

Zika virus and the Chamber of Secrets: unravelling virus-host immune response interactions

Claire Donald
24th February 2017

Zika virus: a historical link to the University of Glasgow



CVR
 Medical Research Council
 University of Glasgow
 Centre for Virus Research

- **Discovery:** 1947, Rhesus macaque, Zika forest
- Involved **Alexander John Haddow**, graduate and Professor at the **University of Glasgow**
- **Further isolation:** 1948, *Aedes africanus*, Zika forest
- **1952—1954:** First human isolation in Nigeria, possible detection in India
- **1954-1981:** Detection in African and SE Asian countries (introduction ca. 1945? First detection late 60s, Malaysia)
- Pioneering work carried out at the Yellow Fever Institute, now Uganda Virus Research Institute, Entebbe (near Kampala and Zika forest)

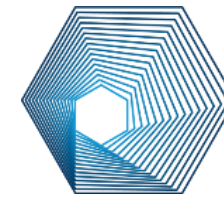


Alexander Haddow
 Zika virus discovery
 1947 UVRI Uganda
 Zika forest

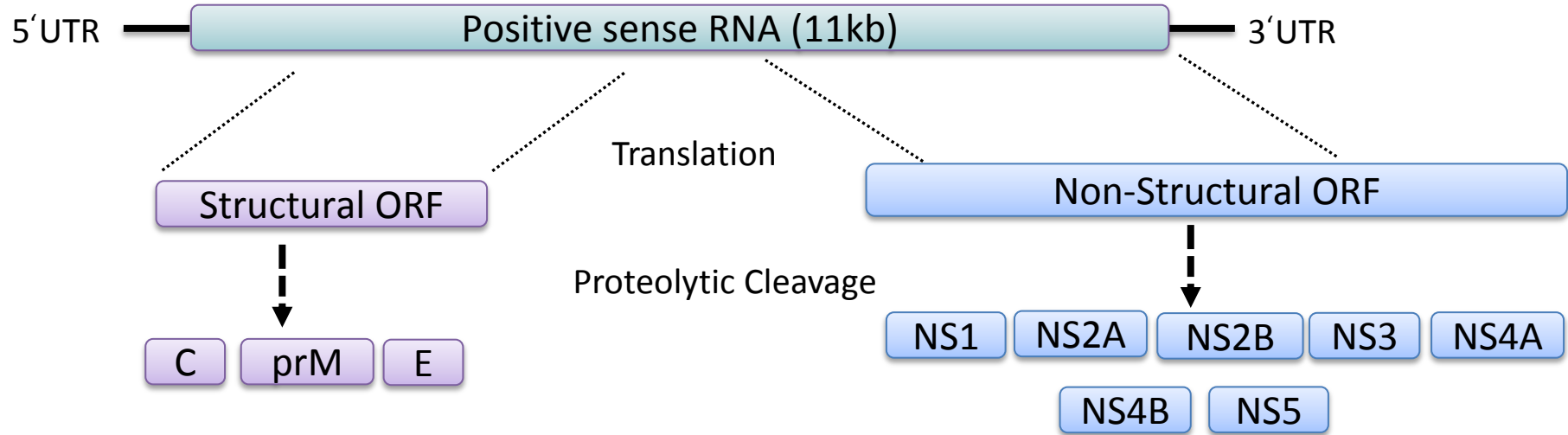


Handwritten field notebook page with a table of mosquito catch data. The table has columns for 'Catch No.', 'Date', 'Time started', 'No. of Catches', 'Dist. Hours', and 'Totals'. The data is handwritten in cursive and includes various mosquito species names and numerical counts.

Catch No.	Date	Time started	No. of Catches	Dist. Hours	Totals
1	11.12.51	16 hours		2.0	1
2				2.1	2
3				2.1	3
4				2.1	4
5				2.1	5
6				2.1	6
7				2.1	7
8				2.1	8
9				2.1	9
10				2.1	10
11				2.1	11
12				2.1	12
13				2.1	13
14				2.1	14
15				2.1	15
16				2.1	16
17				2.1	17
18				2.1	18
19				2.1	19
20				2.1	20
21				2.1	21
22				2.1	22
23				2.1	23
24				2.1	24
25				2.1	25
26				2.1	26
27				2.1	27
28				2.1	28
29				2.1	29
30				2.1	30
31				2.1	31
32				2.1	32



Zika virus (Flavivirus)

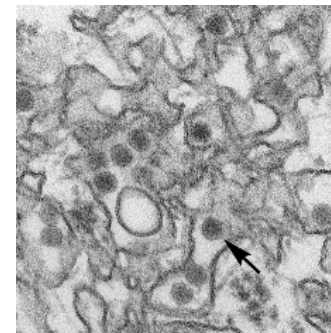


11kb genome, positive stand RNA.

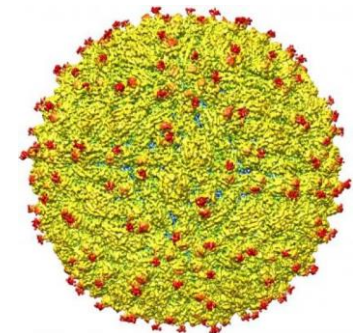
10 Genes: 3 structural (C, prM, E), 7 non-structural.

Non-structural proteins involved in replication and counteraction of host immunity (NS5).

Polyprotein cleaved by viral and host proteases.



CDC, 2005: C. Goldsmith

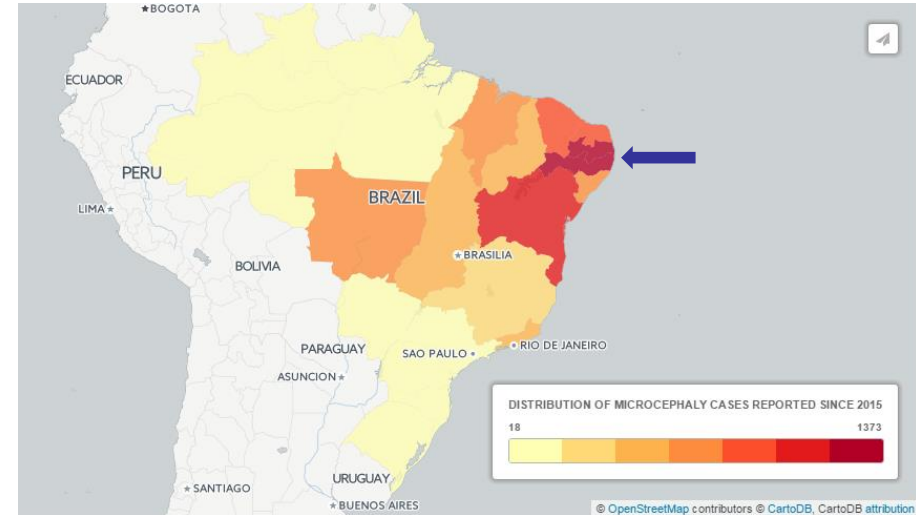


Sirohi *et al.*, 2016 Science



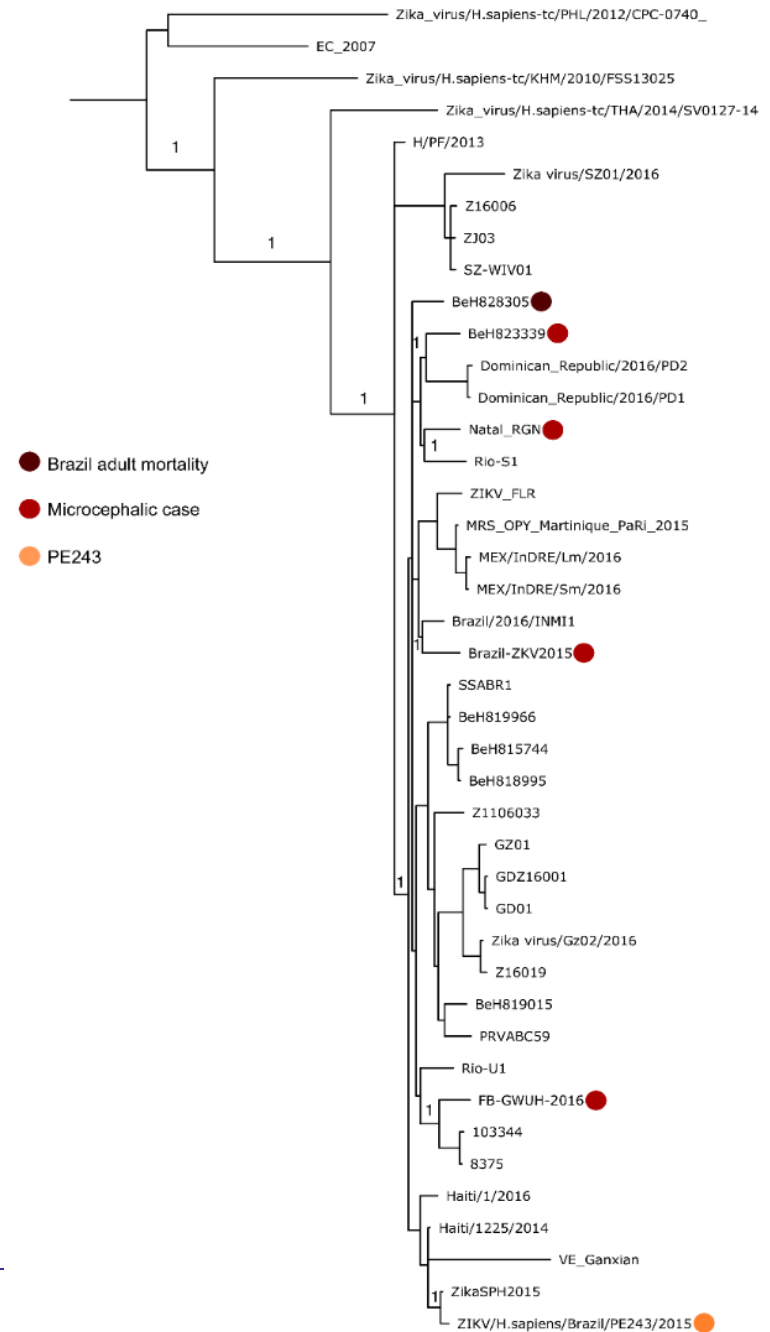
Zika virus from Recife

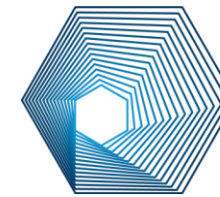
- Obtain a full length ZIKV isolate from Recife, Brazil
- Compare to other American ZIKV isolates
- Identify if subgenomic flavivirus RNA (sfRNA) in ZIKV- infected cells has interferon activity



