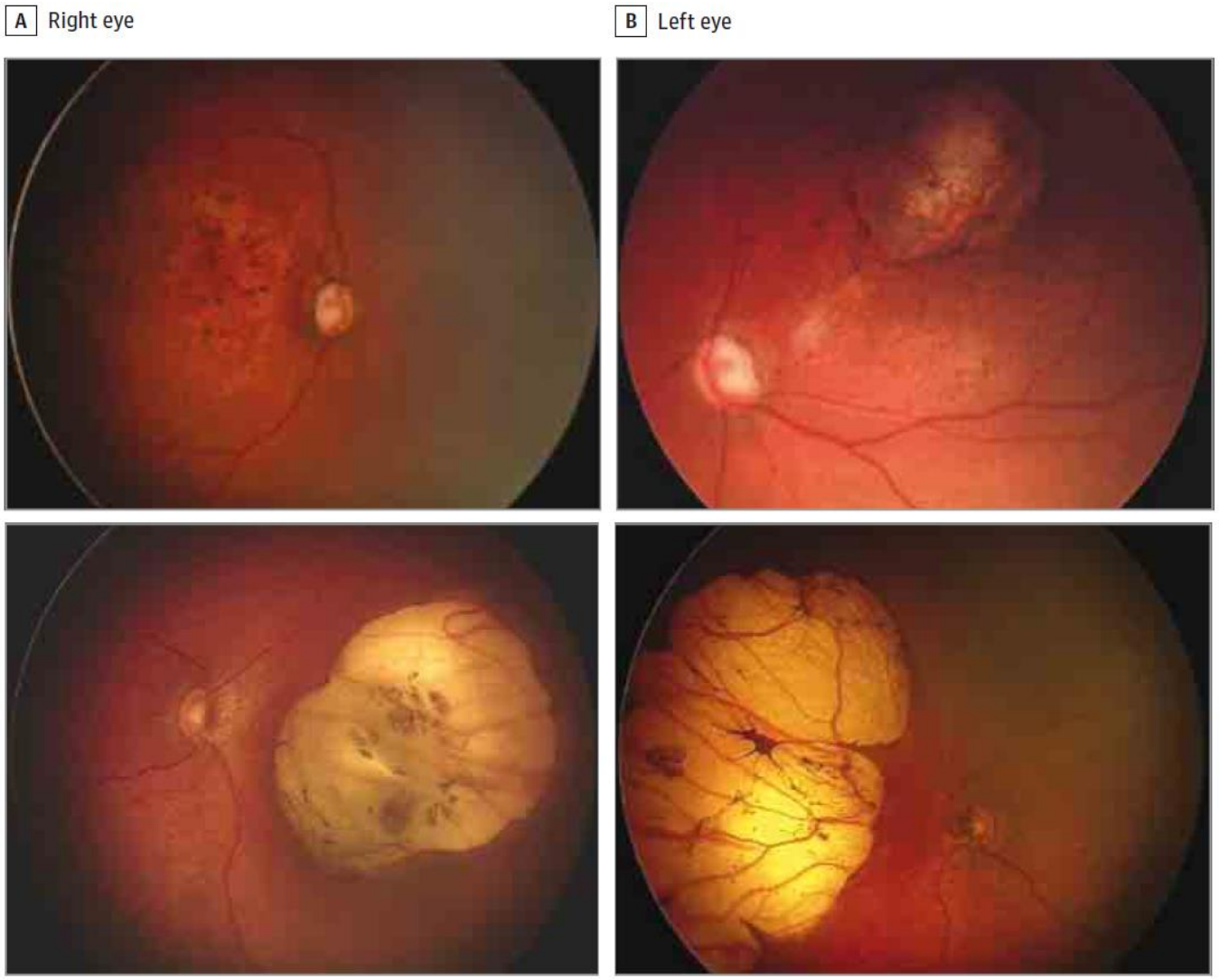
Cortical & subcortical white calcifications & loss of cortical gyration

