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

Claire Donald

24th February 2017

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Zika virus: a historical link to the University of Glasgow

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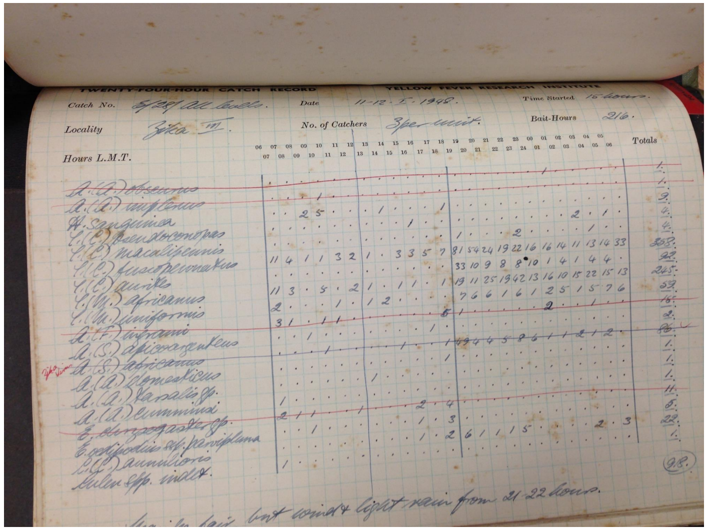
- Discovery: 1947, Rhesus macaque, Zika forest
- Involved Alexander John Haddow, graduate and Professor at the University of Glasgow
- Further isolation: 1948, *Aedes africanus*, Zika forest
- 1952—1954: First human isolation in Nigeria, possible detection in India
- 1954-1981: Detection in African and 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 (Flavivirus)

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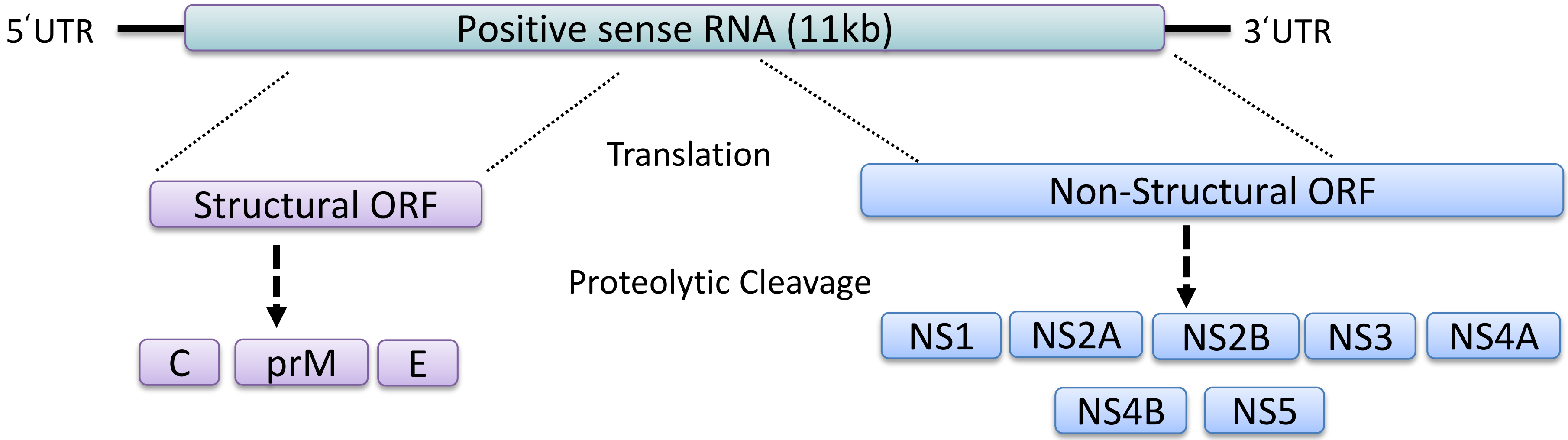
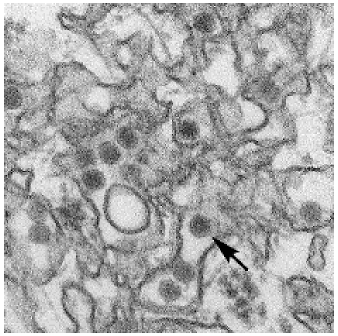


11kb genome, positive stand RNA.

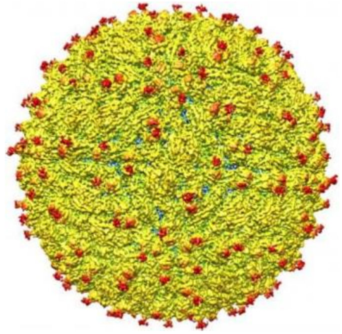
10 Genes: 3 structural (C, prM, E), 7 non-structural.

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

Polyprotein cleaved by viral and host proteases.



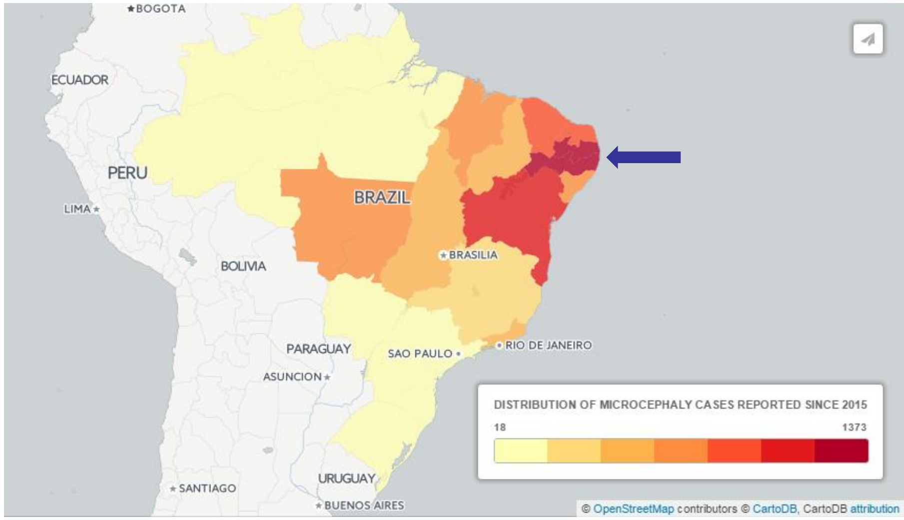
CDC, 2005: C. Goldsmith



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

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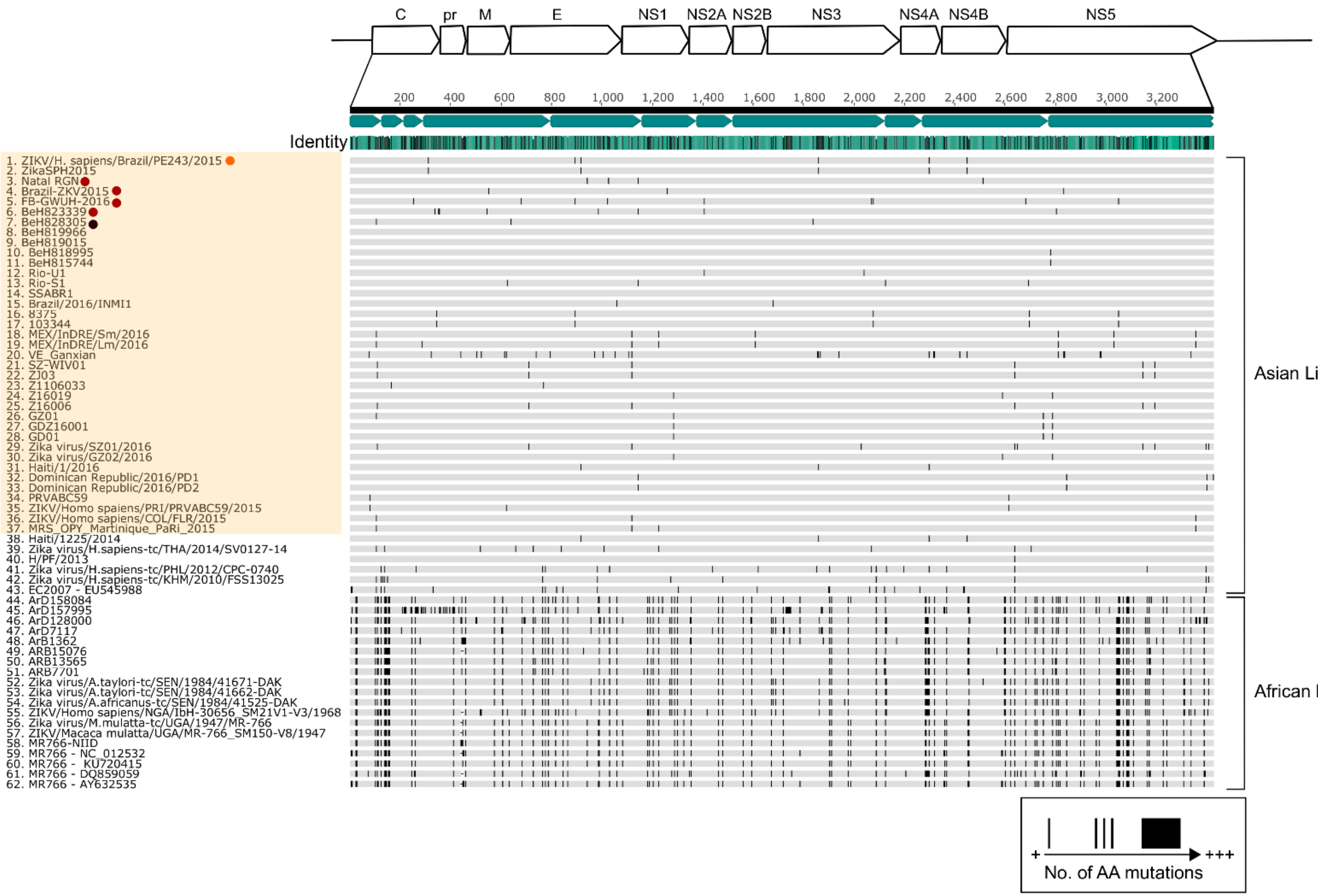
Characterisation of ZIKV

