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Zika Virus (ZIKV)

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ZiKa Virus (ZIKV)

- **Zika virus (ZIKV) is an RNA virus of the Flaviviridae family**
- **Genetically close to Yellow Fever, Dengue, West Nile, and Japanese encephalitis viruses**
- **Mosquito-borne disease transmitted by the Aedes (*Ae. Aegypti*, *Ae. africanus*, *Ae. apicoargenteus*, *Ae. luteocephalus*, , *Ae vitattus*, and*Ae. Furcifer)***

History of the Zika Virus: Africa

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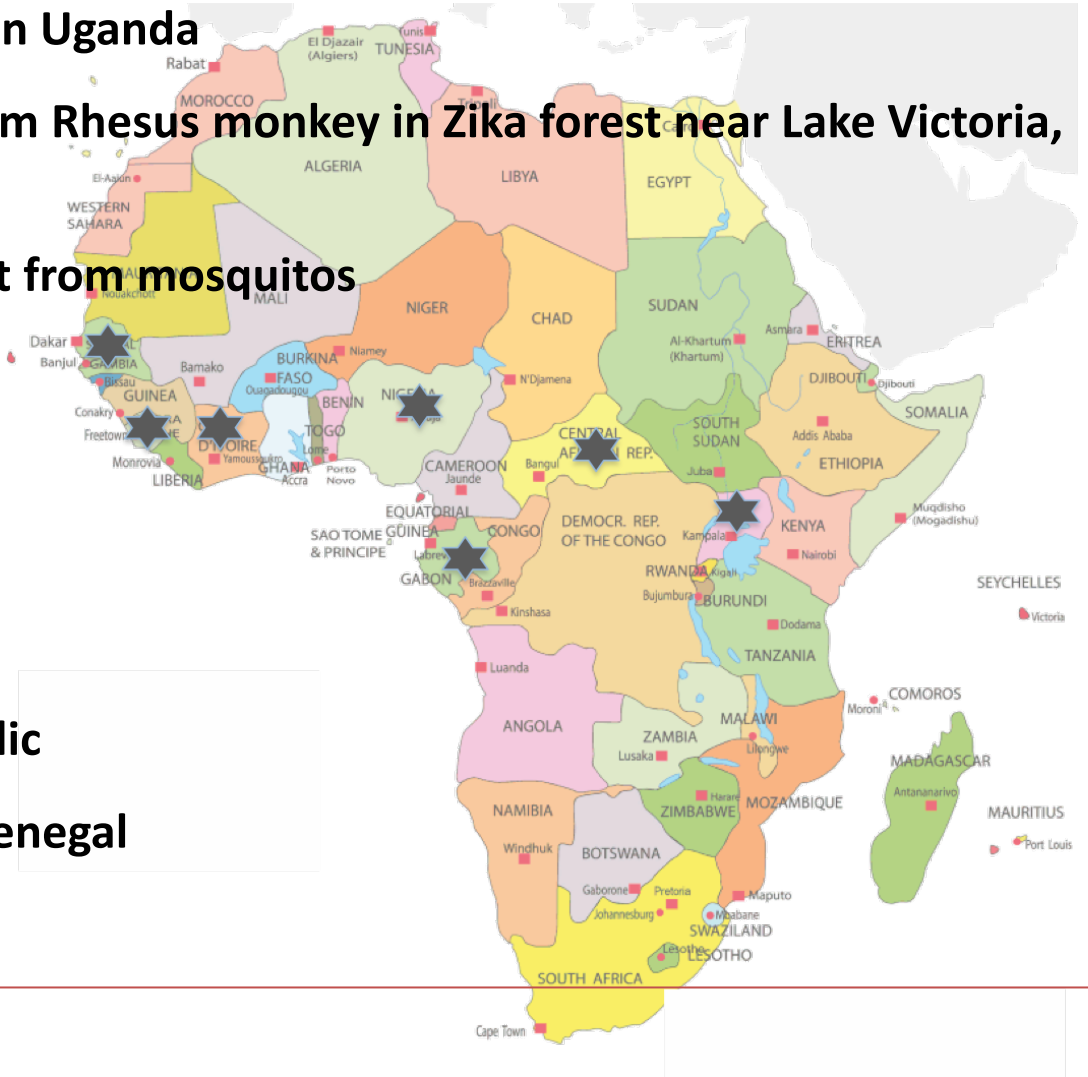
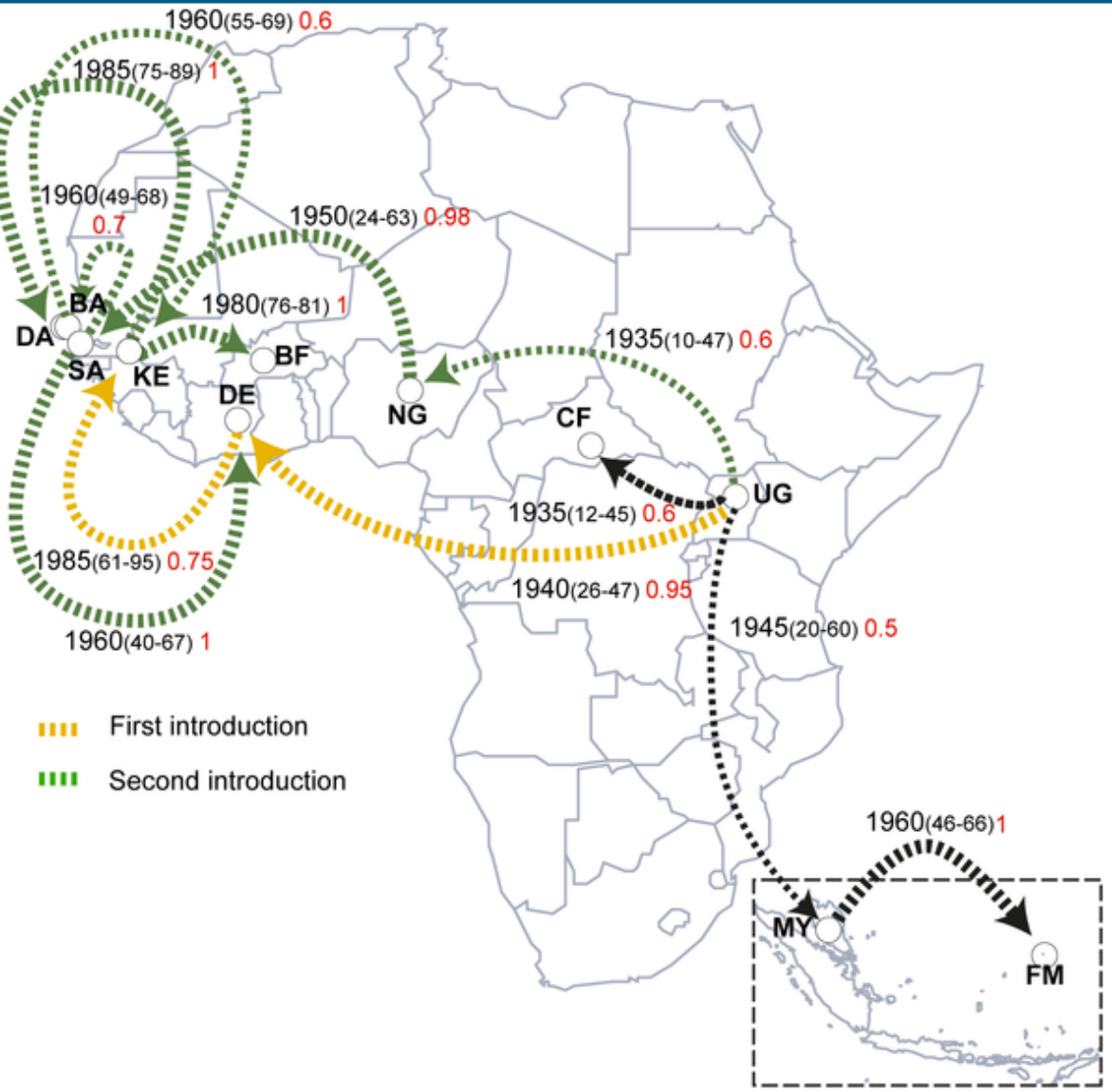
- 1939: West Nile discovered in Uganda
- 1947: First ZIKV isolation from Rhesus monkey in Zika forest near Lake Victoria, Uganda
- 1948: isolated in same forest from mosquitos
- 1969 – 1970: Uganda
- 1971 – 1975: Nigeria
- 1972: Sierra Leone
- 1975: Gabon
- 1979: Central African Republic
- 1988 – 1991; 2011 – 2012: Senegal
- 1999: Cote d'Ivoire



History of the Zika Virus: Asia

- **1977 -1978: Pakistan, Malaysia, Indonesia**
- **2007: Yap Island, Micronesia** (Enfissi et al. Lancet. 2016)
  - 49 confirmed cases
  - 73% of residents > 3 yrs had antibodies to ZIKV
- **2010: Cambodia**
- **2013-14: French Polynesia, population:**270,000 (Cao-Lormeau et al. EID. 2014)
  - 5,895 suspected cases (19,000 suspected cases when extrapolated to include other care centers)