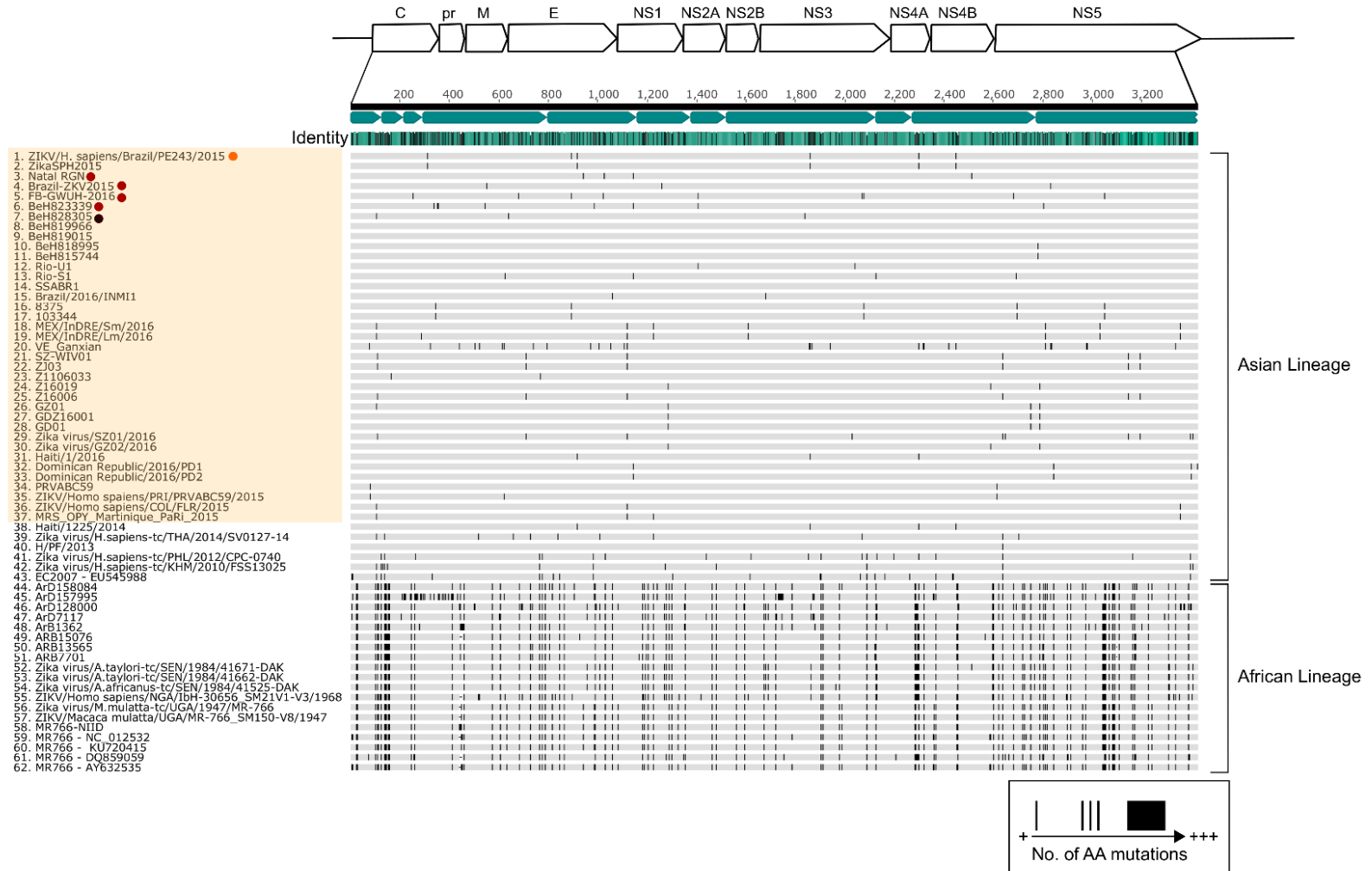
Characterisation of ZIKV

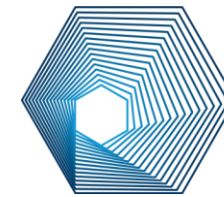
- ZIKV/H.sapiens/
Brazil/PE243/2015
(KX197192)
- Isolated in Recife, Brazil
(2015)
- Patient presented with no
neurological symptoms
- NextSeq500 Illumina platform