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

Characterisation of ZIKV



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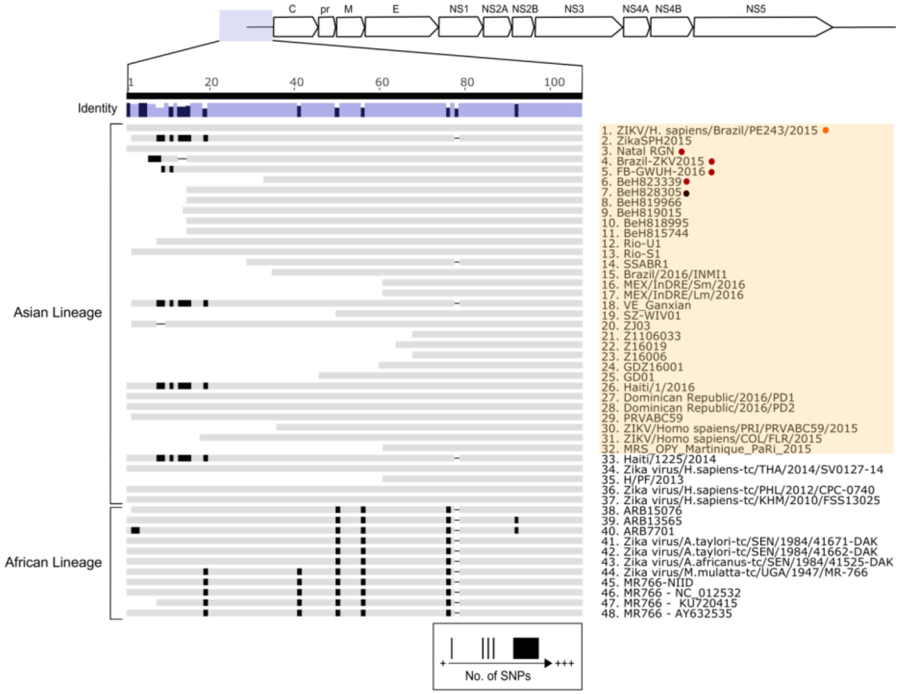
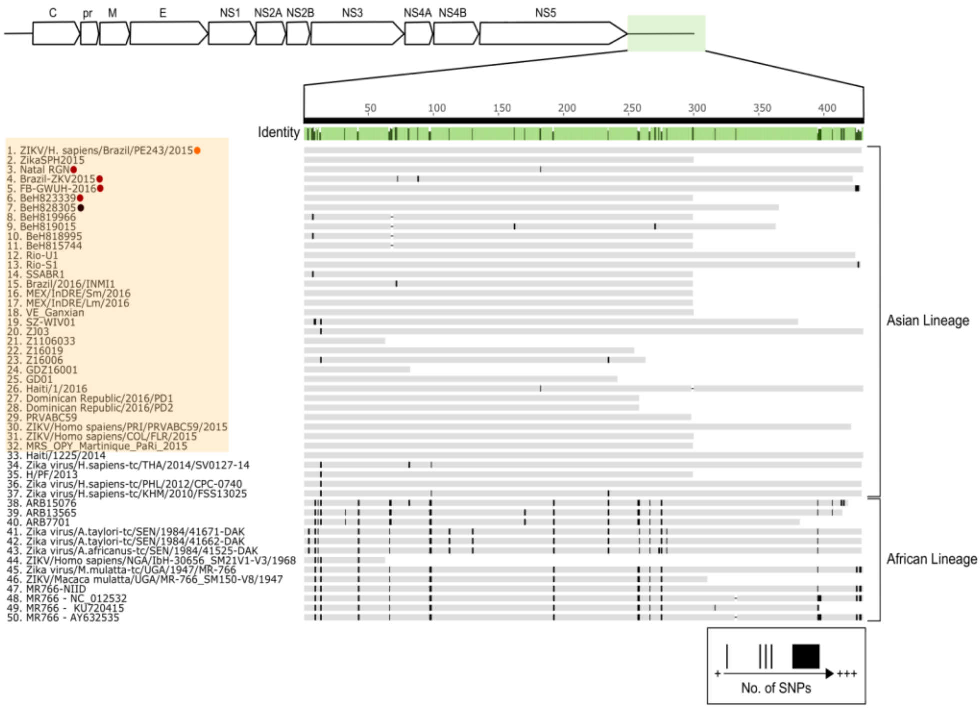
Characterisation of ZIKV

5'UTR



3'UTR



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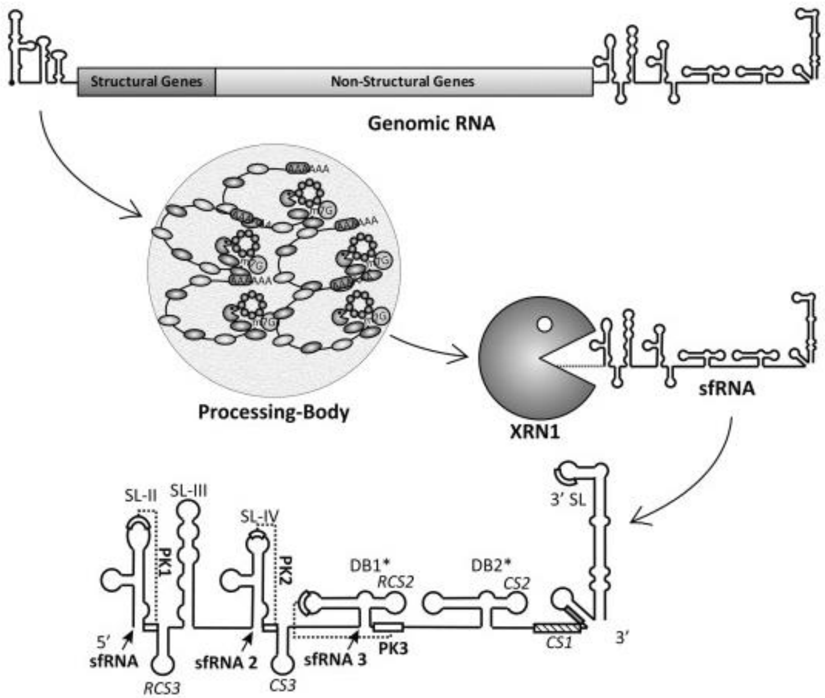
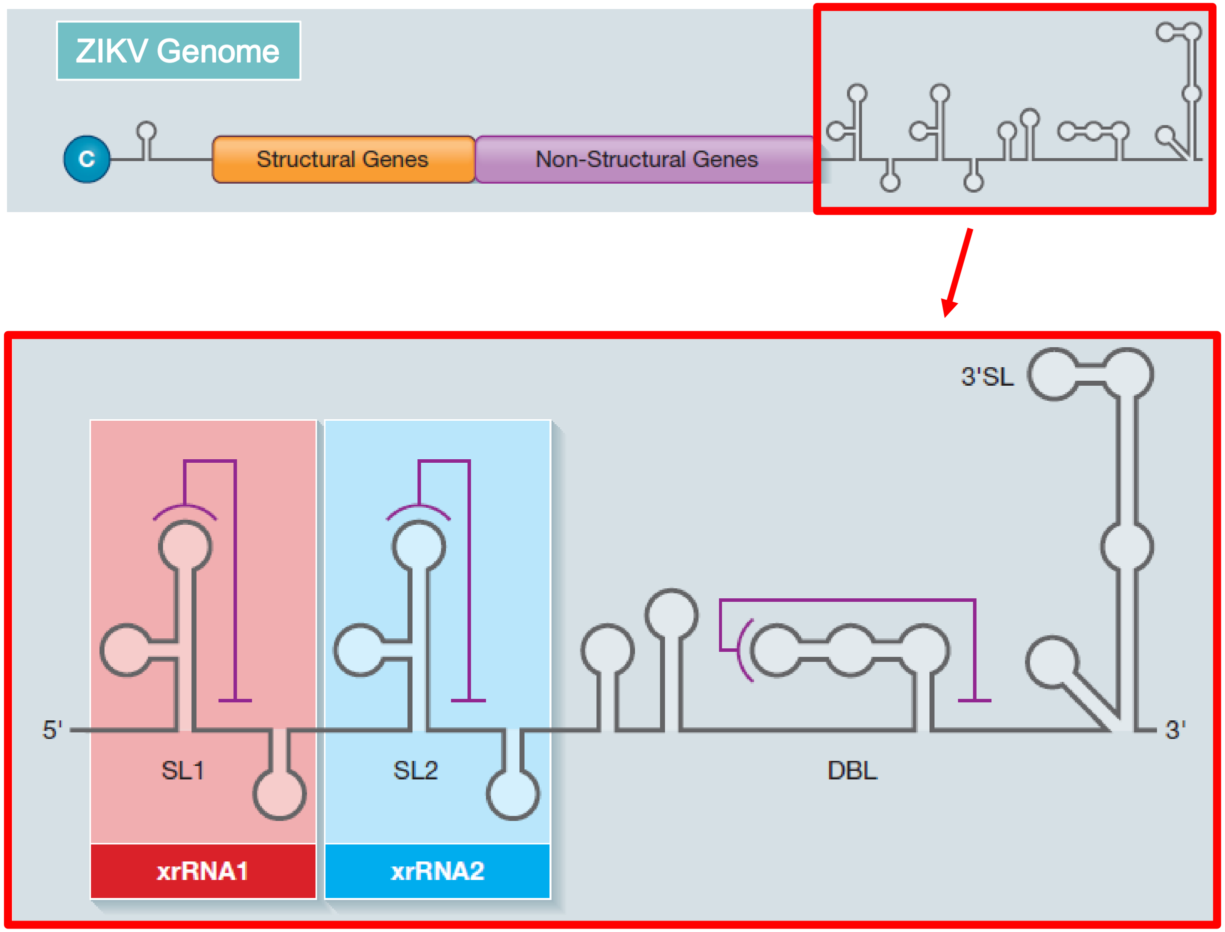
ZIKV sfRNA as an interferon antagonist

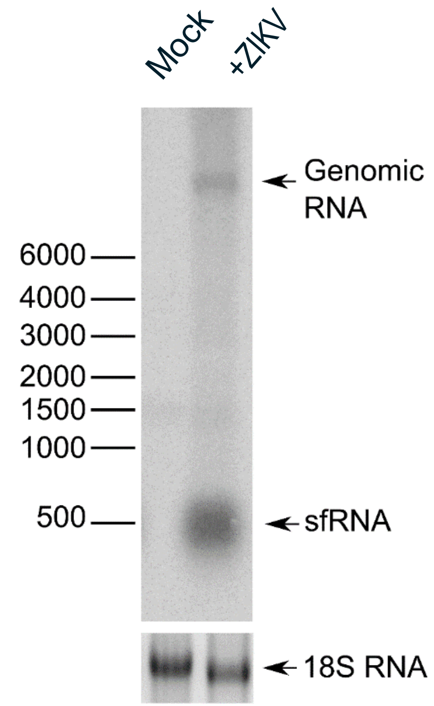


Clarke *et al*., 2015

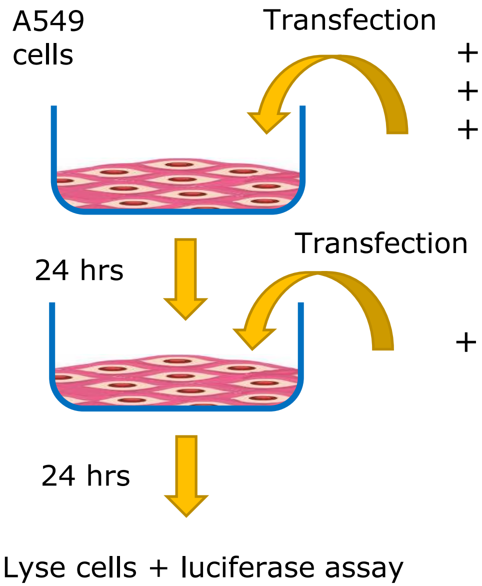
- 0.2-0.6 kb subgenomic flavivirus RNA (sfRNAs)
- Co-linear to the 3′ end of the genome
- Produced through incomplete degradation of the genome by the cellular 5′-3′ exoribonuclease, XRN1
- sfRNA inhibits type I interferon response.

