Towards antivirals for tropical infections

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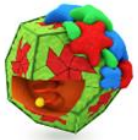
www.antivirals.be & www.facebook.com/NeytsLab

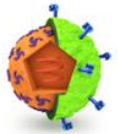
12th Seminar on Travel Medicine, Brussels, January 25th 2018

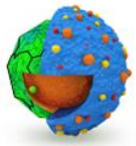

Flaviviridae


Picornaviridae


Norovirus


Respiratory syncytial virus


Chikungunya virus


Hepatitis B virus


Bunyaviridae


Hepatitis E virus


Coronavirus


Rabies virus

What do we have today ….

Herpesvirus

HIV

e.g. tenofovir

HBV (e.g. tenofovir)

HCV

Influenza

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STATE STREET.

If anything kills over 10 million people in the next few decades, it's most likely to be a highly infectious virus rather than a war. Not missiles, but microbes. Now, part of the reason for this is that we've invested a huge amount in nuclear deterrents. But we've actually **invested very little in a system to stop an epidemic**. We're not ready for the next epidemic.

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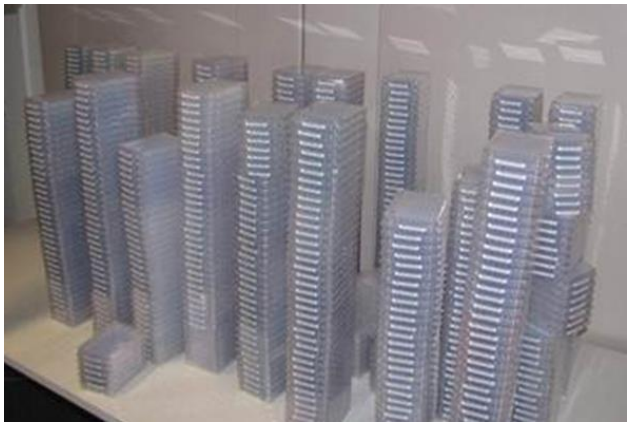
Using cell based/phenotypic antiviral screens



1. CPE based screen for hit identification
2. Hit selection : 100% inhibition of CPE at non cytotoxic conc and >2$\log_{10}$ reduction in viral yield
3. Hit-expansion
4. Hit-optimization
5. Hit-to lead optimization
6. Lead optimization
7. Selection of clinical candidate

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Factors that help to find the needle** in the haystack#?

Large & high quality small molecule libraries

Robust, rapid and convenient phenotypic screenings assays

The infected cell as a black box with many (also many) known targets and interactions between biomolecules

Need equipment to run HTS with highly pathogenic organisms

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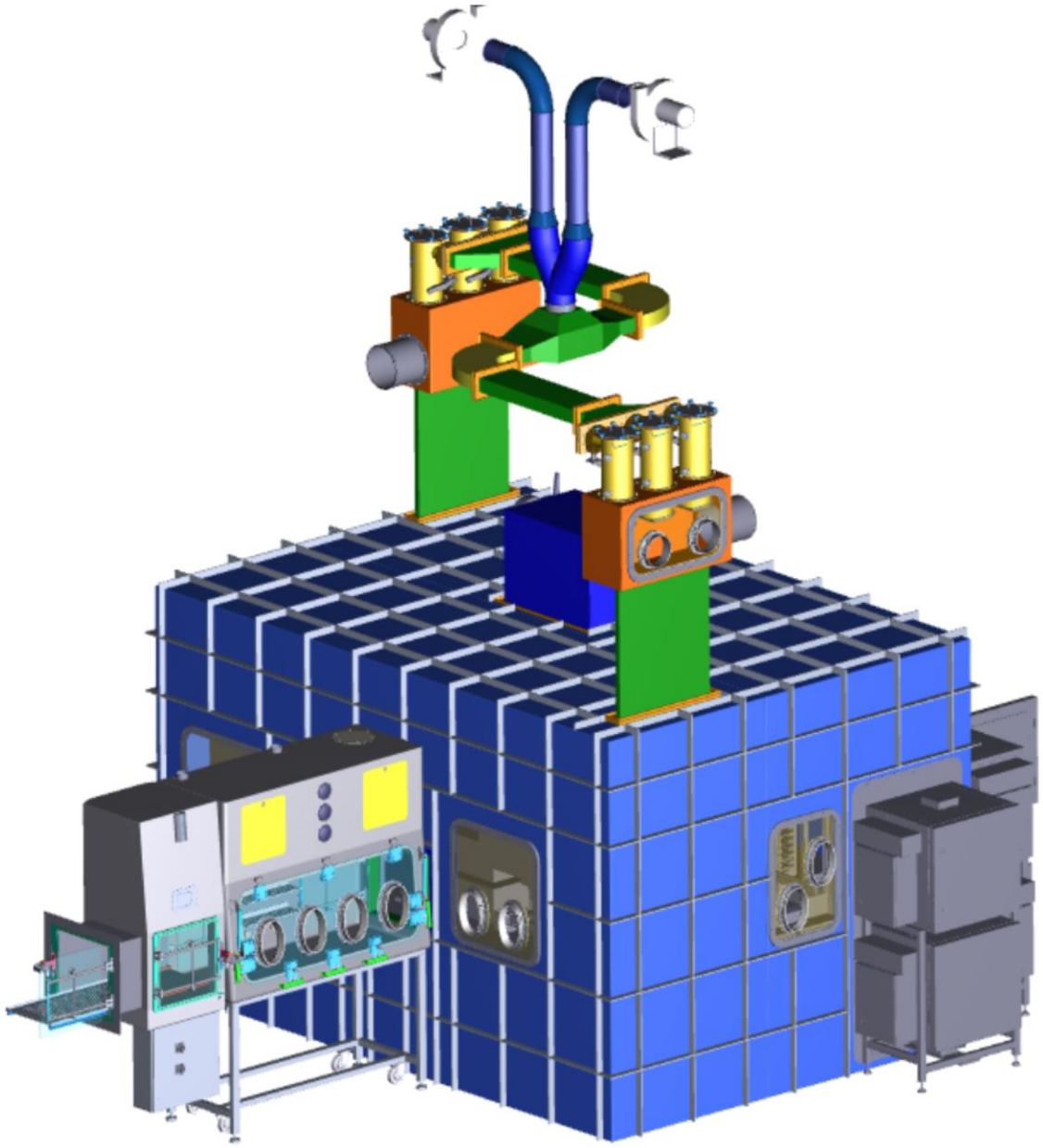
Caps-It a robotized lab-in-a-box...



... and a *human-out-of-the-box* system

Biosafe handling and full containment at all times

HERCULES STICHTING

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Caps-It an automated platform in a BSL3+ environment

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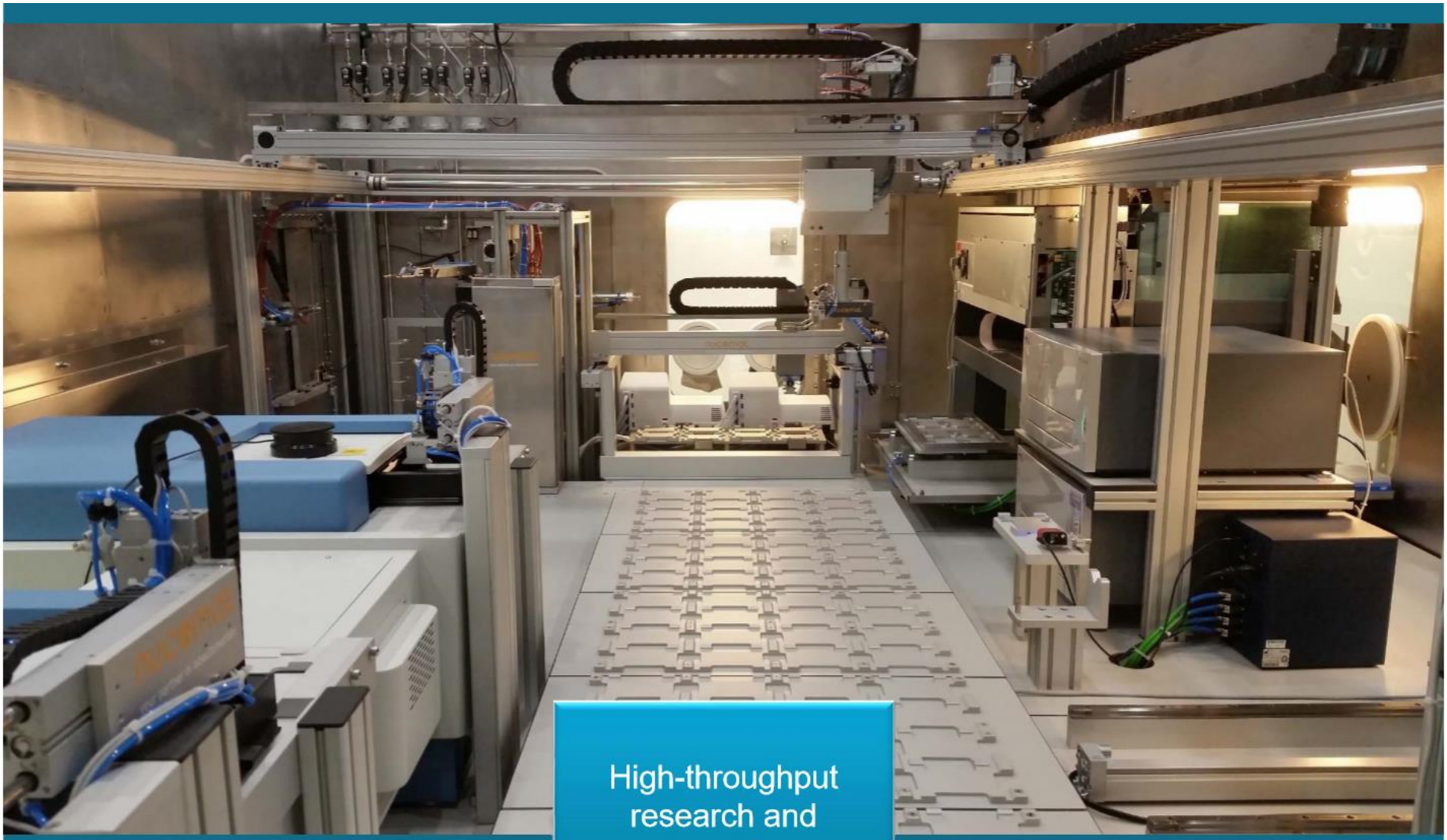