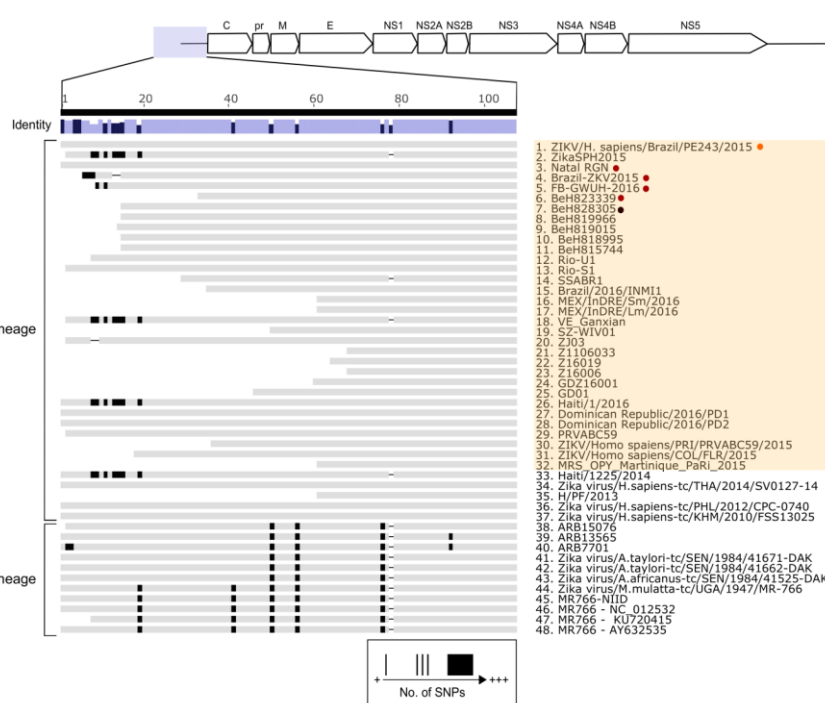
Characterisation of ZIKV



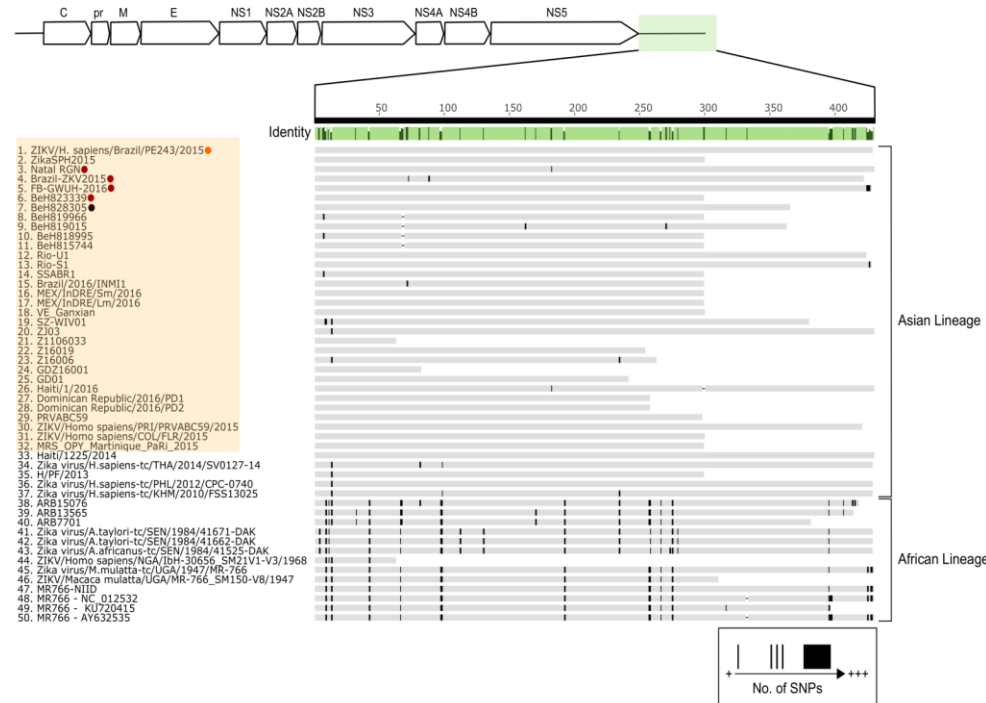


Characterisation of ZIKV

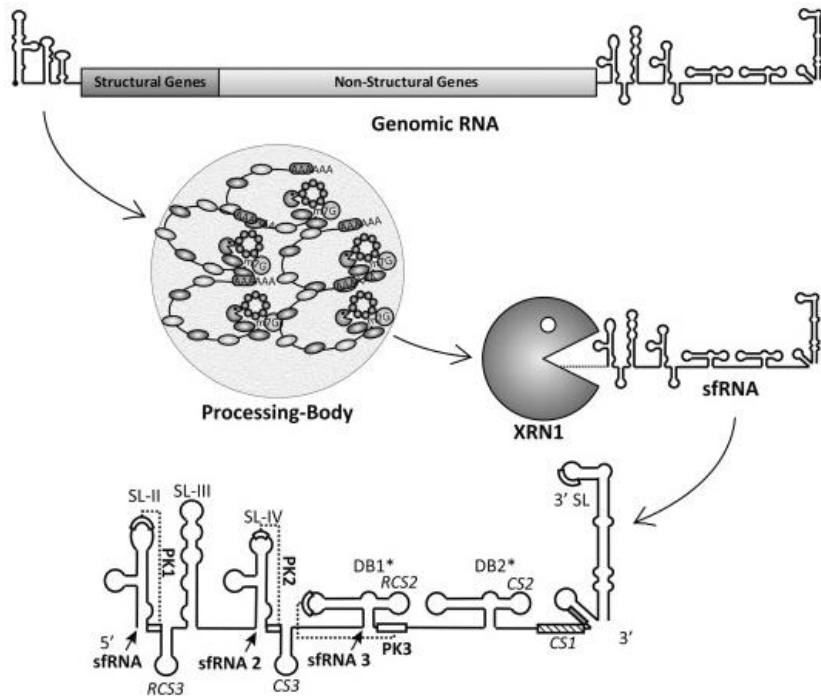
5'UTR



3'UTR



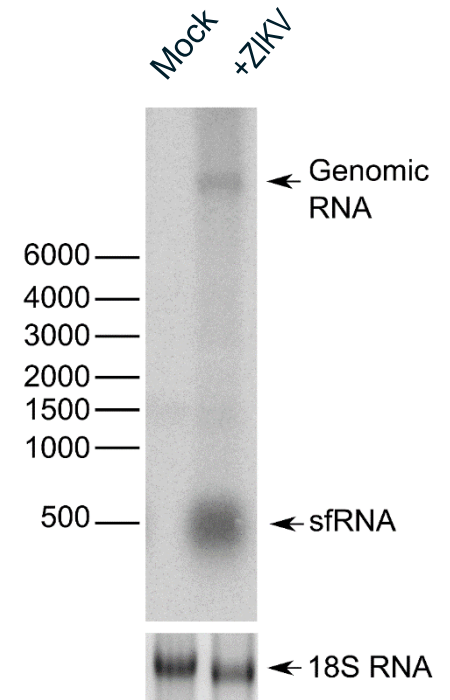
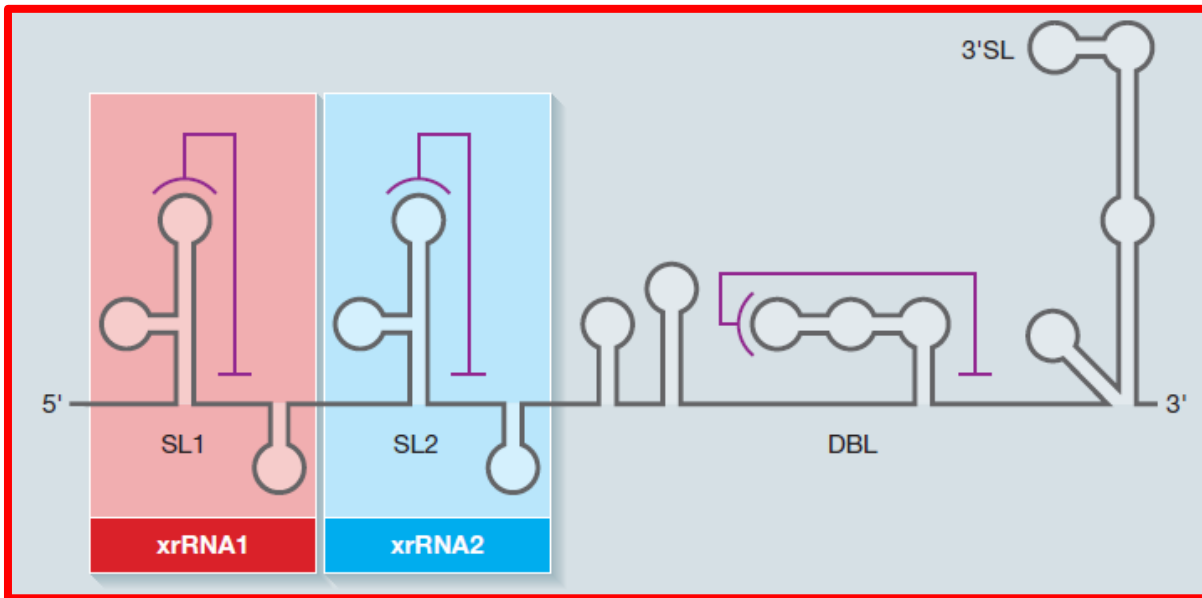
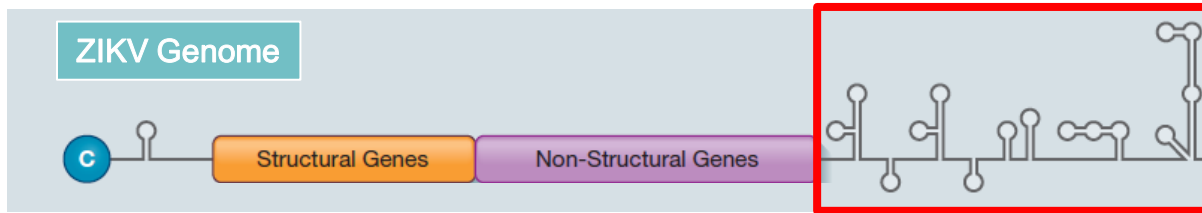
ZIKV sfRNA as an interferon antagonist



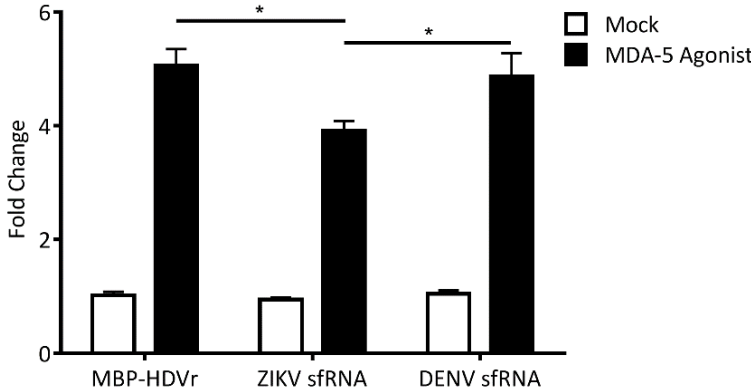
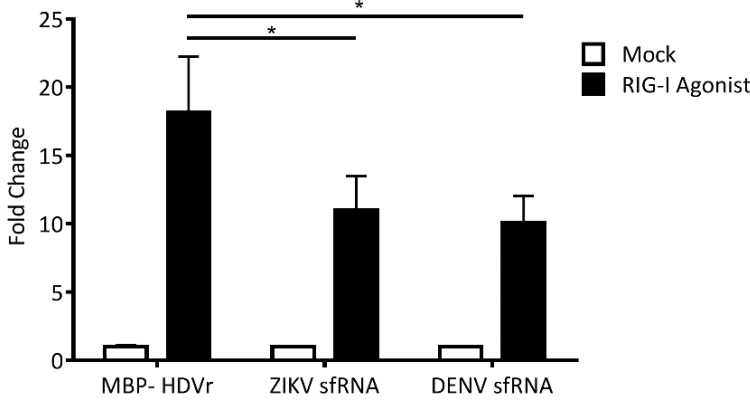
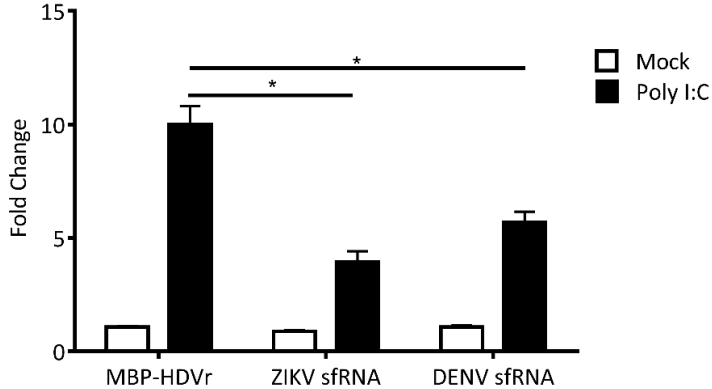
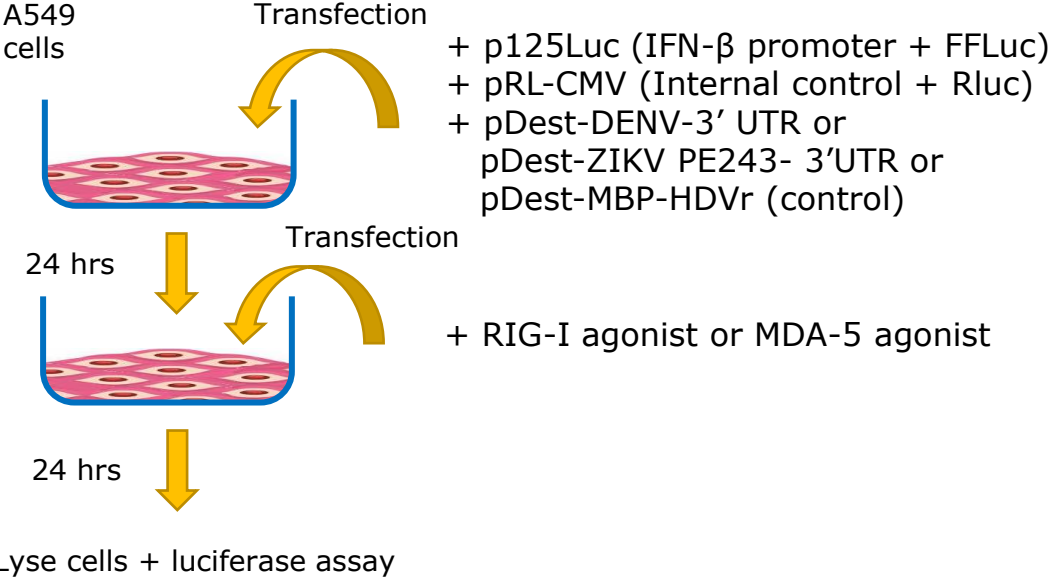
- 0.2-0.6 kb subgenomic flavivirus RNA (sfRNAs)
- Co-linear to the 3' end of the genome
- Produced through incomplete degradation of the genome by the cellular 5'-3' exoribonuclease, XRN1
- sfRNA inhibits type I interferon response.

Clarke *et al.*, 2015

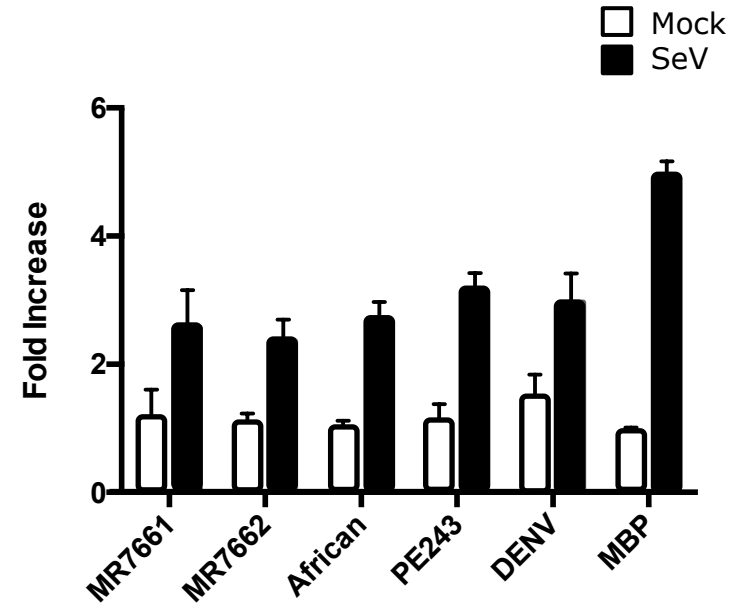
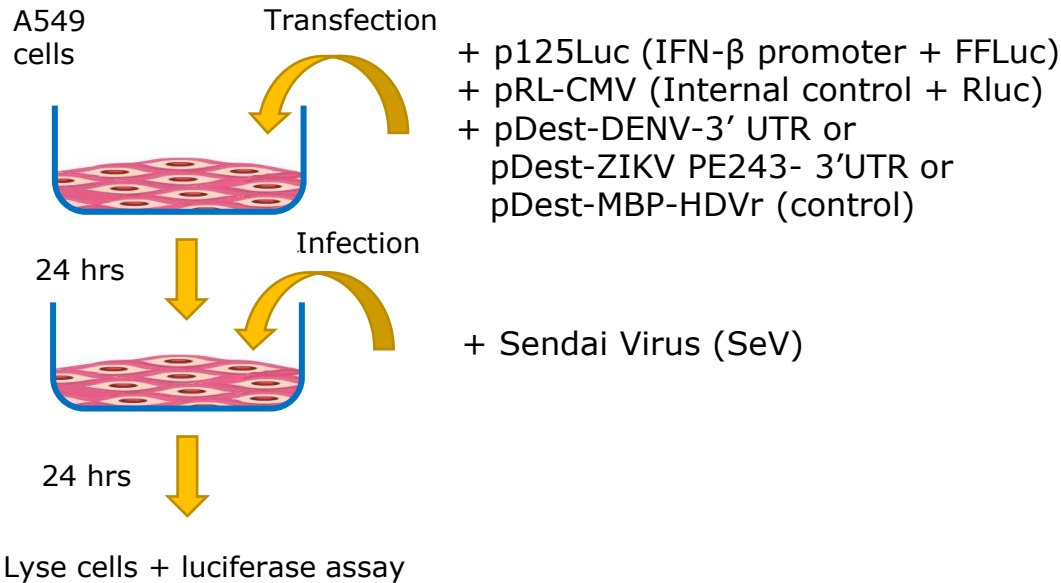
ZIKV sfRNA as an interferon antagonist



ZIKV sfRNA as an interferon antagonist

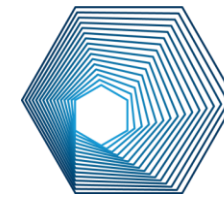


ZIKV sfRNA as an interferon antagonist



ZIKV: Conclusions

- Successfully obtained a full length genome sequence from a Brazilian patient.
- No obvious virological explanation for increased occurrence of neurological diseases associated with current outbreak.
- ZIKV produces sfRNA which acts as an antagonist of RIG-I and MDA5.