ZIKV sfRNA as an interferon antagonist



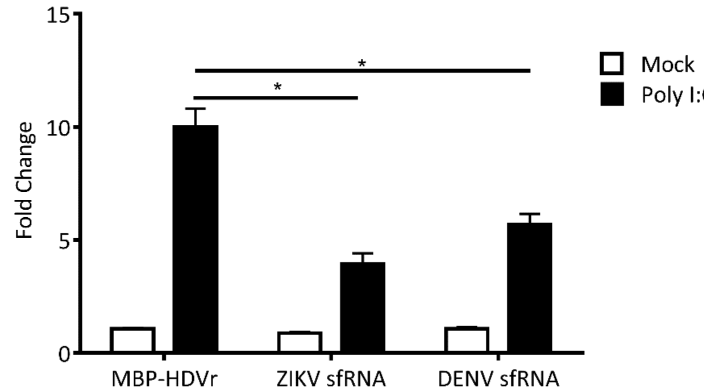


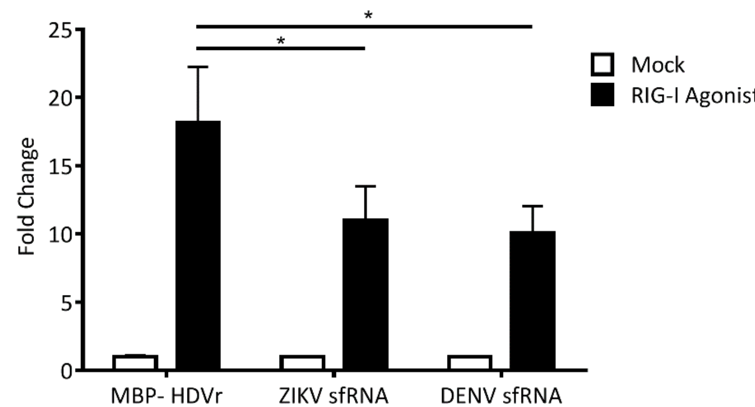
ZIKV sfRNA as an interferon antagonist

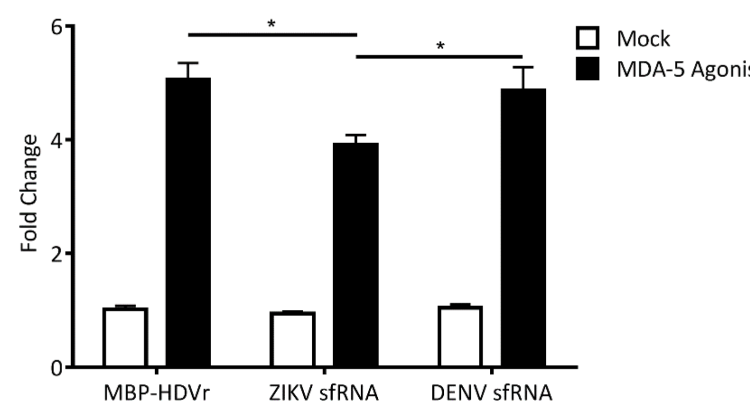


+ p125Luc (IFN-β promoter + FFLuc)
+ pRL-CMV (Internal control + Rluc)
+ pDest-DENV-3′ UTR or
pDest-ZIKV PE243- 3′UTR or
pDest-MBP-HDVr (control)

+ RIG-I agonist or MDA-5 agonist







ZIKV sfRNA as an interferon antagonist

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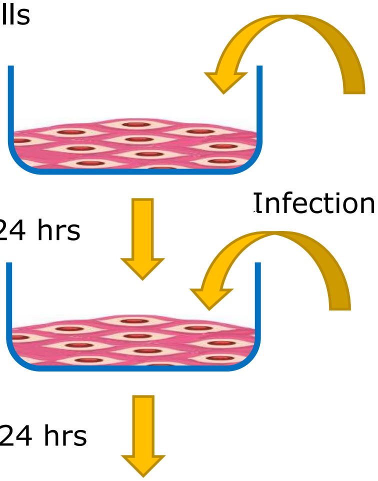
A549 cells

Transfection



+ p125Luc (IFN-β promoter + FFLuc)
+ pRL-CMV (Internal control + Rluc)
+ pDest-DENV-3′ UTR or
  pDest-ZIKV PE243- 3′UTR or
  pDest-MBP-HDVr (control)

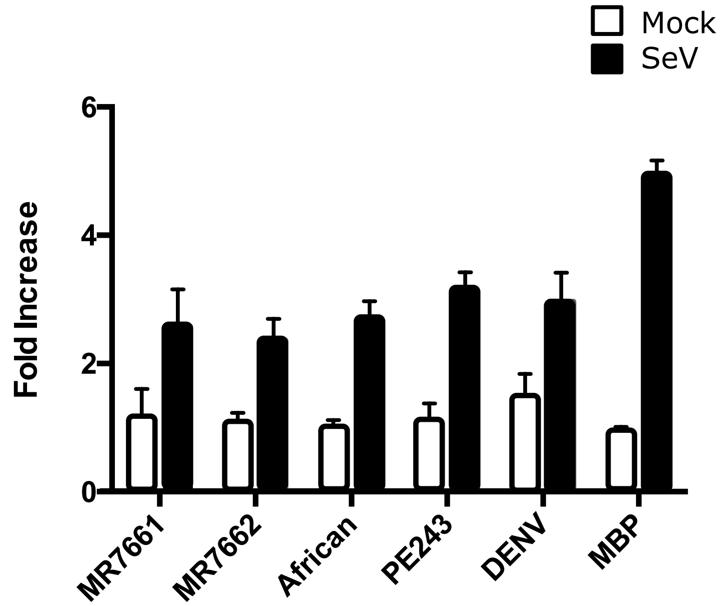
24 hrs

Infection

+ Sendai Virus (SeV)

24 hrs

Lyse cells + luciferase assay



ZIKV: Conclusions

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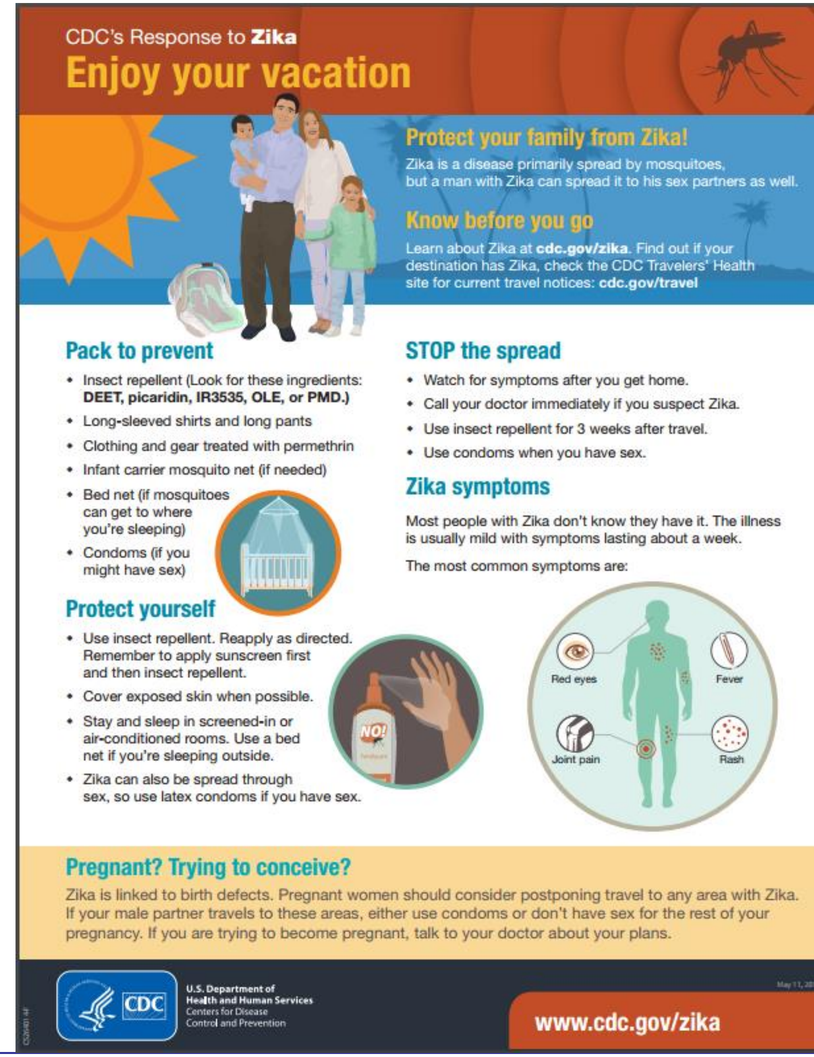
- Successfully obtained a full length genome sequence from a Brazilian patient.
- No obvious virological explanation for increased occurrence of neurological diseases associated with current outbreak.
- ZIKV produces sfRNA which acts as an antagonist of RIG-I and MDA5.