High-throughput research and screening

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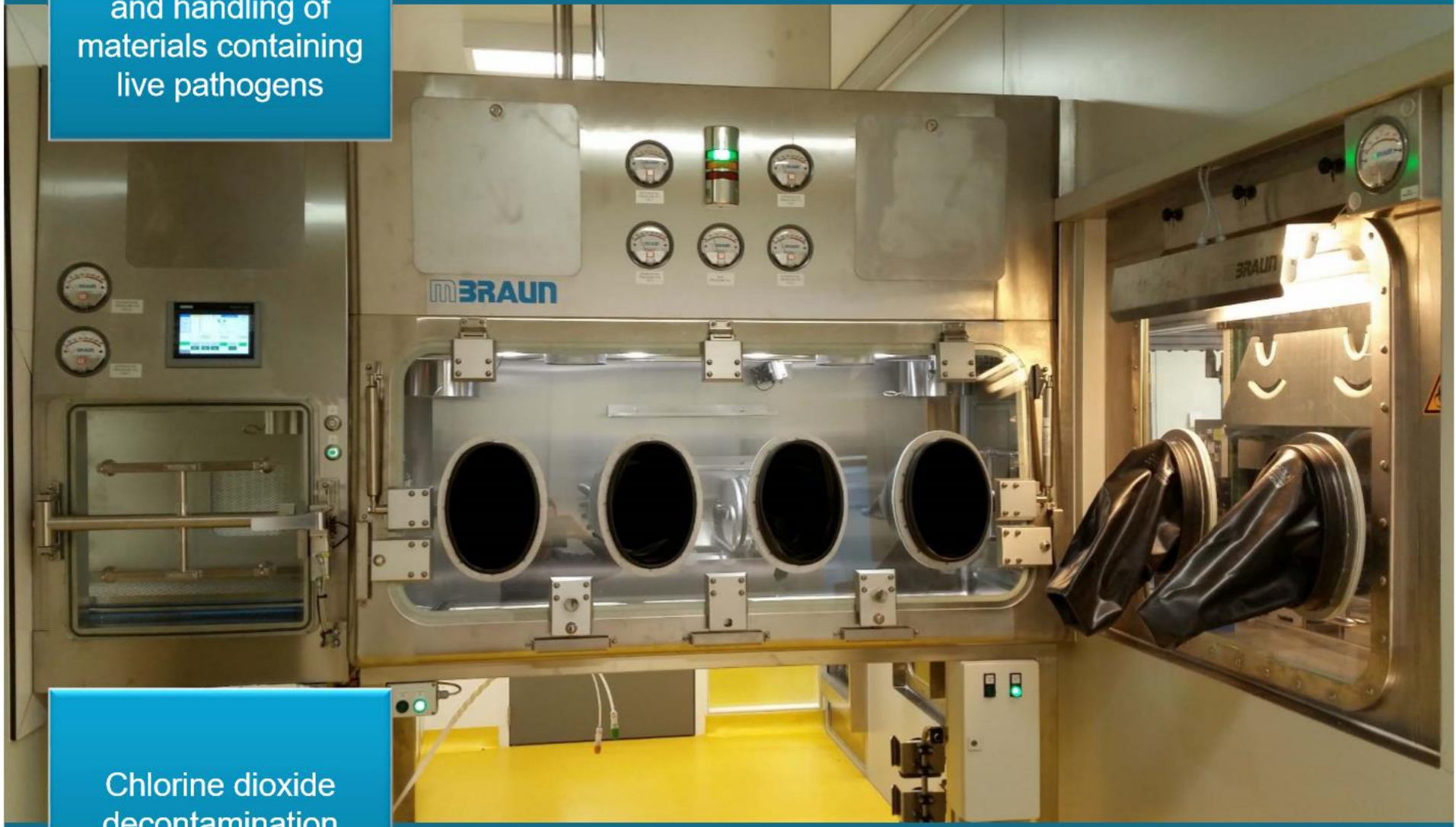
Safe import, export and handling of materials containing live pathogens



Chlorine dioxide decontamination

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Replacement of double HEPA H14 filters without system decontamination

caps-it@kuleuven.be

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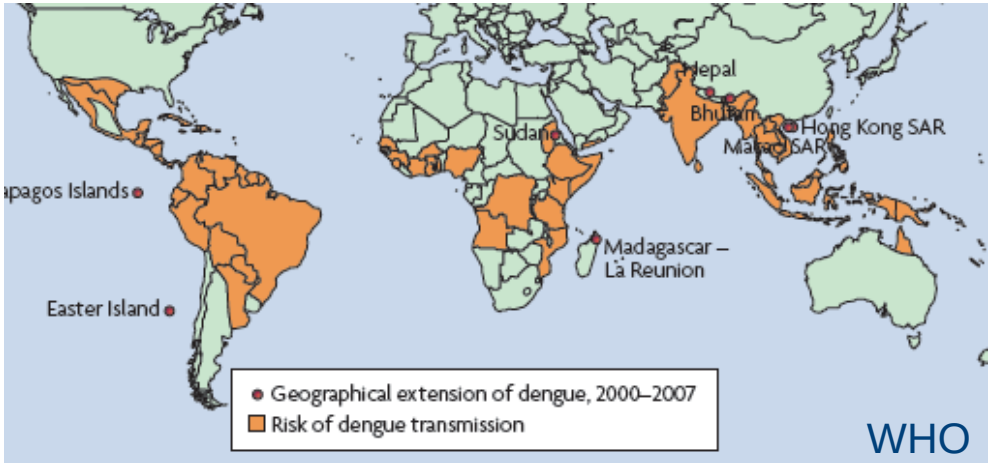
Dengue (hemorrhagic) fever

Second-most prevalent mosquito-borne infection after malaria.
~360 million infections and ~96 million cases/ year.
~ 2/5th of the world's population at risk [WHO]
One hospitalization every minute around the globe



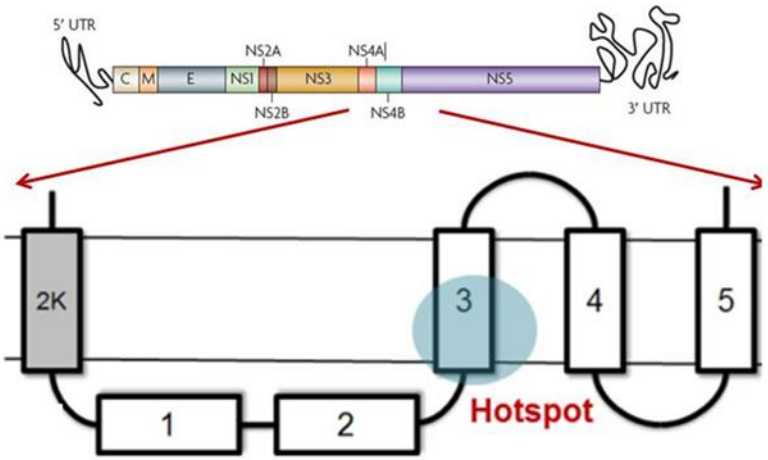
Dengue patients overwhelm hospitals

LAHORE: Dengue outbreak in Lahore is taking a serious turn, as cases are pouring in at such an overwhelming rate that hospitals are forced to lay three patients on one bed, Geo News reported.

A Candidate Dengue Vaccine Walks a Tightrope

Cameron P. Simmons, Ph.D.

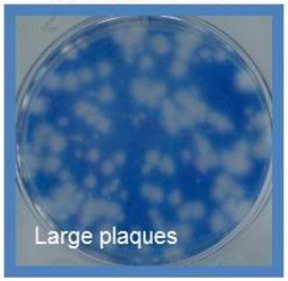
Potent NS4b targeting pan-serotype inhibitors



DENV-2 hit

Med → Chem

Pan-serotype
Low nM to pM activity
Clinical isolates



DENV NS4B

No human homologue – no homologue of HCV NS4B

97% conserved intra-serotype, 78% conserved inter-serotype

Critical for viral replication

Dengue + placebo

Dengue + inhibitor

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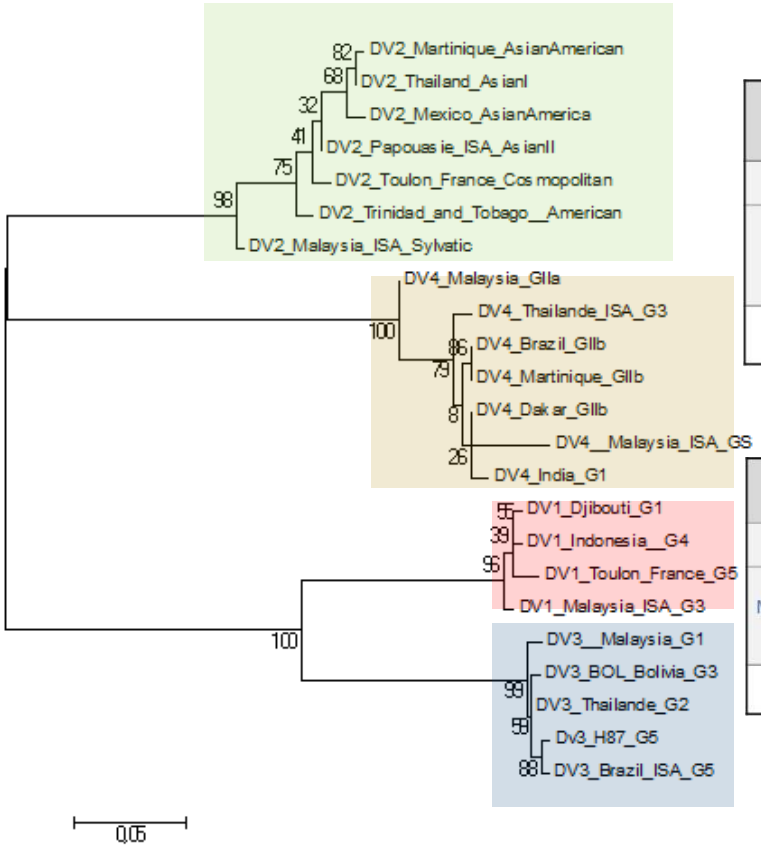
Potent pan-serotype DENV activity



Antiviral activity against DENV: RT-qPCR assay
EC_{50} (nM)

DENV-1				DENV-2						
Djibouti (G1)	Malaysia* ISA (G3)	Indonesia (G4)	Toulon France (G5)	Mexico (Asian America)	Martinique (Asian America)	Trinidad/ Tobago (American	Toulon France (Cosmopol	Thailand (Asian I)	Papouasie (Asian II)	Malaysia (Sylvatic)
7.5	10	6.9	9.6	6.5	11	0.5	<0.04	9.7	0.036	<0.04

Antiviral activity against DENV: RT-qPCR assay
EC_{50} (nM)

DENV-3					DENV-4						
Malaysia (G1)	Thailand (G2)	Bolivia (G3)	H87 (G5)	Brasil ISA (G5)	India (GI)	Malaysia (GIIa)	Dakar (GIIb)	Martiniqu e (GIIb)	Brasil (BIIb)	Thailand (G3)	Malaysia (GS)
10	27	37	29	27	<0.04	9.8	88	10	6.5	>33	8.8

KU Leuven, the Wellcome Trust and Janssen are together joining

the fight against Dengue fever (breakbone fever).