CDC's Response to Zika
Enjoy your vacation

Protect your family from Zika!
Zika is a disease primarily spread by mosquitoes, but a man with Zika can spread it to his sex partners as well.

Know before you go
Learn about Zika at cdc.gov/zika. Find out if your destination has Zika, check the CDC Travelers' Health site for current travel notices: cdc.gov/travel

Pack to prevent

- Insect repellent (Look for these ingredients: **DEET, picaridin, IR3535, OLE, or PMD.**)
- Long-sleeved shirts and long pants
- Clothing and gear treated with permethrin
- Infant carrier mosquito net (if needed)
- Bed net (if mosquitoes can get to where you're sleeping)
- Condoms (if you might have sex)

Protect yourself

- Use insect repellent. Reapply as directed. Remember to apply sunscreen first and then insect repellent.
- Cover exposed skin when possible.
- Stay and sleep in screened-in or air-conditioned rooms. Use a bed net if you're sleeping outside.
- Zika can also be spread through sex, so use latex condoms if you have sex.

STOP the spread

- Watch for symptoms after you get home.
- Call your doctor immediately if you suspect Zika.
- Use insect repellent for 3 weeks after travel.
- Use condoms when you have sex.

Zika symptoms

Most people with Zika don't know they have it. The illness is usually mild with symptoms lasting about a week.

The most common symptoms are:

Pregnant? Trying to conceive?

Zika is linked to birth defects. Pregnant women should consider postponing travel to any area with Zika. If your male partner travels to these areas, either use condoms or don't have sex for the rest of your pregnancy. If you are trying to become pregnant, talk to your doctor about your plans.

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

www.cdc.gov/zika

May 11, 2015

Acknowledgments



CVR

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Amadou Sall

Bernhard Nocht Institute for Tropical Medicine

Esther Schnettler

University of Glasgow

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Yale University

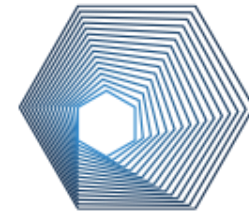
Brett Lindenbach

University of South Bohemia

Martin Selinger

University of Oxford

Jan Rehwinkel



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**FOCUSED MEETING 2017: INTERNATIONAL
MEETING ON ARBOVIRUSES AND THEIR VECTORS**

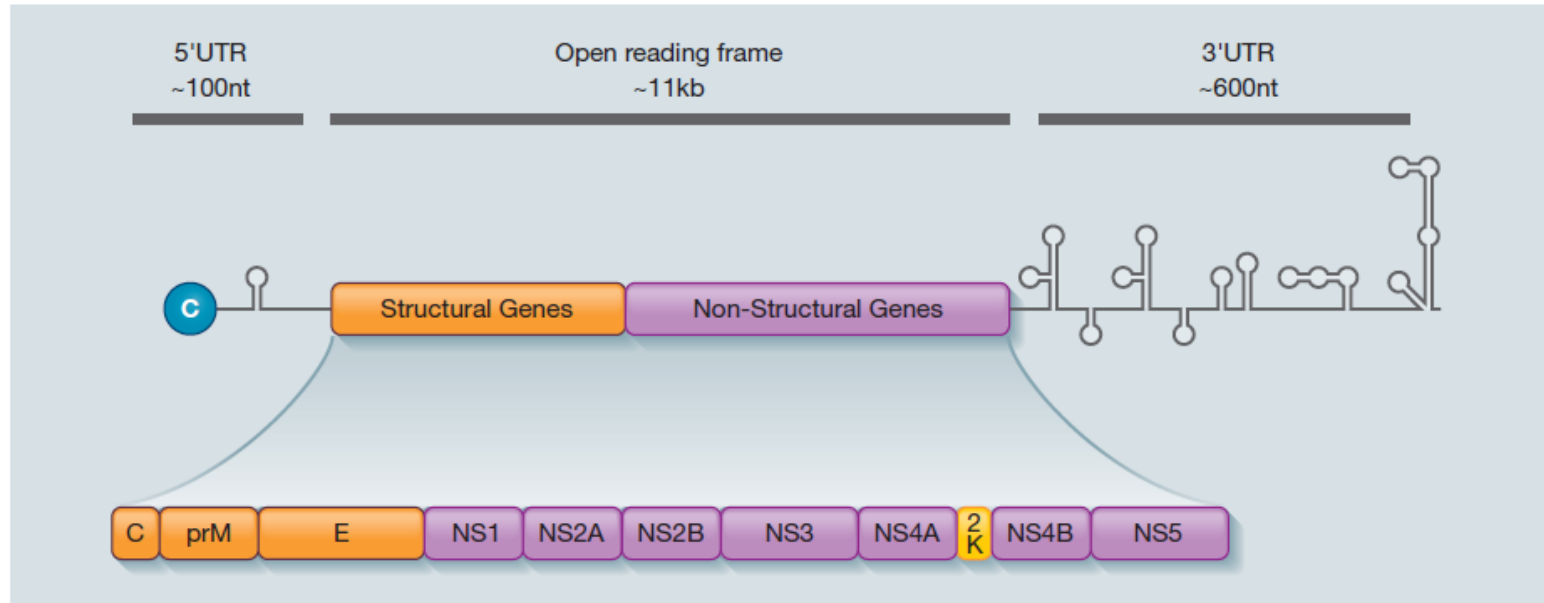
7 - 8 September

2017

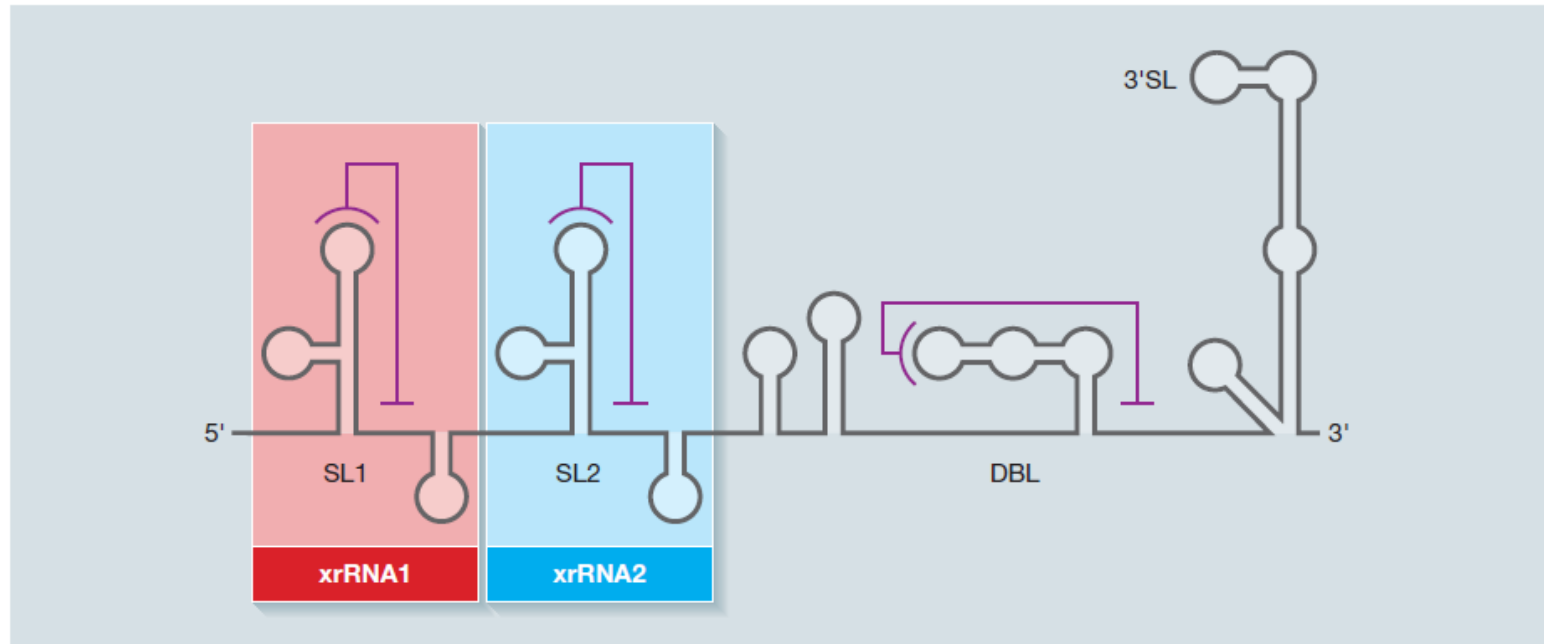
University of Glasgow
Glasgow
UK



**MICROBIOLOGY
SOCIETY**

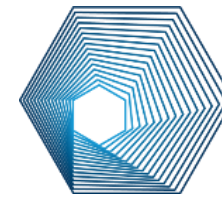


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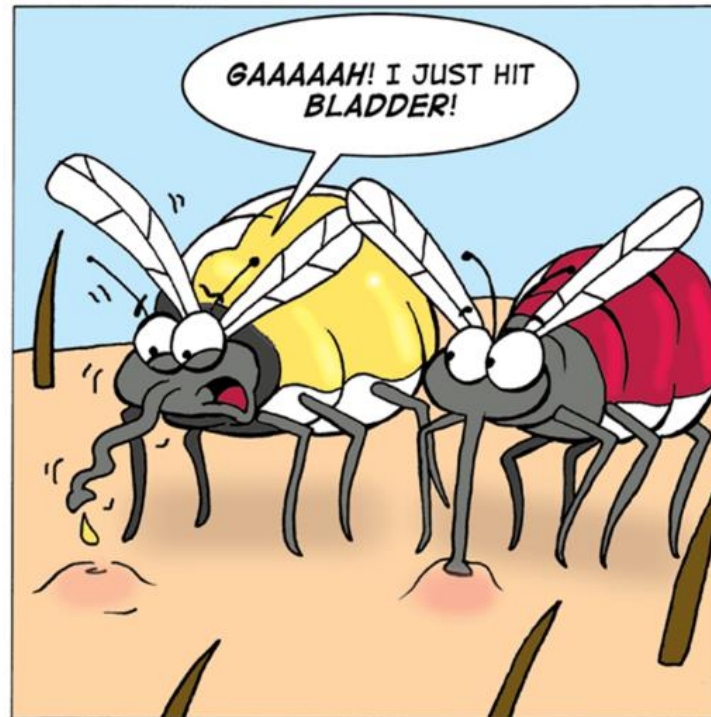


(b)

Thank you!



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Future Work

- Comparisons between lineages
 - Function of sfRNA, NS5 - and others?
- Other interferon pathway interactions
 - TRIM25 or TLR3?
- Reporter ZIKV
 - Expressing nanoluciferase

ZIKA VIRUS

What is Zika?

Zika is a virus transmitted by the *Aedes* mosquito, which also transmits dengue and chikungunya.