Dilated lateral ventricles

EM: enveloped virions

Mlakar J et al. Zika virus associated with microcephaly. N Engl J Med. 2016. [Epub ahead of print]

Suspected congenital ZIKV syndrome



↑cup-disc ratio, macular pigmentary mottling

Optic disc hypoplasia, coloboma

de Paula Freitas B et al. Ocular Findings in Infants With Microcephaly Associated With Presumed Zika Virus Congenital Infection in Salvador, JAMA Ophthalmol. 2016. [Epub ahead of print]

Clinical features & complications of ZIKV infection

Clinical features	Comments
Suspected "congenital Zika syndrome"	
Clinical features	General: • Low birth-weight, reduced foetal movement, excessive/redundant scalp skin, foetal anasarca, polyhydramnios, and arthrogryposis; may be associated miscarriage. Neurological: • Microcephaly, polymalformative syndromes, brainstem dysfunction, and absence of swallowing. Ophthalmological: • Cataract, asymmetrical eye sizes, intraocular calcifications, macular alterations (gross pigment mottling and/or chorioretinal atrophy), optic nerve abnormalities (hypoplasia with double-ring sign, pallor, and/or increased cup-to-disk ratio), iris coloboma, and lens subluxation.
Ultrasonographic features	Brain atrophy, widespread brain calcifications (periventricular, cerebral parenchyma, thalami , and basal ganglia), lissencephaly, pachygyria, dysgenesis of corpus callosum, vermia, and thalami, enlarged cisterna magna, asymmetrical cerebral hemispheres, severe unilateral ventriculomegaly secondary to cortical/subcortical atrophy, displacement of the midline, and thinning of the parenchymal on the dilated side, thin pons and brainstem.
Histopathological features	Brain: • Multifocal collections of filamentous, granular, and neuron-shaped calcifications in the cortex and subcortical white matter with focal involvement of the whole cortical ribbon, occasionally associated with cortical displacement; diffuse astrogliosis with focal astrocytic outburst into the subarachnoid space; activated microglial cells; macrophages expressing HLA-DR; scattered mild perivascular infiltrates composed of T and B cells in the subcortical white matter; Wallerian degeneration of the long descending tracts in the brainstem and spinal cord; granular intracytoplasmic reaction in destroyed neuronal structures (indirect immunofluorescence test); visible virions in damaged cytoplasmic vesicles (electron microscopy). Placenta: • Focal calcifications in villi and decidua of placenta.