Acknowledgments

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Yale University

Brett Lindenbach

University of South Bohemia

Martin Selinger

University of Oxford

Jan Rehwinkel

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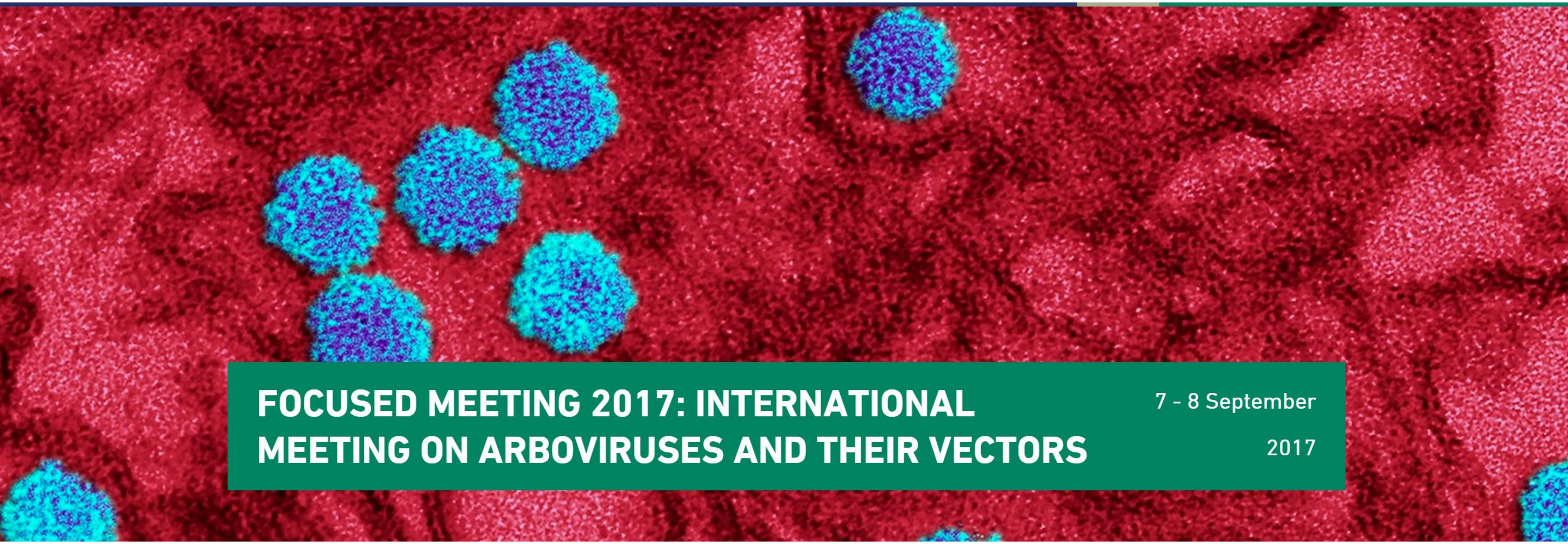

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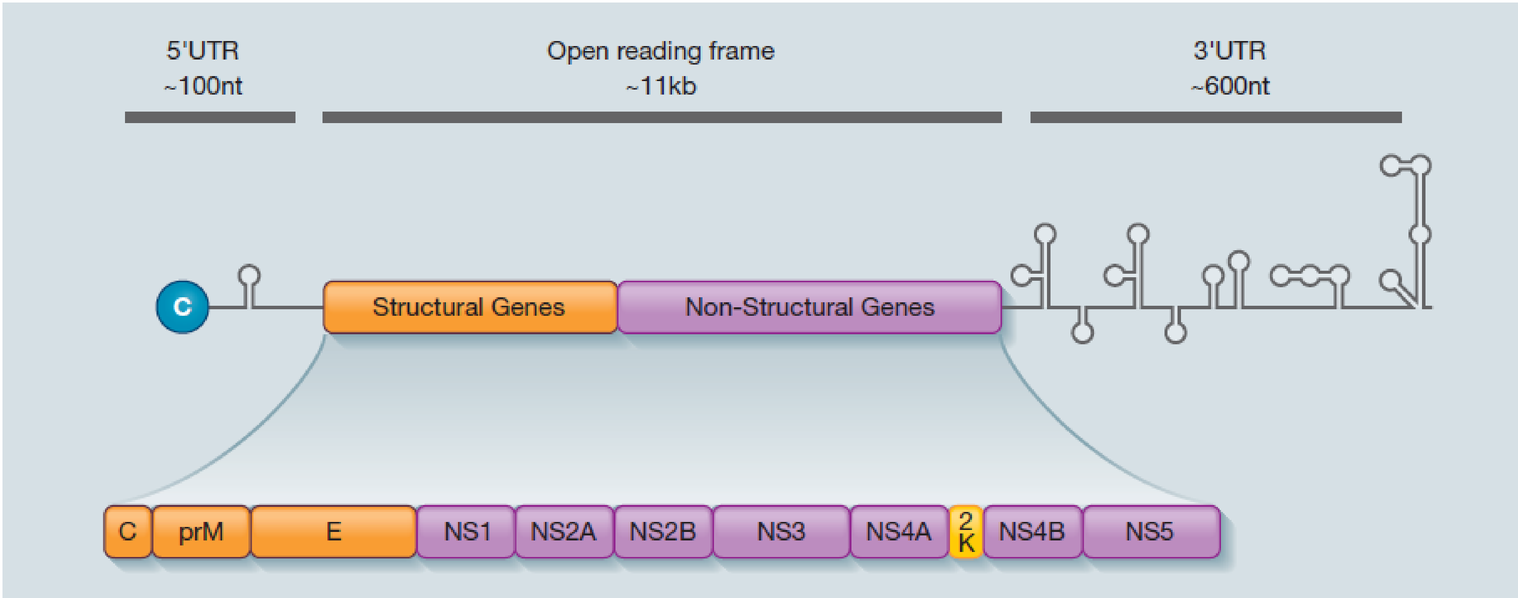
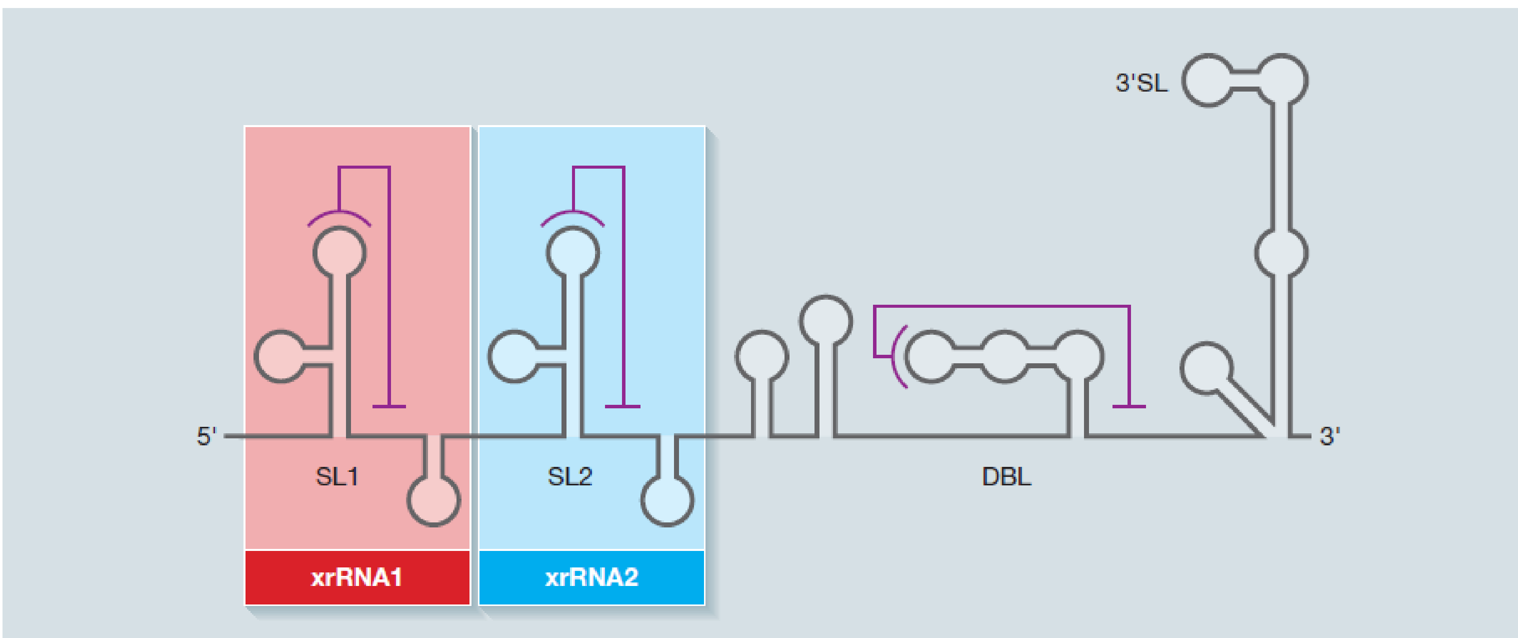
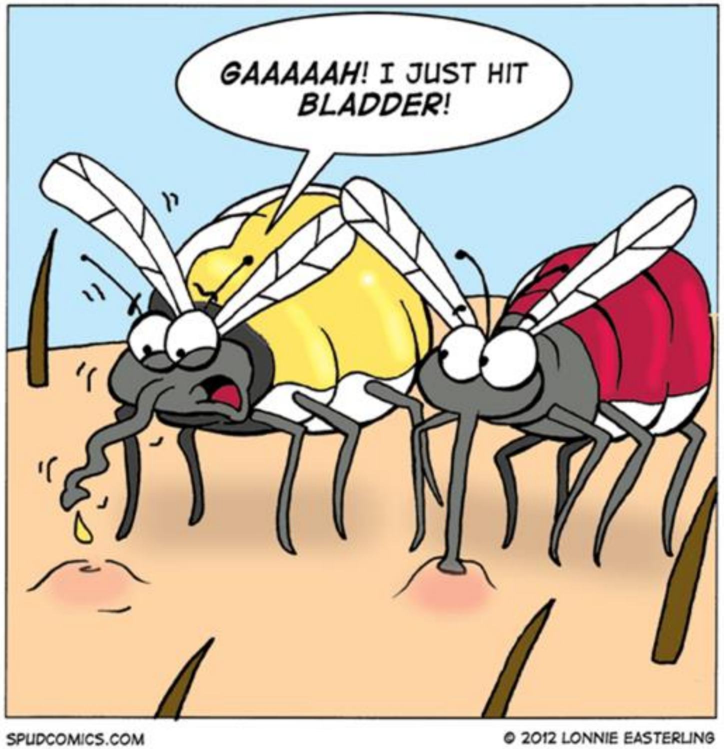

5'UTR
~100nt
Open reading frame
~11kb
3'UTR
~600nt
C
Structural Genes
Non-Structural Genes
C
prM
E
NS1
NS2A
NS2B
NS3
NS4A
2K
NS4B
NS5

(a)