Zika can cause:

- Mild fever
- Conjunctivitis
- Headache and joint pain
- Skin rash

Onset is usually 2-7 days after the mosquito bite

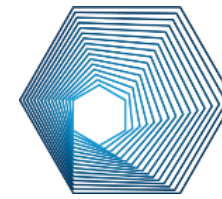
1 in 4 people with Zika infection develops symptoms

A very small number of people can develop complications after becoming ill with the virus

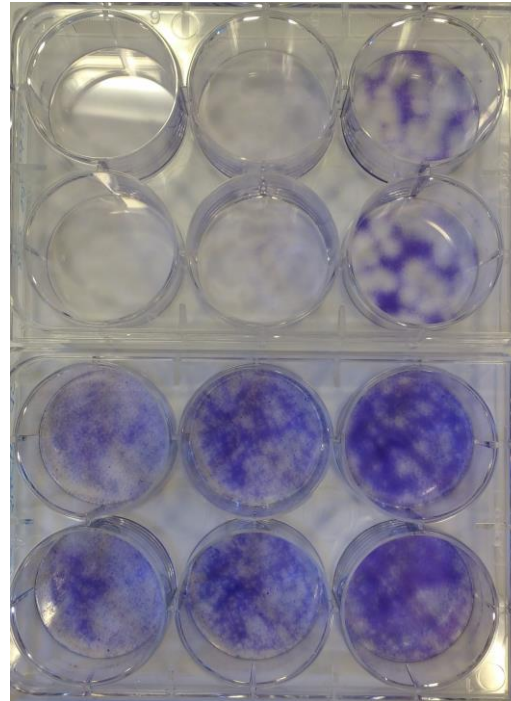
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#zika
#FightAedes
#ZikaVirus
www.paho.org/zikavirus

ZIKV is affected by Interferon



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ZIKV PE243
A549npro (human)
IFN incompetent

PE234
A549 (human)
IFN competent

Plaque size comparisons



Zika virus: a brief history

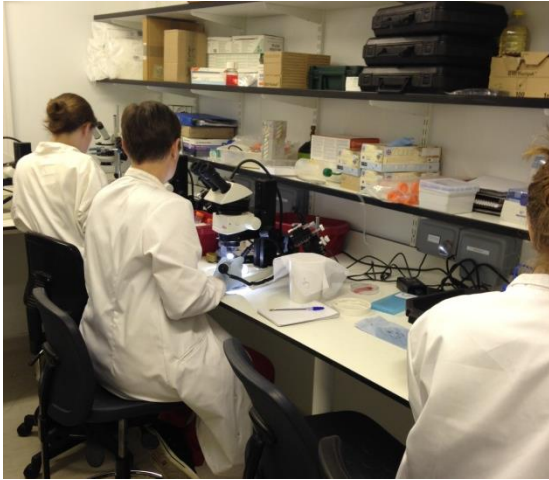
- **Discovery:** 1947, Rhesus macaque, Zika forest
- Involved Alexander John Haddow, graduate and Professor at the University of Glasgow
- **Further isolation:** 1948, *Aedes africanus*, Zika forest
- **1952—1954:** First human isolation in Nigeria, possible detection in India
- **1954-1981:** Detection in African and SE Asian countries (introduction ca. 1945? First detection late 60s, Malaysia)
- Pioneering work carried out at the Yellow Fever Institute, now Uganda Virus Research Institute, Entebbe (near Kampala and Zika forest)



Alexander Haddow
Zika virus discovery
1947 UVRI Uganda
Zika forest



Zika virus: research at the CVR



Dissection/injection lab room



Insectaries

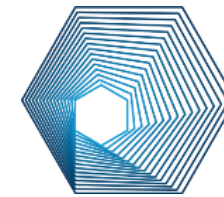
Set up of CL3 insectaries



Embryo micro-injection facility



Zika virus and sexual transmission



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Very unusual for arboviruses, as far as we know!

Increasing number of cases.

Virus has been found in semen.

First description by Foy et al.,
Emerging Infectious Diseases 2011

Can be spread by a man to sexual partners apparently
regardless of symptoms.



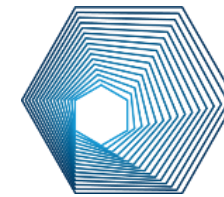
If returning from affected region, Zika compatible symptoms: condoms for 6 months.

If returning from affected region, no symptoms: use condoms for 28 days.

(Public Health England recommendations)



Zika virus: co-infection perspectives



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Zika virus structure solved.

Important data on antibody cross-reactivity between dengue and Zika antibody responses which may impact on pathogenesis, transmission etc.

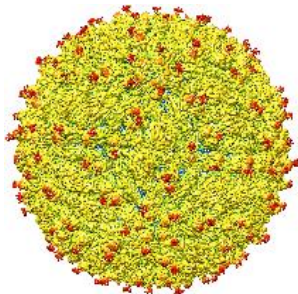
Cross reactivity and/or enhanced ADE observed in some studies which needs to be assessed in the field.

ARTICLE

doi: 10.1038/nature18818

Structural basis of potent Zika–dengue virus antibody cross–neutralization

Giovanna Barba-Spaeth^{1,2*}, Warwisa Dejnirattai^{1,4}, Alexander Rouvinski^{1,2*}, Marie-Christine Vaney^{1,2*}, Iris Medits¹, Arvind Sharma^{1,2}, Etienne Simon-Loriere^{1,4}, Anavaj Sakantabhai^{7,8}, Van-Mai Cao-Lormeau², Ahmed Hassan⁹, Patrick England^{1,2}, Karin Silasny¹, Juthathip Meegkolapanya^{1,2,11}, Franz X. Heitz¹, Gavin R. Screaton¹ & Felix A. Rey^{1,2}



Rossmann Lab

Specificity, cross-reactivity, and function of antibodies elicited by Zika virus infection

Karin Stettler^{1,2}, Martina Beltramello^{1,4}, Diego A. Espinosa^{2,1}, Victoria Graham^{2,1}, Antonino Cassotta^{4,4,1}, Siro Bianchi^{1,1}, Fabrizia Vanzetta^{1,1}, Andrea Minola¹, Stefano Jacoel¹, Federico Mele⁴, Mathilde Foglierini⁴, Mattia Pedotti⁴, Luca Simonelli⁴, Stuart Dowall³, Barry Atkinson³, Elena Percivalle⁵, Cameron P. Simmons^{7,8,9}, Luca Varani⁴, Johannes Blum^{10,11}, Fausto Baldanti⁶, Elisabetta Cameroni¹, Roger Hewson¹, Eva Harris², Antonio Lanzavecchia^{4,5}, Federica Sallusto^{4,1,4}, Davide Corti^{1,1,4}

ARTICLES

nature
immunology

Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with zika virus

Warwisa Dejnirattai¹, Piyaeda Supasa^{1,2}, Wiyada Wongwat¹, Alexander Rouvinski^{1,2}, Giovanna Barba-Spaeth^{1,2}, Thananya Daangchinda¹, Anavaj Sakantabhai^{7,8}, Van-Mai Cao-Lormeau², Prida Malasi^{1,2}, Felix A Rey^{1,2}, Juthathip Meegkolapanya^{1,2} & Gavin R Screaton¹

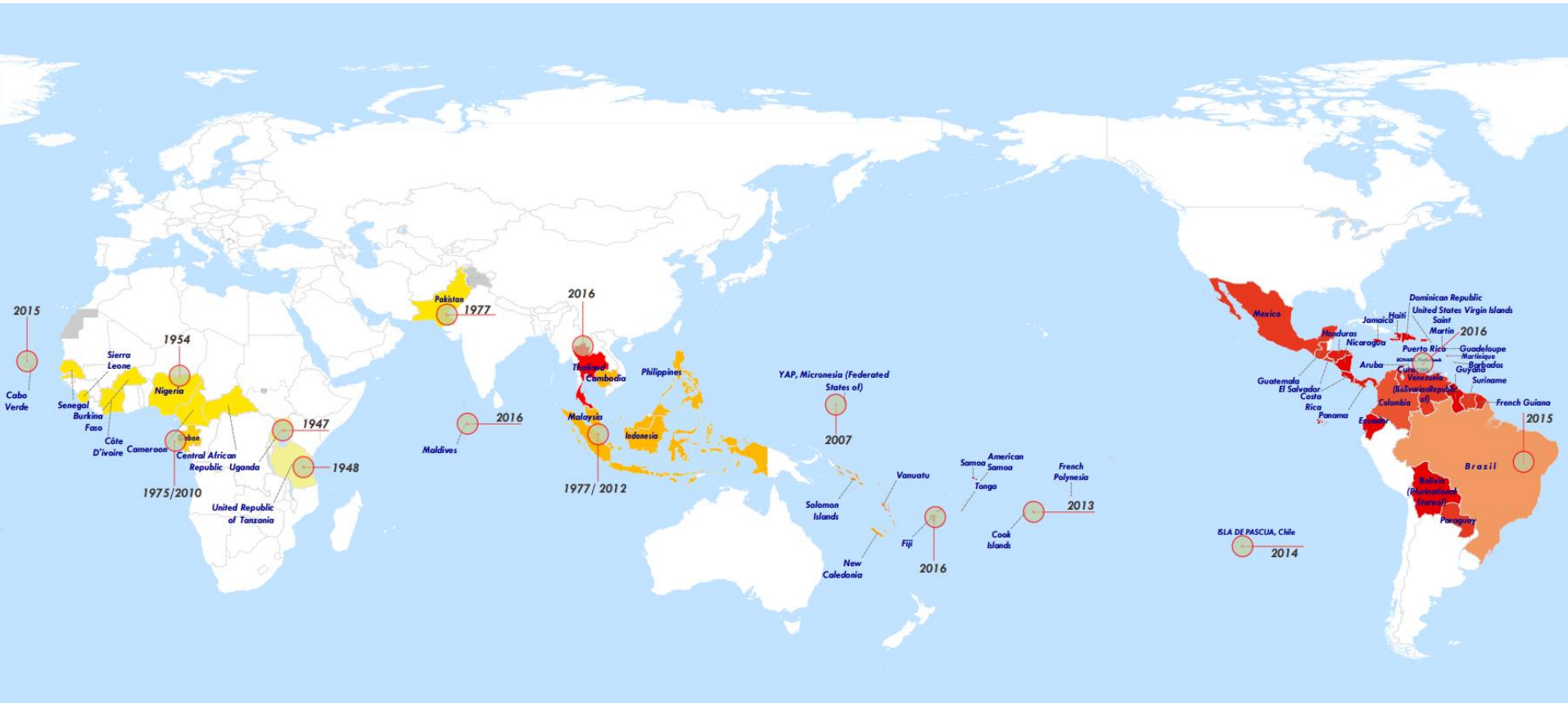


Patient 243

- Sex: F
 - Age: 19
 - Days of symptoms: 2
 - DENV IgM: Negative
 - DENV IgG: Positive
 - ZIKV RT-PCR: Positive
 - ZIKV IgM: Positive (second sample)
- Clinical symptoms
 - No fever (max temp 37°C)
 - Rash on face and limbs for 5 days
 - Arthralgia on hands, fist/wrist and ankle
 - Edema on hands, fist/wrist for 3 days
 - Fully recovered in 16 days



Historical distribution of ZIKV





Clinical Presentation

Incubation period

- Onset of symptoms is 2 – 12 days post infection

Viraemic period

- Short viraemic period allowing for direct virus detection 3 – 5 days after onset of symptoms

Symptoms

- Rash with/without fever
- Arthralgia/ arthritis
- Conjunctivitis

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#zika
#FightAedes
#ZikaVirus
www.paho.org/zikavirus

