

Infectivity and Pathogenicity of Transfusion-transmitted Arboviruses

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IPFA/PEI 26th International Workshop on
Surveillance and Screening of Blood Borne Pathogens

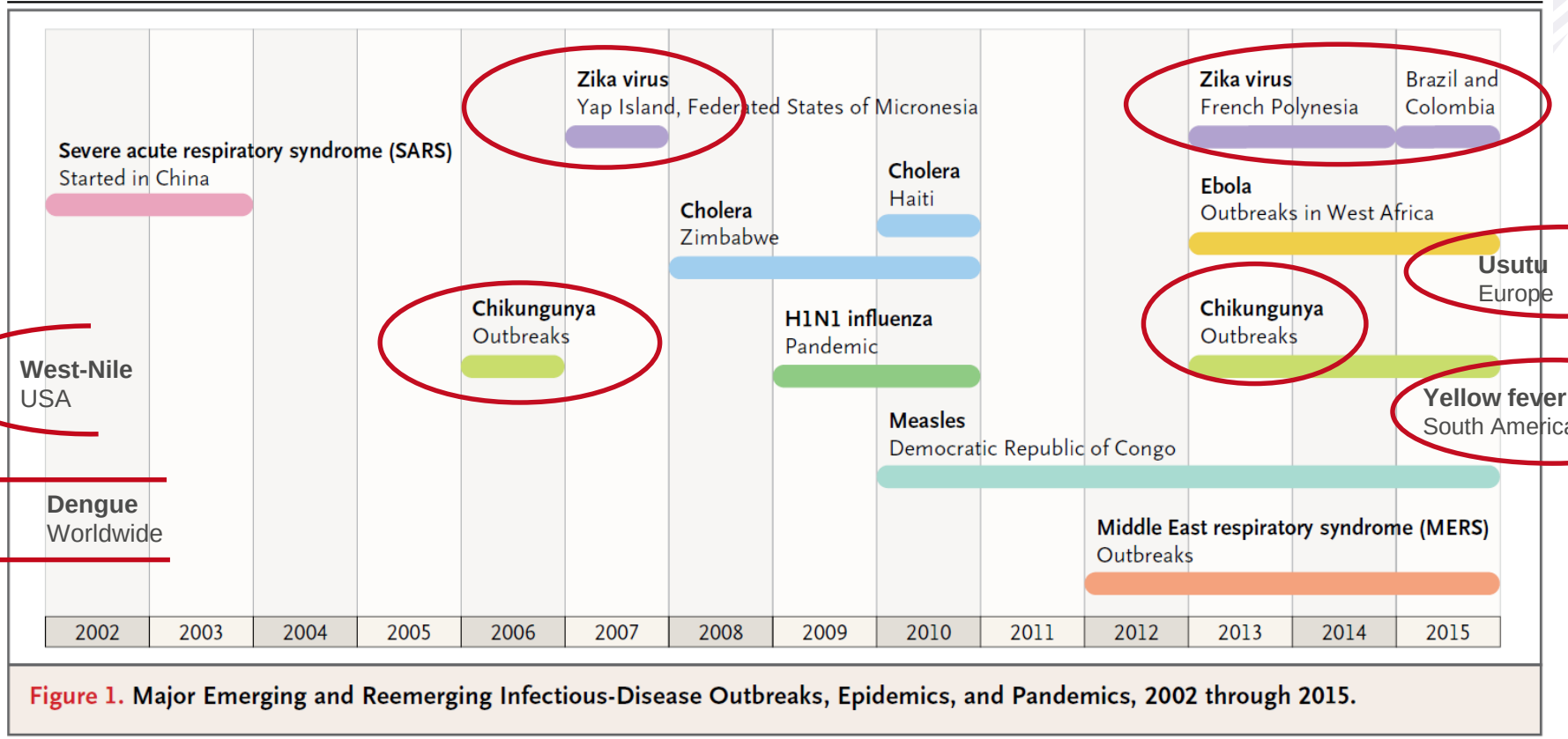
Krakow,

May 22-23, 2019

Conflict of interest disclosure:

None, other than being employed by the Etablissement Français du Sang (EFS), the French transfusion public service

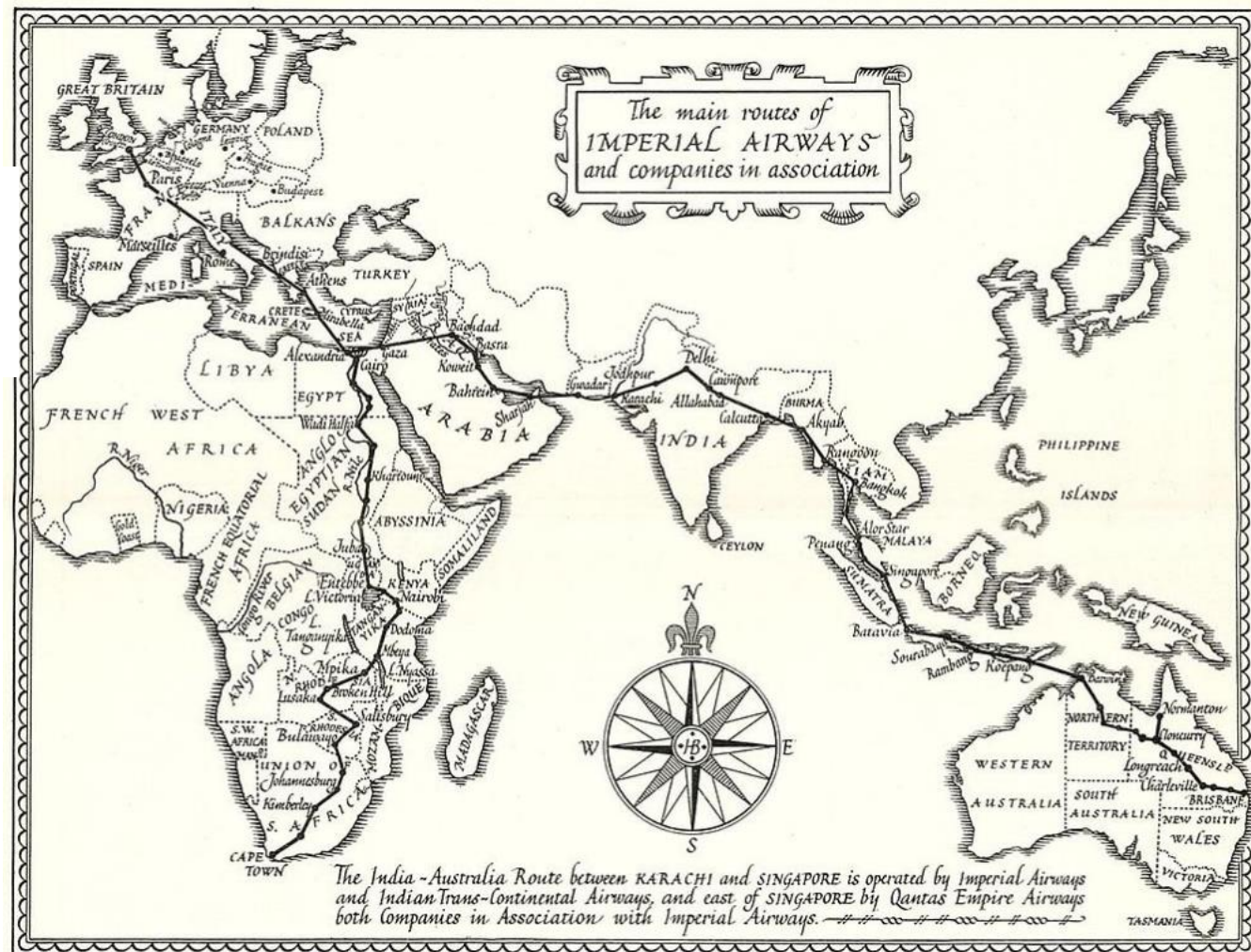
Arboviruses infectious diseases: emerging, reemerging, ...



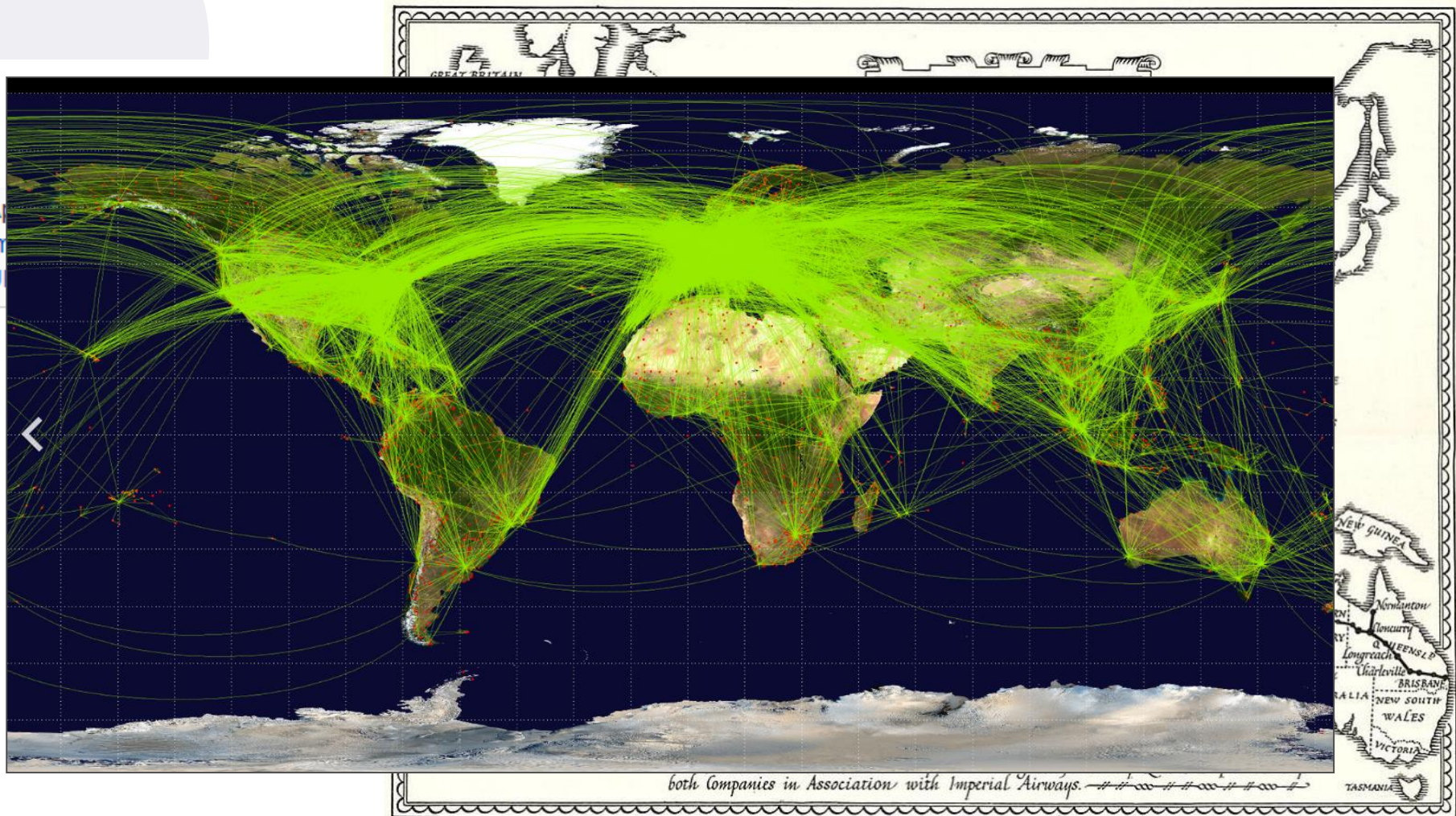
Adapted from Sands P, NEJM, 2016

Arboviruses: taking advantage of a traveled and changing world

April 1935 map showing
Imperial Airways' routes from the
UK to Australia and South Africa