A compound with favorable safety profile and the potential of oral dosing has been identified and is being developed towards FIH studies

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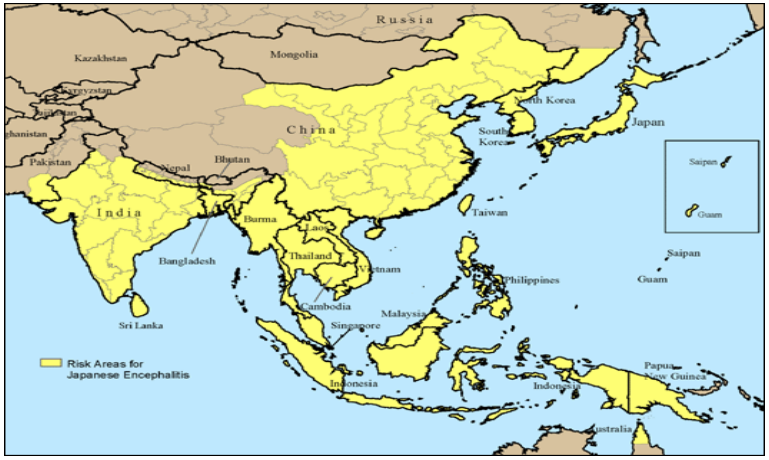
Need for pan-flavivirus antivirals

Japanese encephalitis

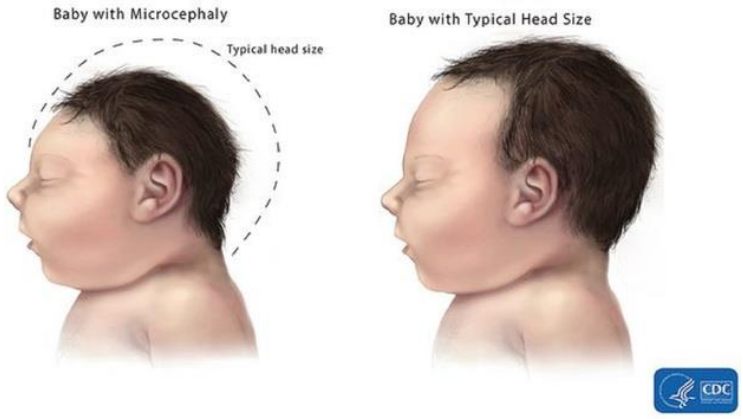
~ 50.000 cases/year (30% mortality)



Yellow fever

200.000 cases/year, 30.000 deaths

Zika virus



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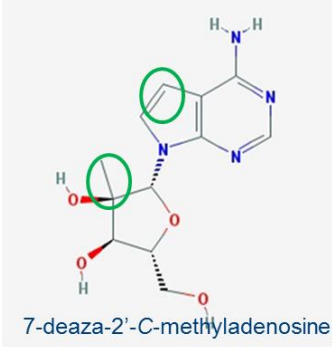
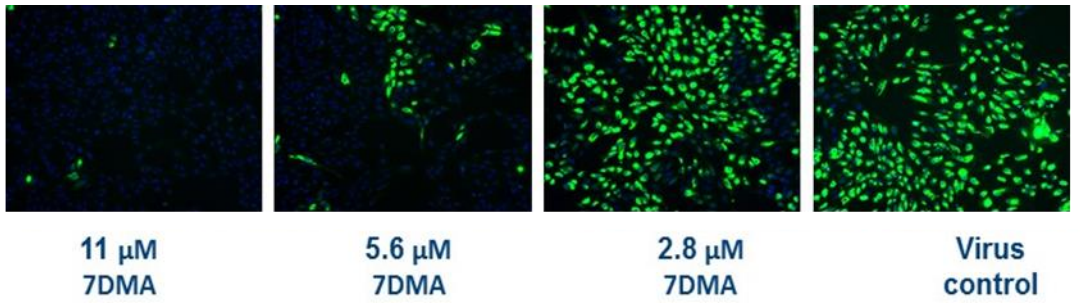
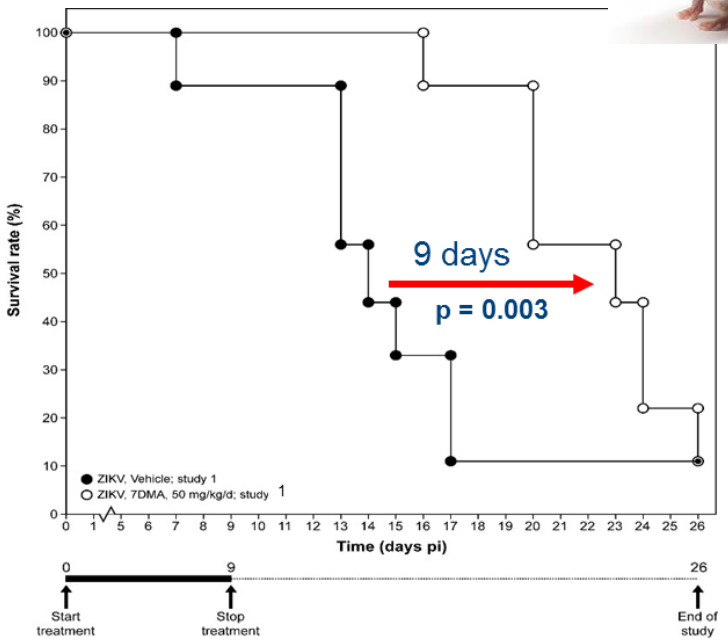
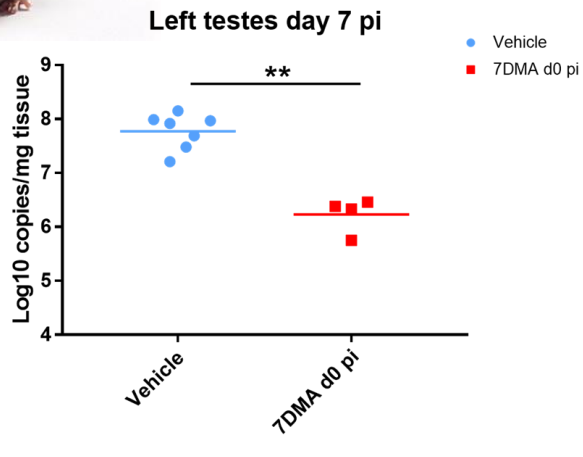
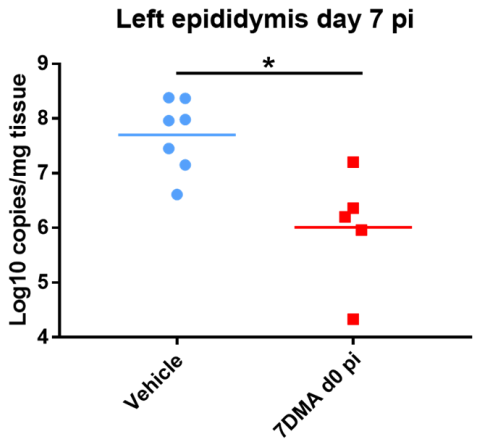
HCV polymerase inhibitor against ZIKV











* p< 0.05 - ** p< 0.01

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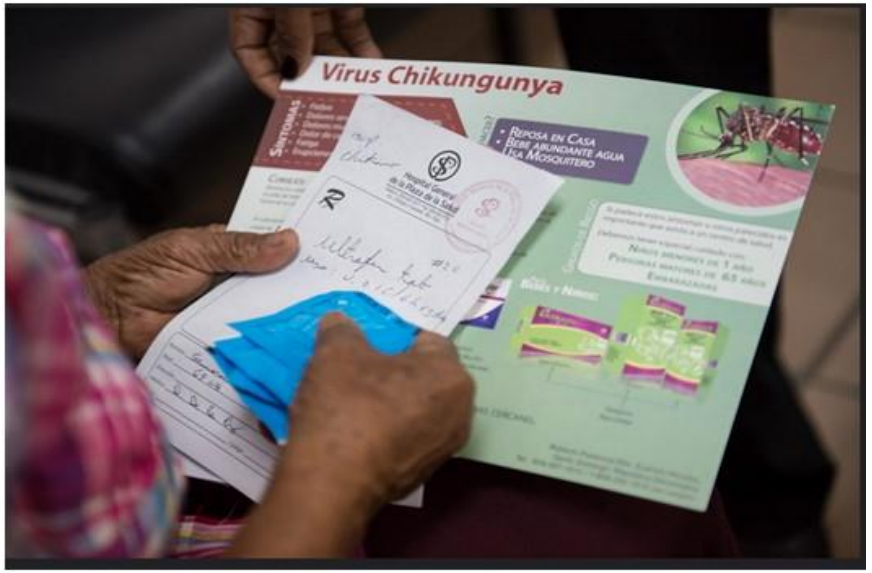
Zmurko *et al.*, PLoS NTD, *2016*

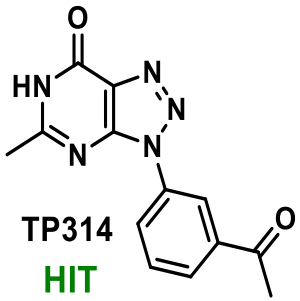
Chikungunya



www.paho.org www.who.org

A novel class of CHIKV inhibitors ….

