Kilimanjaro Christian Medical Centre

Emerging Challenges

Trends and Threats

The 86th KCMC Post Graduate Seminar, 22nd – 24th Oct, 2014

Dengue Fever: Diagnostics

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Dengue Virus

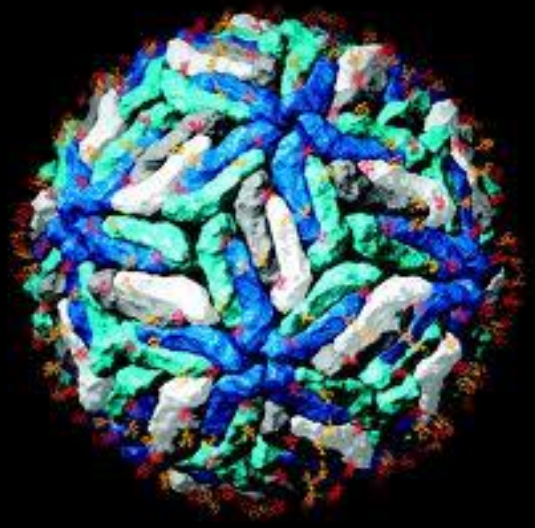
Arbovirus

- Arthropod, Mosquito, born (Aedes aegypti) virus.
- It causes Dengue fever (DF)
  - Swahili phrase "Ka-dinga pepo",
- Dengue hemorrhagic fever (DHF)
- Dengue shock syndrome (DSS)

http://www.denguevirusnet.com/history-of-dengue.html

The Virus

Dengue Virus



The Virus

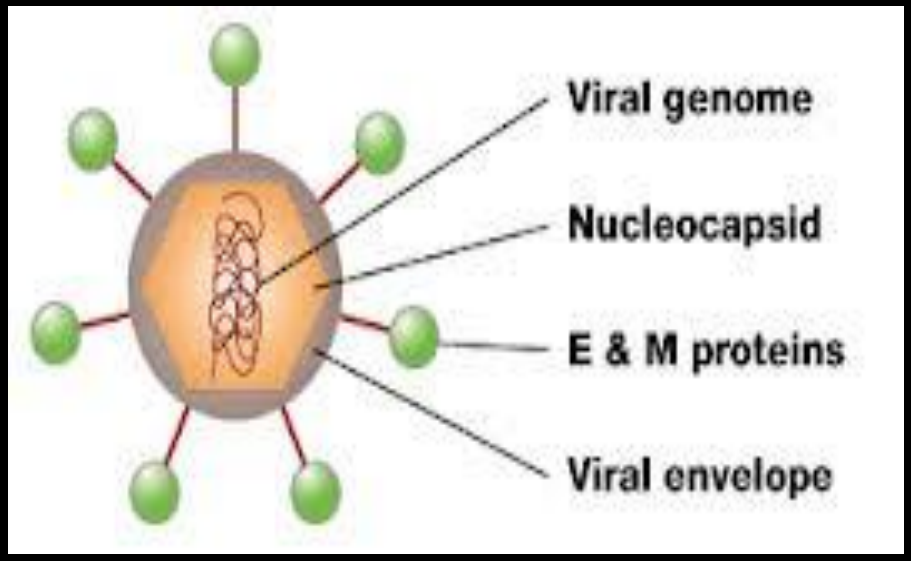
- Has four (4) different serotypes
  - DEN-1, 2, 3, 4
- First reported epidemics in 1780 in Asia, Africa, and North America

Dengue virus,…

- **Four serotypes: DENV-1, 2, 3, 4**
  - All cause full spectrum of disease
  - Infection confers lifelong serotype-specific immunity and short-term (2-3months) cross-immunity
  - Humans can have four infections in a lifetime
- **Genetic variation within serotypes**
  - Some thought to be more virulent