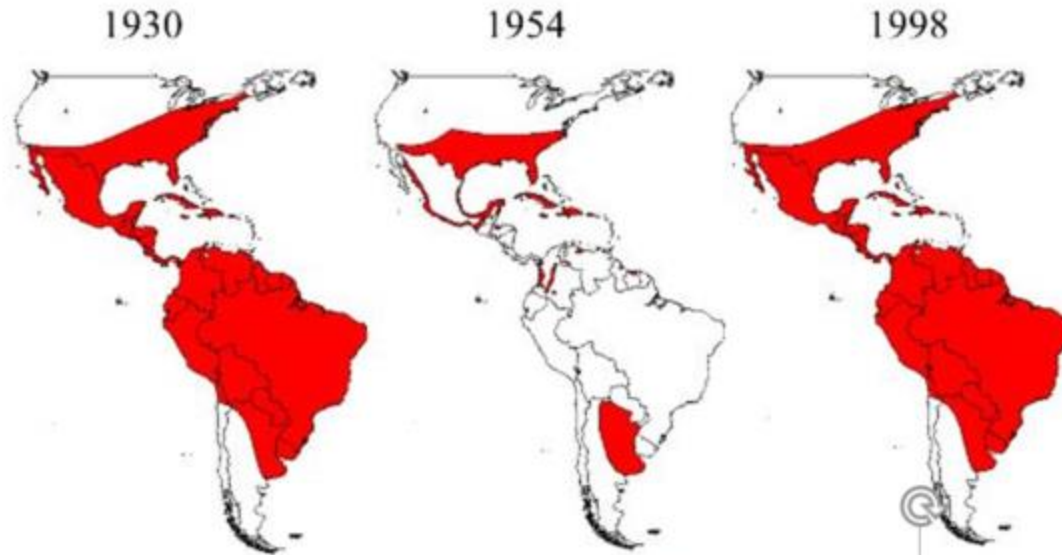


Arboviruses: taking advantage of a traveled and changing world



Arboviruses: taking advantage of a traveled and changing world

Maps showing the geographical distribution of *Aedes aegypti* (presence shown in red) at different times in the Americas.



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Science. Not Hype.

NEWS & VIEWS PUBLICATIONS BROWSE BY TOPIC IN THE MEDIA

Zika Virus Infections in the Americas and the DDT Question
Posted on February 25, 2016 by Don Roberts

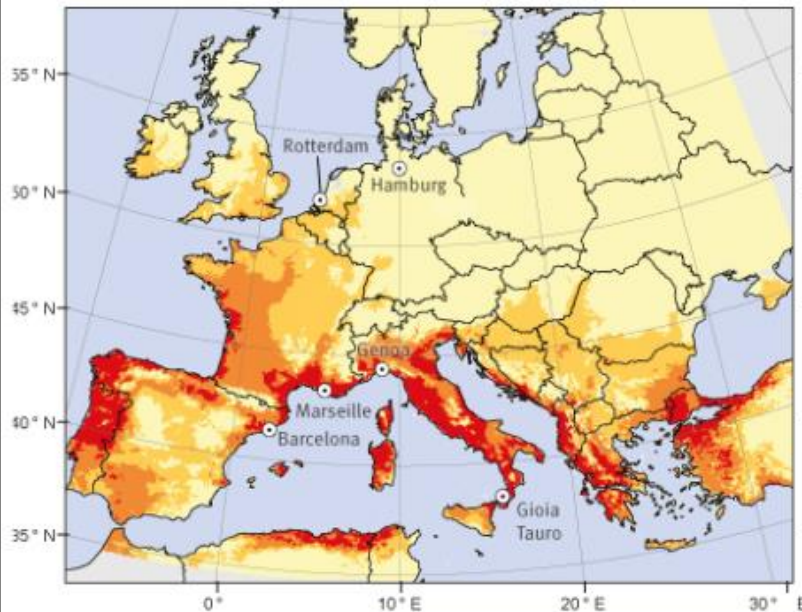
both Companies in Association with Imperial Airways.

Arboviruses: taking advantage of a traveled and changing world

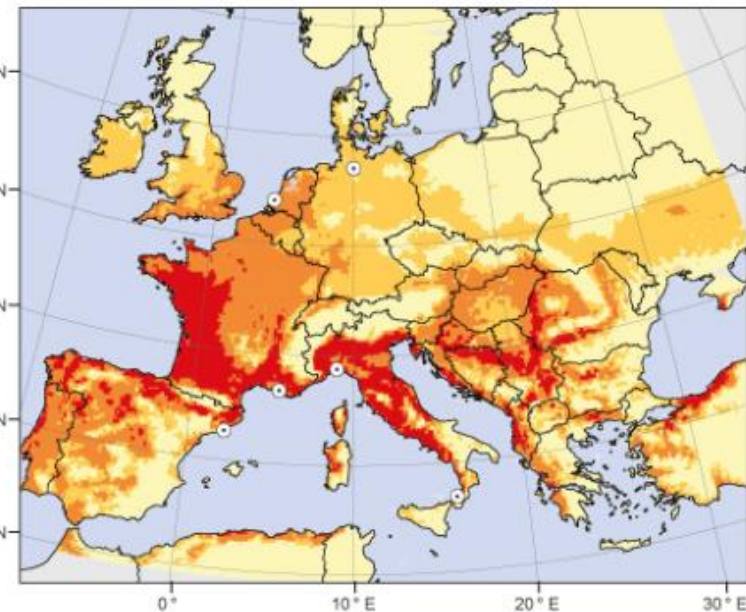
Maps showing the geographical distribution of *Aedes aegypti* (presence shown in red) at different times in the Americas.

End of the 20th century (A) and projected (2011–2040) (B) climatic suitability of *Aedes albopictus* in Europe, with locations of important harbours

A. End of the 20th century



B. 2011–2040



Climatic suitability for *A. albopictus*

0.00–0.20 0.21–0.40 0.41–0.60 0.61–0.80 Ⓞ Harbours

The emergence of arboviruses as a threat to transfusion safety

Transmission of West Nile Virus from an Organ Donor to Four Transplant Recipients

Iwamoto M et al, N Engl J Med, 2002

The emergence of arboviruses as a threat to transfusion safety

Transmission of West Nile Virus from an Organ

Transmission of West Nile Virus through Blood Transfusion in the United States in 2002

Pealer L et al, New Engl J Med, 2003

The emergence of arboviruses as a threat to transfusion safety

Transmission of West Nile Virus from an Organ

Transmission of West Nile Virus through Blood

West Nile Virus among Blood Donors
in the United States, 2003 and 2004

Stramer SL et al, New Engl J Med, 2005

The emergence of arboviruses as a threat to transfusion safety

Transmission of West Nile Virus from an Organ

Transmission of West Nile Virus through Blood

West Nile Virus among Blood Donors

Screening the Blood Supply for West Nile Virus

CDC Home



Centers for Disease Control and Prevention
CDC 24/7: Saving Lives. Protecting People.™

Morbidity and Mortality Weekly Report (*MMWR*)

Fatal West Nile Virus Infection After Probable Transfusion-Associated Transmission — Colorado, 2012

Weekly

August 9, 2013 / 62(31);622-624

The emergence of arboviruses as a threat to transfusion safety

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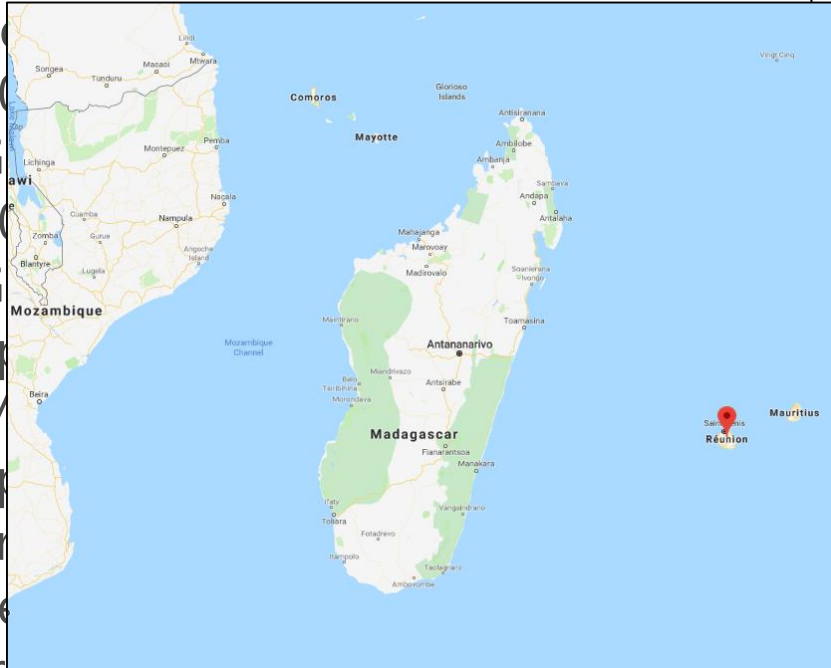
August 9, 2013 / 62(31);622-624

France, EFS and arbovirus outbreaks

- 2005-2006, **Chikungunya**, La Réunion
- 2014, **Chikungunya**, Martinique, Guadeloupe
- 2016, **Zika**, Martinique, Guadeloupe, French Guyana
- Episodically, **Dengue** , La Réunion, Mayotte, Martinique, Guadeloupe
- Episodically, **West Nile**, southern France
- Permanently, **Arbovirus**, returning travelers

France, EFS and arbovirus outbreaks

- 2005-2006, **Chikungunya**, La



Chikungunya virus outbreak on Reunion Island, 2005-2006

- Interruption of whole blood collection, introduction of pathogen reduced (apheresis) platelets, RBC and plasma from mainland
- Over the course of the outbreak, the mean risk of was estimated at 1,3 per 1000 donations.
- The risk peaked at 1,5 % donations at the height of the outbreak in February 2006.
- During this period, an estimated 312,500 of 757,000 inhabitants had been infected by mosquito-borne transmission.