MADTP



TP314
HIT

Virus (Strain)	EC_{50} (µM) MADTP-372
Chikungunya virus 899 (lab)	2.6
Venturini (Italy 2008)	1.4
Congo 95 (2011)	0.75
St Martin (2013)	4.3
VEEV TC83	6.2

Venezuelan Equine Encephalitis Virus

Delang et al., Sci Reports 2016

Gigante et al, J Med Chem 2014.

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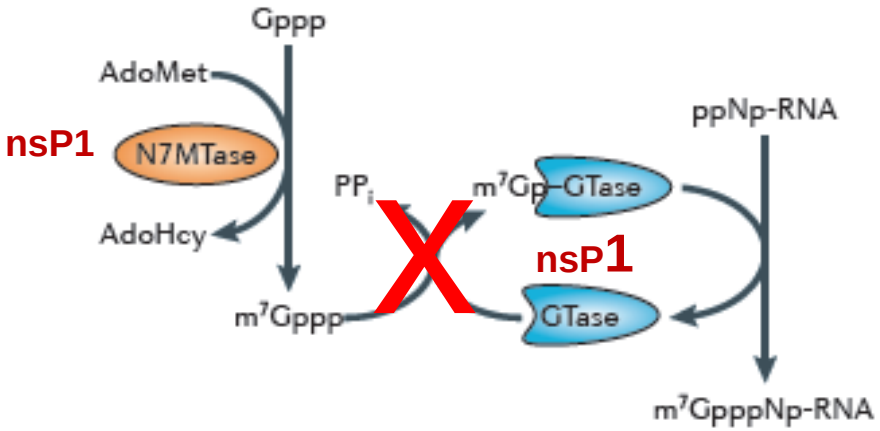
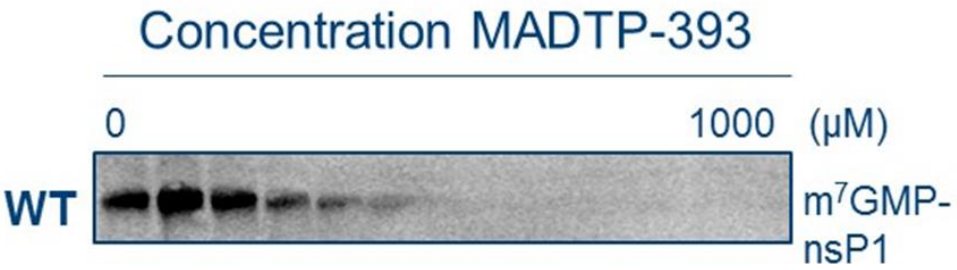
…. that target viral capping

Resistant CHIKV clones : P34S mutation in NS1
Introduction in WT background confirms resistant phenotype

Decroly et al, Nature Reviews Microbiology 2012



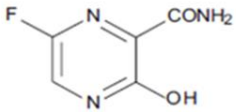


VEEV GTase

Block formation of m7Gppp-nsP1 and thus capping

Favipiravir (T-705)

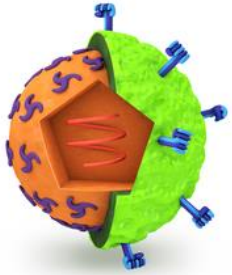
is a broad-spectrum antiviral that has been approved in Japan for treatment of influenza virus infections.



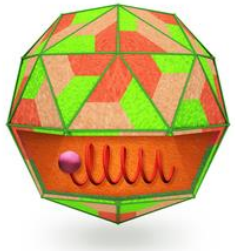
T-705

Toyama Pharm.

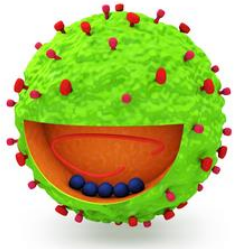
+ssRNA viruses:



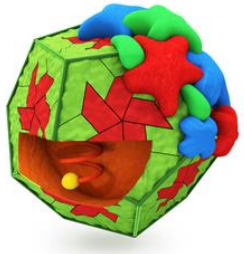
Alphaviruses (e.g. CHIKV)



Enteroviruses

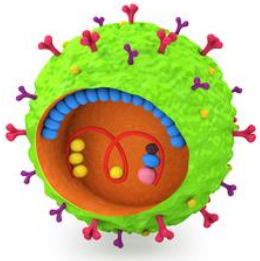


Flaviviruses (e.g. ZIKV)

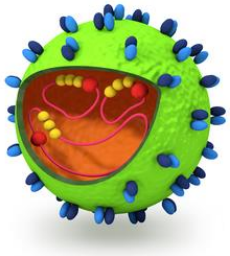


Norovirus

-ssRNA viruses:



Paramyxoviruses



Hantaviruses

Ebola virus

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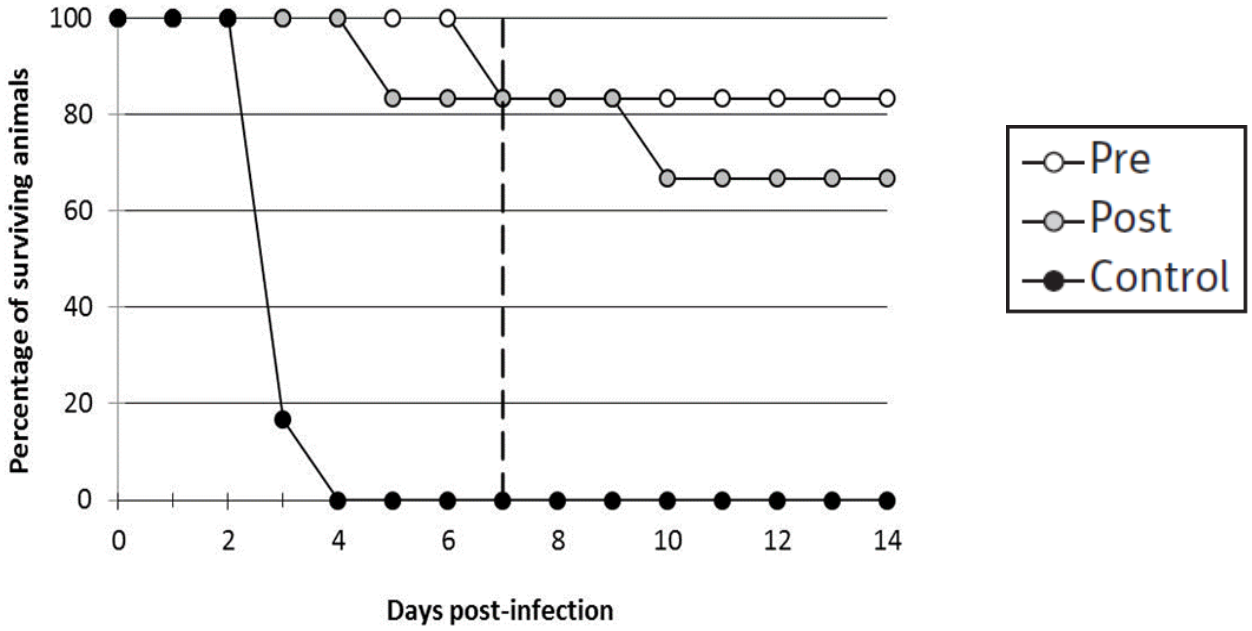
Favipiravir (T-705)

- Favipiravir inhibits CHIKV replication *in vitro*.
- Treatment of infected AG129 mice **(acute infection model)** with favipiravir increased survival by more than 50%.



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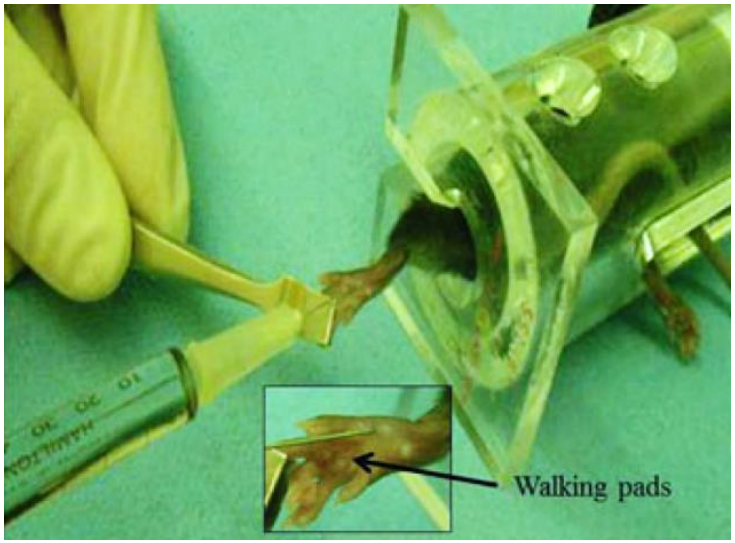
Delang *et al.*, J. Antimicrob. Chemother. (2014)

Infection protocol

- C57BL/6 mice, 3 weeks old.
- Infected with 10^3 PFU of CHIKV-899 in the left hind footpad.
- Tissues were collected up to 14 weeks p.i.