Structure of the virus

- Family: *Flaviviridae*
- Genus: *Flavivirus*

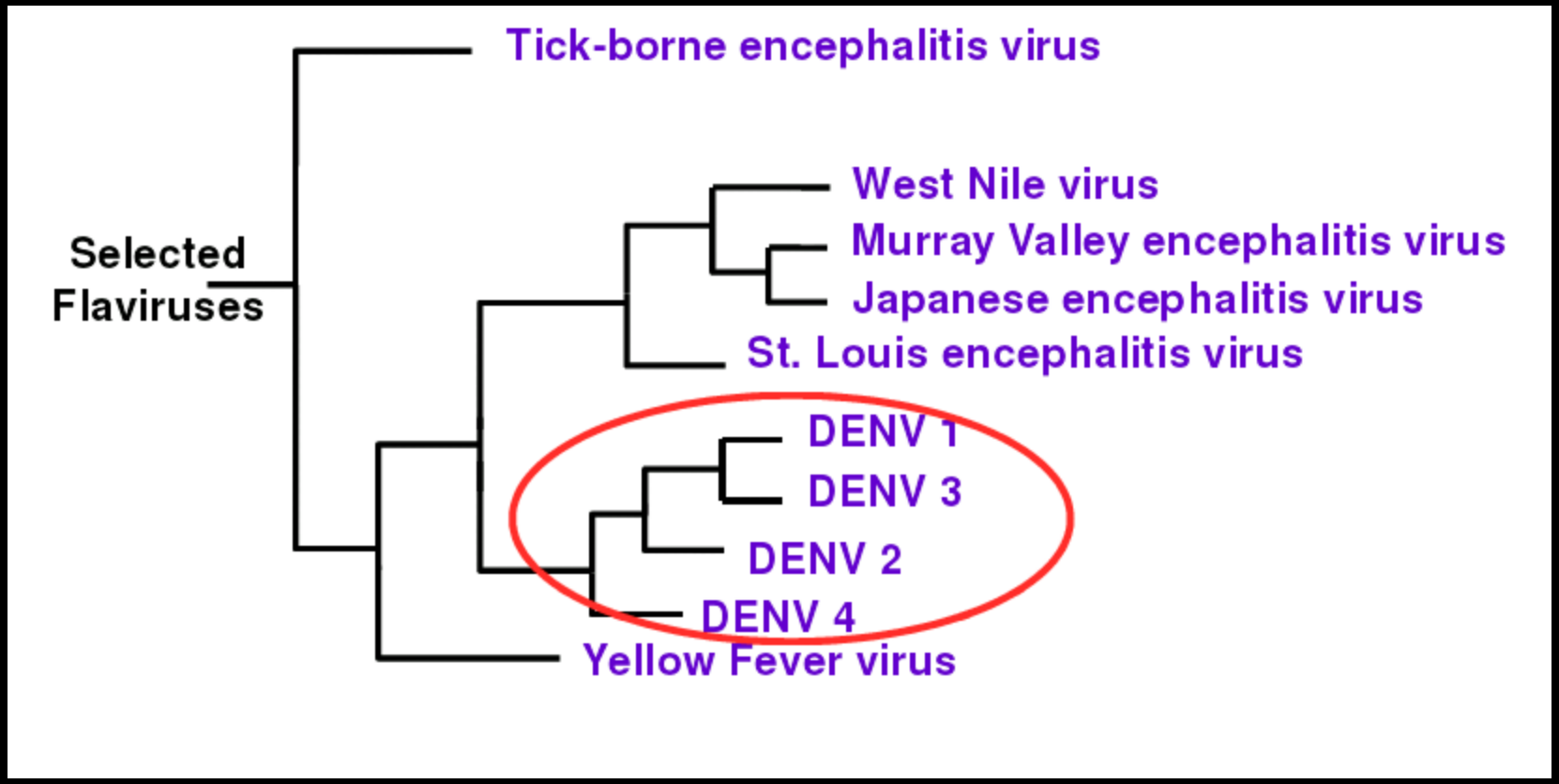


Dengue virus

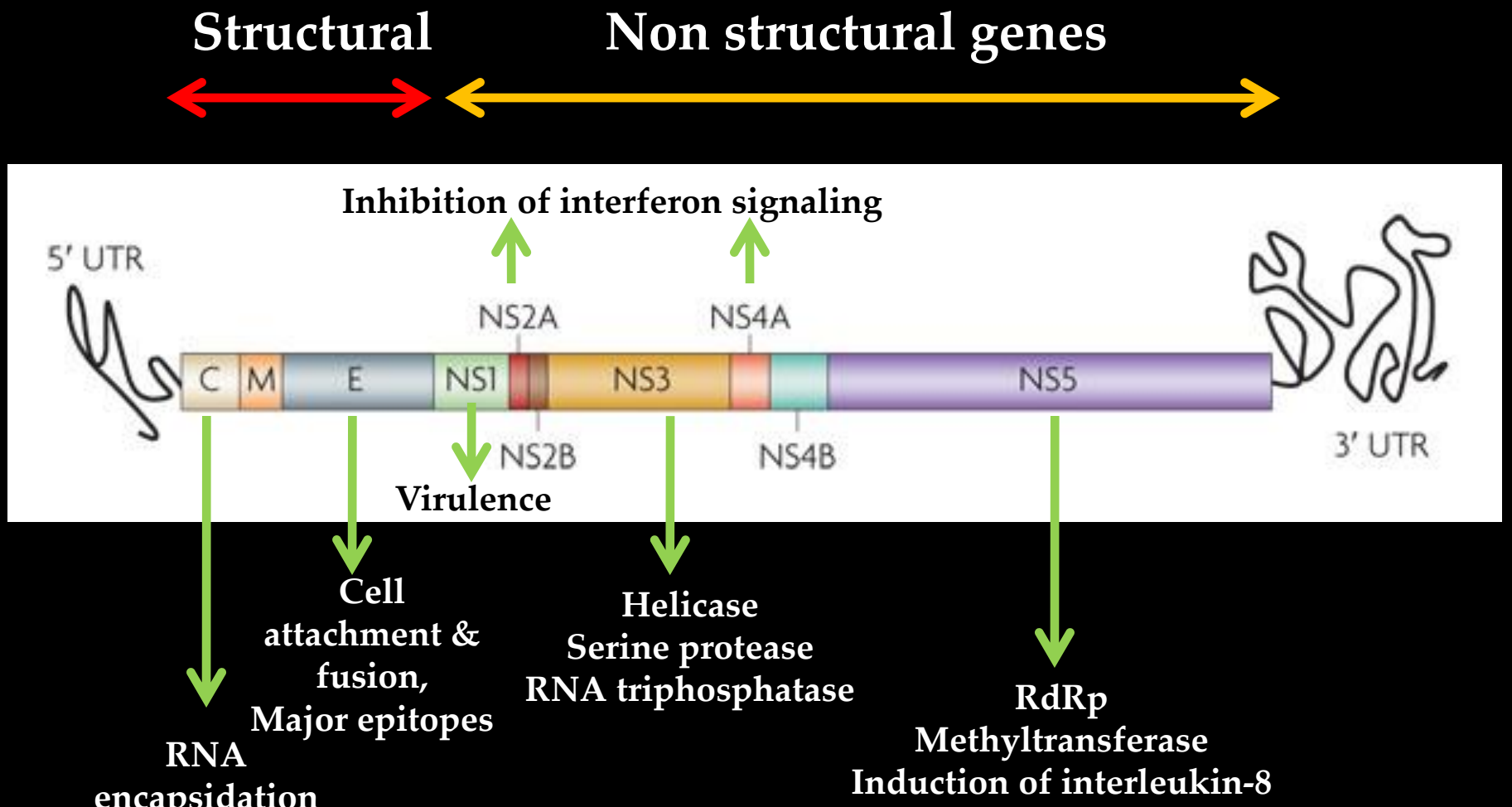