Brouard et al, Transfusion 2009

Chikungunya virus outbreak in the Caribbean's, 2014

- Risk prevention measures included:

- detection of CHIKV RNA

(individual NAT in arboviral laboratory)

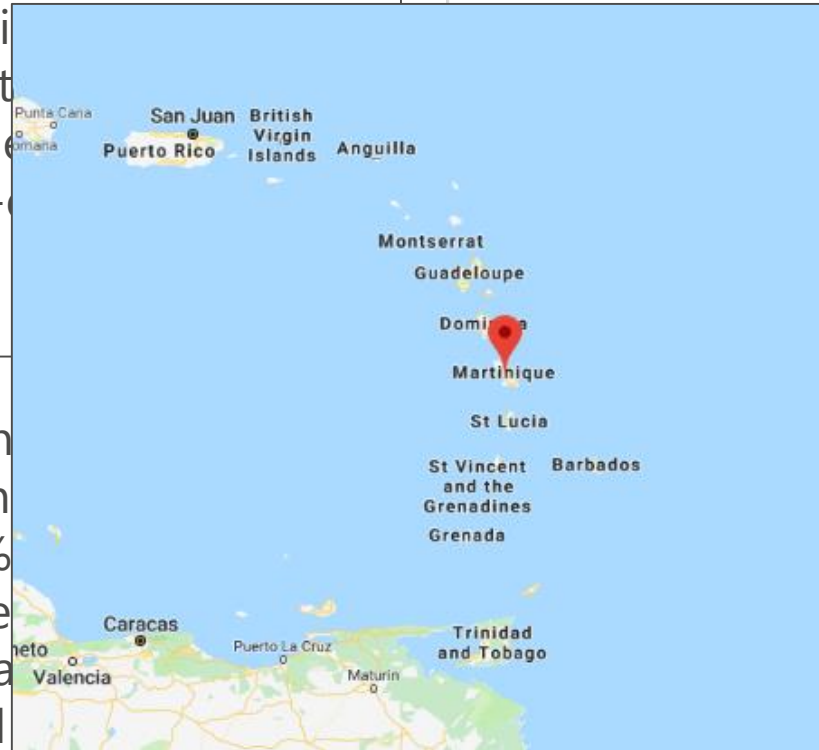
- 3-day quarantine and reinforced post-donation information

- use of Intercept

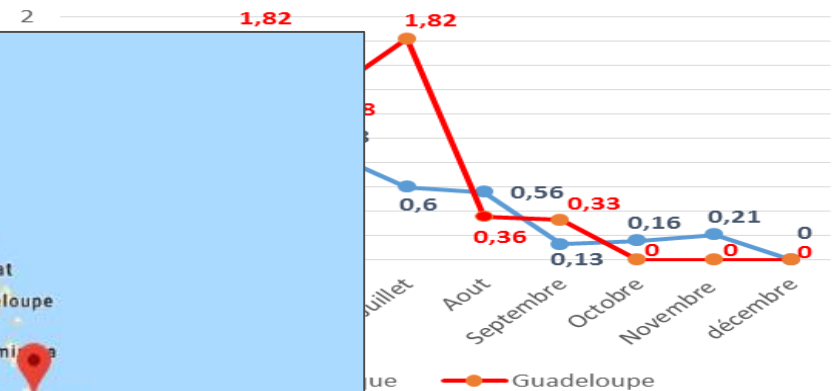
- Individual NAT screening of platelet donations (0.4%, with

- A large majority (75% after donation, suggested with a 72 hours quarantine of CHIKV positive blood

- Intercept platelets from CHIKV positive donations were transfused to 10 recipients with no clinical evidence for transmission.



Prévalence (%) mensuelles des dons ARN CHIK-V positifs

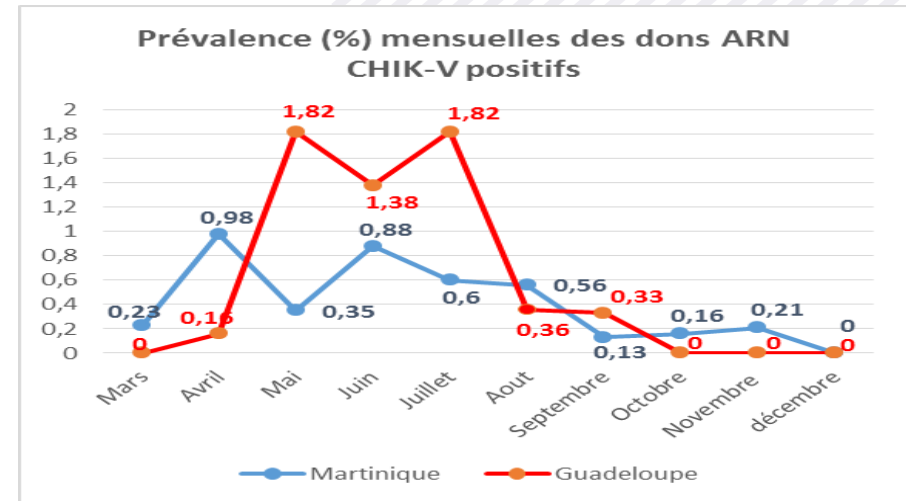


CHIKV positive

symptoms within 3 days of transfusion combined with information combined with a lack of transfusing a

Chikungunya virus outbreak in the Caribbean's, 2014

- Risk prevention measures included:
 - detection of CHIKV RNA (individual NAT in dedicated arboviral laboratory in Marseille)
 - 3-day quarantine combined with reinforced post-donation information
 - use of Intercept platelets



- Individual NAT screening of 16 269 donations identified 63 CHIKV positive donations (0.4%, with 1 to 2% at the epidemic peak).
- A large majority (75%) of CHIKV positive donors reported symptoms within 3 days after donation, suggesting that enhanced post donation information combined with a 72 hours quarantine may significantly mitigate the risk of transfusing a CHIKV positive blood product
- Intercept platelets from CHIKV positive donations were transfused to 10 recipients with no clinical evidence for transmission.

Zika virus outbreak in the Americas in 2016

Martinique, Guadeloupe, French Guyana

Risk mitigation measures

Pre-existing:

- RBC: 3 day quarantine and enhanced post-donation information request
- Platelets: Intercept treated
- Plasma: from mainland France

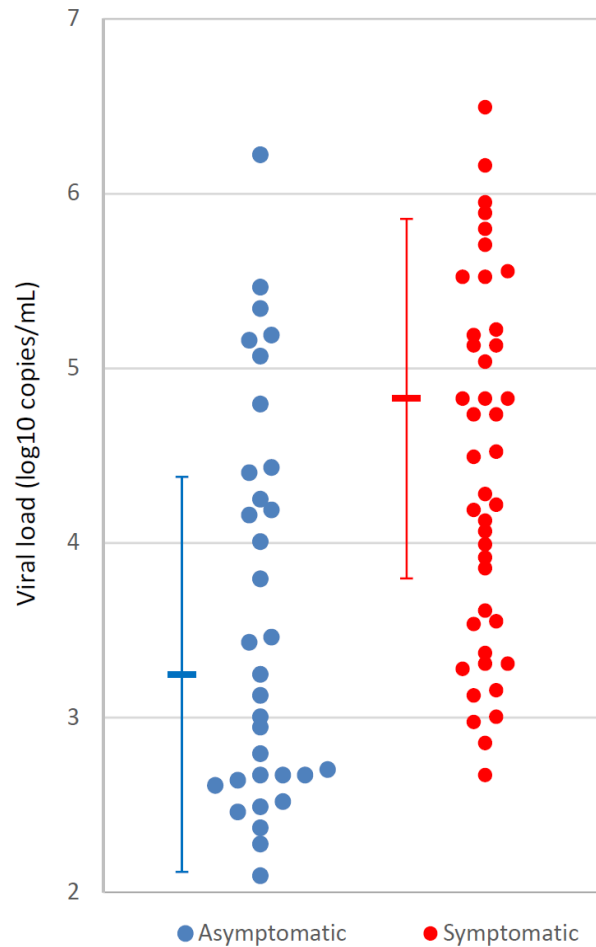
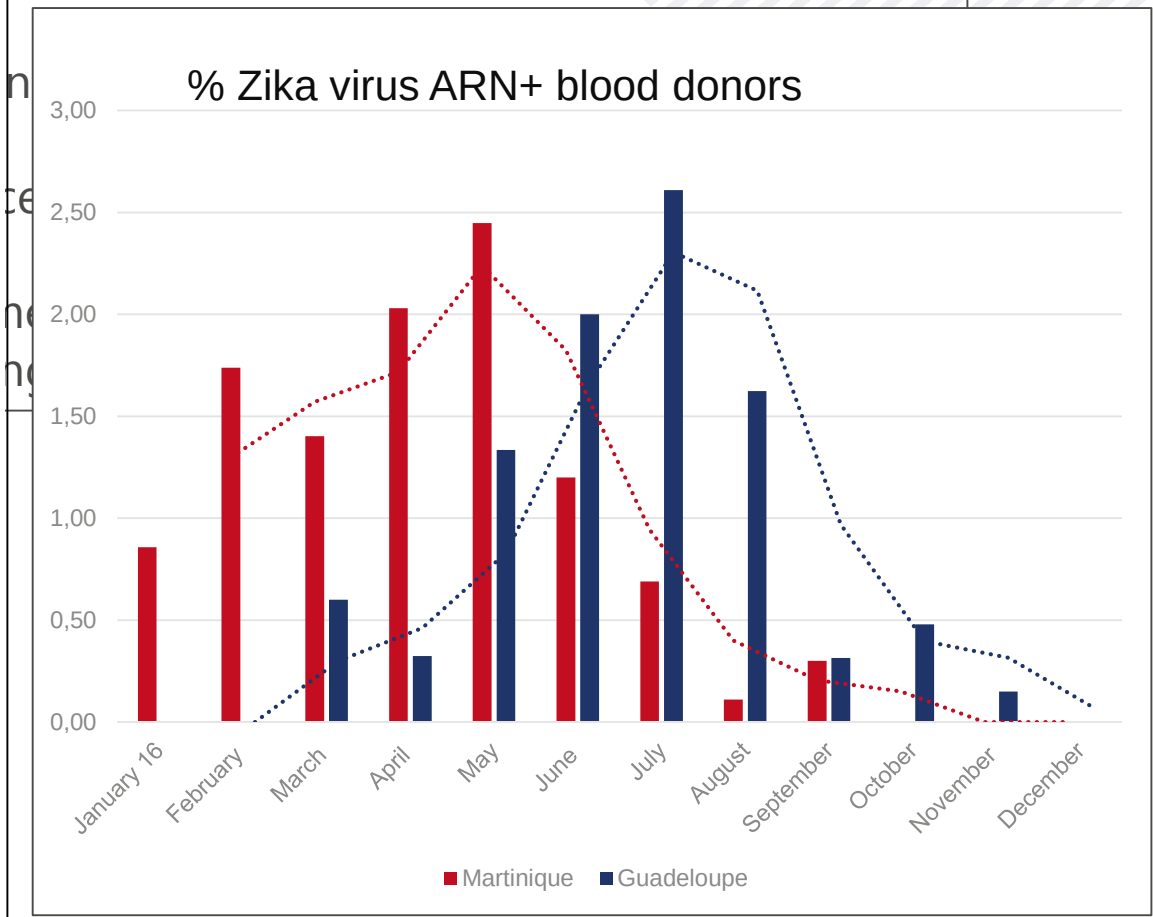
New:

- Transfusion of pregnant women with RBC from mainland France
- Individual NAT ZiKV screening (2/15/16 to 1/3/17)

Gallian P et al, Blood 2017

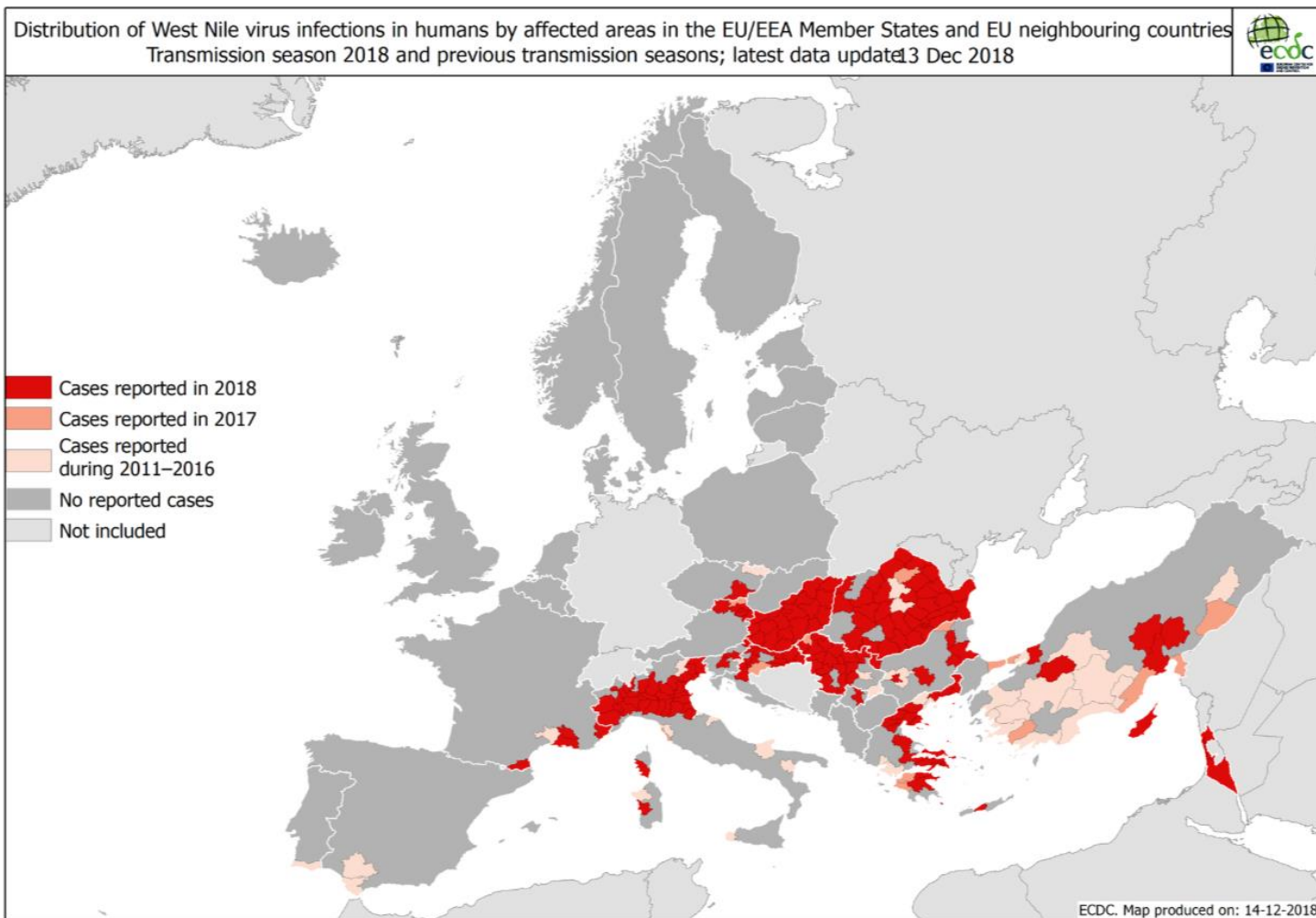
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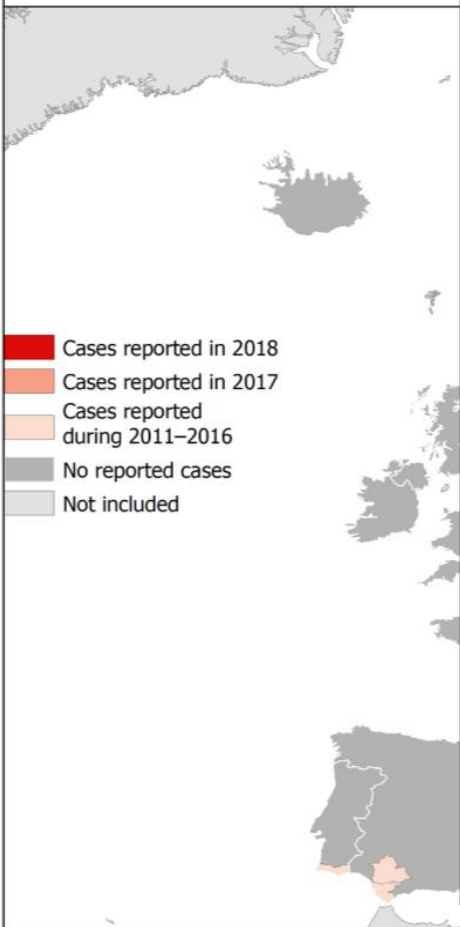
Gallian P et al, Blood 2017

West Nile circulation in Europe

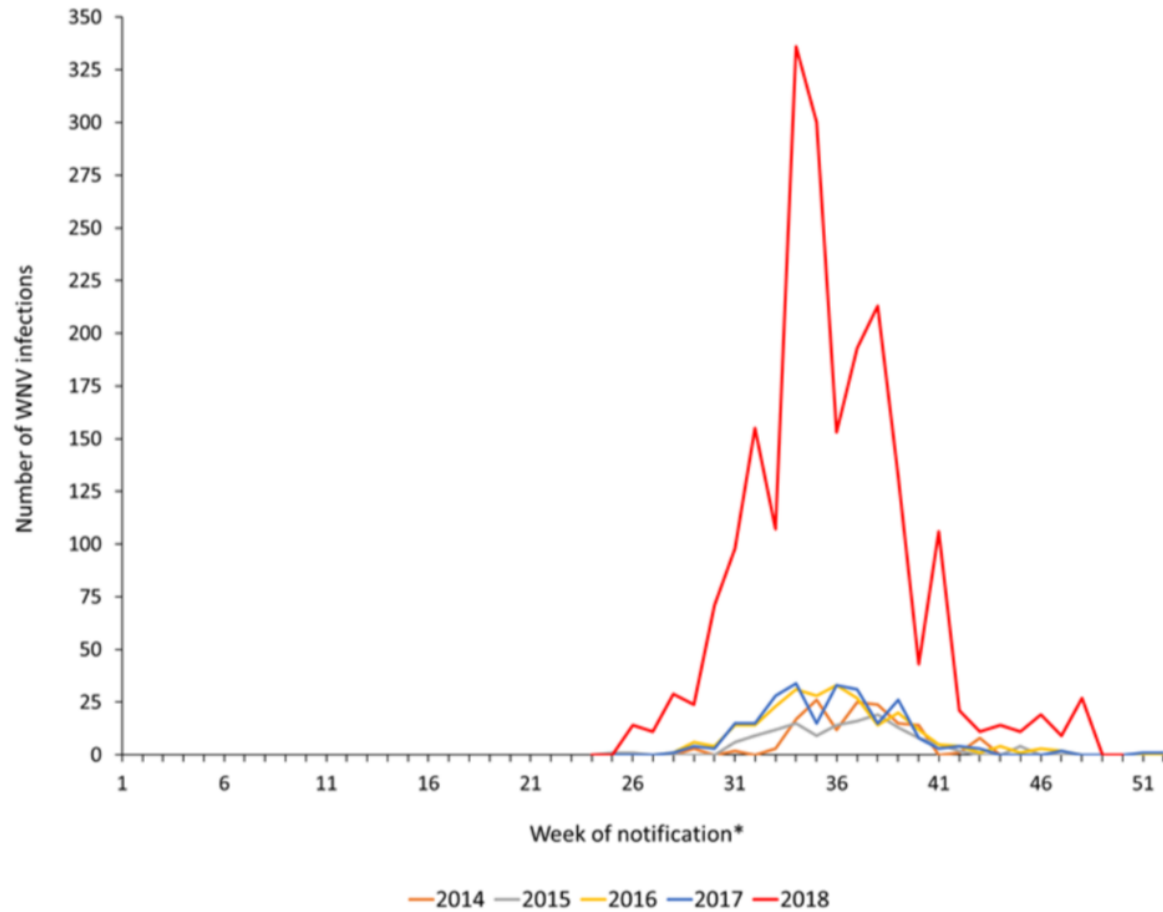


West Nile circulation in Europe

Distribution of West Nile virus infections in Europe by country, transmission season 2018 and previous years



Number of WNV infections in EU/EEA and EU enlargement countries by epidemiological week of notification*, 2014-2018.