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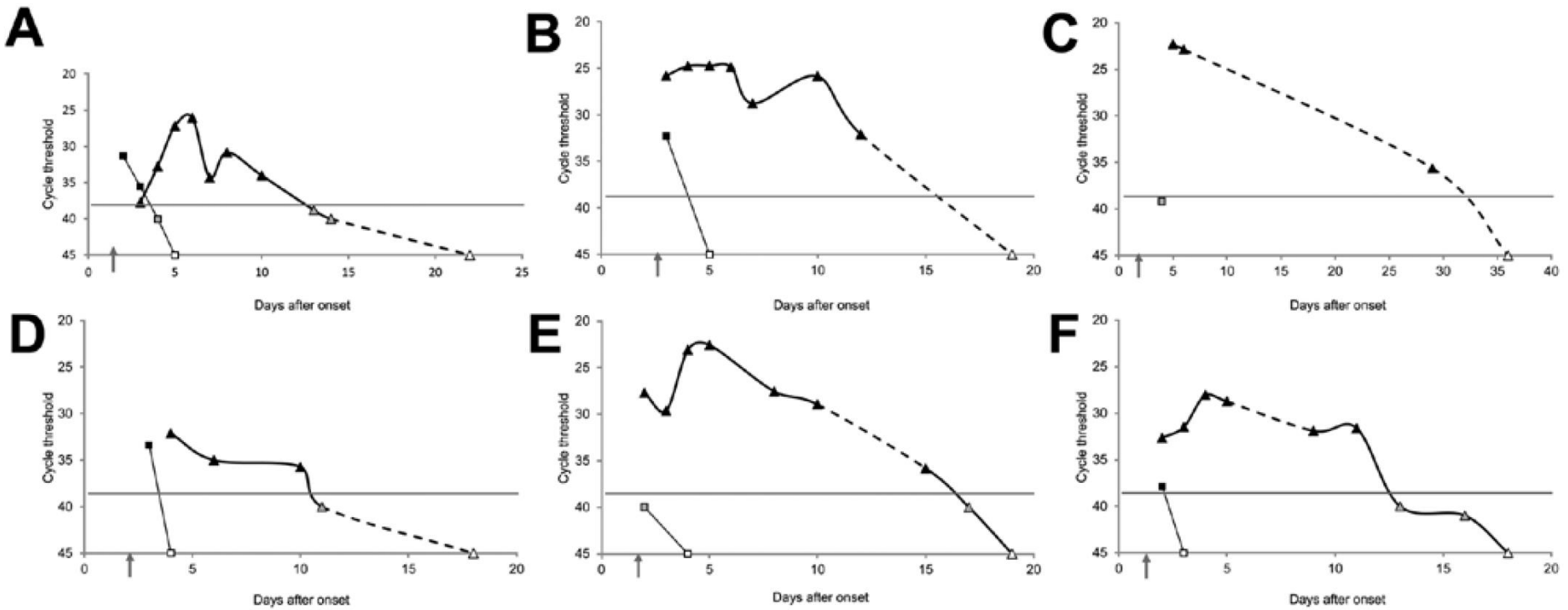
Who needs testing after recent travel to ZIKV-affected areas?

1. **Fever** with or without other symptoms; with or without pregnancy
2. **Complications:** GBS, meningoencephalitis, thrombocytopenic purpura
3. **Congenital CNS abnormalities:** microcephaly, intracranial calcifications, macular atrophy, chorioretinitis/scarring, etc.
4. Organ (kidney) or blood donors
5. Women symptomatic after sexual intercourse with men with travel Hx (lactating mother)

Laboratory diagnosis: RT-PCR (blood & urine)



Blood: usually **<5 days**; Urine: may be **>35 days**

Gourinat AC et al. Detection of Zika virus in urine. Emerg Infect Dis. 2015; 21:84-6.

Laboratory diagnosis: RT-PCR (saliva)