- Enveloped
- + Sense
- ss RNA genome
- Icosahedral

Dengue virus,…

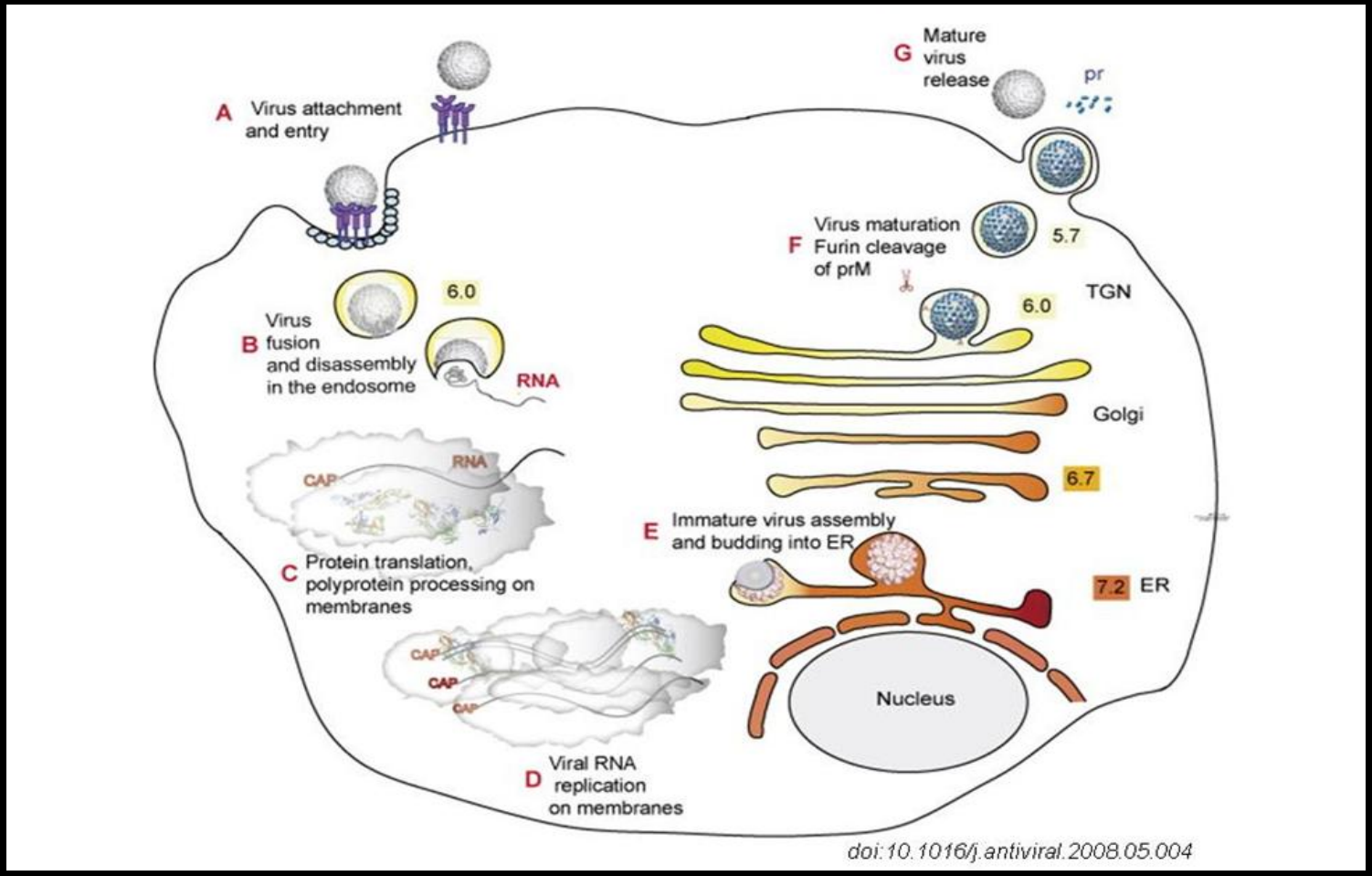
Relationship,…..