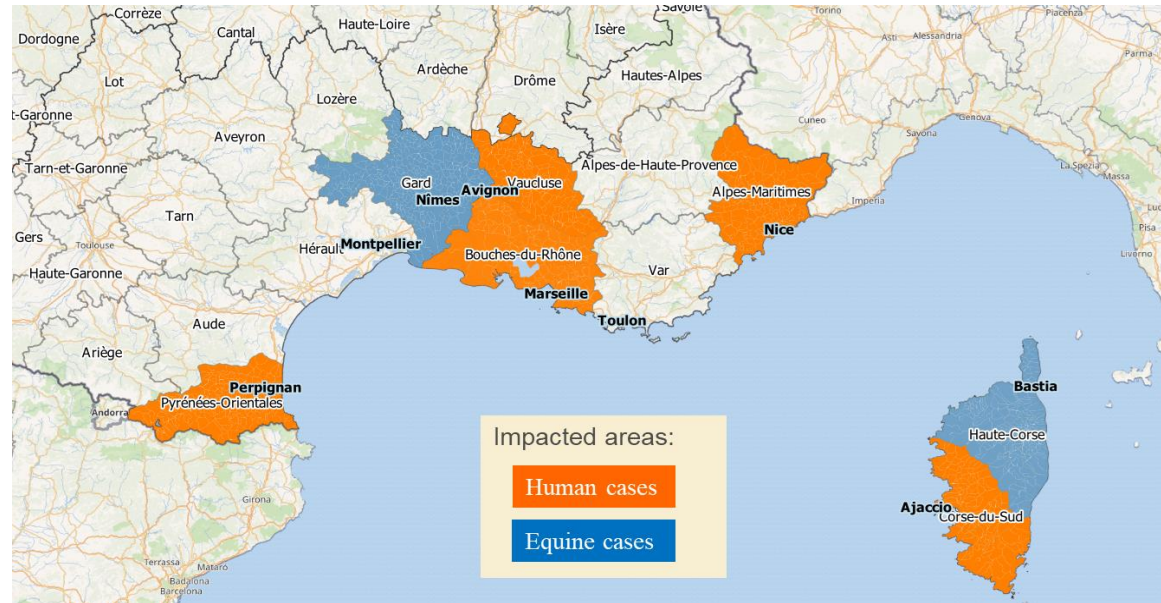


* Week of notification to national authorities or if missing, week of notification to ECDC.

ECDC. Map produced on: 14-12-2018

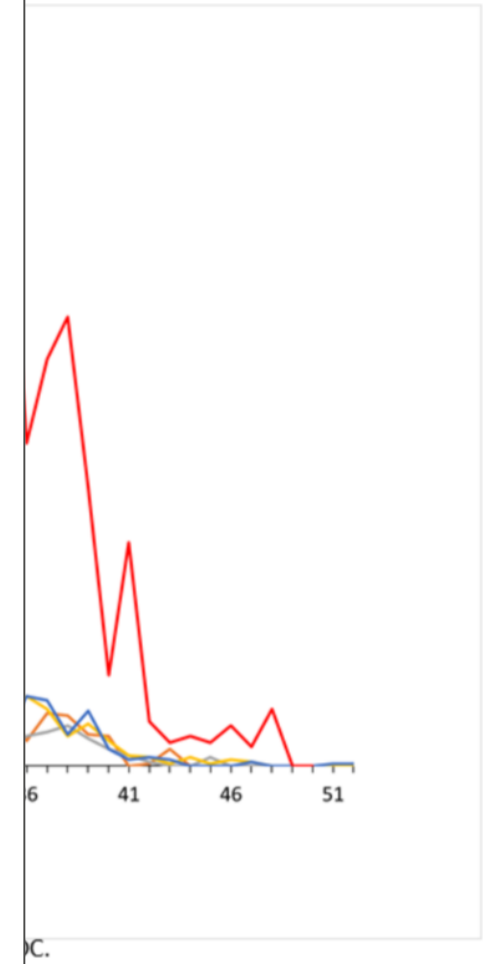
West Nile circulation in Europe

West Nile virus circulation, Mainland France, 2018



- Early and extended viral circulation Alpes Maritimes region
- Twenty five cases symptomatic West Nile infections (5 meningo-encephalitis, 2 meningitis)
- NAT screening, 44 500 donations tested (August-December), one positive donations (whole blood donations); Intercept platelets nation-wide since November 2017

ement countries by



ECDC. Map produced on: 14-12-2018

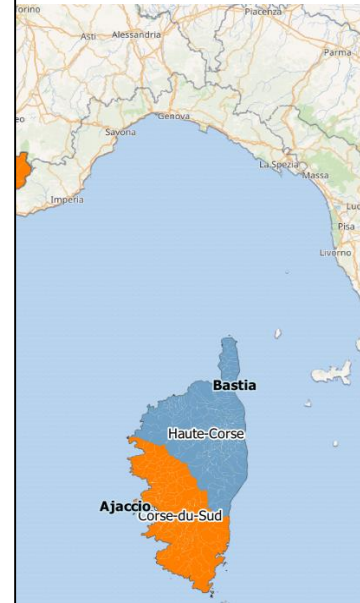
West Nile circulation in Europe

West Nile virus circulation, Mainland France, 2018

France, EFS and arbovirus outbreaks

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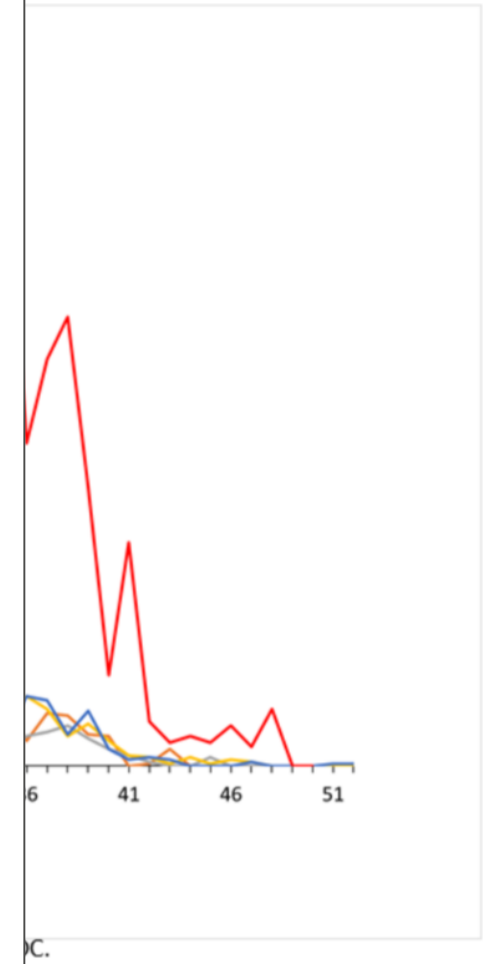


pes Maritimes

Nile infections (5

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whole blood

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ECDC. Map produced on: 14-12-2018

Transfusion-relevant arboviruses (2019)

Arbovirus	West Nile	Dengue	Chikungunya	Zika	Usutu	Others (Yellow fever, ..)
Family	Flaviridae	Flaviridae	Togaviridae	Flaviridae	Flaviridae	...
Arthropode	Culex mainly	Aedes Aegypti / Aedes Albopictus	Aedes Aegypti / Aedes Albopictus	Aedes Aegypti mainly	Culex mainly	...
Nb of cases / (estimates)	2018 USA: 2544 EU: 2083 clinical cases	390 million / year world-wide	> 1,4 million during the 2015 outbreak in the Americas	> 800 000 cases in the Americas 2015-2018	?	...
Nb of reported TTI	35 (USA)	< 15	0	4 (asymptomatic)	0	0?
Risk to blood supply	High	Mild	None?	Unknown (likely mild)	Unknown	Mild to unknown

Adapted from Jimenez A et al, *Transf Med Rev*, 2016

Transfusion-transmitted arbovirus can cause disease

Transmission of West Nile Virus through Blood Transfusion in the United States in 2002

Pealer L et al, New Engl J Med, 2003

Dengue virus–induced hemorrhage in a nonhuman primate model

Onlamoon N et al, Blood 2009

Development of classic dengue hemorrhage in 6 out of 6 rhesus monkeys 3 to 5 days after intravenous injection of dengue virus serotype 2 at $1.10E7$ PFU/ animal (high dose) in 6 out of 6 rhesus monkeys.

Transfusion-transmitted arbovirus can cause disease

Transmission of West Nile Virus by Transfusion in the United States

Pealer L et al, *New Engl J Med*, 2001

Dengue virus–induced hemophagocytosis

Onlamoon N et al, *Blood* 2009

Development of classic dengue fever in monkeys 3 to 5 days after intravenous inoculation with serotype 2 at $1.10E7$ PFU/ animal.

Transfusion-Transmitted Zika Virus Infection in Pregnant Mice Leads to Broad Tissue Tropism With Severe Placental Damage and Fetal Demise

Tai W et al, *Frontiers in microbiology* 2019

- Transfused-transmitted ZIKV at the early infection stage led to significant viremia and broad tissue tropism in the pregnant Zika-susceptible A129 mice (type I interferon receptor deficient)
- Pregnant mice transfused with early-stage, but not later stages, ZIKV plasma also exhibited severe placental and fetal infection, resulting in fetal and pup death.

Potential contributors to the low number of reported transfusion-transmitted arbovirus infections

- **Difficulty to differentiate vector borne from transfusion transmission; low-resource settings where arboviruses are commonly encountered;**
- **Frequent asymptomatic or mild infection in recipients (and in donors);**

Transfusion-Transmitted Dengue and Associated Clinical Symptoms During the 2012 Epidemic in Brazil

Sabino EC, JID, 2016

During a large epidemic of DENV-4 infection in Brazil:

- > 0,5% of donations were RNA positive
- 37,5% (95%CI, 15,2%-64,6%) of RNA positive blood donations transmitted the DENV-4 to the recipients
- **No significant differences in symptoms and mortality between cases and controls**

Potential contributors to the low number of reported transfusion-transmitted arbovirus infections

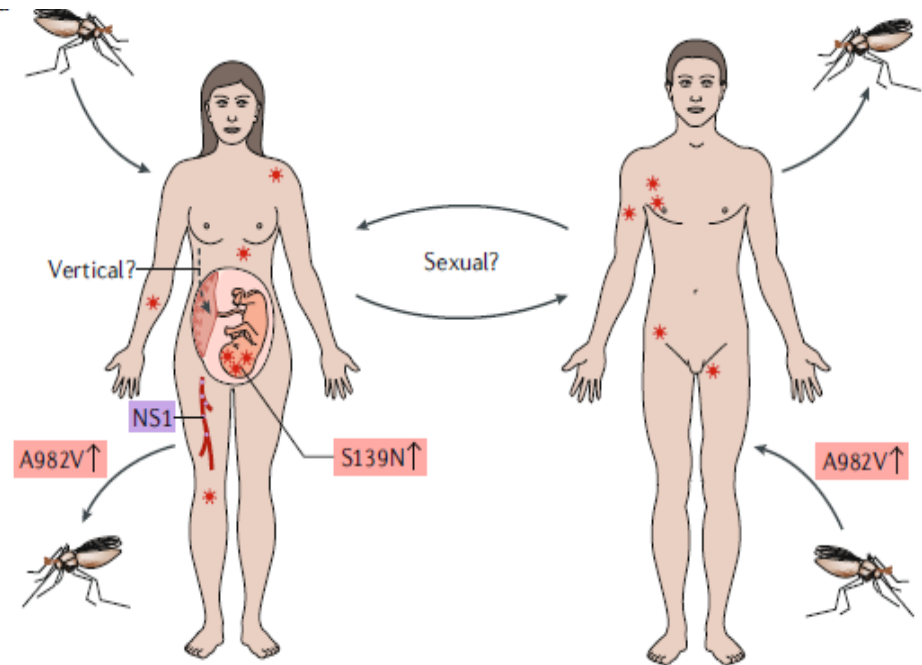
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- **Evolving pathogenicity over time / outbreak-specific pathogenicity**

Potential contributors to Zika virus transmission-transfusion-transmission

- Difficulty to differentiate vertical transmission; low-resource countries commonly encountered;
- Frequent asymptomatic or donors);
- **Evolving pathogenicity of Zika virus**

The evolution of Zika virus from Asia to the Americas

Lui Z-Y et al. *Nature Rev Microbiol*, 2019



- The S139N substitution enhances the tropism of ZIKV for human neural progenitor cells and increases fetal microcephaly in mice.
- The A982V substitution in NS1 enhances the uptake of viruses by mosquitoes, and leads to increased interferon inhibition by NS1.