Geographic Spread of ZiKV in Africa and Asia



BA: Bandia, Senegal
BF: Burkina Faso
CF: Central African Republic
DA: Dakar, Senegal
DE: Cote d'Ivoire
FM: Fed. States of Micronesia
MY: Malaysia
NG: Nigeria
SA: Senegal
UG: Uganda

Faye et al. PLOS NTD. 2014

Epidemic in Yap Micronesia

Yap is a small group of small islands

Population 7500 people

First outbreak of ZikV

Lasted from April to July

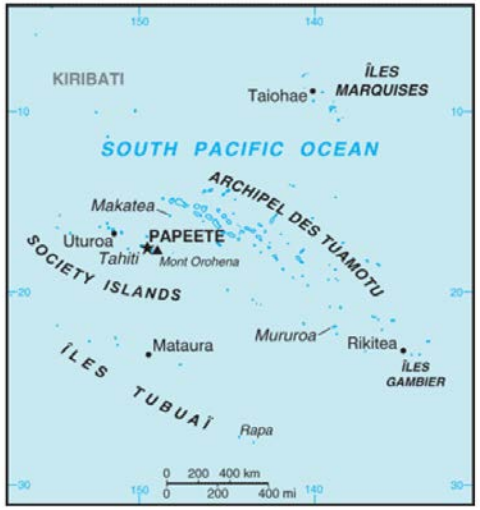
Extensively studied by CDC (clinically, Elisa, neutralizing, RT-PCR)

Estimated: Seropositivity 78%

Subclinical infections 4 to 1

Zika Virus Outbreak on Yap Island, Federated States of Micronesia Mark R. Duffy, et al. N Engl J Med 2009; 360:2536-2543June 11, 2009DOI: 10.1056/NEJMoa0805715

Epidemic in French Polynesia



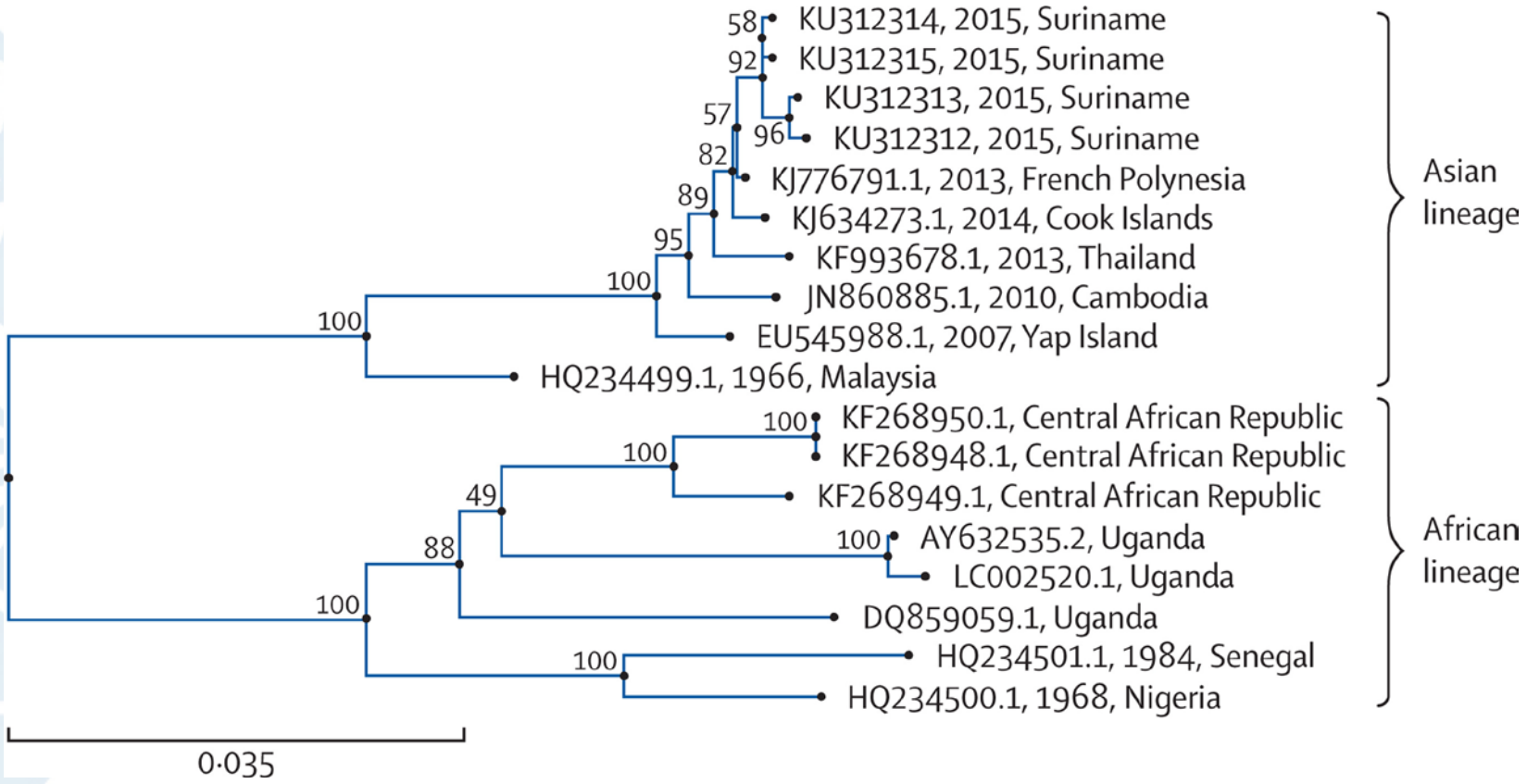
- Sept 13 – March 14 epidemic in French Polynesia
- 270 000 population – estimated 29 000 clinical Zika – @10% attack rate (some isolation) – described Guillain-Barré syndrome and other neurological symptoms in adults and mother to child transmission but no microcephaly in first reports
- After Microcephaly in Brazil (25th Nov 2015)- look back and found 15 newborn/fetuses with neurological malformations (12 microcephaly) whose mothers were pregnant during the epidemic – several had had terminations