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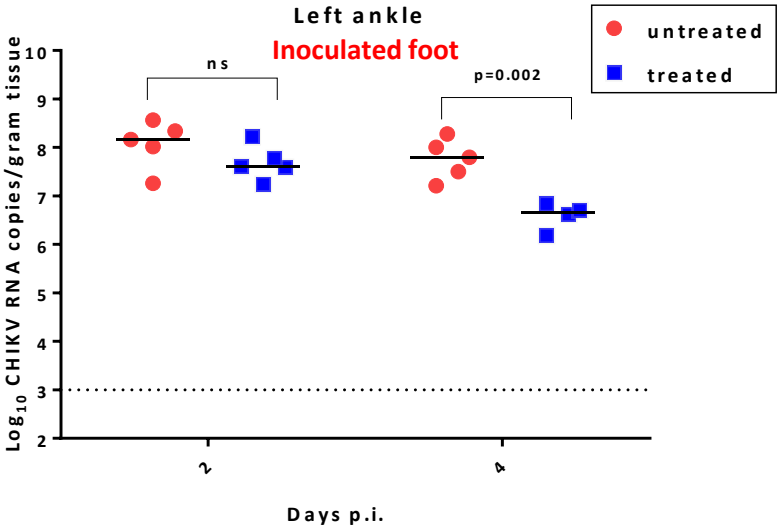
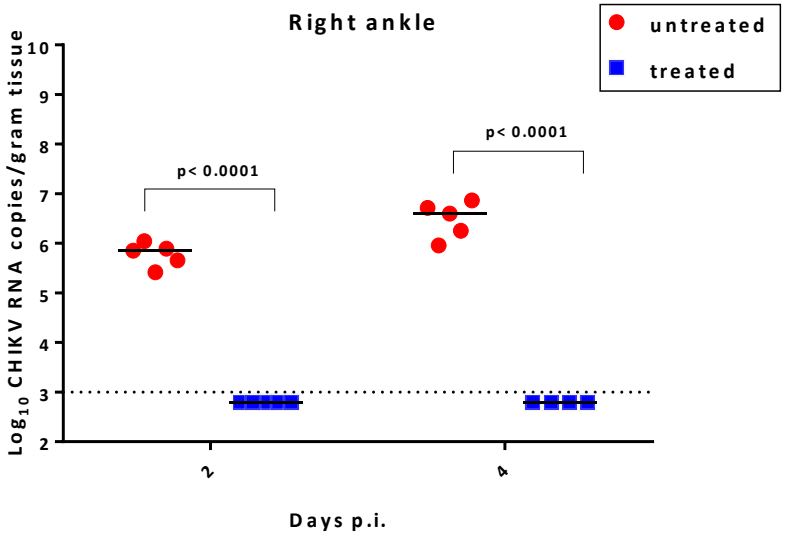
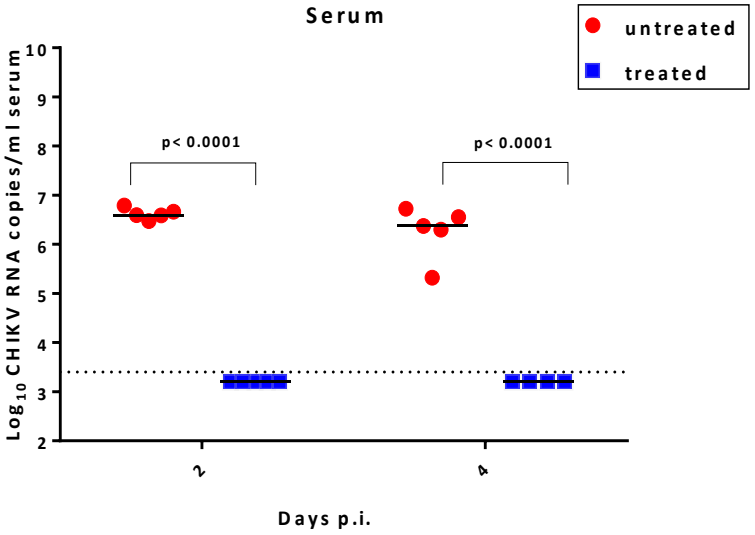
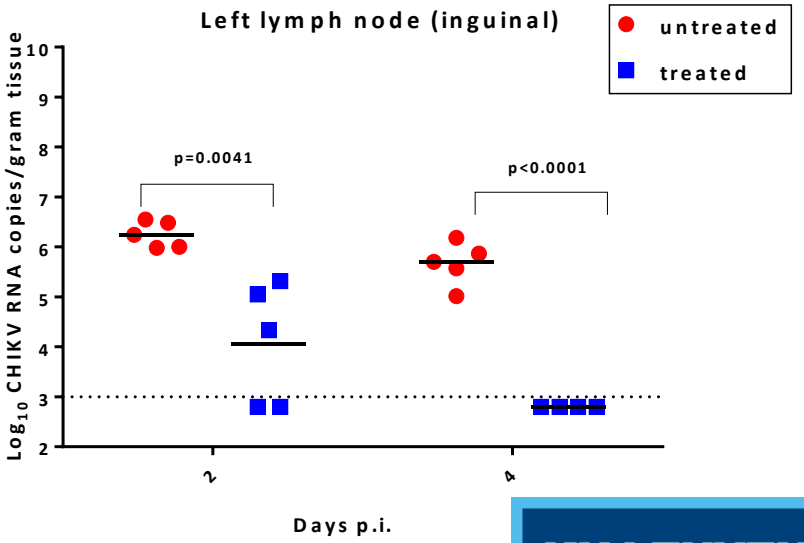
Untreated vs. treated mice (viral RNA)









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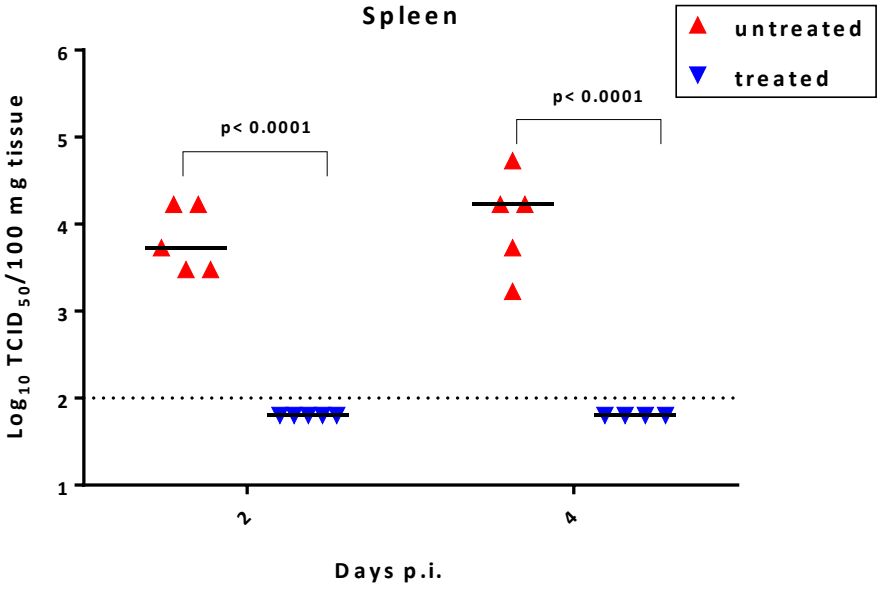
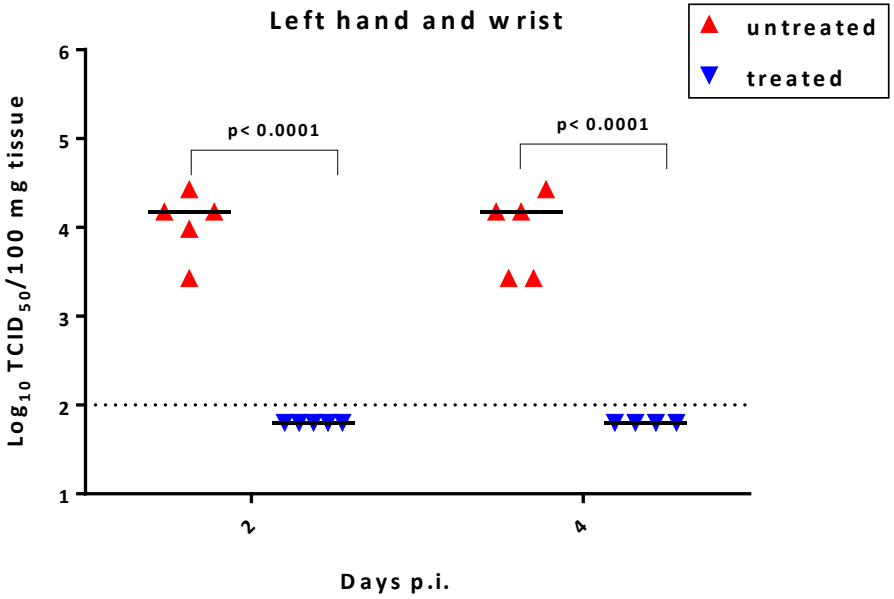
Abdelnabi *et al*. Antiviral Res. (2018);149:113-117.

Untreated vs. treated mice (infectious virus)





Treatment with T-705 during the acute phase inhibited systemic viral spread.

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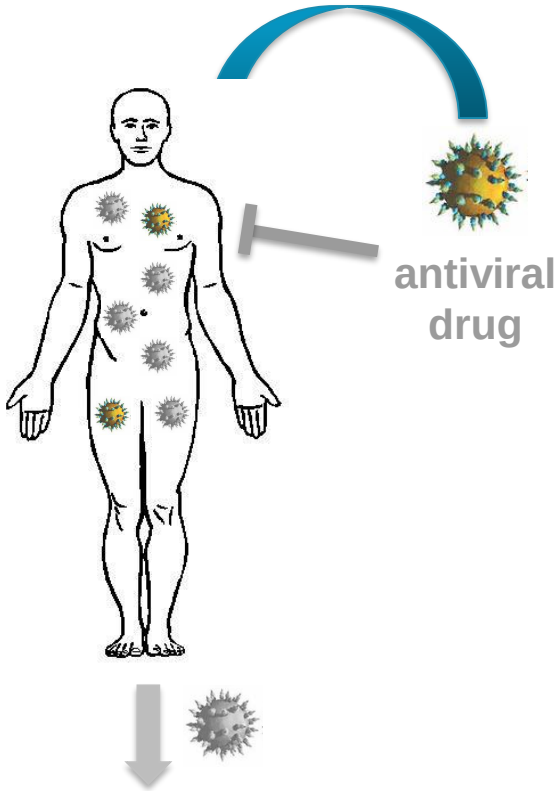
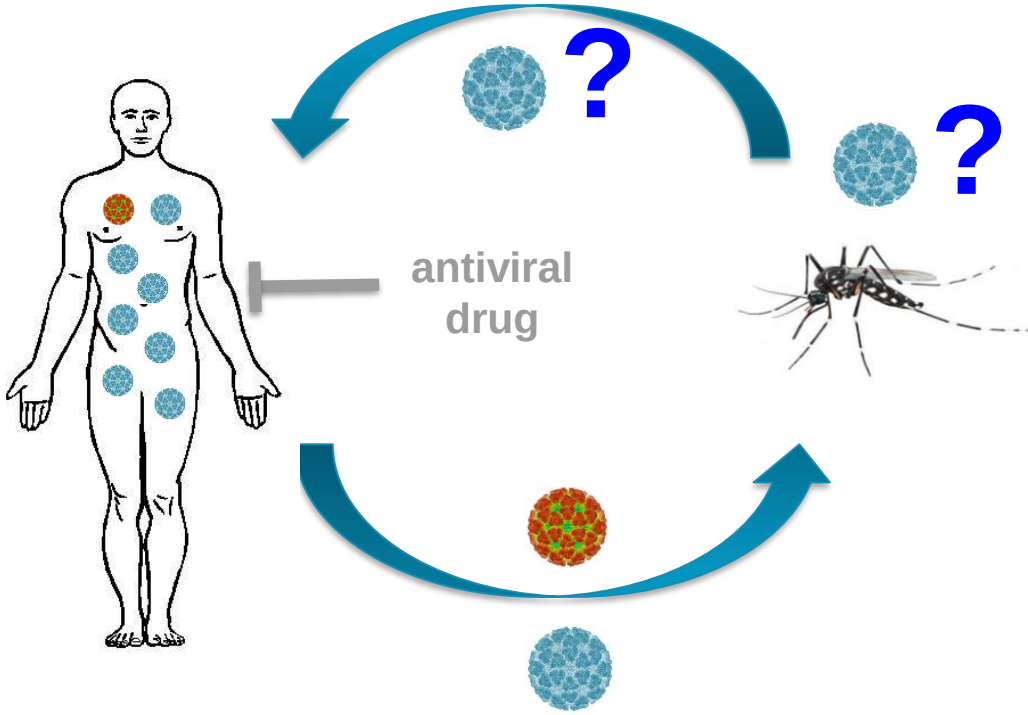
Antiviral drug resistance: relevant for transmission?