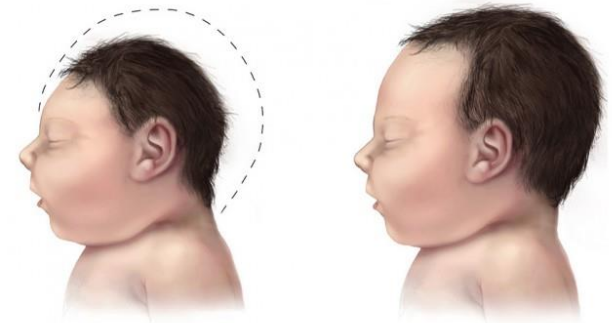
Pan American Health Organization | World Health Organization
REGIONAL OFFICE FOR THE AMERICAS



Potential complications

Microcephaly in foetuses and newborns

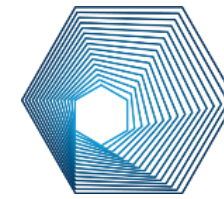
- ZIKV linked to severe congenital central nervous system malformations and microcephaly
- ZIKV can pass from a pregnant woman to her child



Guillain-Barré syndrome (GBS)

- Temporal association between ZIKV outbreaks and increases in the incidence of GBS

Zika virus and microcephaly



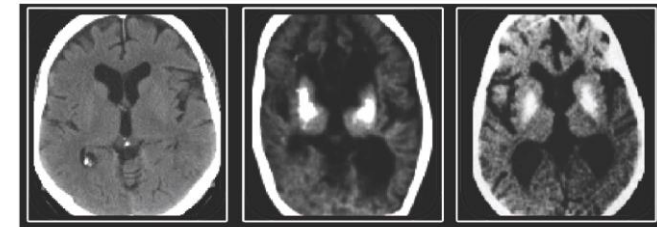
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History: IMIP, non profit hospital Recife

- September 2015: increase in cases of microcephaly - 29 cases born between August and September 2015 (usually a dozen cases per year)
- October (27.10.2015): Notification State Department of Health & Protocol development – CIEVS
- November 2015 : Ministry of Health decrees Public Health Emergency in Brazil



Defined as occipito-frontal circumference $< -2sd$ of mean for age/sex



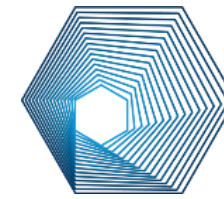
Normal

AGS

CMV/Rubella

Source: CDC

Zika congenital syndrome



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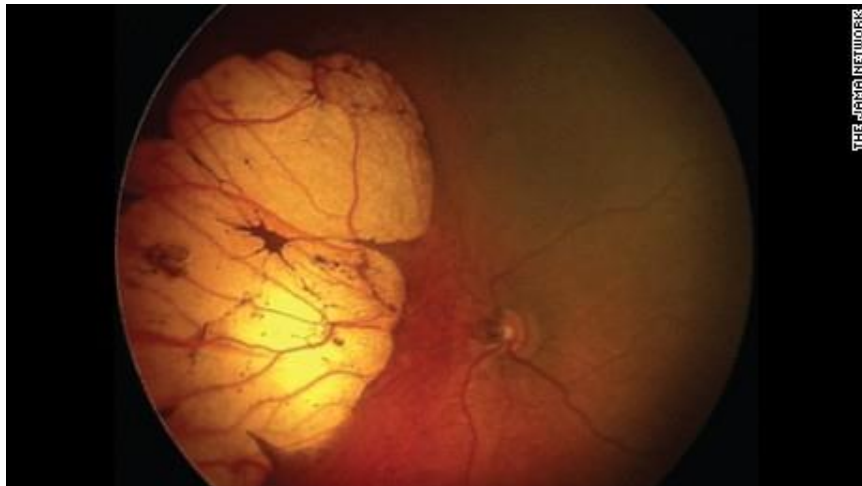
Beyond microcephaly- other malformations:

Craniofacial disproportion, spasticity, seizures, irritability and brainstem dysfunction including feeding difficulties

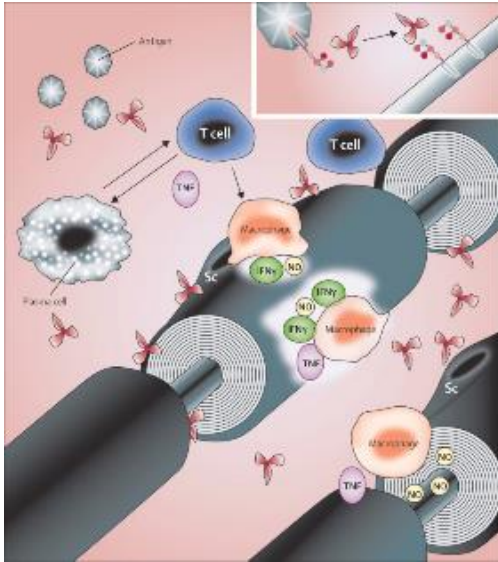
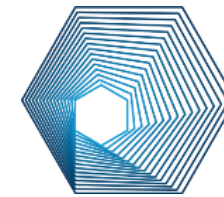
Ocular abnormalities

Neuroimaging such as calcifications, cortical disorders and ventriculomegaly

Zika-induced macular atrophy



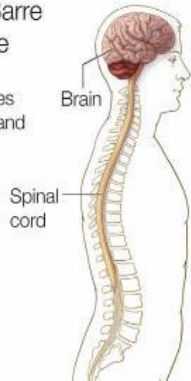
Zika virus and Guillain-Barré syndrome



- Muscle weakness due to immune system attacking the peripheral nervous system; plateau phase typically a week before improvement.
- *Can be triggered by viral or bacterial infection (flu, C. jejuni).*
- *Countries, territories or areas reporting GBS potentially related to Zika virus infection: Brazil, El Salvador, French Polynesia, Suriname, Venezuela (source: WHO).*
- Bahia (Brazil): 42 GBS cases in 2015; 26 (62%) had history of symptoms consistent with Zika virus infection. 19% increase from the previous year nationwide (source: WHO).

Guillain-Barre Syndrome

Affects nerves in the brain and spinal cord



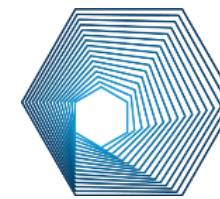
Normal nerve



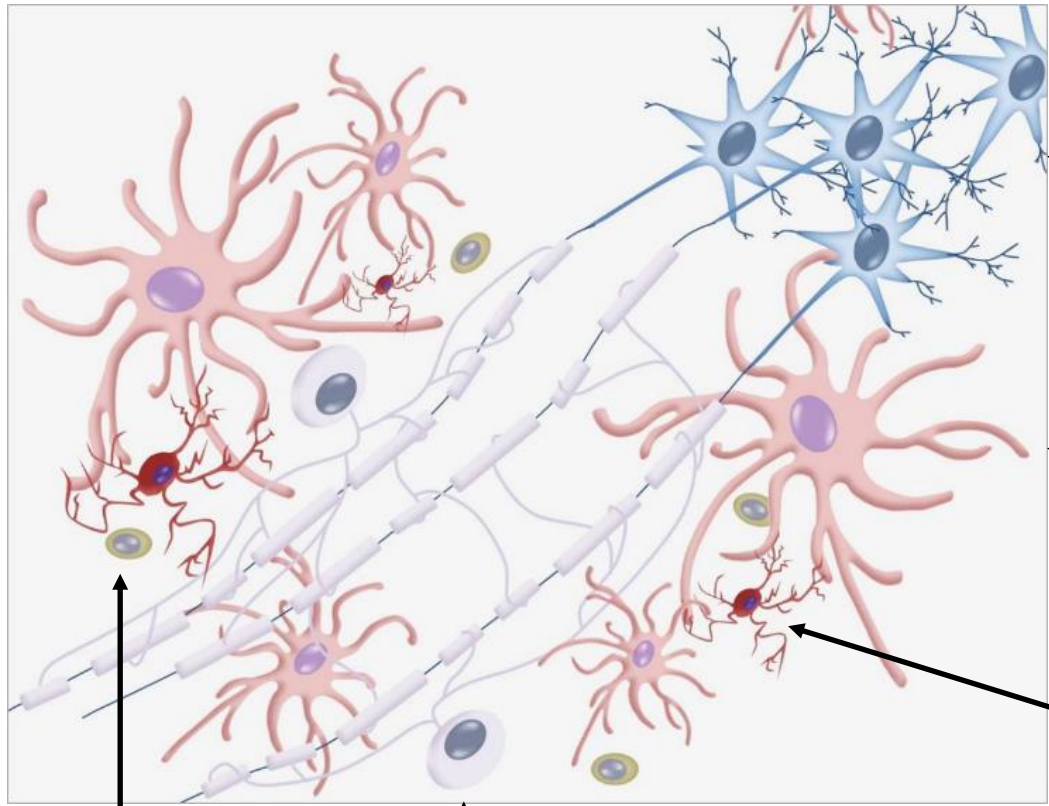
Affected nerve



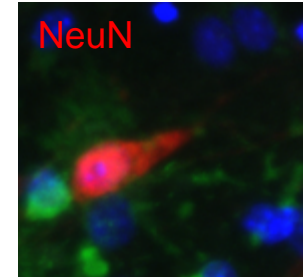
CNS co-culture composition



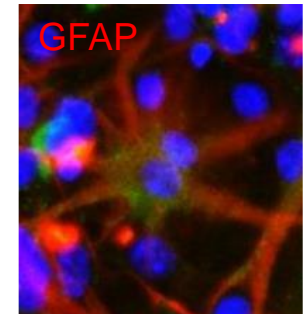
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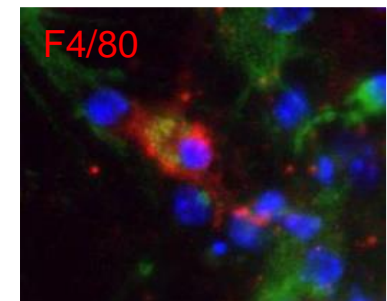
Neuron
NeuN