3'SL
5'
3'
SL1
SL2
DBL
xrRNA1
xrRNA2

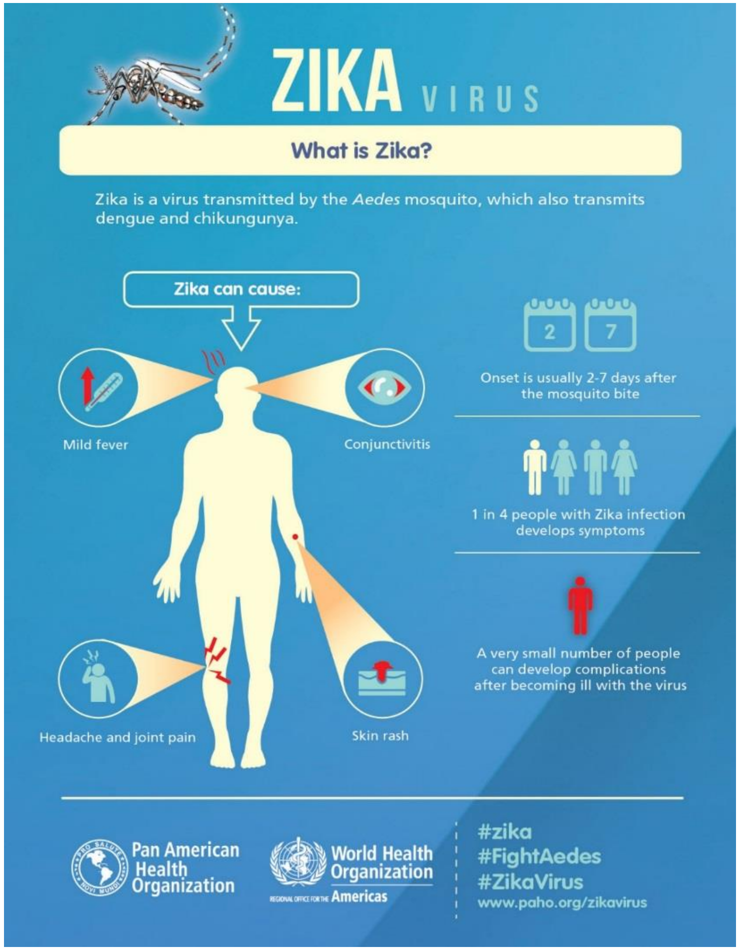
(b)

Thank you!

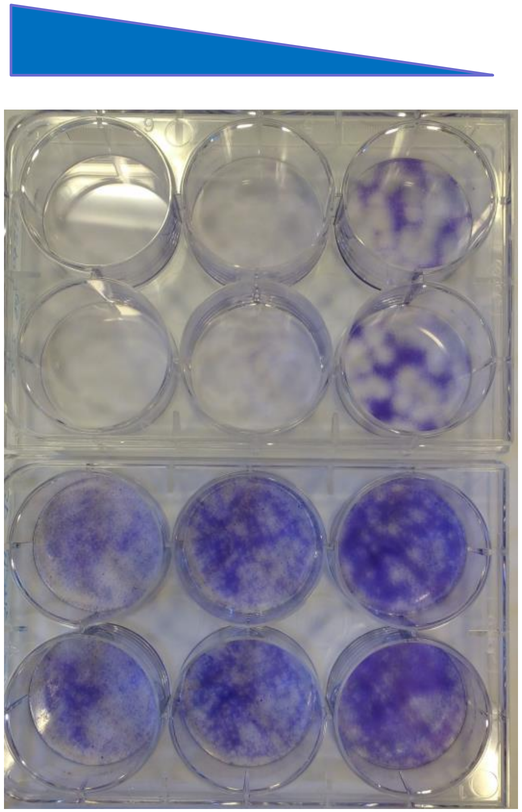

GAAAAAH! I JUST HIT BLADDER!
SPUDCOMICS.COM
© 2012 LONNIE EASTERLING

Future Work

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



ZIKV is affected by Interferon



ZIKV PE243
A549npro (human)
IFN incompetent

PE234
A549 (human)
IFN competent

Plaque size comparisons

Zika virus: a brief history

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- 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

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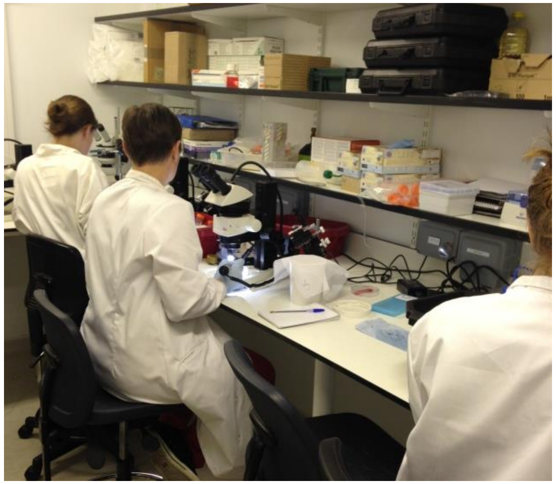
- Pioneering work carried out at the Yellow Fever Institute, now Uganda Virus Research Institute, Entebbe (near Kampala and Zika forest)

Alexander Haddow
Zika virus discovery
1947 UVRI Uganda
Zika forest

Zika virus: research at the CVR

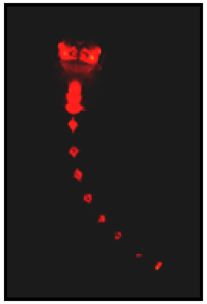


Dissection/injection lab room

Insectaries

Embryo micro-injection facility

Set up of CL3 insectaries



Zika virus and sexual transmission

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 symptons: 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

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

Giovanna Barba-Spaeth, Warwisa Dejnirattisai, Alexander Rouvinski, Marie-Christine Vaney, Iris Medits, Arvind Sharma, Etienne Simon-Lorière, Anavaj Sakuntabhai, Van-Mai Cao-Lormeau, Ahmed Haouz, Patrick England, Karin Stiasny, Juthathip Mongkolsapaya, Franz X. Heinz, Gavin R. Screaton & Félix A. Rey

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

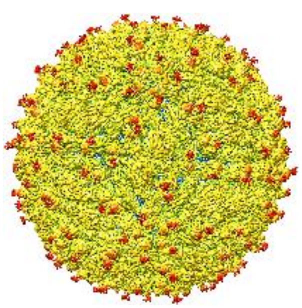
Karin Stettler[1,*], Martina Beltramello[1,*], Diego A. Espinosa[2,†], Victoria Graham[3,†], Antonino Cassotta[4,5,†], Siro Bianchi[1,†], Fabrizia Vanzetta[1,†], Andrea Minola[1], Stefano Jaconi[1], Federico Mele[4], Mathilde Foglierini[4], Mattia Pedotti[4], Luca Simonelli[4], Stuart Dowall[3], Barry Atkinson[3], Elena Percivalle[6], Cameron P. Simmons[7,8,9], Luca Varani[4], Johannes Blum[10,11], Fausto Baldanti[6], Elisabetta Cameroni[1], Roger Hewson[3], Eva Harris[2], Antonio Lanzavecchia[4,5], Federica Sallusto[4,‡,§], Davide Corti[1,‡,§]

ARTICLES

nature immunology

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

Wanwisa Dejnirattisai, Piyada Supasa, Wiyada Wongwiwat, Alexander Rouvinski, Giovanna Barba-Spaeth, Thaneeya Duangchinda, Anavaj Sakuntabhai, Van-Mai Cao-Lormeau, Prida Malasit, Felix A Rey, Juthathip Mongkolsapaya & Gavin R Screaton



Rossmann Lab

MRC Medical Research Council

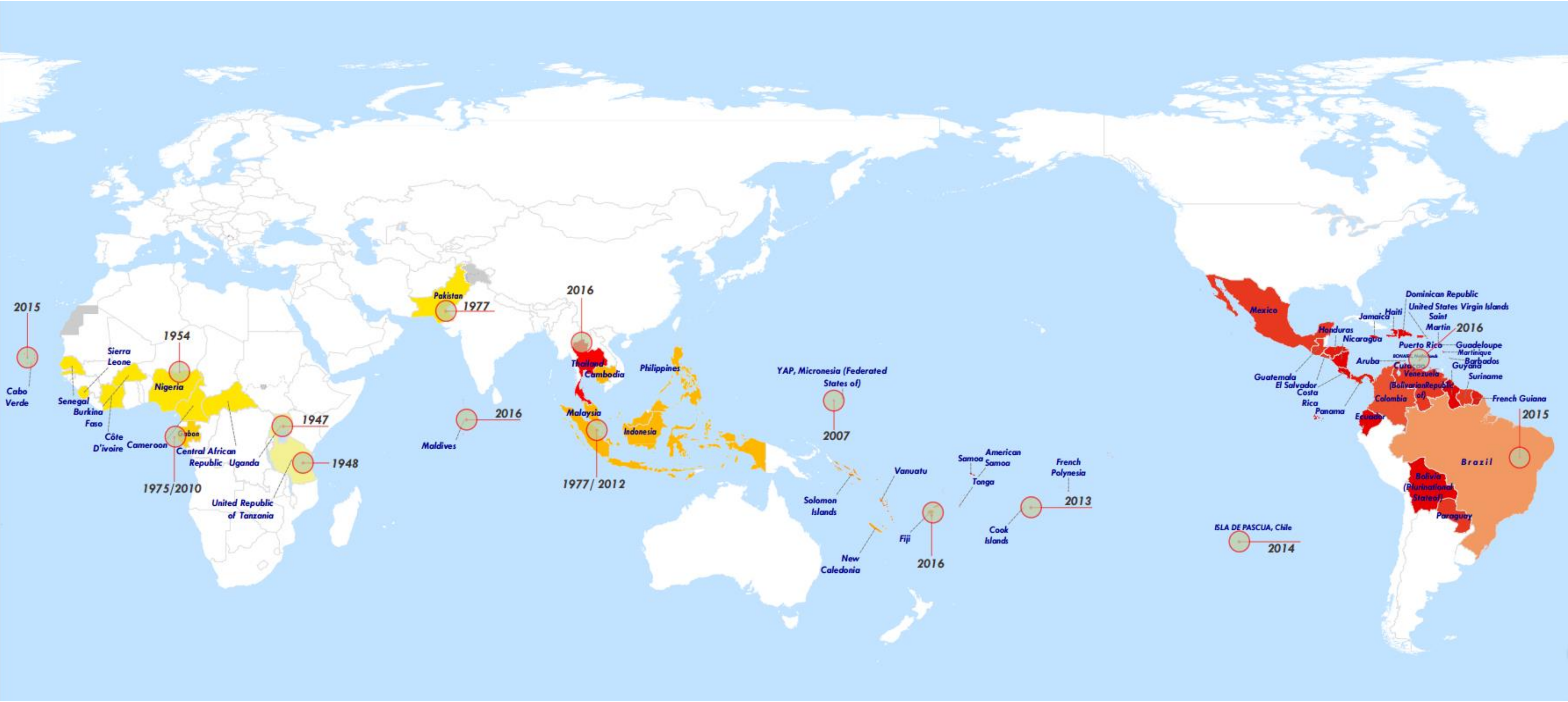
University of Glasgow

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 symtoms
  - 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