Viral genome (10.7kb)



Viral lifecycle



Laboratory Diagnostics

Tests that can be done,..!

- Routine blood test
- Tests to check the clotting process
- Urine to check protein leak
- Special tests to identify the **DV** or its **foot marks**

Lab warning signs

Leucopenia

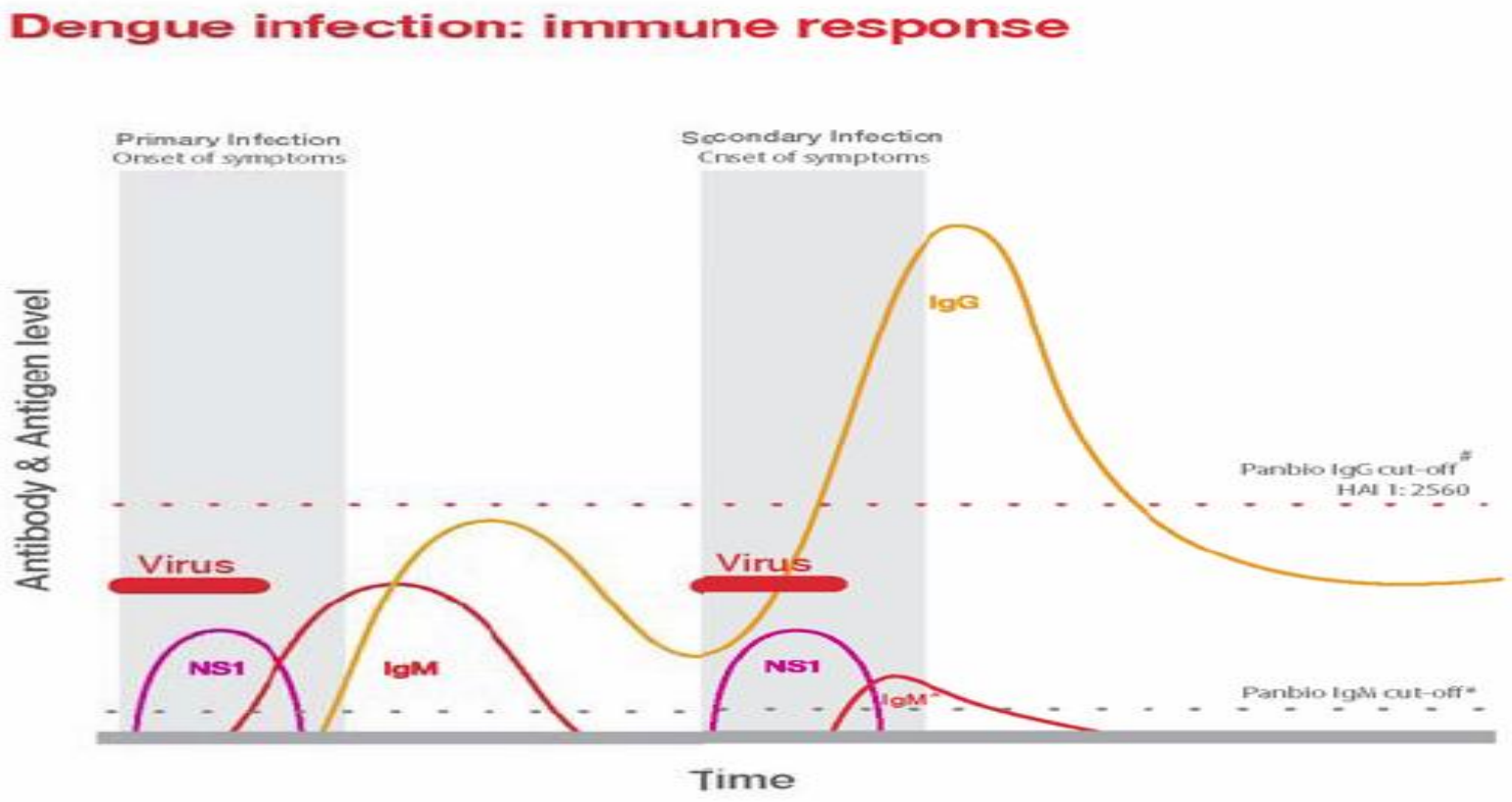
- Occurs 24 hours before rapid decrease in platelet count
- Not predictive of plasma leakage
- Good indicator that patient could have dengue