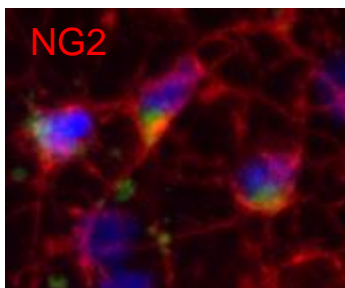
Astrocyte
GFAP



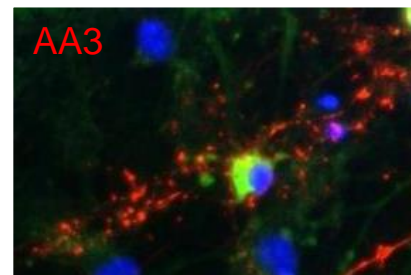
Microglia
(macrophage-like)
F4/80



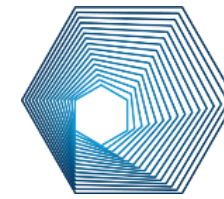
Precursor cell



Oligodendrocyte
(myelin-producing)
AA3



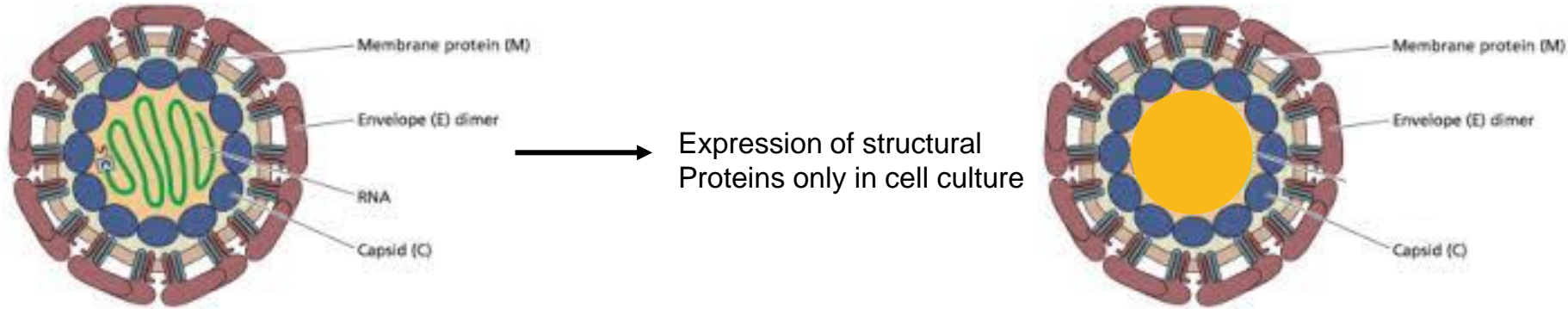
Zika virus: vaccine candidates



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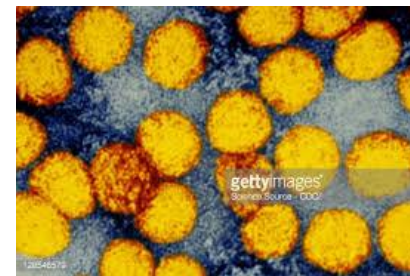
Strategies- examples:

- **Virus-like particles: virus shell missing genetic information but enough to stimulate immunity**



- **Attenuated viruses (example: 17D yellow fever vaccine, close relative of Zika):**

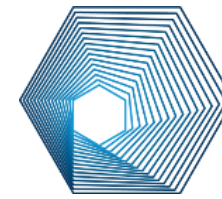
Viruses with mutations that make them less virulent! These can be in proteins that are Related to virulence, replication, spread....



Efforts are underway to test various options!

Problem: other flaviviruses such as dengue are co-circulating and vaccination MIGHT make dengue infections through inefficient antibodies as these viruses are very similar.

Zika virus PE243 & phylogenetic data: tracing outbreaks and virus spread!

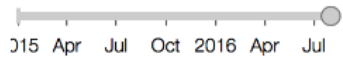


nextstrain / Zika ▾

Real-time tracking of Zika virus evolution

Phylogeny

2016 Jul 14



Region

- French Polynesia
- American Samoa
- China
- Brazil
- Colombia
- Suriname
- Venezuela
- Panama
- Honduras
- Guatemala
- Mexico
- Martinique
- Puerto Rico
- Dominican Republic
- Haiti

Color by geographic region

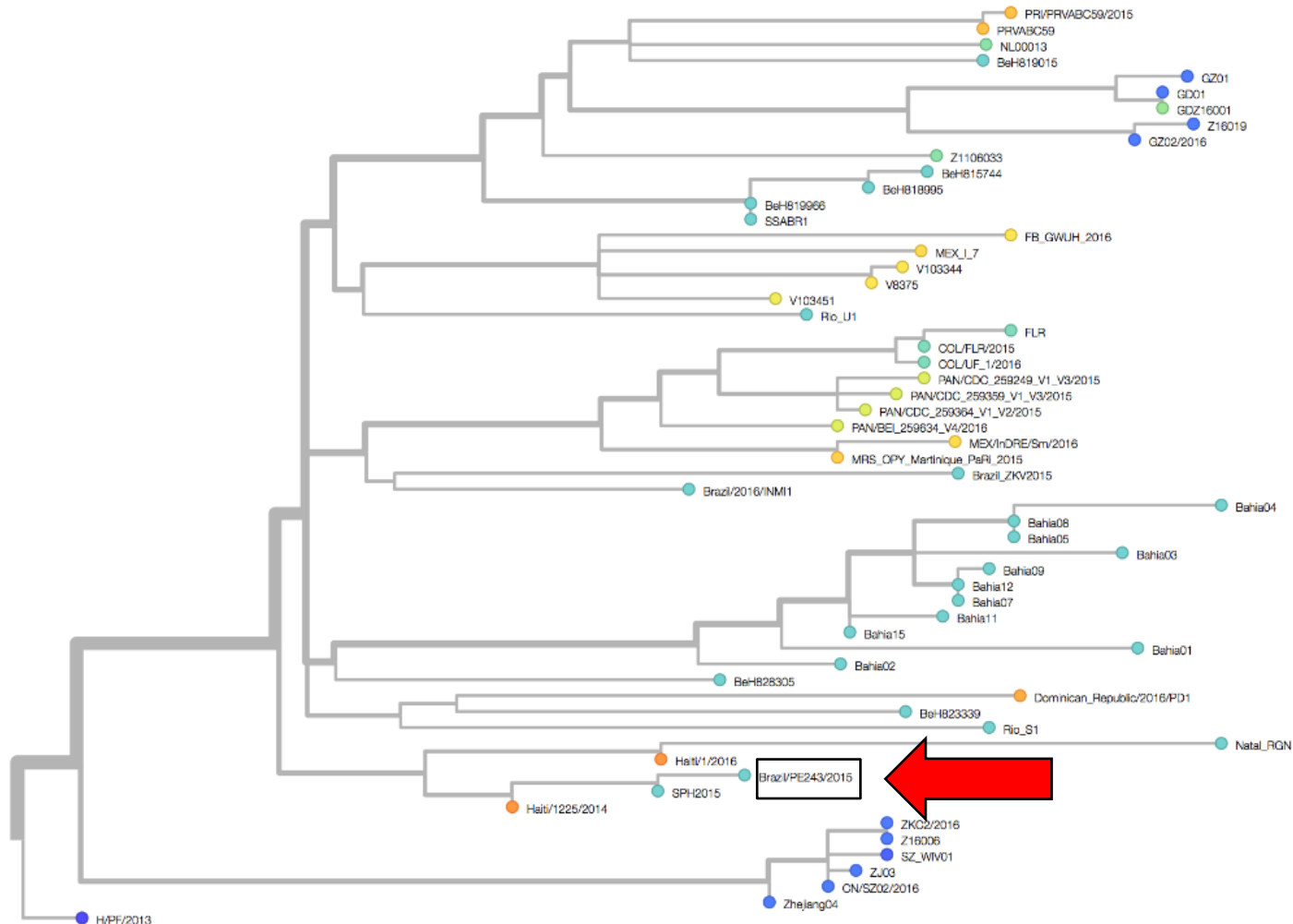
Or Genomic position

branch labels

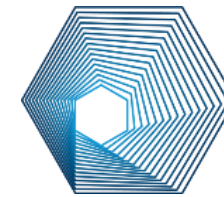
search strains...

clear

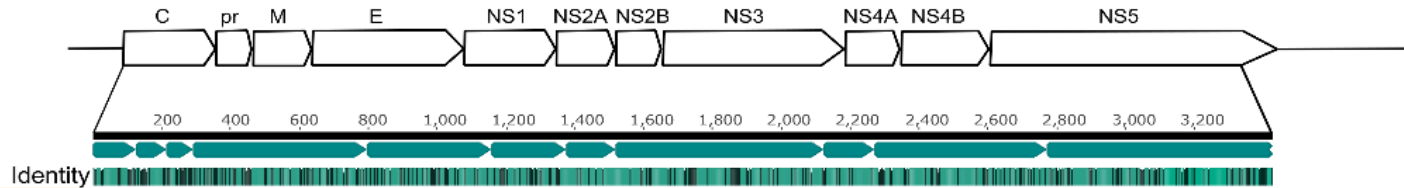
reset layout



Zika virus PE243 properties



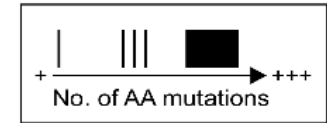
Full genome including 5'/3' termini sequenced! Genbank KX197192

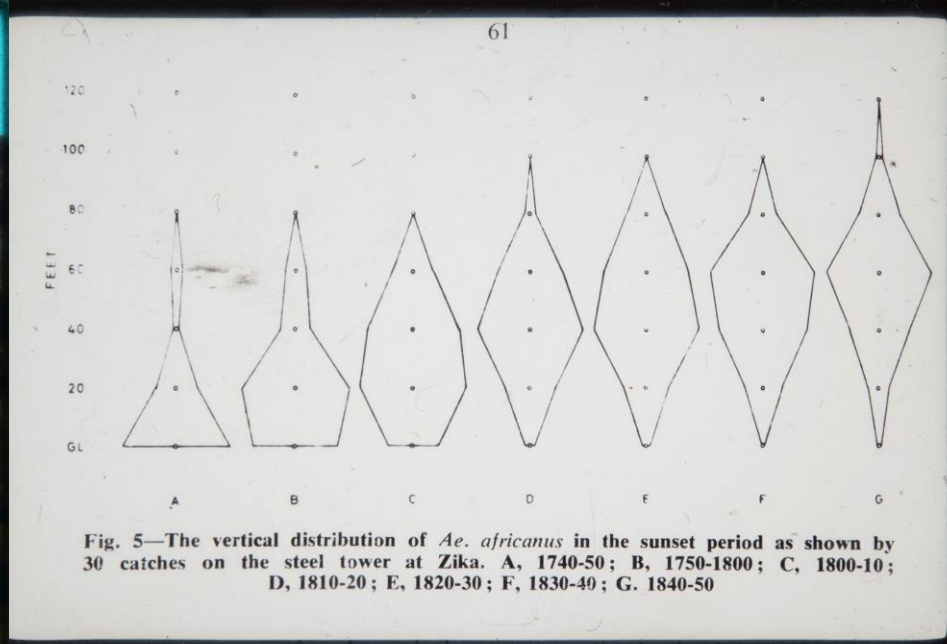


1. ZIKV/H. sapiens/Brazil/PE243/2015 ●
2. ZikaSPH2015 ●
3. Natal RGN ●
4. Brazil-ZKV2015 ●
5. FB-GWUH-2016 ●
6. BeH823339 ●
7. BeH828305 ●
8. BeH819966 ●
9. BeH819015 ●
10. BeH818995 ●
11. BeH815744 ●
12. Rio-U1 ●
13. Rio-S1 ●
14. SSABR1 ●
15. Brazil/2016/TNMI1 ●
16. 8375 ●
17. 103344 ●
18. MEX/INDRE/Sm/2016 ●
19. MEX/INDRE/Lm/2016 ●
20. VE_Ganxian ●
21. SZ-WIV01 ●
22. Z103 ●
23. Z1106033 ●
24. Z16019 ●
25. Z16006 ●
26. GZ01 ●
27. GDZ16001 ●
28. GD01 ●
29. Zika virus/SZ01/2016 ●
30. Zika virus/GZ02/2016 ●
31. Haiti/1/2016 ●
32. Dominican Republic/2016/PD1 ●
33. Dominican Republic/2016/PD2 ●
34. PRVABC59 ●
35. ZIKV/Homo sapiens/PRI/PRVABC59/2015 ●
36. ZIKV/Homo sapiens/COL/FLR/2015 ●
37. MRS_OPY_Martinique_PaRI_2015 ●
38. Haiti/1225/2014 ●
39. Zika virus/H.sapiens-tc/THA/2014/SV0127-14 ●
40. H/PF/2013 ●
41. Zika virus/H.sapiens-tc/PHL/2012/CPC-0740 ●
42. Zika virus/H.sapiens-tc/KHM/2010/FSS13025 ●
43. EC2007 - EU545988 ●
44. ArD158084 ●
45. ArD157995 ●
46. ArD128000 ●
47. ArD711 ●
48. ARB1362 ●
49. ARB15076 ●
50. ARB13565 ●
51. ARB7701 ●
52. Zika virus/A.taylori-tc/SEN/1984/41671-DAK ●
53. Zika virus/A.taylori-tc/SEN/1984/41662-DAK ●
54. Zika virus/A.africanus-tc/SEN/1984/41525-DAK ●
55. ZIKV/Homo sapiens/NGA/IbH-30656_SM21V1-V3/1968 ●
56. Zika virus/M.mulatta-tc/UGA/1947/MR-766 ●
57. ZIKV/Macaca mulatta/UGA/MR-766_SM150-V8/1947 ●
58. MR766-NLD ●
59. MR766 - NC_012532 ●
60. MR766 - KU720415 ●
61. MR766 - DQ859059 ●
62. MR766 - AY632535 ●

Asian Lineage

African Lineage





TWENTY-FOUR-HOUR CATCHES AT ZIKA, NEAR ENTEBBE, JANUARY 1948.
YELLOW FEVER RESEARCH INSTITUTE.