http://ecdc.europa.eu/en/publications/Publications/Zika-virus-French-Polynesia-rapid-risk-assessment.pdf

http://www.eurosurveillance.org/images/dynamic/EE/V19N13/art20751.pdf

- http://www.hygiene-publique.gov.pf/IMG/pdf/note_malformations_congenitales_cerebrales.pdfFrench Polynesia

ZIKV: Phylogenetic Analysis



(Enfissi et al. Lancet. 2016)

Clinical Presentation

Zika:

- Rash, mild dengue-like low grade fever, asthenia, wrist and finger arthralgia, headache, swollen ankles and mouth ulcers

Dengue:

- Fever, malaise, headache, body pain, rash

Table. Characteristics of 24 patients with positive and negative results for infection with Zika virus, Brazil, 2015

Reverse transcription PCR result for Zika virus (no.)	Mean (SD) patient age, y	Patient sex, F/M	No. (%)			
			Rash	Fever	Myalgia	Headache
Positive (7)	33 (15)	6/1	6 (85.7)	3 (43)	4 (57.1)	3 (43)
Negative (17)	31 (8.5)	12/5	12 (70.6)	6 (35.3)	9 (53)	11 (64.7)

(Campos et al. EID. 2015)

Dengue

- Pain behind eyes
- Dizziness
- Red rash
- Nausea and vomiting
- Pain at articulations
- Weight loss
- Weakness
- Headache
- High fever
- Bleeding from nose and gums

Chikungunya

- Intense pain in foot and hand articulations
- Red rash
- Headache
- High fever
- Muscular pain

ZikaV

- Back pain
- Red eyes
- Headache
- Lesions with white dots and reddish skin
- Pain at articulations
- Low fever
- Muscular pain

Differential Diagnosis of Dengue Fever

Conditions that mimic the febrile phase of dengue infection	
Flu-like syndromes	Influenza, measles, Chikungunya, infectious mononucleosis , HIV seroconversion illness
Illnesses with a rash	Rubella, measles, scarlet fever, meningococcal infection, Chikungunya, drug reactions
Diarrhoeal diseases	Rotavirus, other enteric infections
Illnesses with neurological manifestations	Meningo/encephalitis Febrile seizures
Conditions that mimic the critical phase of dengue infection	
Infectious	Acute gastroenteritis, malaria, leptospirosis, typhoid, typhus, viral hepatitis, acute HIV seroconversion illness, bacterial sepsis, septic shock
Malignancies	Acute leukaemia and other malignancies
Other clinical pictures	Acute abdomen – acute appendicitis – acute cholecystitis – perforated viscus Diabetic ketoacidosis Lactic acidosis Leukopenia and thrombocytopaenia ± bleeding Platelet disorders Renal failure Respiratory distress (Kussmaul's breathing) Systemic Lupus Erythematosus

Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control, WHO 2009

Microcephaly: A Public Health Emergency

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- Concomitant co-circulation with dengue in both Brazil and French Polynesia associated with increases in severe neurologic complications. (Enfissi et al. Lancet. 2016)
  - Rash, headaches, fever
  - Guillain-Barre syndrome
  - Congenital neurological malformation

- 3,000 cases of microcephaly

In Brazil in 2015, 20x more than In 2014

Use of Diagnostic Tests for Dengue

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Patient Management:

confirm clinical diagnosis

- **Confirmed diagnosis**:
  - Virus isolation
  - Nucleic acid detection
  - Antigen detection
  - Seroconversion for IgM
  - 4-fold rise in IgG titres
- **Highly suggestive**:
  - IgM positive

Surveillance/measure impact of interventions:

- IgM positivity
- virus isolation/nucleic acid detection

Outbreak investigations:

- IgM positivity
- virus isolation/nucleic acid detection and to identify genotype

Vaccine/drug trials:

- Best/most feasible diagnostic methods to define a dengue infected patient (and to identify the genotype)

Laboratory Diagnosis: Dengue

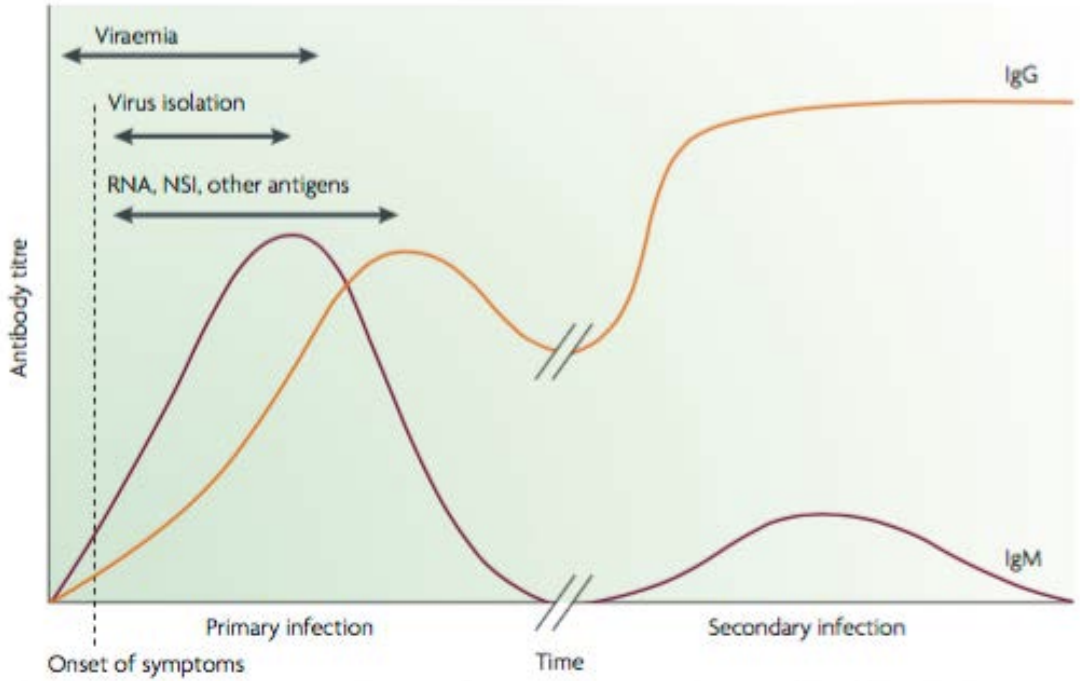
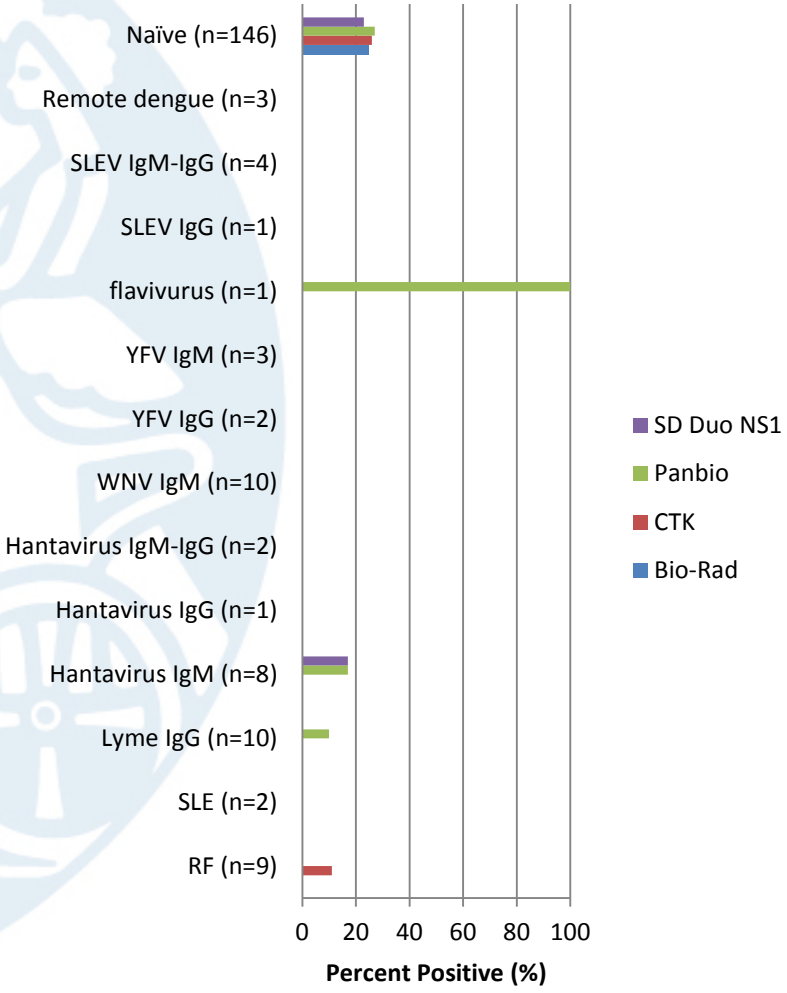
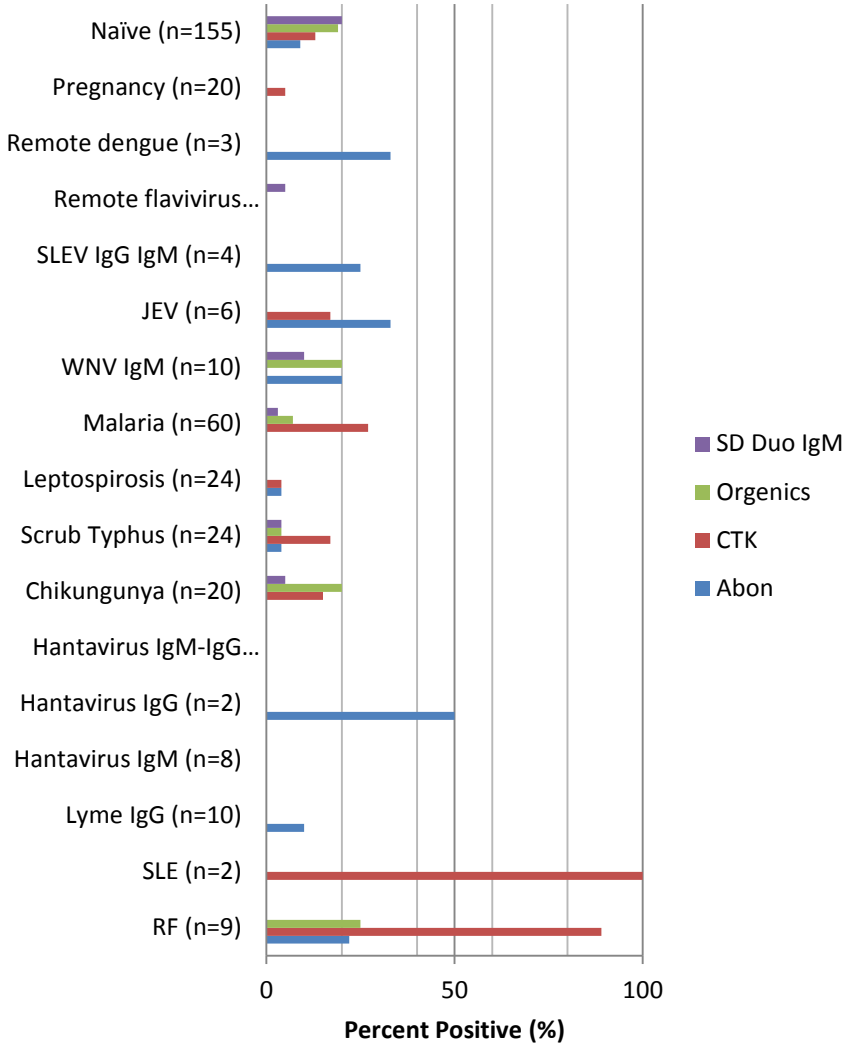


Figure 2 | **Major diagnostic markers for dengue infection.** The titre of the IgM and IgG response varies, depending on whether the infection is a primary or secondary infection.