human virus (HCV, HIV)



transmission of drug-resistant virus

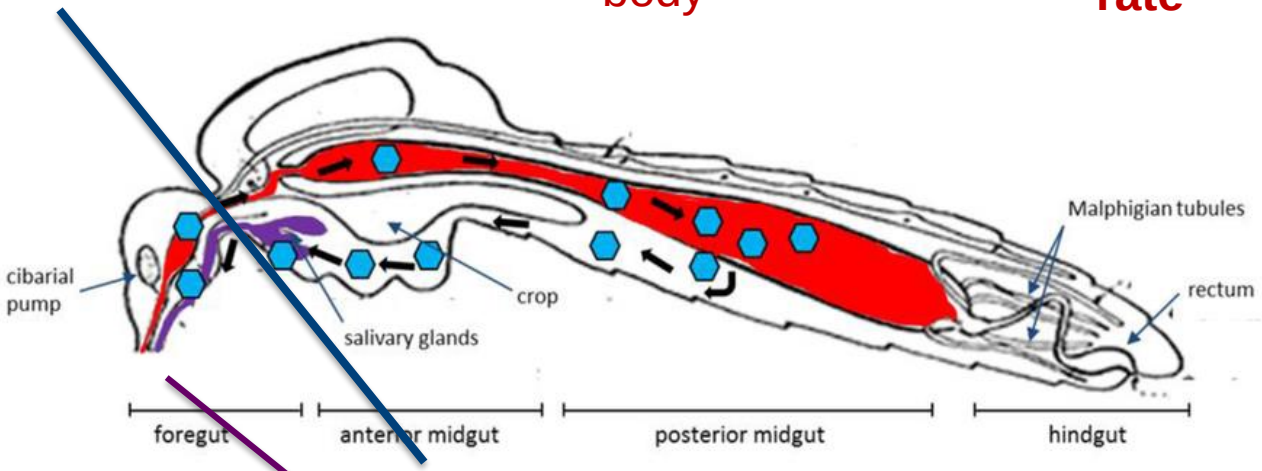
arbovirus



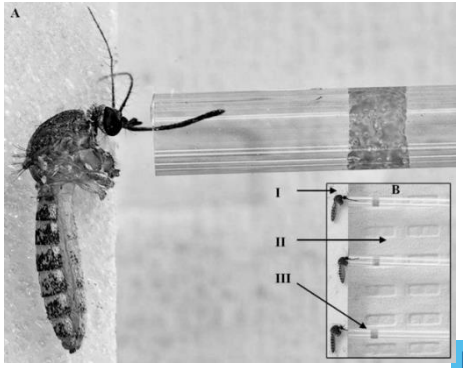
Vector competence

= ability of an insect vector to acquire, replicate and transmit an infectious agent

mosquito body → **infection rate**

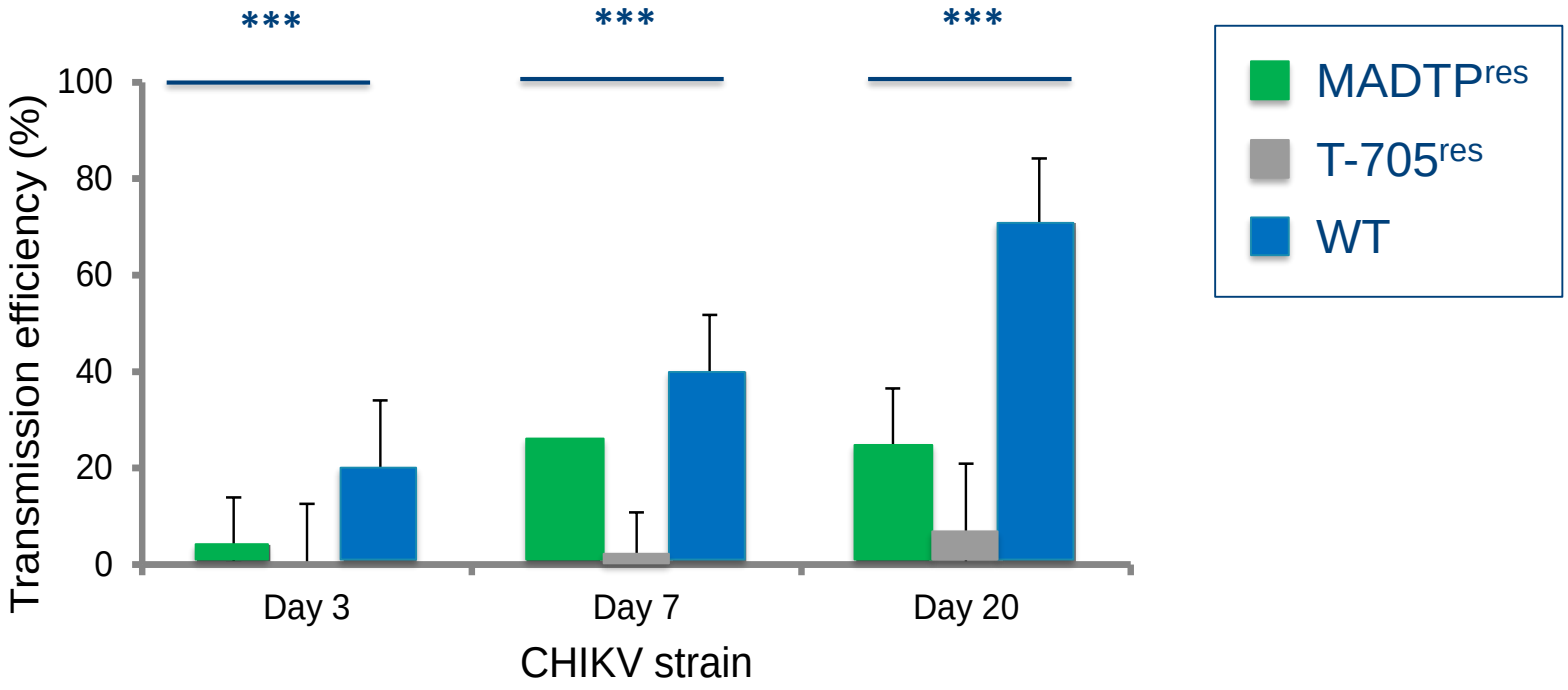


saliva → **transmission efficiency**



Drug-resistant CHIKV: transmission efficiency

Transmission efficiency (TE): *proportion of mosquito females with infectious saliva among tested ones.*



average results of two independent blood meals
 # mosquitoes per condition = ± 50

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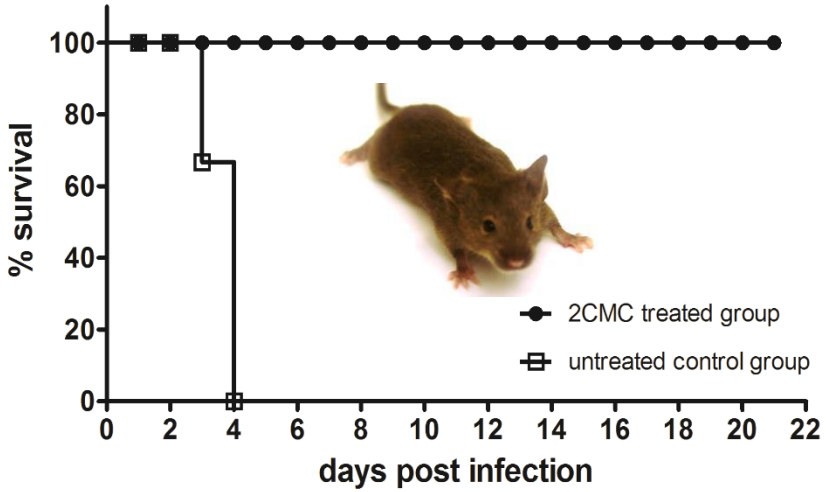
NOROVIRUS INFECTIONS

4 min flow of the Niagara Falls

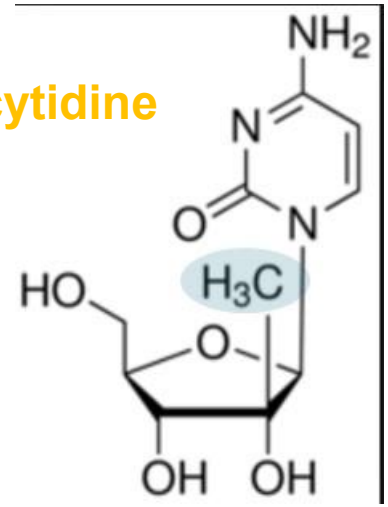
~ 4 billion cases of diarrhea worldwide/year (www.who.int)