Rapid decrease in platelet count + rising trend in haematocrit

- Occur shortly before or at defervescence
- May precede changes in blood pressure and pulse pressure
- Indicate an increase in vascular permeability

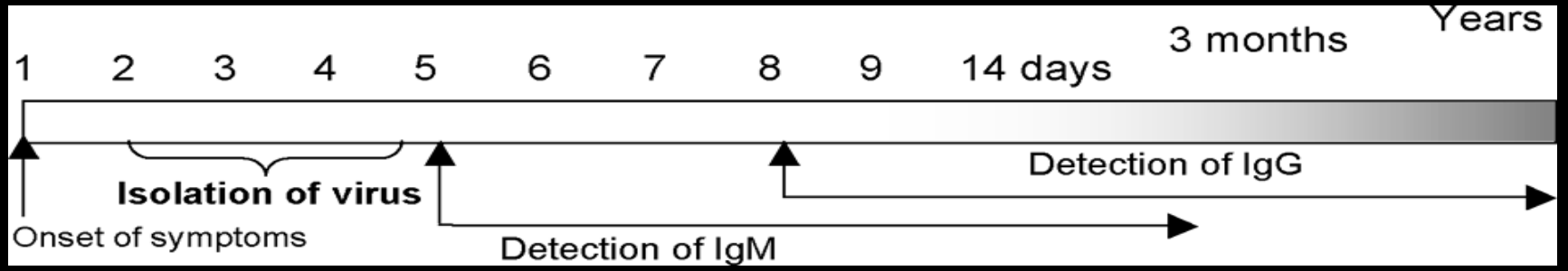
NOTE: Changes in haematocrit may be masked by IV fluid therapy

Diagnostic markers



Samples

- Serum–Rapid test, ELISA, PCR
- Whole blood–Rapid test, PCR, Viral isolation
- Tissue–IHC, viral isolation, PCR



Sample preparation

1. 2 tubes required
    1. Plain
    2. EDTA tube
2. Separation (Serum)

Sample preparation

3. Sample storage at least -20º C
    - Whole blood 2-8ºC

Special Tests: Dengue Virus

1. IgG/IgM (Ag NS1) Rapid Test

2. ELISA IgG/IgM or Ag ELISA (NS1)

3. RT-PCR – **CONFIRMATORY**

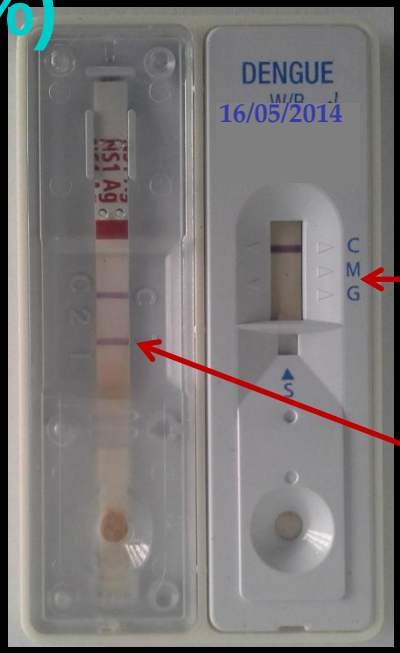
4. DNA Sequencing

5. Viral Isolation (Cell Culture/Inoculation)

KCMC Confirmed Samples

A total of 33 samples tested, 6 were POSITIVE (18.2%)

- F, 86yrs, Majengo
- Cas, (M-I)