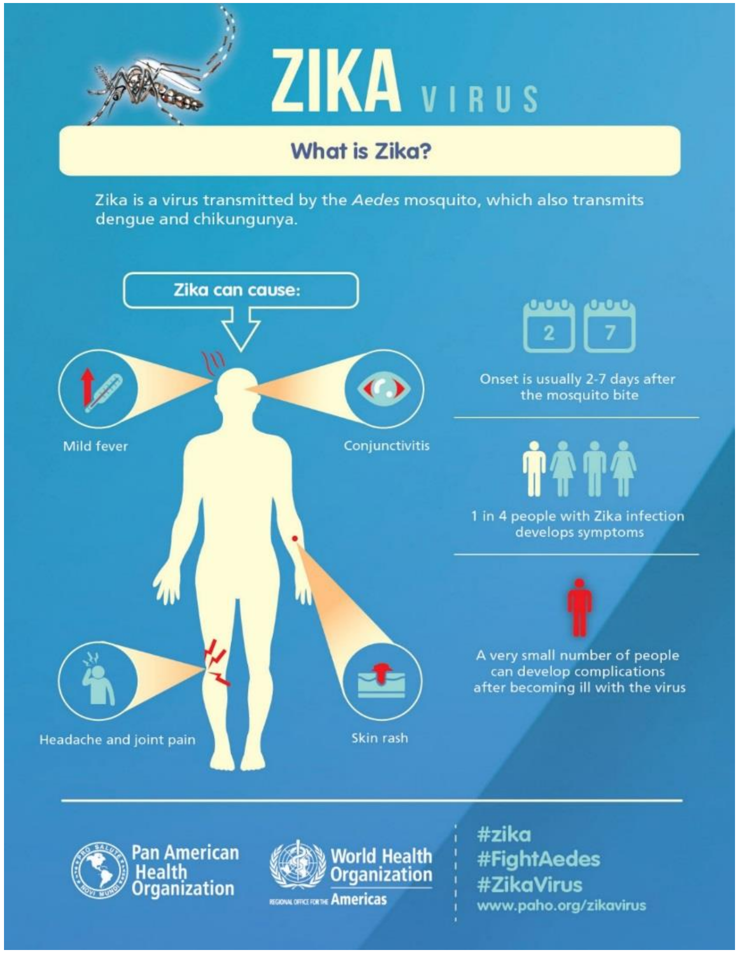
- Onset of symptoms is 2 – 12 days post infection

Viraemic period

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

Symptoms

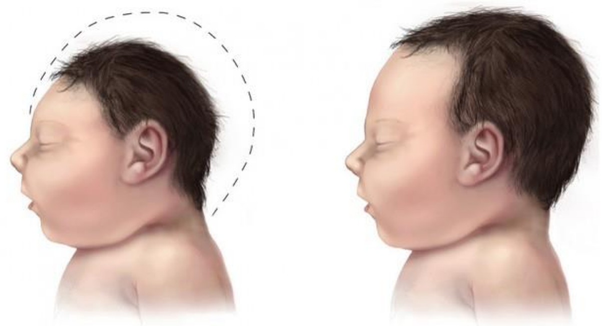
- Rash with/without fever
- Arthralgia/ arthritis
- Conjunctivitis



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

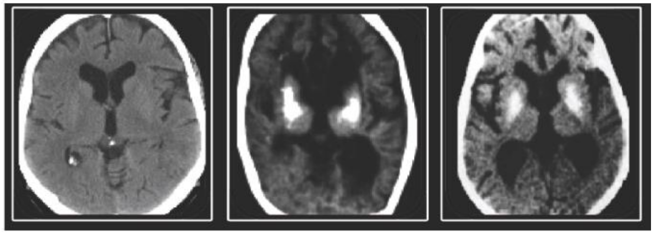
Zika virus and microcephaly

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

Source: CDC



Normal AGS CMV/Rubella

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Zika congenital syndrome

Beyond microcephaly- other malformations:

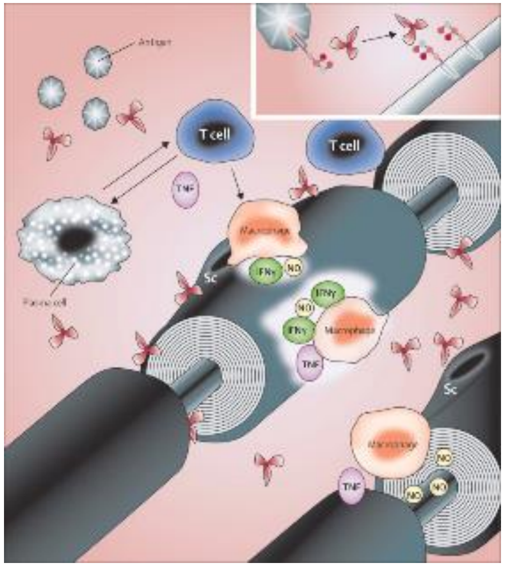
Craniofacial disproportion, spasticity, seizures, irritability and brainstem dysfunction including feeding difficulties

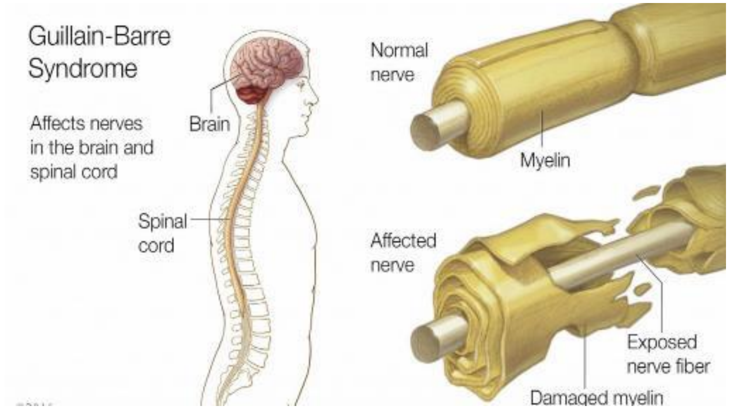
Ocular abnormalities

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).

CNS co-culture composition

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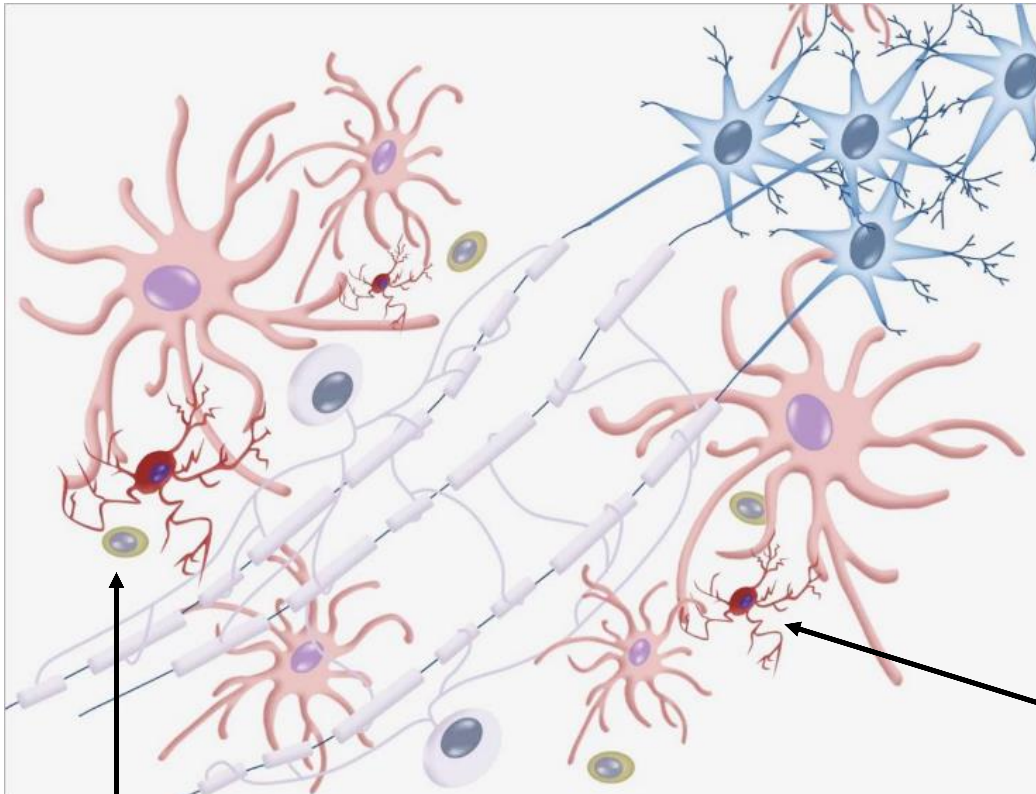
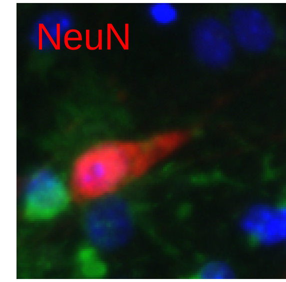
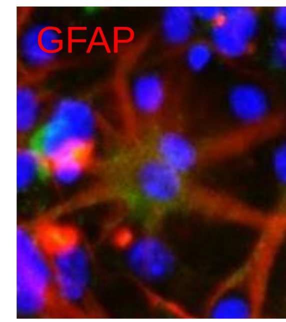