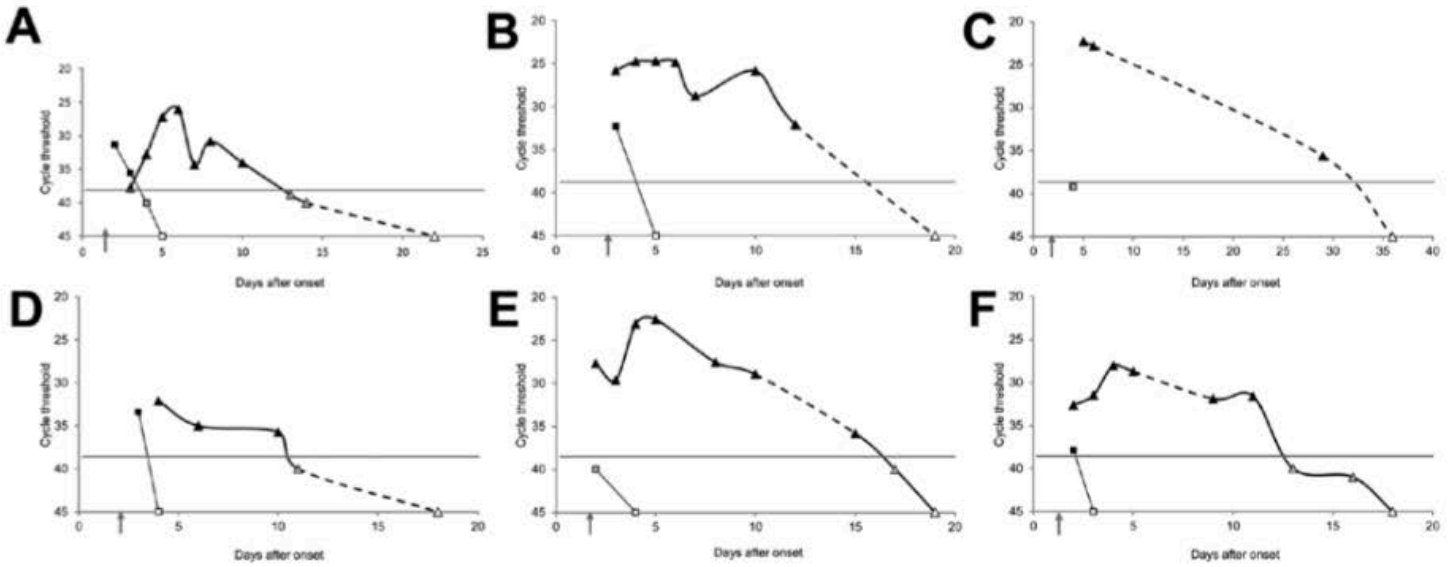
NS1 TESTS		Day 0-5	Day 6-14
	Company	Sensitivity*	Sensitivity**
ELISA	Bio-Rad	60%	29%
	Panbio	75%	19%
	SD	70%	31%
RDT	Bio-Rad	52%	19%
	CTK	40%	19%
	Panbio	60%	12%
	SD duo IgM/NS1	59%	59%

Dengue IgM RDTs: Cross reactivity





Zika Virus Diagnosis: Serum and Urine



Detection of Zika virus in blood and urine specimens of 6 patients by using real-time reverse transcription PCR with primers/ probe 1086/1162c/1107-Cy5 (11) New Caledonia, 2014. A) Patient 1; B) Patient 2; C) Patient 3; D) Patient 4; E) Patient 5; F) Patient 6. Triangles indicate urine samples and squares indicate serum samples. The cutoff cycle threshold (Ct) value is 38.5, as previously reported (11) and is indicated by horizontal lines. Black symbols indicate amplifications with Ct <38.5, gray symbols indicate amplifications with Ct ≥38.5, and white symbols indicate negative amplifications. Onset of disease (day 0) was defined retrospectively after questioning patients about initial symptoms. Dashed lines indicate a period >2 days without a sample being obtained. Arrows indicate onset of rash.