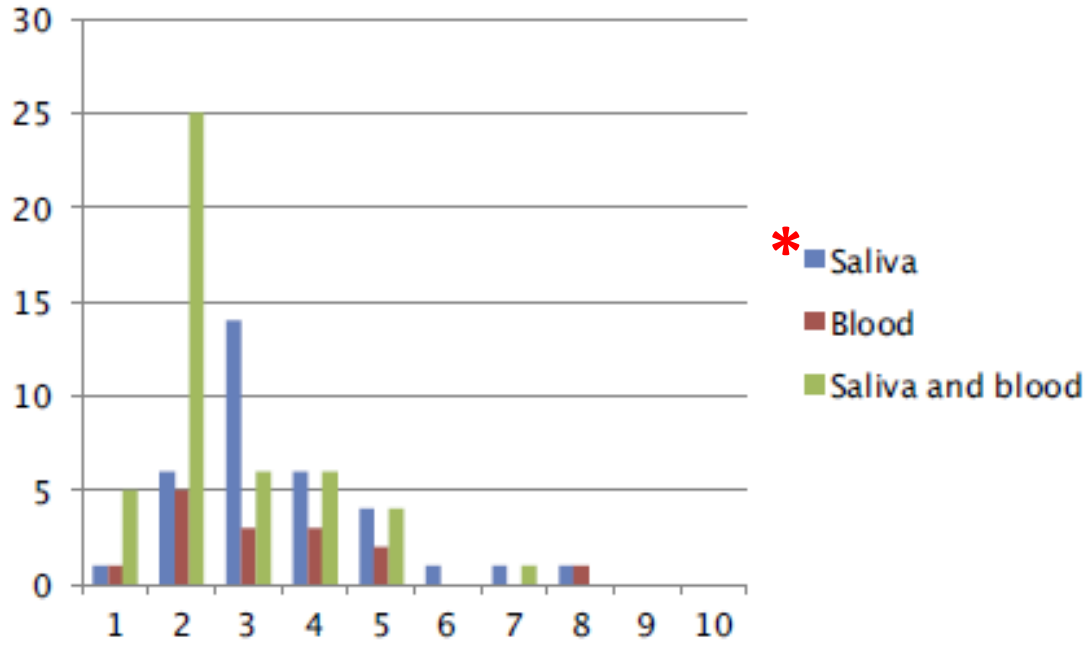


Fig. 1. Proportion of positive samples (Y axis in %) according to the number of days after symptoms onset (X axis) for the 182 patients with saliva, blood or both samples tested by ZIKV RT-PCR.

Table 1
ZIKV RT-PCR results for patients with both samples collected.

		Saliva	
		Positive	Negative
Blood	Positive	52 (28.6%)	16 (8.8%)
	Negative	35 (19.2%)	79 (43.4%)

Musso D et al. Detection of Zika virus in saliva. J Clin Virol. 2015; 68:53-5.

Specimen types for laboratory diagnosis of ZIKV infection

Specimen types	Laboratory diagnostics	
	RT-PCR	Viral culture
Serum (<5d; may be ≥11d)	• Most cases have short-lived (≤5 days of symptom onset) and low-level viraemia • Rarely, viral RNA may be detected in serum on as late as 11 days of symptom onset	• Infectious ZIKV has been detected in human blood collected on as early as the day of symptom onset (Vero cells) • 3/34 (8.8%) of archived serum samples which were RT-PCR-positive for ZIKV yielded infectious viral particles in Vero cells
Urine (≥36d)	• Higher viral load than concomitant serum samples • Positive from day 2-3 to after day 30 of symptom onset	• Successful isolation in Vero E6 cells; may be especially useful in patients with genitourinary symptoms.
Semen ***haematospermia; ≥62d**	• Higher viral load (10^7 copies/ml) than concomitant urine (10^3 copies/ml) and serum (undetectable) samples • May be positive for ≥62 days of symptom onset	• Successful isolation Vero cells; may be especially useful in patients with genitourinary symptoms.
Nasopharyngeal swab	• Positive in a patient whose concomitant serum and wound (monkey bite) samples were negative	• May be complimentary to serum and urine for suspected ZIKV infection.
Saliva	• Viral RNA is more frequently detected in saliva than blood • Positive in both neonates and adults • Complimentary but cannot replace serum samples	• May be complimentary to serum and urine for suspected ZIKV infection.
Amniotic fluid	• Positive in two pregnant women whose foetuses had ultrasounographic evidence of microcephaly	• May be useful in infants with suspected congenital ZIKV infection.
Foetal / placental / umbilical cord tissue	• Positive in a neonate with congenital anomalies (microcephaly, foetal anasarca, and polyhydramnios) who died within the first 5 minutes of life • Positive in the brain of 4 full-term infants (2 as miscarriage and 2 with microcephaly) with suspected congenital ZIKV infection	• May be useful in infants with suspected congenital ZIKV infection.
Cerebrospinal fluid	• May be useful in infants with suspected congenital ZIKV infection or patients with neurological complications	• May be useful in infants with suspected congenital ZIKV infection or patients with neurological complications.
Skin biopsy	• May be useful to exclude concomitant infections in patients with persistent or atypical rash	• May be useful to exclude concomitant infections in patients with persistent or atypical rash.
Joint fluid	• May be useful to exclude concomitant infections in patients with persistent or recurrent arthritis	• May be useful to exclude concomitant infections in patients with persistent or recurrent arthritis.
Bone marrow	• May be useful to exclude concomitant infections in patients with unusually persistent or severe cytopenia	• May be useful to exclude concomitant infections in patients with unusually persistent or severe cytopenia.
Other tissues	• Brain, liver, spleen, and pooled visceral (kidney, lung, and heart) tissues were positive in a fatal case (an adult male with co-morbidities and immunosuppressive treatment)	• May be useful to exclude concomitant infections in patients with unusually severe or fatal infection.