- F, 26yrs, Moshi, KCMC?
- M-II
- rtRT-PCR confirmed

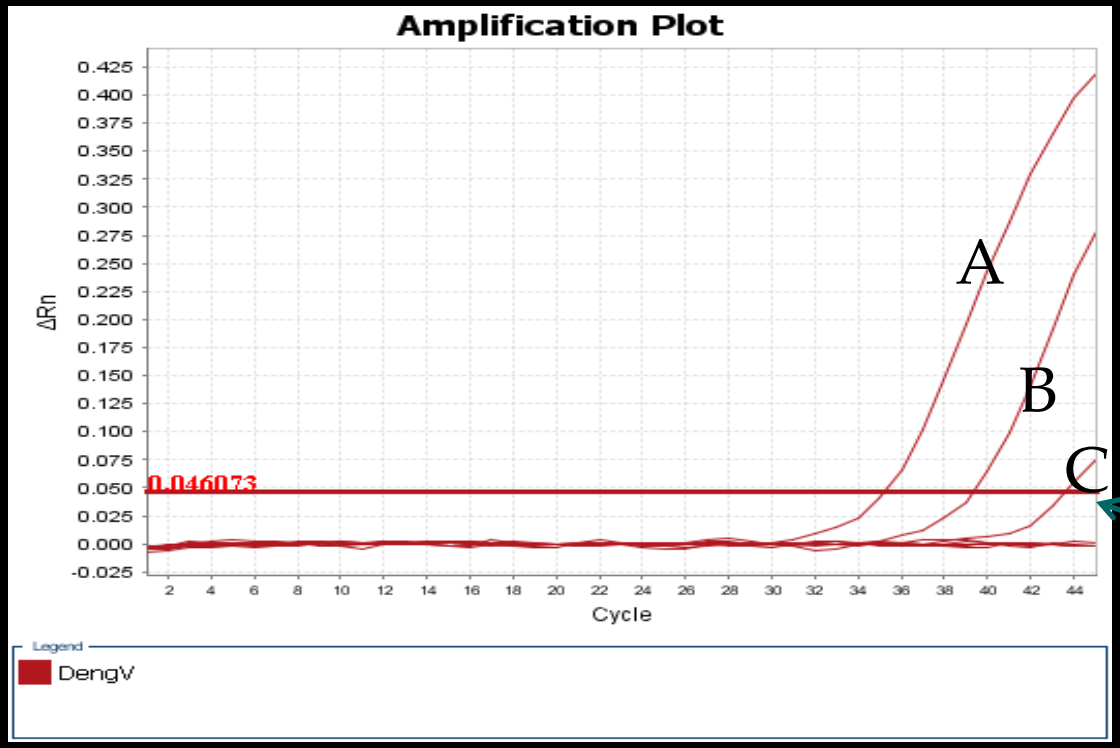
Kifaro *et al.*, 2014-Unpublished data

Pattern of SD Bioline results

	Laboratory results			No of Pts
Pattern of results	NS1	IgM	IgG	
1	Reactive	Reactive	Reactive	2
2	Reactive	Non R	Non R	4

- NS1- first marker to released by infected cells
- IgM and IgG - depends on time of sample collection

Amplification Plot-rtRT-PCR



- F, 26yrs, Moshi, KCMC?
- M-II,
- rtRT-PCR confirmed

10019-10695
676nt 3′ UTR

0.046073 ct value

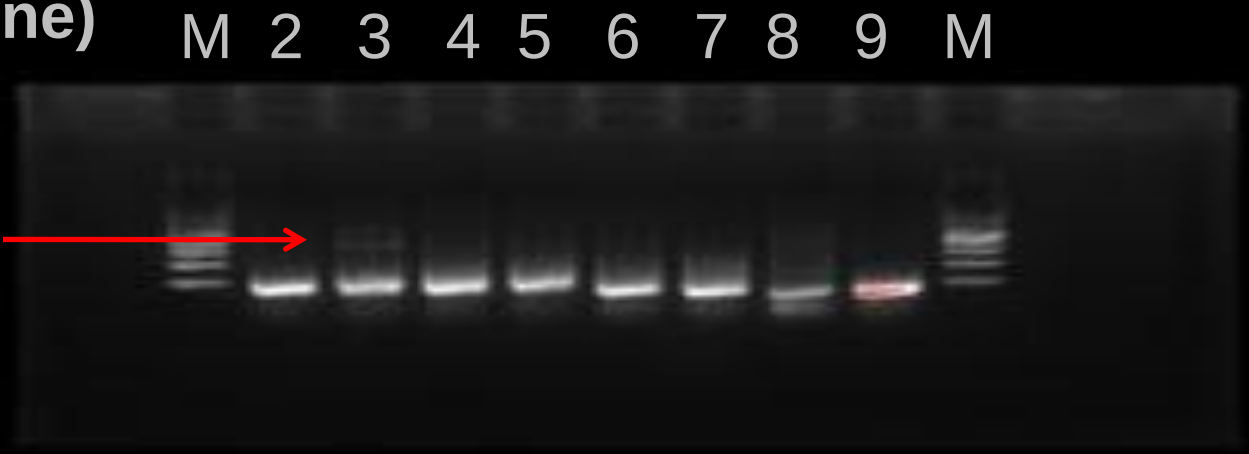
A & B = Pos control

C = Sample 002, Amplification

Kaunara *et al*., 2014 (Unpublished data-KCMU College)