This series of 5 catches was carried out in secondary lakeshore forest with little understorey & fairly dense undergrowth. The forest area is narrow & is bordered on the lake side by an extensive papyrus swamp.

Prominent trees are Albizia, Piptadenia, Morospis, Canarium, Ficus & Phoenix

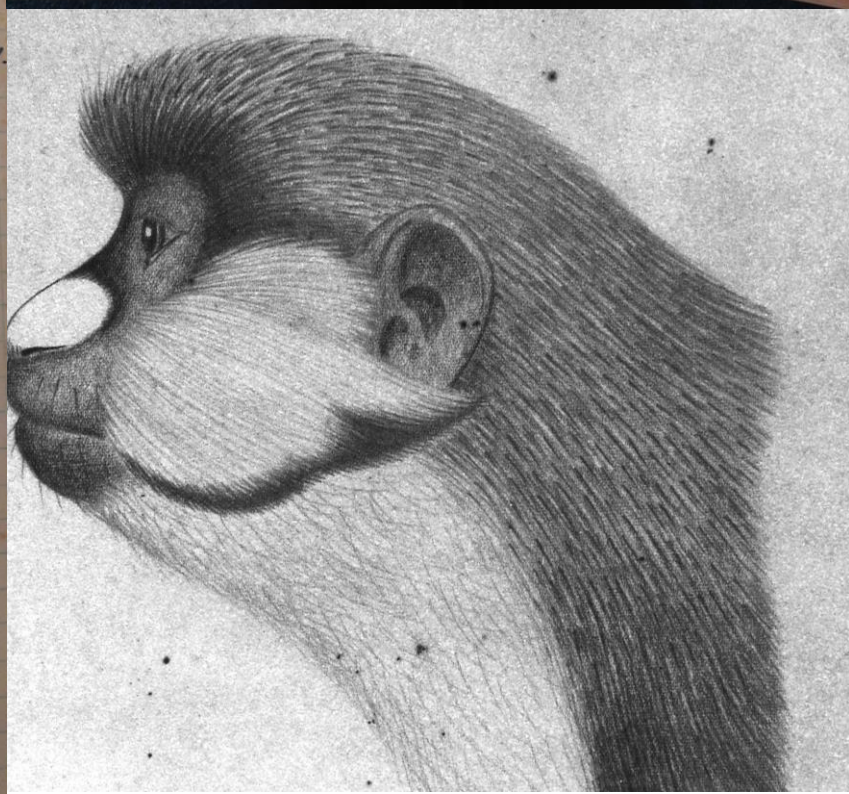
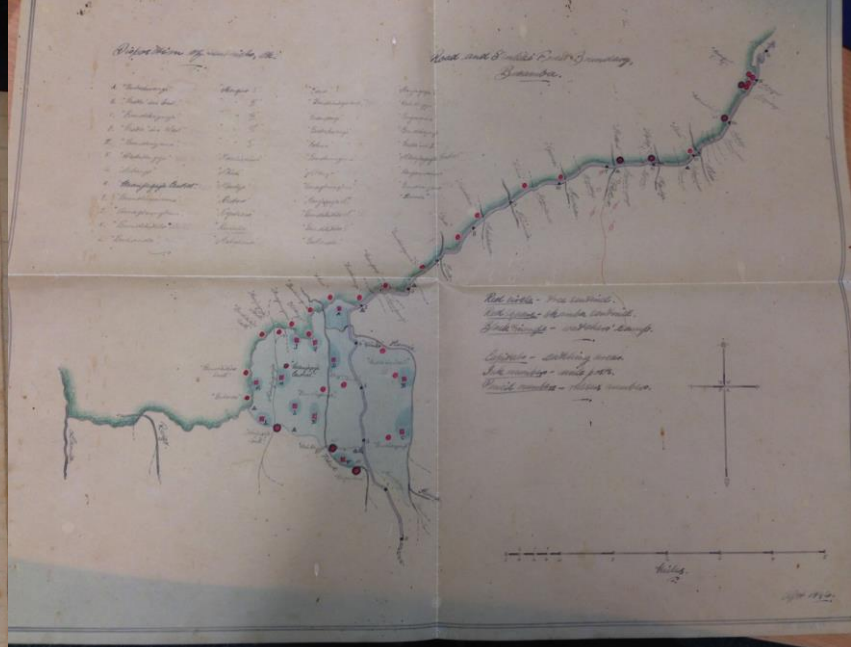
The tree unit occupied platforms at 38 & 55 feet respectively, the tree being Strombosia grandifolia. The ground unit sat below the tree.

The serial numbers of these catches are 27-28-29-30-31.

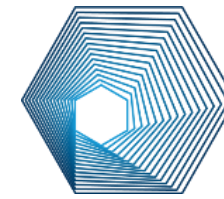
The nearest banana plantation is about 350 yards from the catching station.

The nearest hut is also about 350 yards from the catching station.

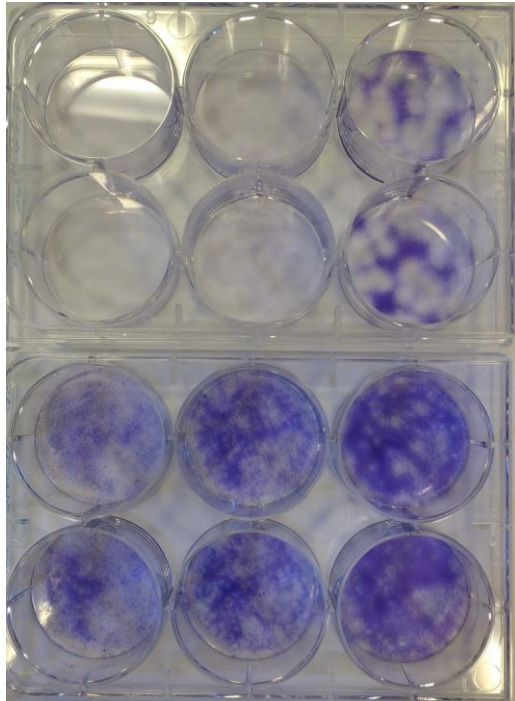
Err.:- For A. sanguinea read A. egypticus.



ZIKV: Classical Virology



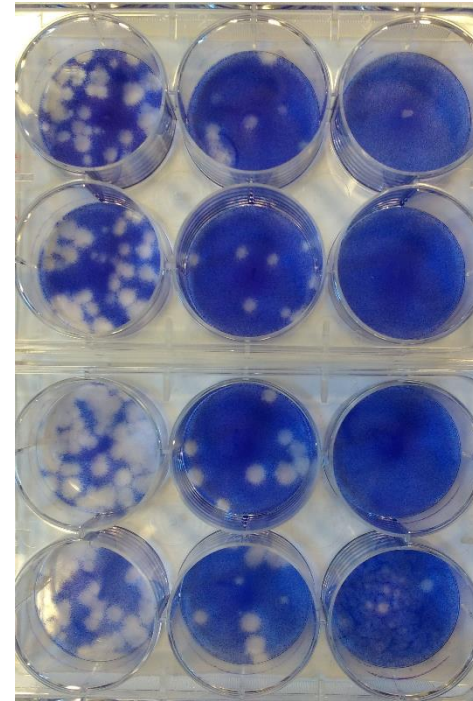
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PE243 (Brazilian)
A549npro (human)
IFN incompetent

PE234 (Brazilian)
A549 (human)
IFN competent

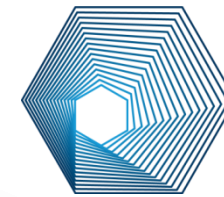
Plaque size
comparisons



MR766 (African)
Vero (monkey)
Titre 1.8×10^7 pfu/ml

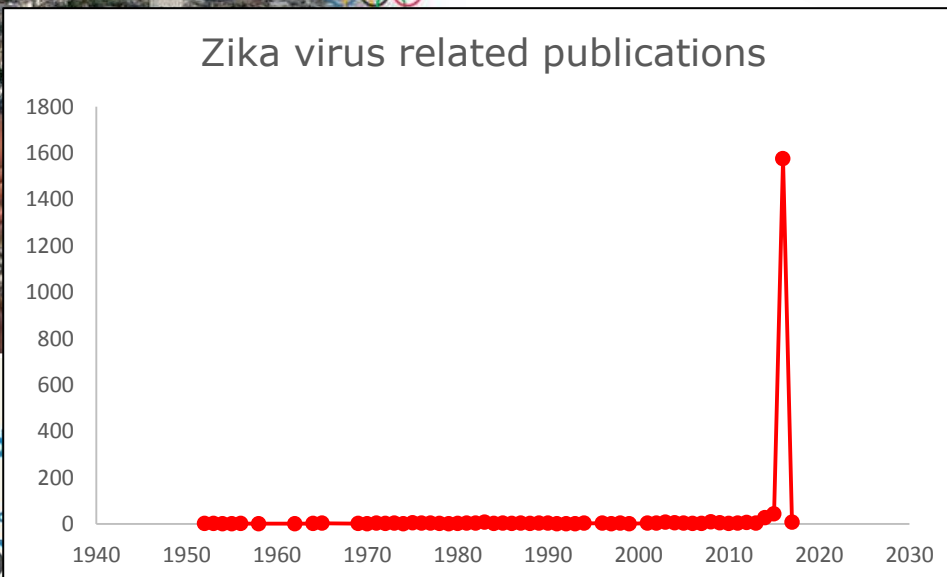
PE234 (Brazilian)
Vero (monkey)
Titre 2.8×10^7 pfu/ml

Virus Titrations



Hurricane Zika and Glasgow

Zika virus threat to Rio Olympics: What you **MUST** do to stay safe



Zika prevention

Use physical barriers: screens, closed doors

Use insect repellent

Sleep under a bed net

Wear light-coloured, long-sleeved shirts and trousers

TO ZIKA

1 in 4
Infected people show symptoms

2 weeks
How long the virus stays in the blood stream



his daughter Luiza, who was born with microcephaly

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at you need to know

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