3 days with each 4 “events” of 200 ml = 9.600.000.000 liter

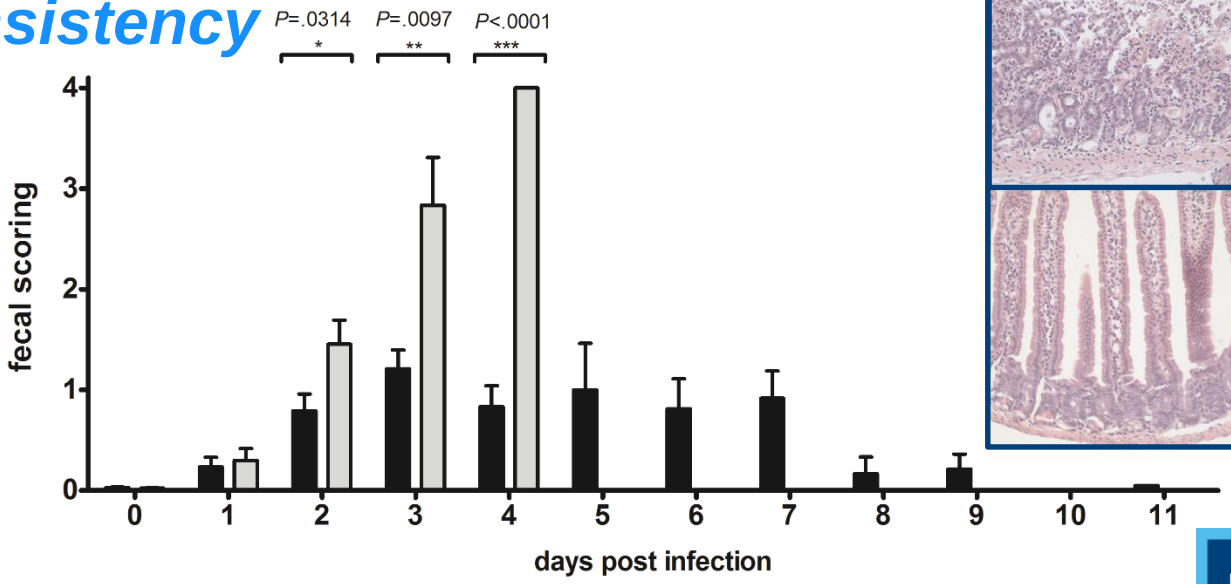
Antiviral treatment of mouse norovirus infection

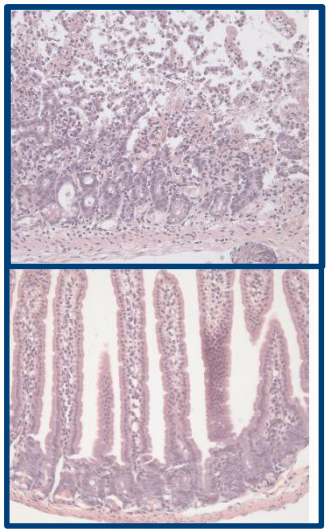


2'C methylcytidine (2CMC)



Fecal consistency



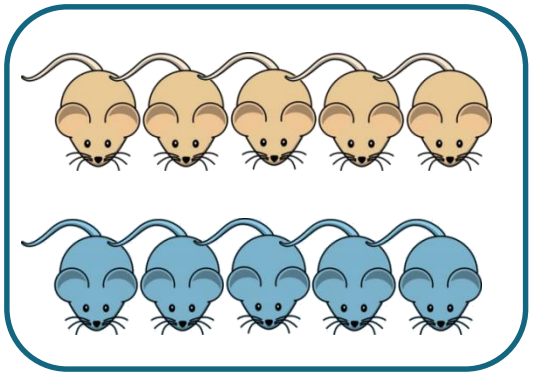


Infected

Infected & treated

Rocha -Pereira et al., J. Virol , 2013

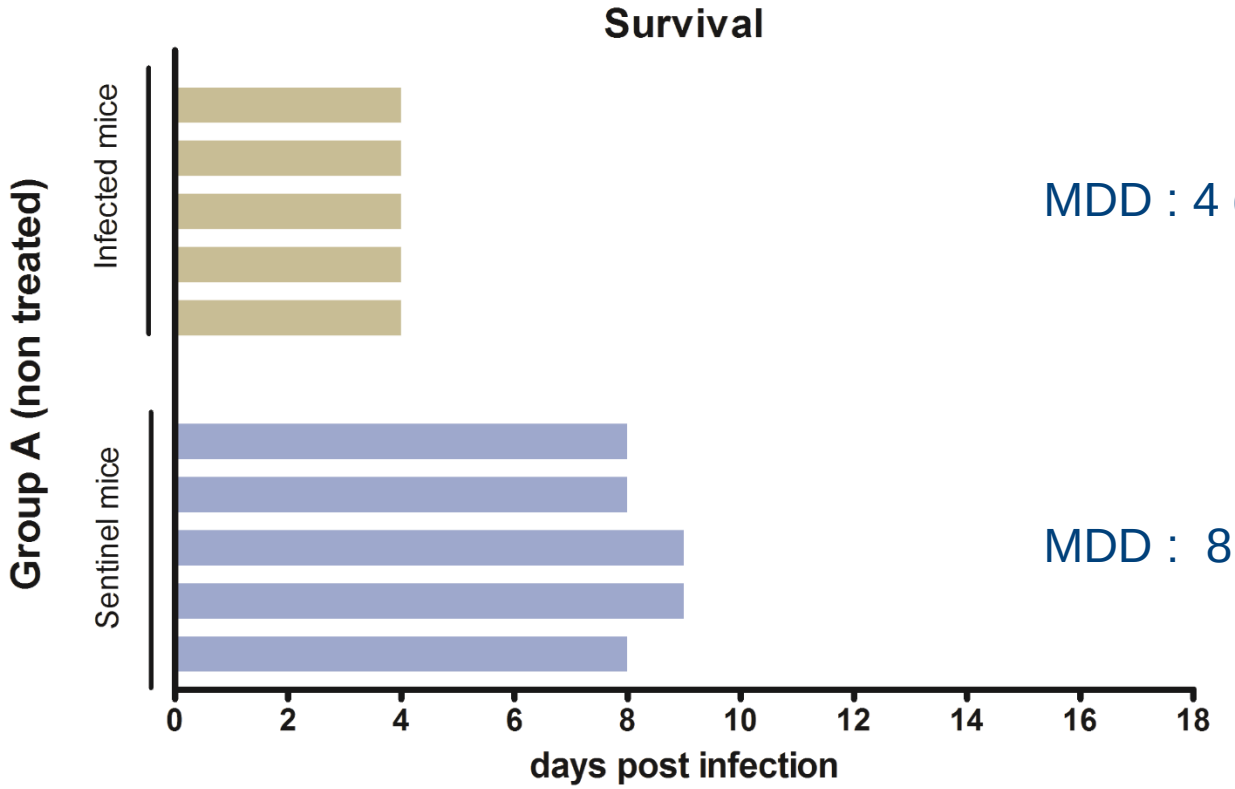
Transmission of mouse norovirus



5 infected animals

+

5 non infected sentinels

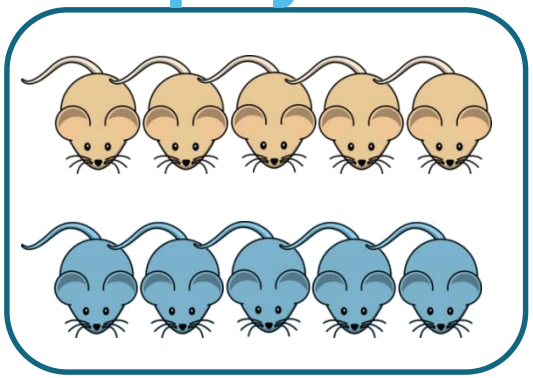


MDD : 4 d pi

MDD : 8.4 ± 0.5 d pi

Rocha -Pereira et al., J. Antimicrob Chemother (2014)