KCMC Confirmed Samples

rt-PCR Gel electrophoresis (Polyprotein gene)



Dl 5'-TCAATATGCTGAAACGCGCGAGAAACCG-3' (134-161)
D2 5'-TTGCACCAACAGTCAATGTCTTCAGGTTC-3' (616-644)

Rapid test = REACTIVE
PCR = POSITIVE

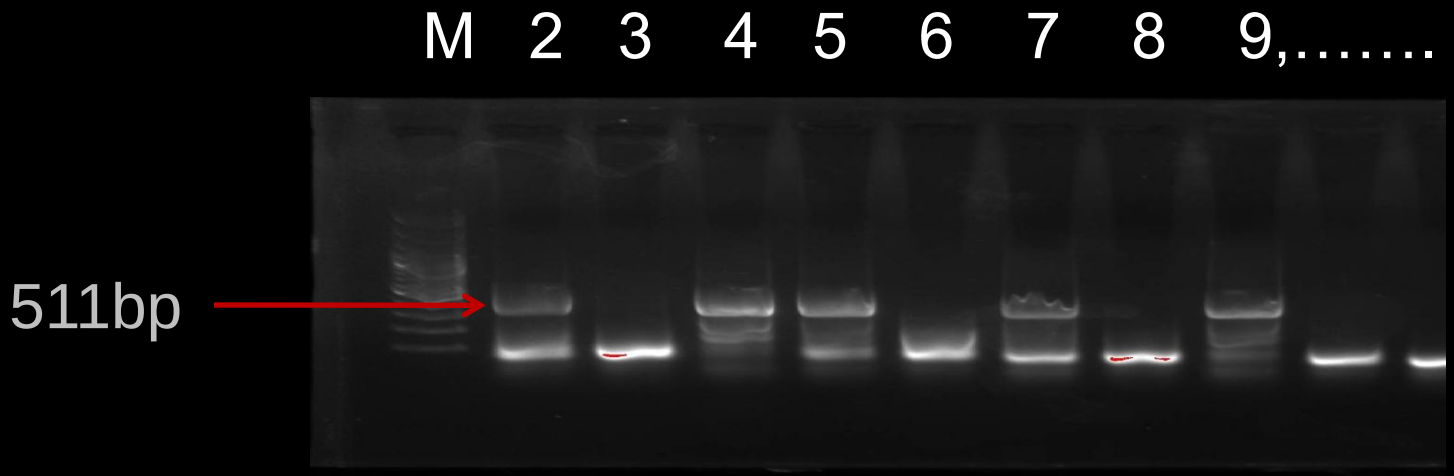
Lanes 1 = Marker,
Lanes 2, 3, 4 and 5 are samples 1, 2, 3 and 4,
Lanes 6 and 7 are negative controls during RNA extraction,
Lanes 8 and 9 are negative controls during RT-PCR
Lane 10 is a DNA marker.

Female 26 years collected 16.05.2014, DV is POSITIVE.

Kifaro *et al*., 2014-Unpublished data

KCMC Confirmed Samples

rt-PCR Gel electrophoresis (Polyprotein gene)



Dl 5'-TCAATATGCTGAAACGCGCGAGAAACCG-3' (134-161)
D2 5'-TTGCACCAACAGTCAATGTCTTCAGGTTC-3' (616-644)

Agarose gel analysis of the product from RT-PCR. Lanes: MW, molecular size markers (in base pairs); N, uninfected mosquitoes; 2 to 8, RNAs isolated from patient samples

Genetic Characterization

- We did serotype specific PCR
  - Preliminary results - Serotype II
- DNA Sequencing – waiting results
  - Phylogenetic analysis – predict origin of the virus

Discussion

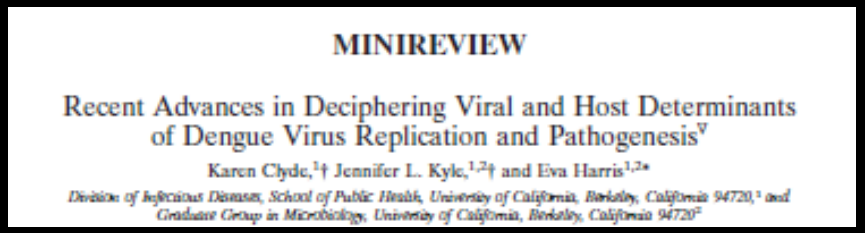
- All cases had classical dengue fever.
- No recorded mortality related to DF.
- The virus was imported from Dar es Salaam.