Gourinat et al. EID. 2015

Zika Virus Diagnosis: Saliva

French Polynesia, Oct 2013-Mar 2014:

- 1,067 samples from 885 patients with fever
- ZIKV RNA positive in 210/748 (28%) in blood and 182/319 (57%) in saliva
- Use of saliva increased rate of detection at the acute stage of disease, but did not enlarge the window of detection of ZIKV RNA

		Saliva		
		+	-	**Total**
Blood	+	52 (29%)	16 (9%)	**68 (38%)**
	-	35 (19%)	79 (43%)	**114 (62%)**
Total		**87 (48%)**	**95 (52%)**	**182**

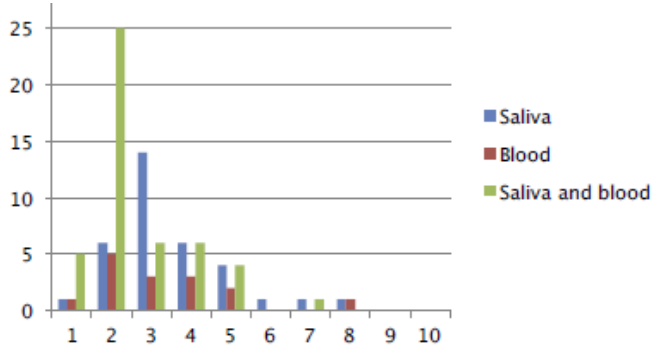


Fig. 1. Proportion of positive samples (Y axis in %) according to the number of days after symptoms onset (X axis) for the 182 patients with saliva, blood or both samples tested by ZIKV RT-PCR.

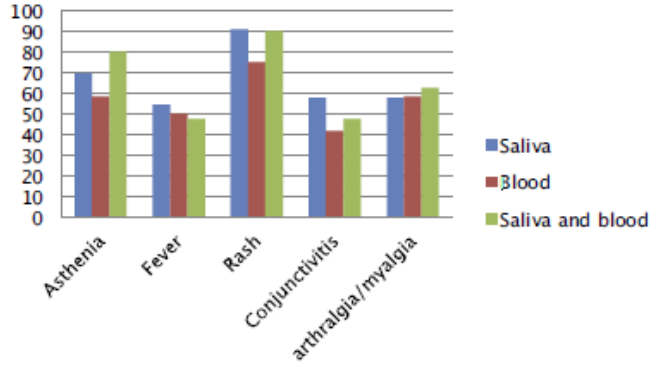


Fig. 2. Proportion (Y axis in %) of symptoms (X axis) reported for the 182 patients with saliva, blood or both samples tested positive by ZIKV RT-PCR.

Mussi et al J Clin Virol 2015

1st Zika case in a returning Canadian Traveler

- Jan 2013: Patient left Canada, stayed 8 days in Bangkok; 5 days in Phuket; 3 days in Bangkok; returned to Canada via Hog Kong
- Day 1 on return: Intermittent fever and chills
- Day 3: Mouth sore, oral blisters
- Day 5: Papular rash - spread to extremities including her palms, lasting 4 days with retro-orbital headache, fever, mild conjunctivitis
- Day 7: significant joint and muscle tenderness fpr 2 days and then become episodic for another 4 days
- Day 16: resolution of symptoms

TABLE 1

Summary of samples collected and testing performed relative to onset of illness*

Sample type	No. days after onset†	Dengue EIA (IgM/IgG)	RT-PCR gel-based assay result	CDC results, Zika virus IgM EIA or PRNT‡
Blood	6	NT	Positive	IgM EIA: Equivocal
Urine and nasopharyngeal swab specimen	6	NA	Positive	
Blood	9	Negative/negative	Positive	IgM EIA: Equivocal, PRNT titer < 10
Blood	10	Positive (2.5)/negative	Negative	
Blood	41	Positive (1.5)/negative	Negative	
Blood	77	Positive (1.5)/negative	NT	IgM EIA: Strongly positive, PRNT titer = 1,280
Blood	114	Negative/negative	NT	–

*EIA = enzyme immunoassay; RT-PCR = reverse transcription polymerase chain reaction; CDC = Centers for Disease Control and Prevention; PRNT = plaque reduction neutralization test; NT = not tested; NA = not available.

†Number of days when samples were collected after onset of illness.

‡See text for description of testing.

Fonseca et al. Am J Trop Med Hyg. 2014

Diagnosis of ZikV infection

Low level of virus in blood or urine

Detection of viral RNA in blood or urine samples
RT-PCR (and viral isolation) in blood or urine samples
collected less than five days after the onset of symptoms .

Serology Paired IgM/IgG very low in early infection
Cross reaction with dengue (confirmed by neutralization assay).

No reliable commercial kit for detection of specific antibodies to ZikV.

Moving Forward

- Development and evaluation of more accurate diagnostic tests to confirm Zika infection, especially ones that can be used on a population basis

- Development and evaluation of multiplex tests to distinguish fever associated with dengue, chikungunya and Zika virus infections

- Studies to determine the association between microcephaly and Zika virus infection in pregnancy

Thank you

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