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Laboratory diagnosis: summary

1. Specimens: **serum, urine**, **saliva**, semen, NPA/S, fetal tissue, amniotic fluid & cerebrospinal fluid.

2. **RT-PCR** for viral RNA (especially on first week post-symptom onset).

3. **EIA & indirect immunofluorescent antibody** tests with acute and convalescent titre: possible cross-reactivity with other flaviviruses such as DENV and WNV.
   - IgM: positive on d3; last for ≥d77

     (Shinohara K et al. Zika fever imported from Thailand to Japan, and diagnosed by PCR in the urines. J Travel Med 2016;23. Fonseca K et al. First case of Zika virus infection in a returning Canadian traveler. Am J Trop Med Hyg. 2014;91:1035-8.)
   - IgG: positive on d6; last for ≥d67

     (Tappe D et al. First case of laboratory-confirmed Zika virus infection imported into Europe, November 2013. Euro Surveill 2014;19.)

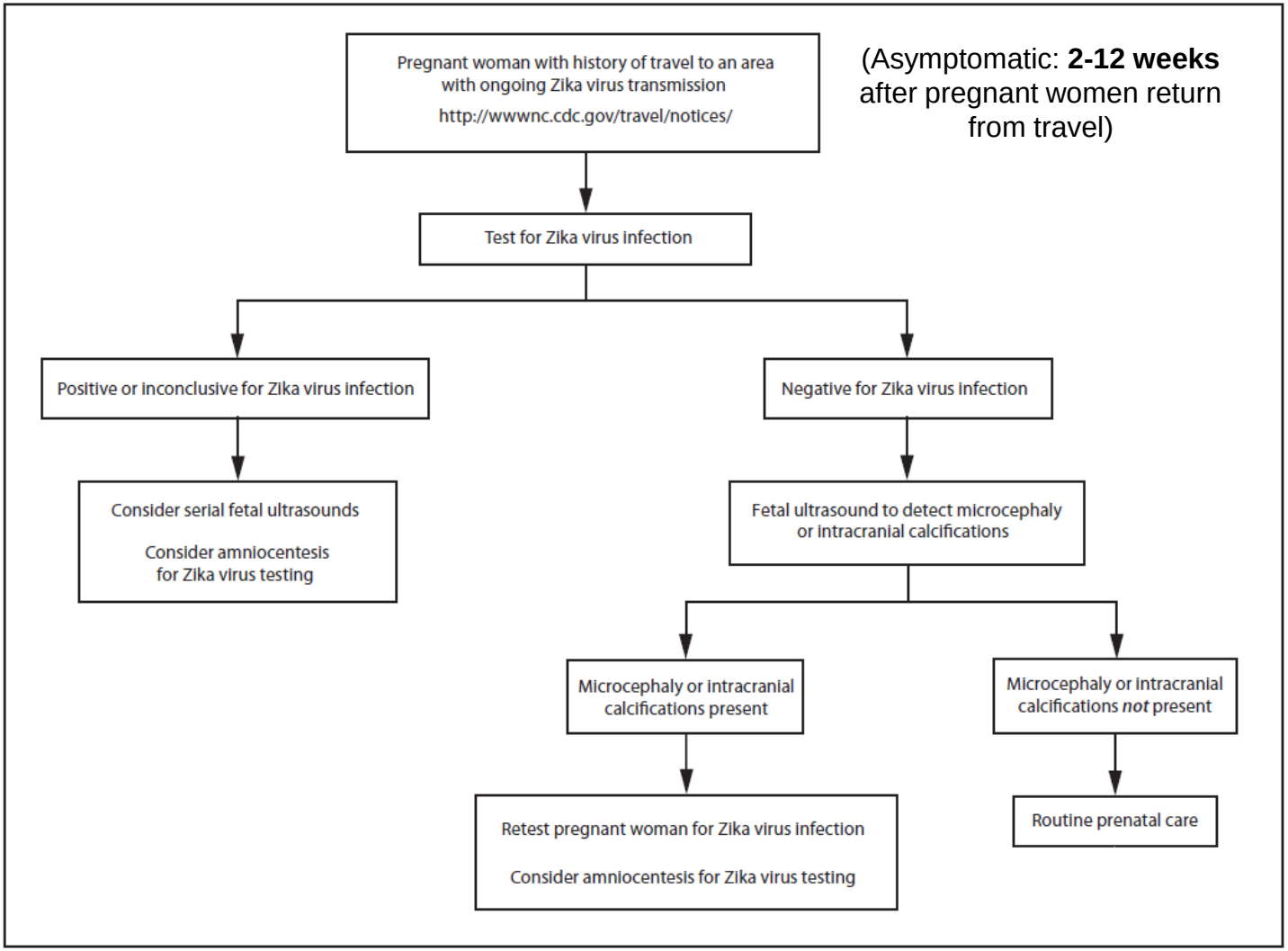
4. Viral culture: Vero, VeroE6, C6/36

5. No FDA approved tests are available yet.

Clinical management

- Zika fever:
  - **Supportive:** no specific antivirals
  - **Acetaminophen:** fever, arthralgia
  - **Anti-histamines:** pruritis
  - **Avoid aspirin:** bleeding, Reye's syndrome (<12 y/o)
  - **Avoid NSAID:** bleeding (coinfection with DENV / CHIKV)
  - **GBS:** IVIG (?immune enhancement), plasmapheresis
  - **CNS infection:** ?minocycline

Recommended testing algorithm for pregnant women with travel Hx to ZIKV-affected areas



Oduyebo T et al. Update: Interim guidelines for health care providers caring for pregnant women and women of reproductive age with possible Zika virus exposure – United States, 2016. MMWR Morb Mortal Wkly Rep. 2016;65:122-7.

Epidemic & infection control: control at the source (mosquitoes)!

1. **Education of self protection to travelers (especially pregnancy)**