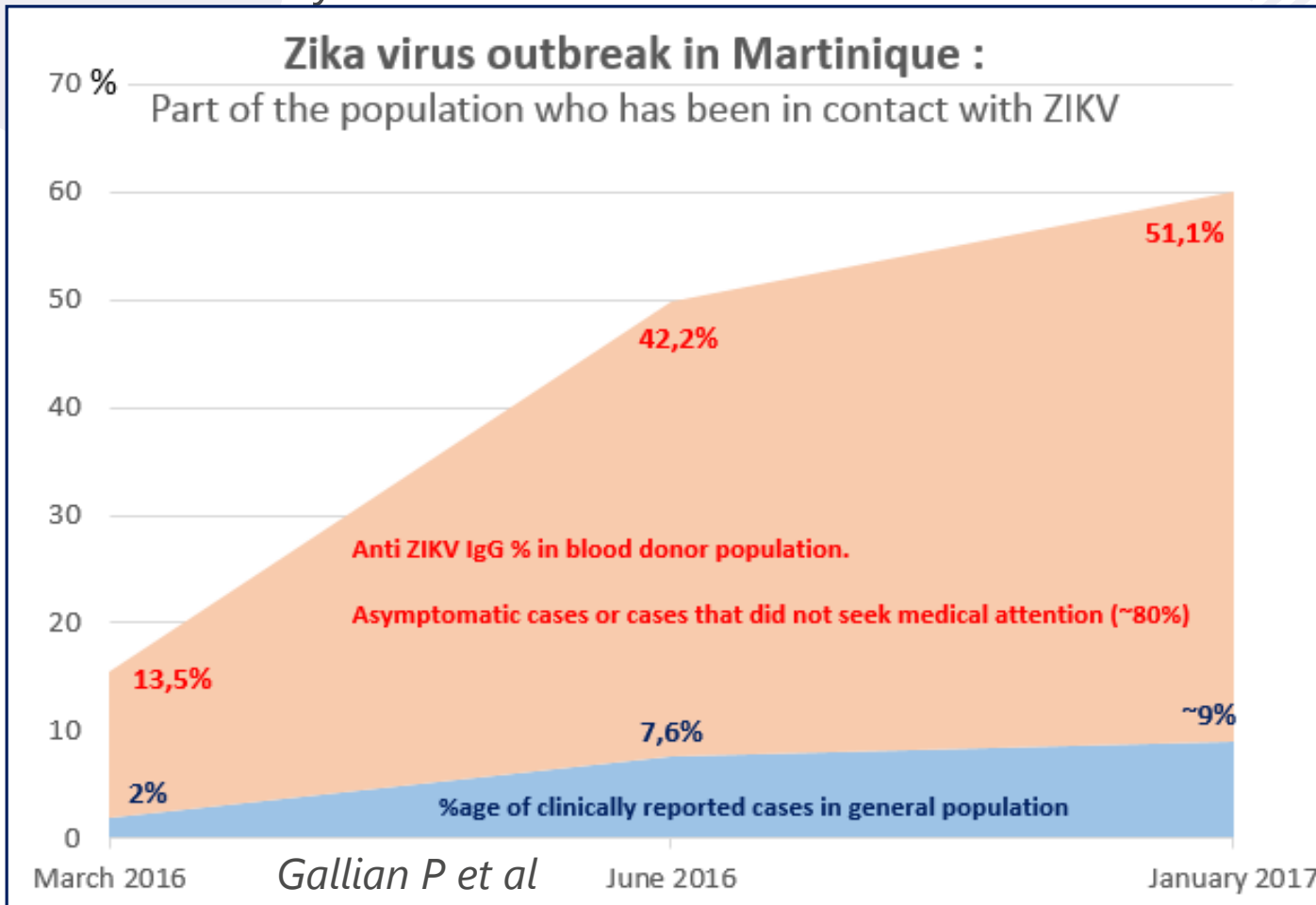
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- **Prior exposure in transfusion recipients;**

Gallian P et al

Potential contributors to the low number of reported transfusion-transmitted arbovirus infections

- Difficulty to differentiate vector-borne from transfusion



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Potential contributors to the low number of reported transfusion-transmitted arbovirus infections

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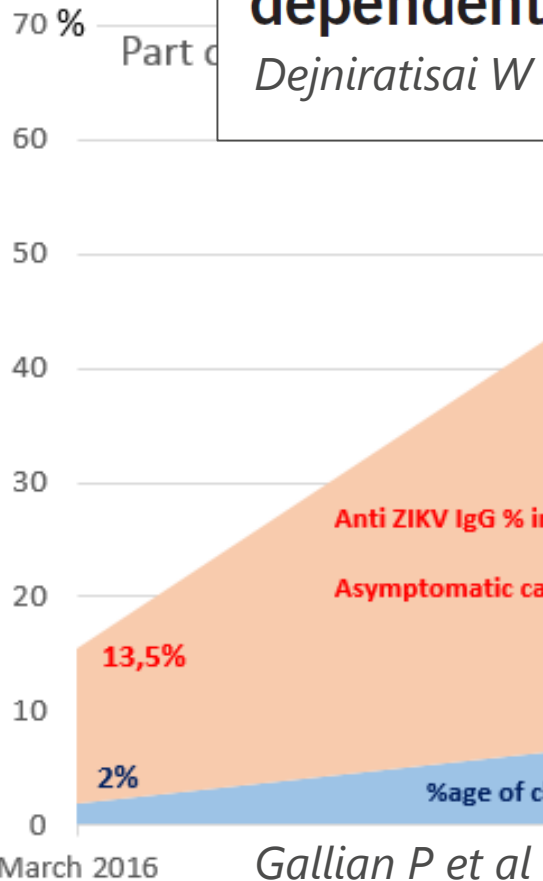
Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with Zika virus

Dejniratisai W et al. Nature Immunol, 2016

Prior Dengue Virus Exposure Shapes T Cell Immunity to Zika Virus in Humans

Grifoni A et al, J Virol, 2017

- ZIKV-reactive T cells in the acute phase of infection are detected earlier and in greater magnitude in DENV-immune patients.
- The frequency of ZIKV-reactive T cells continues to rise in the convalescent phase in DENV-naïve donors but declines in DENV-preexposed donors, compatible with more efficient control of ZIKV replication and/or clearance of ZIKV antigen.



Potential contributors to the low number of reported transfusion-transmitted arbovirus infections

- Difficulty to differentiate vector-borne from transfusion transmission; low-resource settings where arboviruses are commonly encountered;
- Frequent asymptomatic or mild infection in recipients (and in donors);
- Evolving pathogenicity over time / outbreak-specific pathogenicity
- Prior exposure in transfusion recipients;
- **Low viral titer and/or IgM seropositivity in the donor;**

Potential contributors to the low number of reported transfusion-transmitted arbovirus infections

- Difficulty to differentiate vector-borne from transfusion transmission; low-resource settings where arboviruses are commonly encountered;
- Frequent asymptomatic donors); Minimal infectious dose may increase after IgM seroconversion (*Simmons, Busch et al, ISBT 2018*)
- Evolving pathogen pathogenicity Frequency of IgM positive donations among RNA positive donations:
- Prior exposure
- **Low viral titre**
 - Chikungunya (Martinique, 2014): 7% (5/63)
 - Zika (Martinique, 2016) 14% (10/67)
 - Zika (USA, 2016-2017): 56% (5/9) (*Saa P et al, NEJM 2018*)

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- Frequent asymptomatic or mild infection in recipients (and in donors);
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- **Modulation of infectious virulence in relation with blood products manufacturing and storage;**
- **Transfusion-specific mitigation strategies (detection / pathogen reduction).**

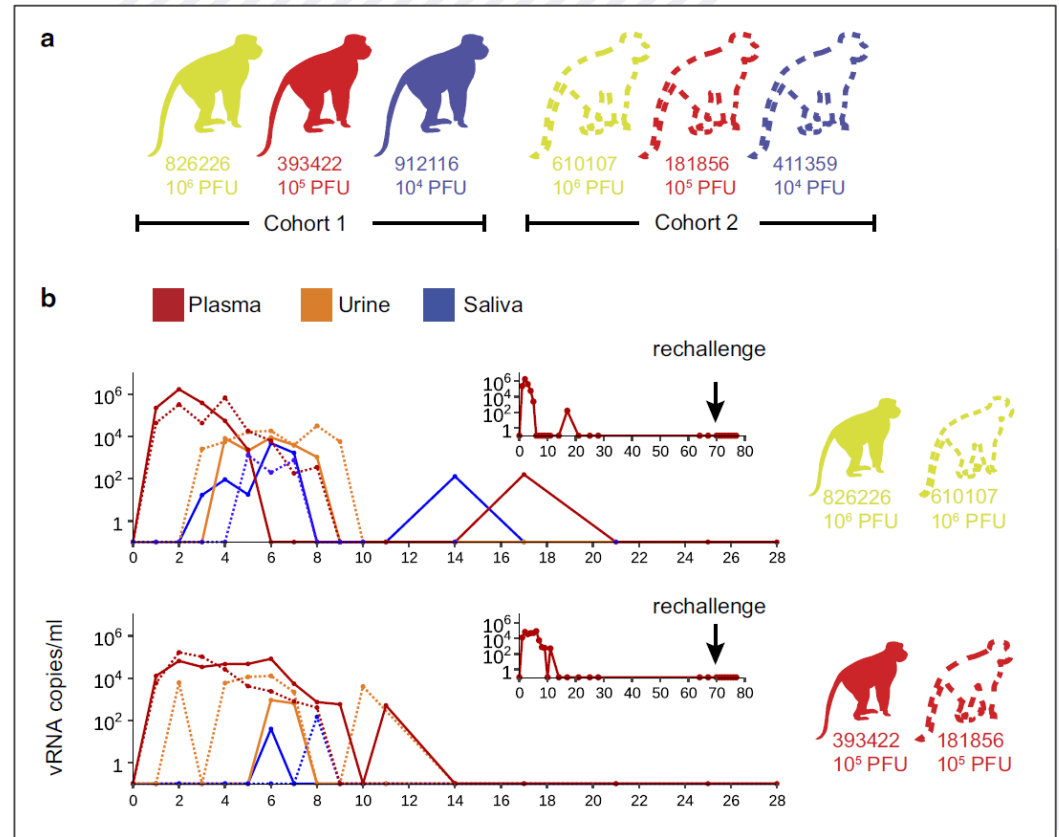
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- Low viral titer and/or IgM seropositivity in the donor;
- Modulation of infectious virulence in relation with blood products manufacturing and storage;
- Transfusion-specific mitigation strategies (detection / pathogen reduction);
- **Differing infectivity, pathogenicity and phenotypic expression of the disease between transfusion- and vector-borne transmissions.**