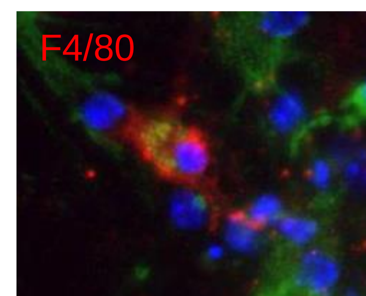
Neuron
NeuN



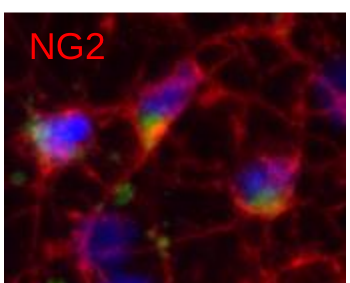
Astrocyte
GFAP



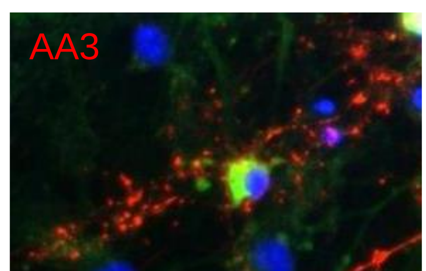
Microglia
(macrophage-like)
F4/80



Precursor cell



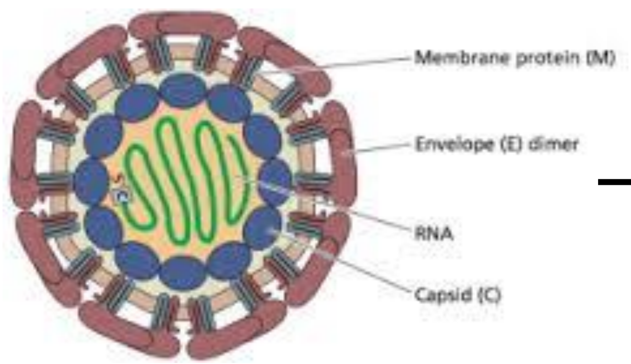
Oligodendrocyte
(myelin-producing)
AA3



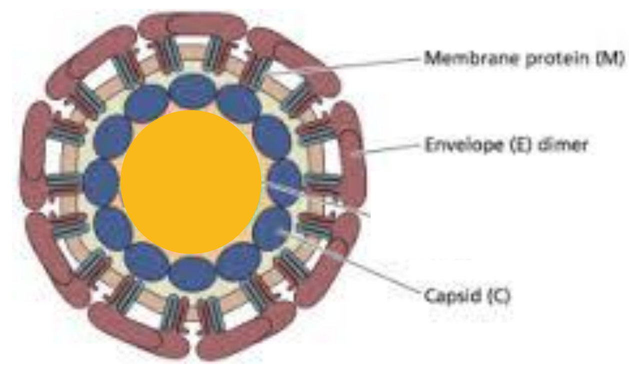
Zika virus: vaccine candidates

Strategies- examples:

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

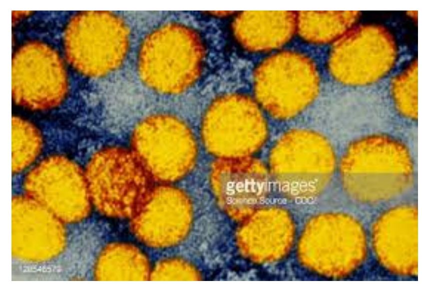


Expression of structural
Proteins only in cell culture



- 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!

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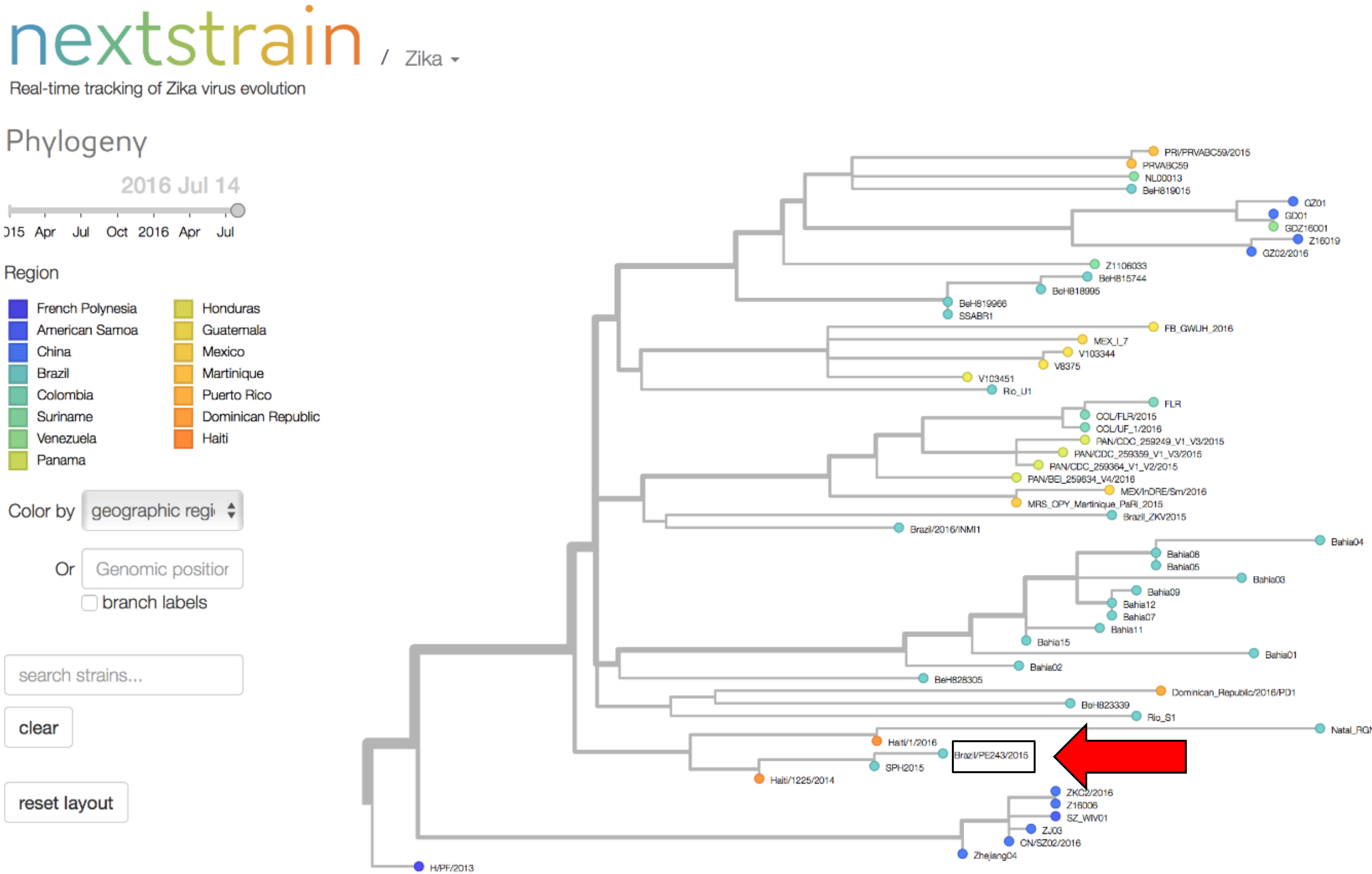
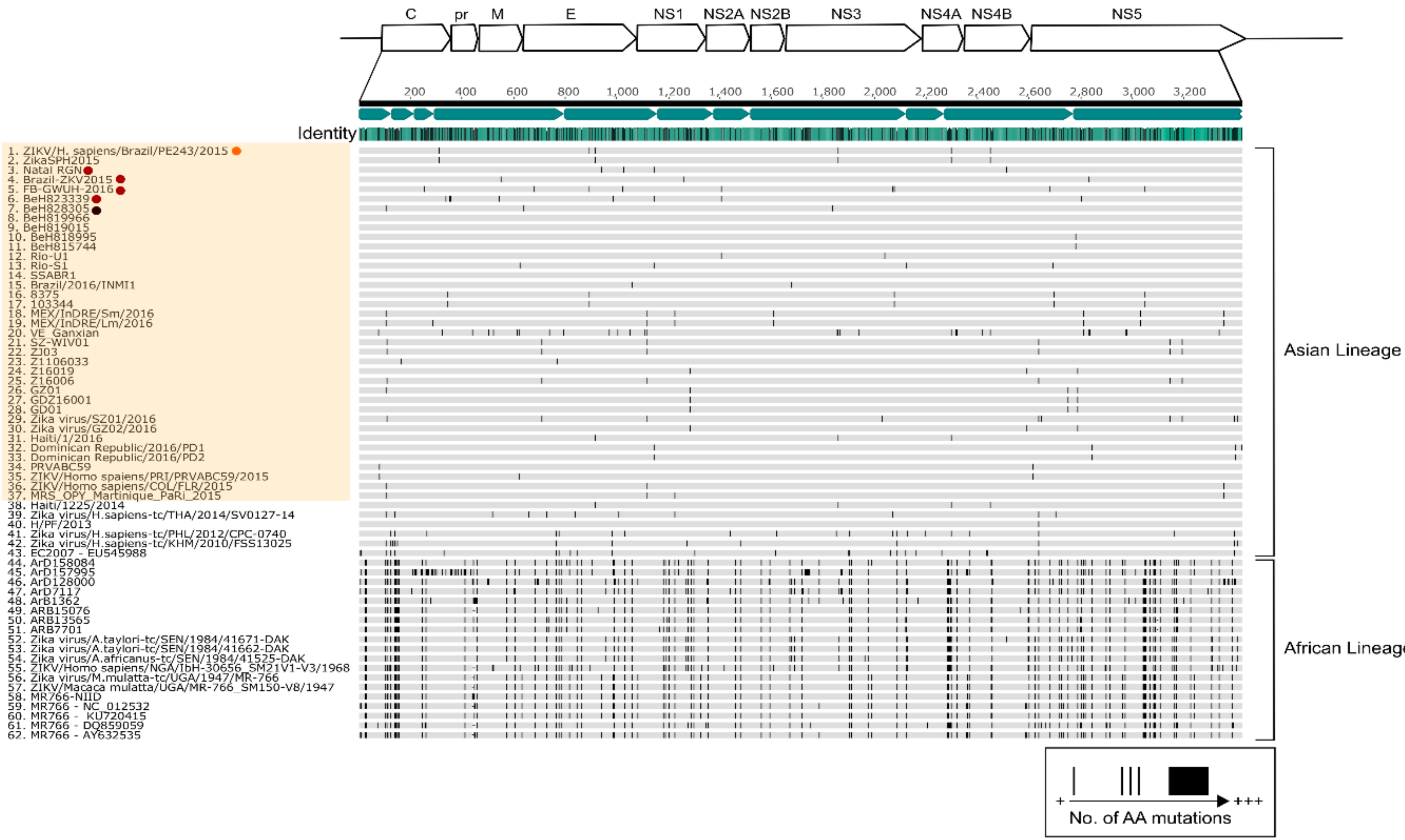


Zika virus PE243 properties

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



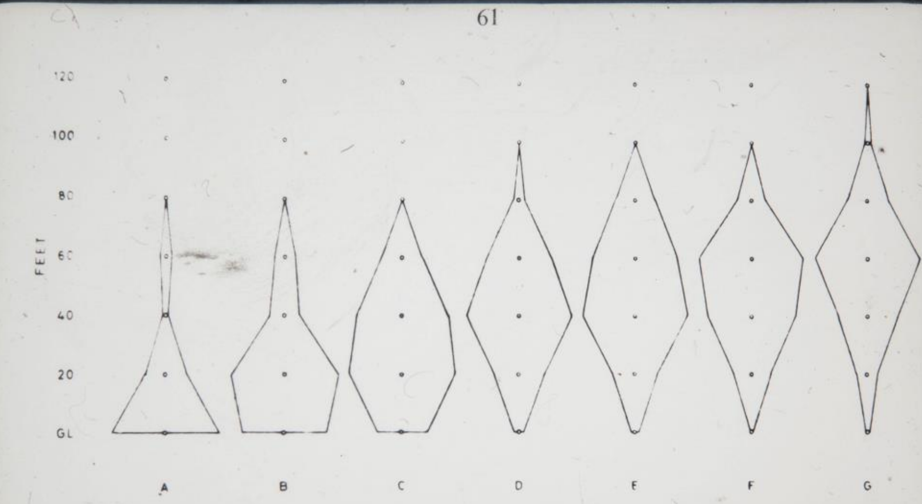


Fig. 5—The vertical distribution of *Ae. africanus* in the sunset period as shown by 30 catches on the steel tower at Zika. A, 1740-50; B, 1750-1800; C, 1800-10; D, 1810-20; E, 1820-30; F, 1830-40; G. 1840-50

TWENTY-FOUR-HOUR CATCH RECORD — **YELLOW FEVER RESEARCH INSTITUTE**

Catch No. E/28/ All levels. *Date* 11-12-I-1948. *Time Started* 15 hours.