2. **Returning travelers** from ZIKV-affected areas: **testing** if symptomatic; self-Rx with insect repellant for **≥14 days**

3. **Targeted mosquito control** at **cargo terminals** to stop entry of infected mosquito in cargo

4. Local mosquito control at **black spots**: construction sites; illegal dumping grounds; invalid car fields; refuse recollection point for recycling

Control measures for non-mosquito-borne ZIKV transmission

Transmission routes	Active transmissions	No active transmission (HK)
Blood transfusion	• Universal nucleic acid testing of blood donors. • Temporary discontinuation of blood donation (importation of blood products).	• Pre-donation questionnaire (identify donors with recent travel Hx). • Deferral of blood donors with travel Hx for ≥14 days. • Self-reporting of symptoms after blood donation (usually symptomatic 3-10 days after blood donation).
Organ transplantation	• Donated organs (**kidneys) from individuals with travel Hxhistory to affected areas: test for ZIKV.	
Semen	• Use barrier methods unless trying to conceive.	• Recent travel Hx: use barrier methods.
Perinatal / Transplacental	• Avoid mosquito bites. • Interval USG: early detection of intra-uterine complications. • ?delay becoming pregnant.	• Avoid/defer travelling to affected areas. • Avoid mosquito bites.
Breastfeeding	• Defer breastfeeding in infected mothers until virus clearance in breast milk & bodily fluids (eg: blood, urine, and saliva).	
Saliva	• Avoid exposure to saliva of infected patients until virus clearance.	
Mucocutaneous (laboratory & patient-care procedures)	• Avoid mucocutaneous exposure to infected patients' blood and bodily fluids.	
Haemodialysis	• Questionnaire to identify patients with recent travel Hx to affected areas. • Virological testing +/- use of a separate haemodialysis machine.	
Monkey bite	• Avoid contact with infected animals.	

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