A rhesus macaque model of Asian-lineage Zika virus infection

Dudley DM et al, Nature Communications, 2016

- Following **subcutaneous inoculation**, ZIKV RNA is detected in plasma 1 day post infection (d.p.i.) in all animals and is also present in saliva, urine and cerebrospinal fluid.
- Mice remain viremic for up to 21 days.
- Neutralizing antibodies are detected by 21 d.p.i.



Zika Virus Tissue and Blood Compartmentalization in Acute Infection of Rhesus Macaques

Coffey LL et al, PLOS one, 2017

- **Intravenous inoculation** of 2 non-pregnant rhesus macaques with a 2015 Brazilian ZIKV strain.
- No clinical disease
- Short-lived plasma viremias (up to day 6) that clears as neutralizing antibody develops.

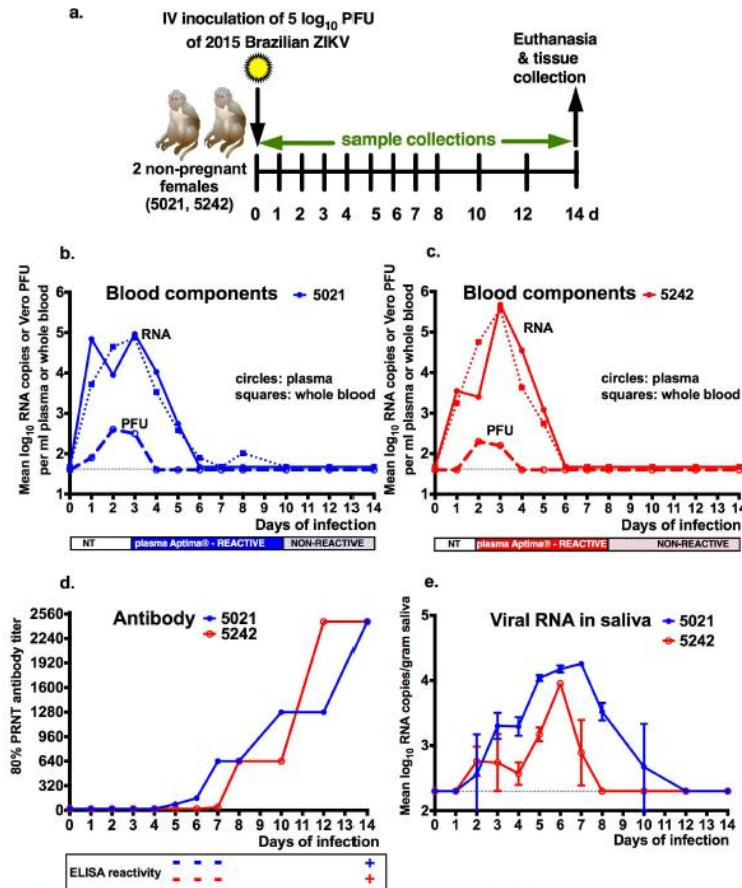
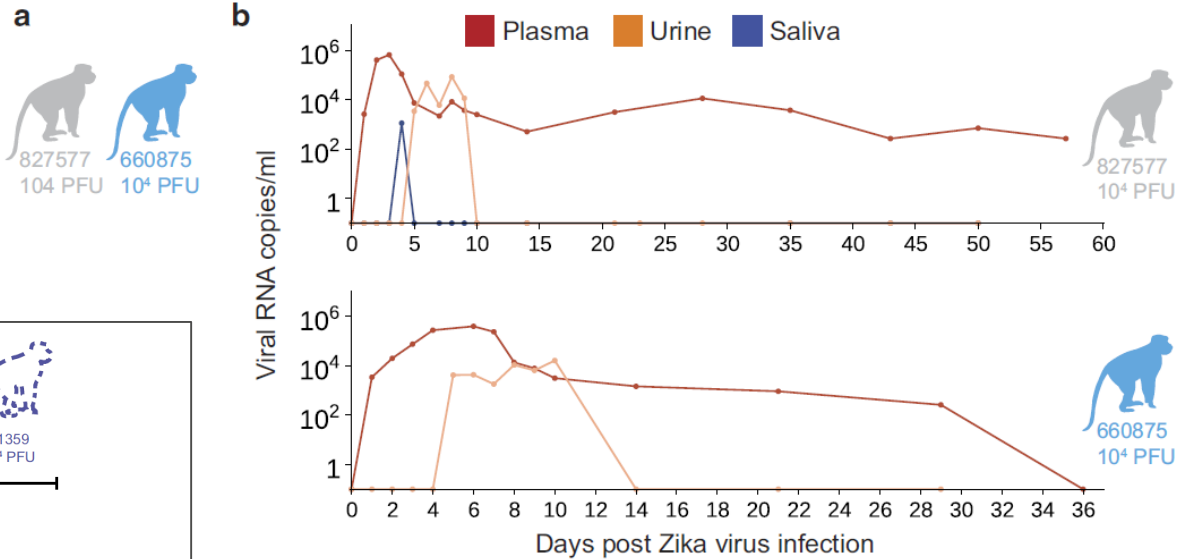
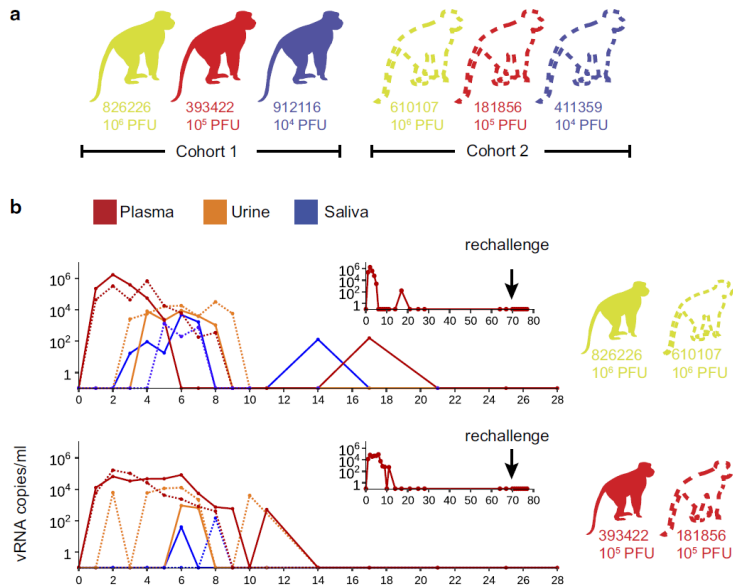


Fig 1. ZIKV infection of 2 adult macaques. a) Virus inoculation and sampling timeline. Two West Nile virus-antibody negative non-pregnant female macaques received an intravenous inoculation of 5.0 log₁₀ PFU of a 2015 Brazilian ZIKV strain on day 0. Signs of clinical disease were recorded twice daily. Animals were anesthetized daily from 1 to 8 then on 10, 12 and 14 dpi for blood collection and euthanized for tissue collection

- Similar inoculation doses, different inoculation mode, shorter duration of viremia:
- intravenous inoculation likely resulted in antigen presentation to many lymph nodes simultaneously, possibly promoting faster innate immune responses with clearance of circulating vRNA
 - differences in the 2015 Brazil and 2013 French Polynesia ZIKV genomes
 - Intra-animal differences

A rhesus macaque model of Asian-lineage Zika virus infection

Dudley DM et al, Nature Communications, 2016



Persistent viremia after **subcutaneous inoculation** in **pregnant** macaques

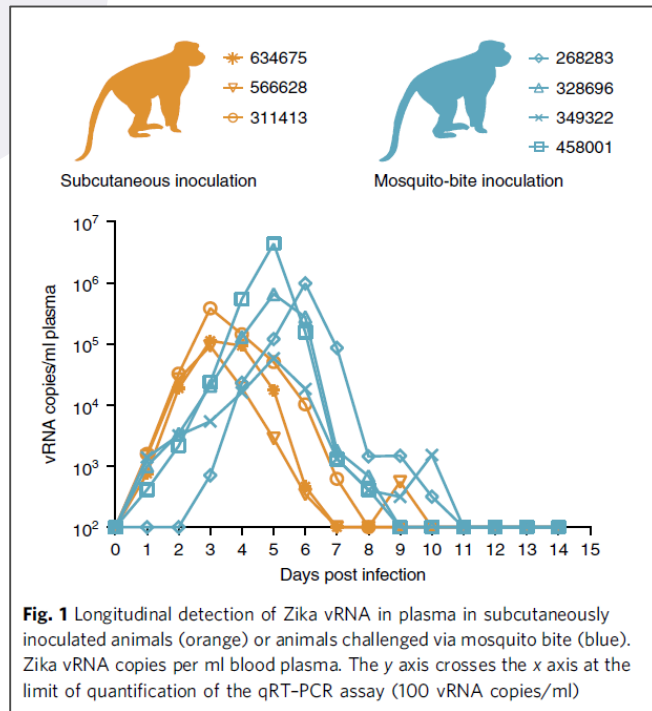
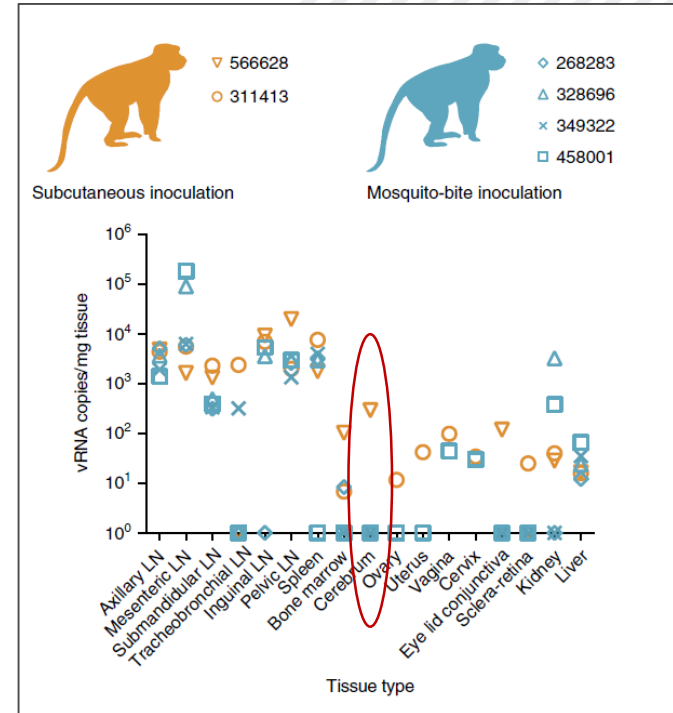
Mosquito bite