Locality Zika III. *No. of Catchers* 3 per unit. *Bait-Hours* 216.

Hours L.M.T.

Species	06-07	07-08	08-09	09-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	17-18	18-19	19-20	20-21	21-22	22-23	23-24	24-01	01-02	02-03	03-04	04-05	05-06	Totals
~~A. (A.) obscurus~~	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	1
~~A. (A.) [illegible]~~	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
H. sanguinea	.	.	2	5	.	.	.	1	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	9
C. (C.) pseudoconopas	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	2	.	1	.	4
C. (C.) maculipennis	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	2	.	.	.	.	1	.	.	4
C. (C.) fuscopennatus	11	4	1	1	3	2	1	.	3	3	5	7	81	54	24	19	22	16	16	14	11	13	14	33	358
C. (C.) aurites	.	.	.	.	.	.	.	.	.	.	.	.	33	10	9	8	8	10	1	4	1	4	4	.	92
C. (M.) africanus	11	3	.	5	.	2	1	.	1	1	.	1	19	11	25	19	42	13	16	10	15	22	15	13	245
C. (M.) uniformis	2	.	.	.	1	.	1	2	.	.	.	.	7	6	6	1	6	1	2	5	1	5	7	6	59
~~A. (F.) ingrami~~	3	1	.	1	1	.	.	.	.	.	.	5	1	.	.	.	.	.	2	.	.	1	.	.	18
A. (S.) apicoargenteus	.	.	1	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	2
~~A. (S.) africanus~~ Zika Virus	.	.	.	1	.	.	.	.	1	.	.	1	49	4	4	5	8	6	1	1	2	1	2	.	86
A. (A.) domesticus	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	1
A. (A.) tarsalis sp.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
A. (A.) cumminsi	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
~~E. chrysogaster sp.~~	2	1	1	.	.	1	.	.	.	2	.	4	.	.	.	.	.	.	.	.	.	.	.	.	11
E. oedipodius ssp. paviplumia	.	.	1	.	.	.	.	.	.	1	.	3	.	.	.	.	.	.	.	.	.	.	.	.	5
C. (C.) annulioris	.	.	.	.	.	.	.	.	.	1	.	2	6	1	1	1	5	.	.	.	.	2	.	3	22
Culex spp. indet.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
																									918

[illegible] bait but wind & light rain from 21-22 hours.

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 Albizzia, Piptadenia, Maesopsis, Canarium, Ficus & Phoenix

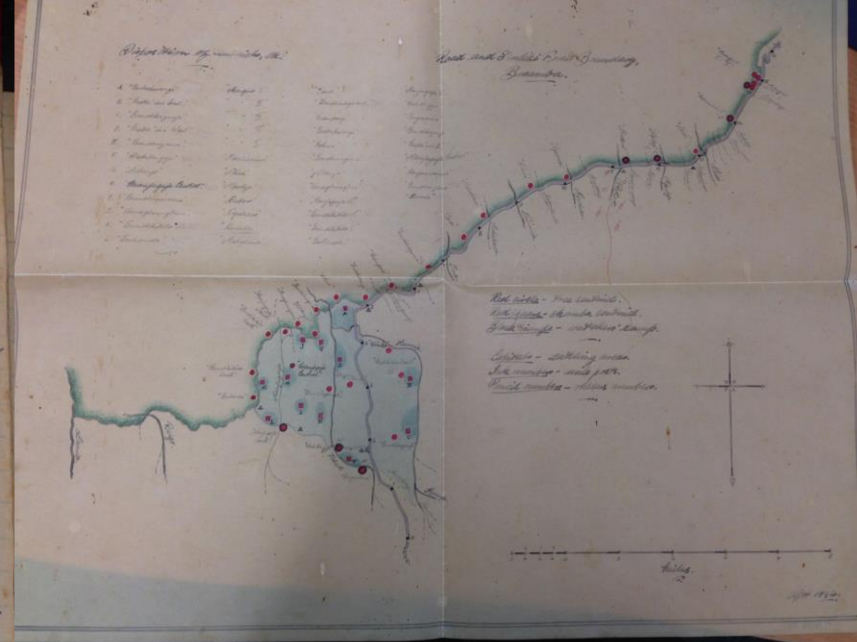
The tree units occupied platforms at 38 & 55 feet respectively, the tree being Strombosia grandifolia. The ground unit sat below the tree.

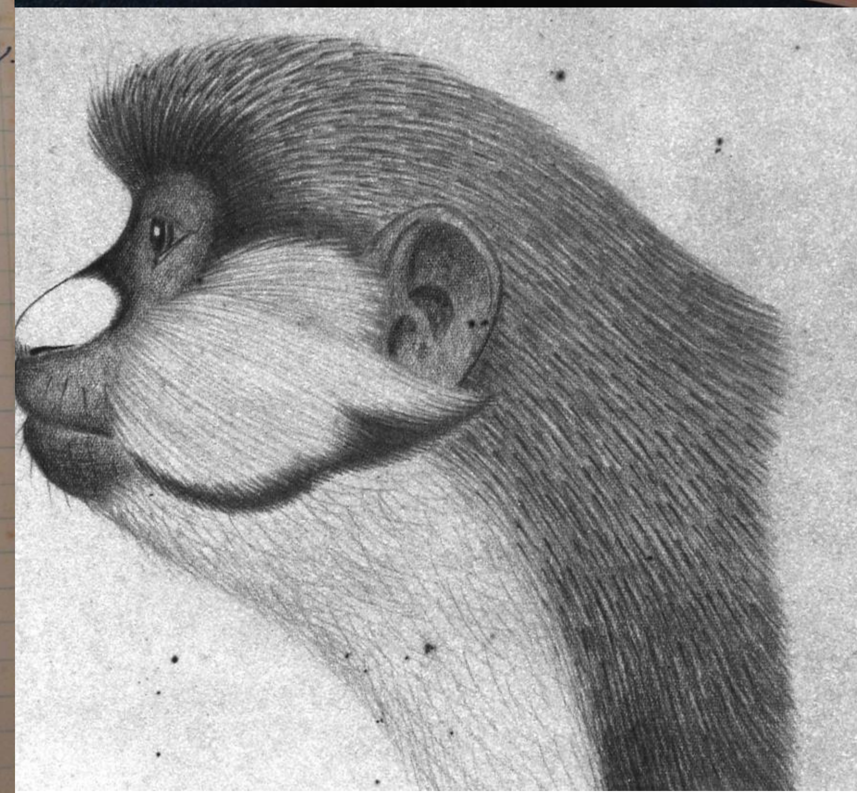
The serial numbers of these catches are E/27-28-29-30-31.

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

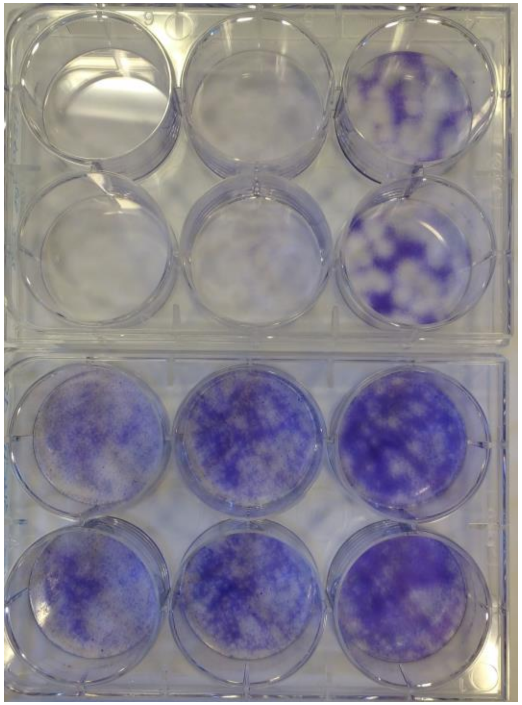
The nearest hut is also about 350 yards from the catching station.

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





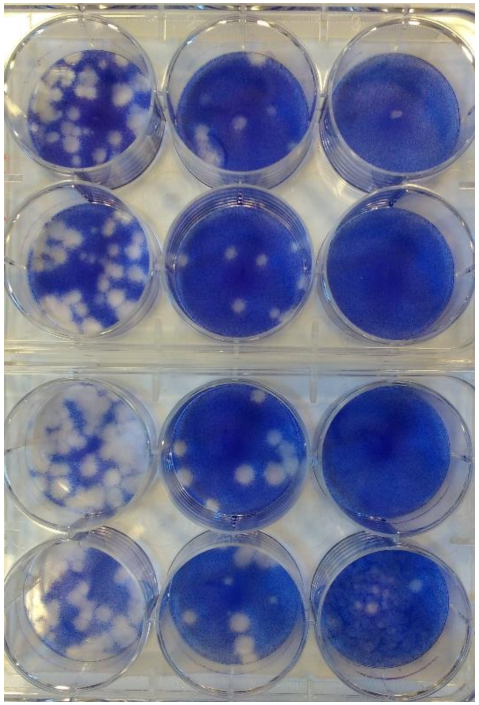
ZIKV: Classical Virology



PE243 (Brazilian)
A549npro (human)
IFN incompetent

PE234 (Brazilian)
A549 (human)
IFN competent

Plaque size comparisons



MR766 (African)
Vero (monkey)
Titre 1.8x10^7 pfu/ml

PE234 (Brazilian)
Vero (monkey)
Titre 2.8x10^7 pfu/ml

Virus Titrations

Hurricane Zika and Glasgow

Medical Research Council
University of Glasgow
Centre for Virus Research