Prophylaxis with 2CMC prevents transmission

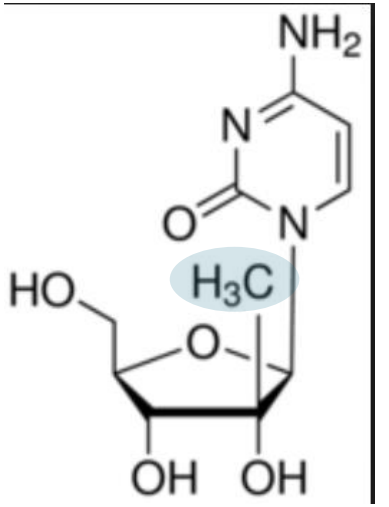


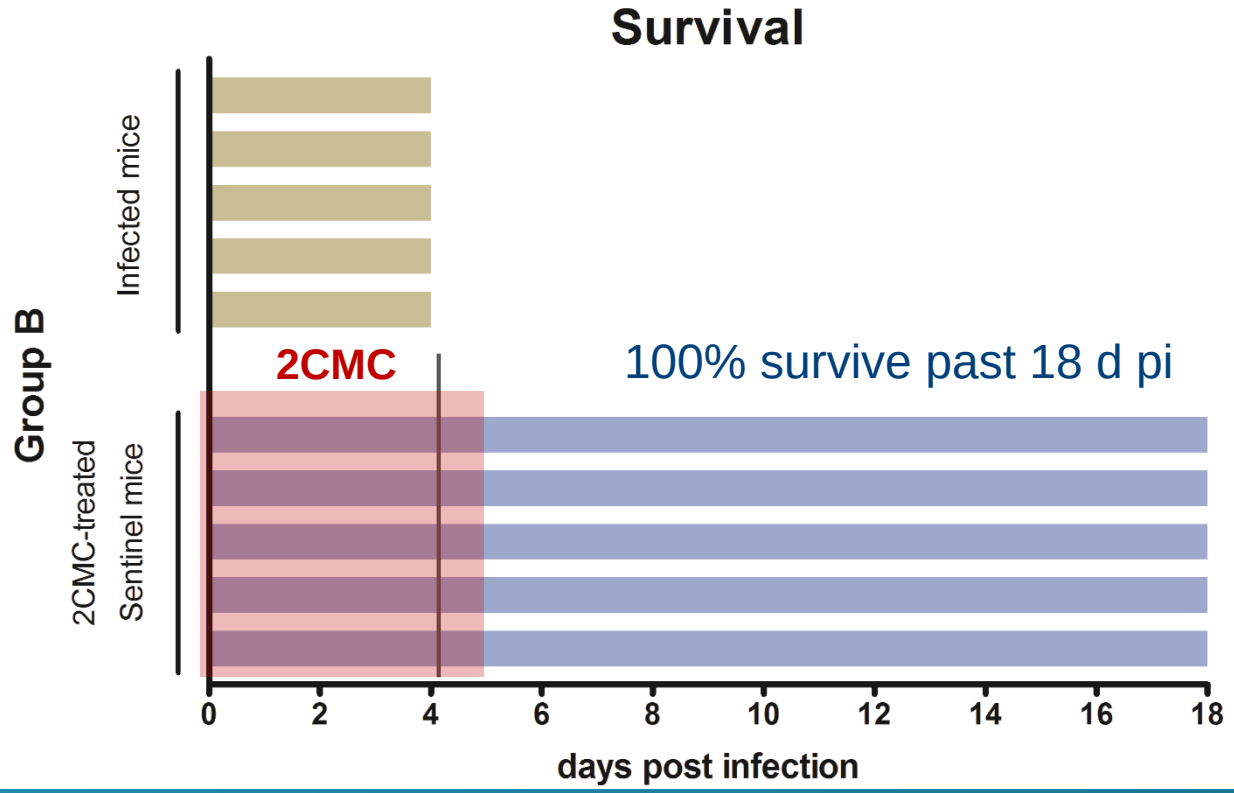
5 infected animals

+

5 sentinels (non infected)

2CMC





100% survive past 18 d pi

Bedding was replaced at 4 d pi

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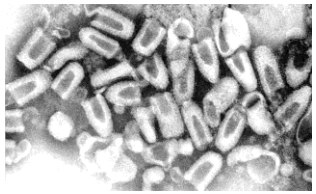
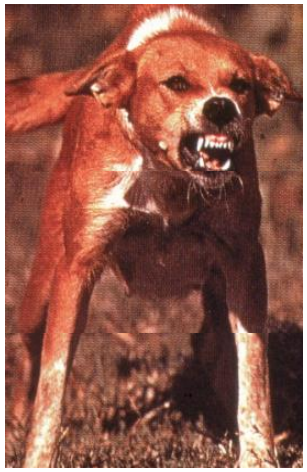


Rabies

160 people die of rabies every day, says major new study

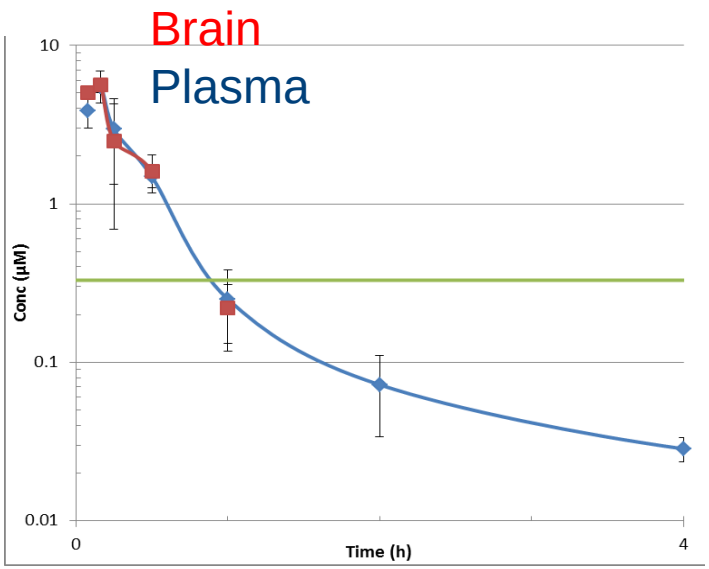
17 April 2015

A global study on canine rabies, published today (16 April 2015), has found that **160** people die every single day from the disease. The report is the first study to consider the impact in terms of deaths and the economic costs of rabies across all countries. Even though the disease is preventable, the study says that around **59,000** people die every year of rabies transmitted by dogs.

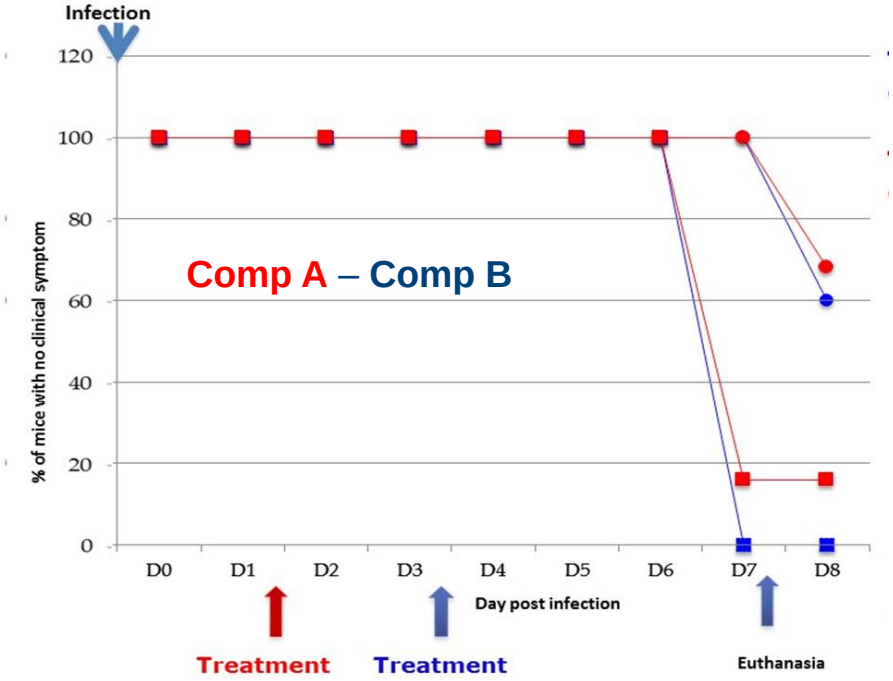


Antivirals against rabies

Once class of partially optimized inhibitors (EC50 ~ 1 µM)



Single dose PK in mice



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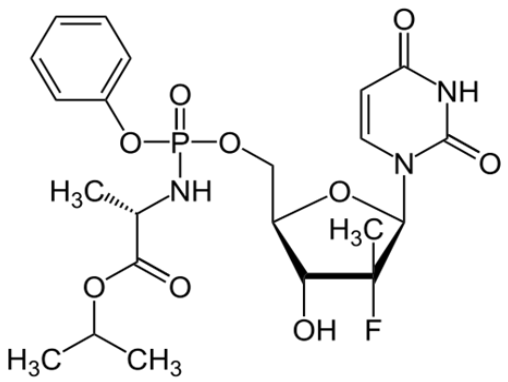
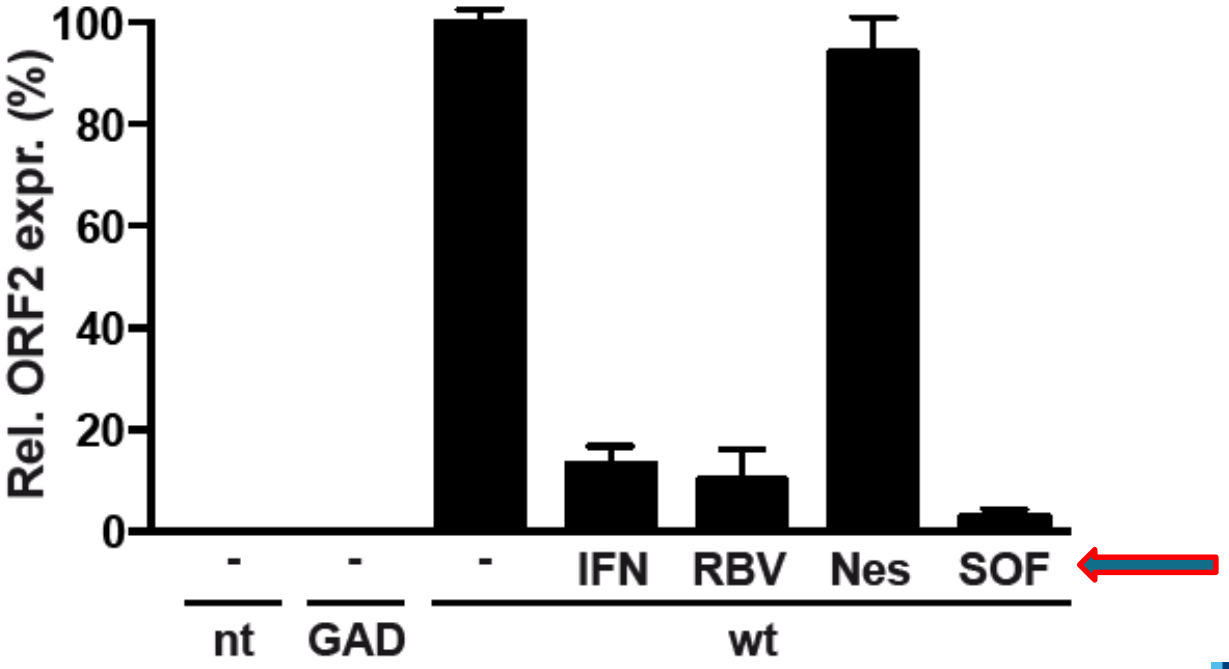
The HCV drug sofosbuvir inhibits HEV





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Gouttenoire et al., Gastroenterol (2015)

Conclusion

Antivirals still needed against many viral infections

Excellent molecular targets to be further explored such as

- flavivirus NS4B
- enterovirus 2C
- alphavirus nsP1

Broad-spectrum RNA virus inhibitors needed

- pan-genus
- pan-family
- multi-family
  - e.g. PI of picorna & noroviruses
  - T-705-like?
  - nucleoside analogues

Off-label use (e.g. Sofosbuvir against HEV)

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THANK YOU

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