Conclusion

- This study has confirmed, for the first time, the presence of the DV in Kilimanjaro region, northern-Tanzania.

- Since, the first recorded dengue fever outbreak by the end of April and May, 2010 in Dar es Salaam region, Tanzania.

Important References


MINIREVIEW

Recent Advances in Deciphering Viral and Host Determinants of Dengue Virus Replication and Pathogenesis[▽]

Karen Clyde,[1]† Jennifer L. Kyle,[1,2]† and Eva Harris[1,2]*

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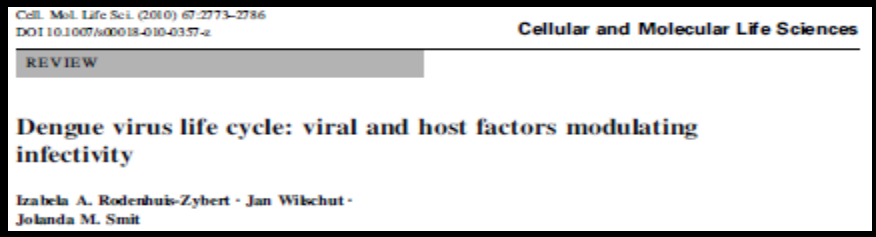
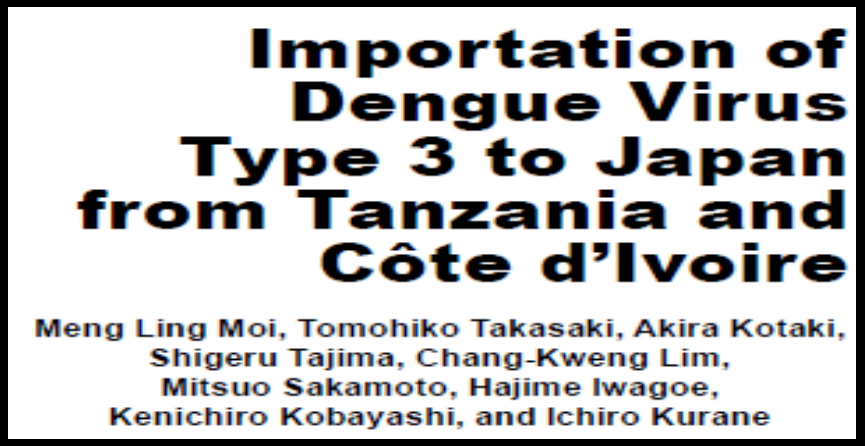

Cell. Mol. Life Sci. (2010) 67:2273–2786
DOI 10.1007/s00018-010-0357-z

Cellular and Molecular Life Sciences

REVIEW

Dengue virus life cycle: viral and host factors modulating infectivity

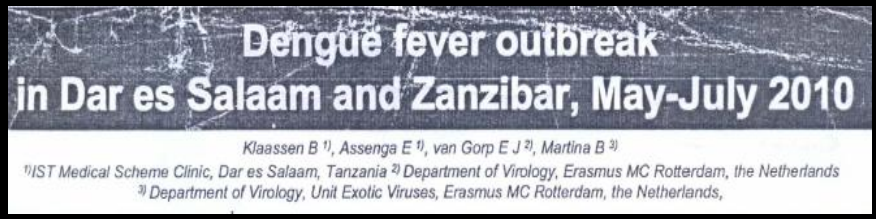
Izabela A. Rodenhuis-Zybert · Jan Wilschut · Jolanda M. Smit


Importation of Dengue Virus Type 3 to Japan from Tanzania and Côte d'Ivoire

Meng Ling Moi, Tomohiko Takasaki, Akira Kotaki, Shigeru Tajima, Chang-Kweng Lim, Mitsuo Sakamoto, Hajime Iwagoe, Kenichiro Kobayashi, and Ichiro Kurane


2012–2020

GLOBAL STRATEGY FOR DENGUE PREVENTION AND CONTROL


Dengue fever outbreak in Dar es Salaam and Zanzibar, May-July 2010

Klaassen B [1], Assenga E [1], van Gorp E J [2], Martina B [3]

[1]IST Medical Scheme Clinic, Dar es Salaam, Tanzania [2] Department of Virology, Erasmus MC Rotterdam, the Netherlands [3] Department of Virology, Unit Exotic Viruses, Erasmus MC Rotterdam, the Netherlands,

Kaunara *et al.*, 2014 (Unpublished data-KCMU College)

Dengue health topic [http://www.who.int/topics/dengue]

Masembe Tambwe, 8th Feb, 2014 Daily News

http://www.denguevirusnet.com/history-of-dengue.html

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Gerald Misinzo Ph.D (Virologist)
SUA & SACIDS

Thank You !