- The majority of the inoculum delivered by a mosquito is deposited extravascularly and only a small amount ($\sim 10^2$ PFU)) is deposited intravascularly.
- Throughout this process, a mosquito injects saliva into the host.
- The saliva of hematophages, including mosquitoes, is a cocktail of potent components that prevents clotting and causes vasodilation, as well as alters the inflammatory and immune response, to help facilitate blood feeding



Infection via mosquito bite alters Zika virus tissue tropism and replication kinetics in rhesus macaques

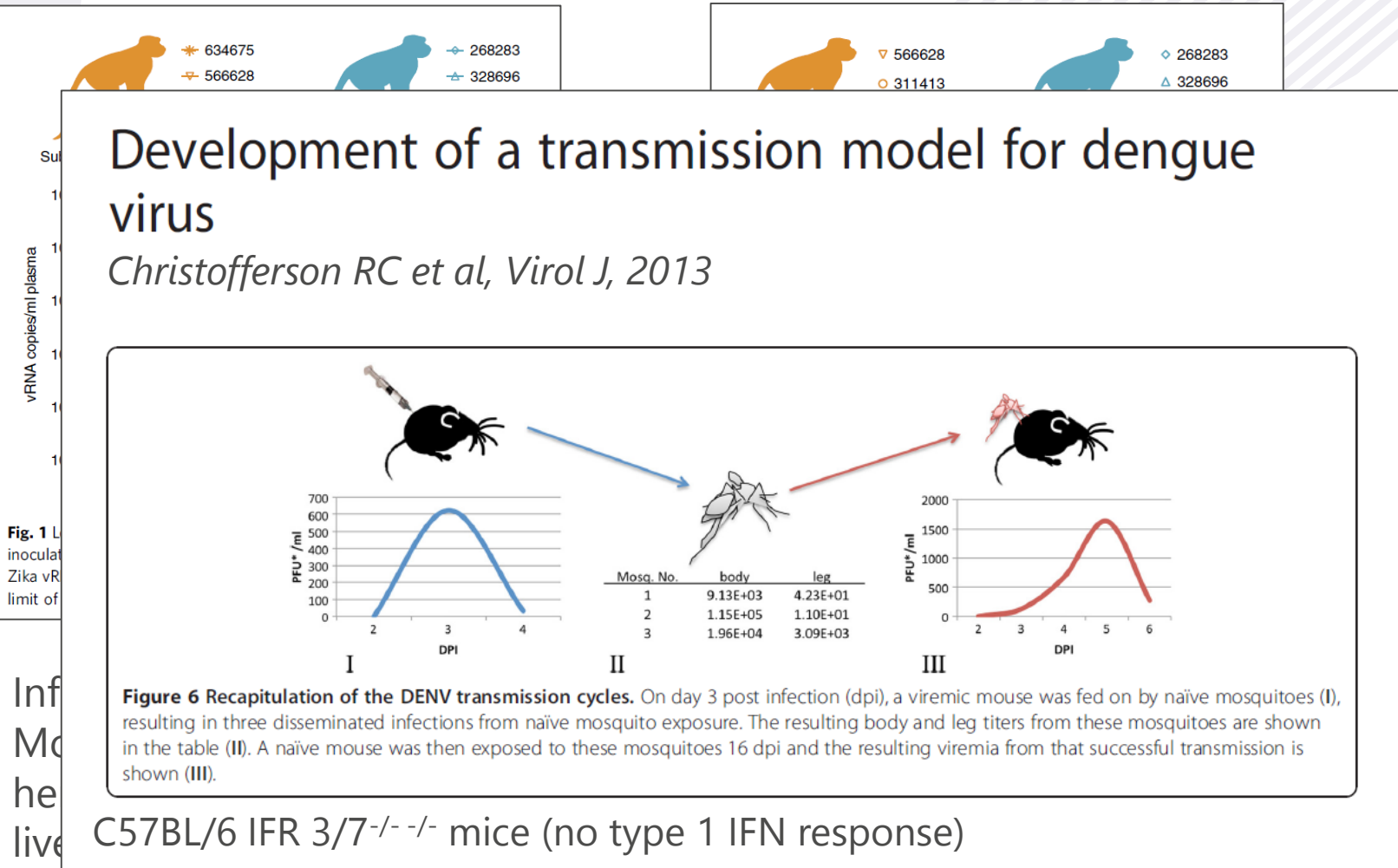
Dudley DM et al, *Nature communications*, 2017



- Infection via mosquito bite delays ZIKV replication to peak viral loads
- Mosquito-infected animals ZIKV tissue distribution was limited to hemolymphatic tissues, female reproductive tract tissues, kidney, and liver

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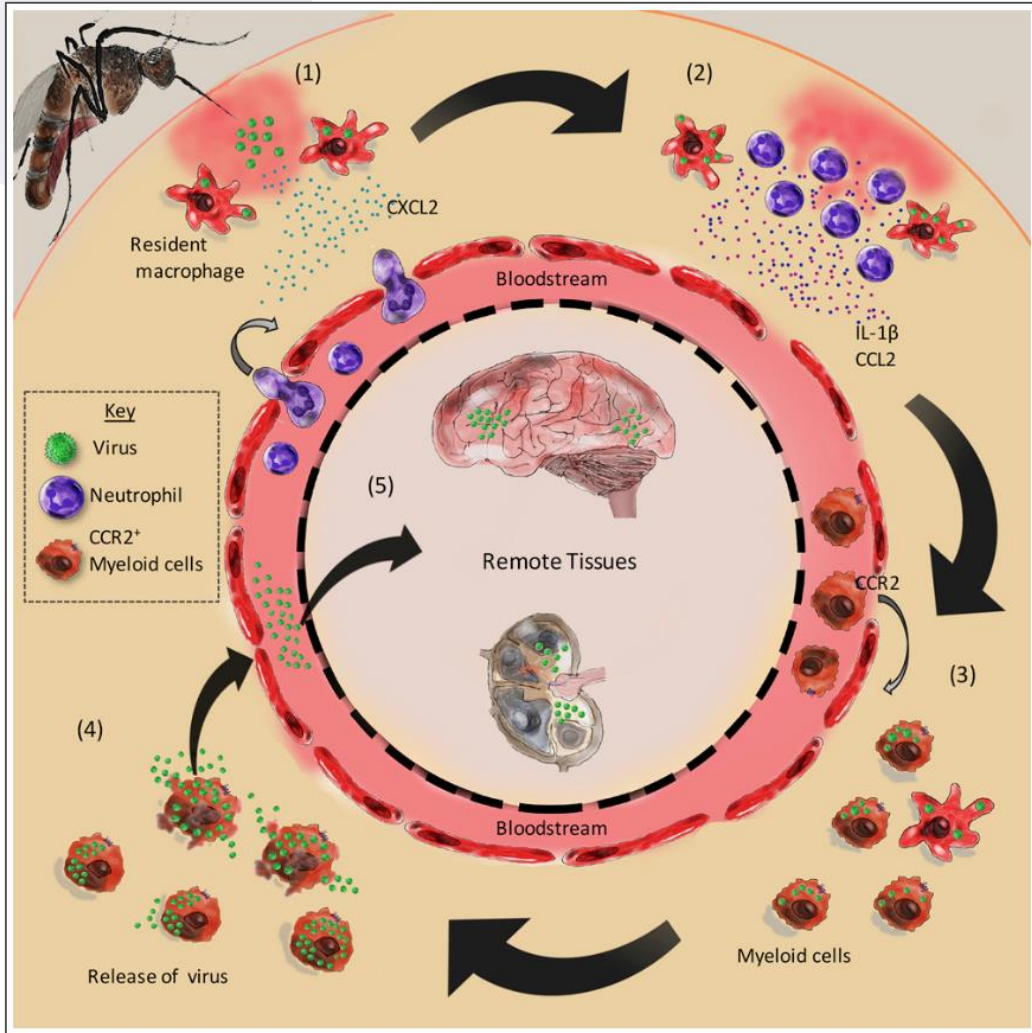
Dudley DM et al, *Nature communications*, 2017



C57BL/6 IFR 3/7^{-/-} mice (no type 1 IFN response)

Host Inflammatory Response to Mosquito Bites Enhances the Severity of Arbovirus Infection

Pingen M et al, *Immunity*, 2016



- Mosquito bites enhance virus replication and dissemination and increase host mortality
- Neutrophil-driven inflammation retains virus in skin to drive macrophage recruitment
- Recruited and resident myeloid cells become infected and replicate virus
- Blocking leukocyte recruitment to bite site inhibits viral infection

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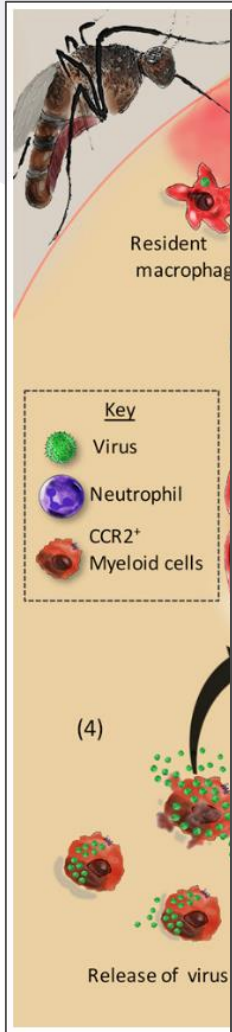
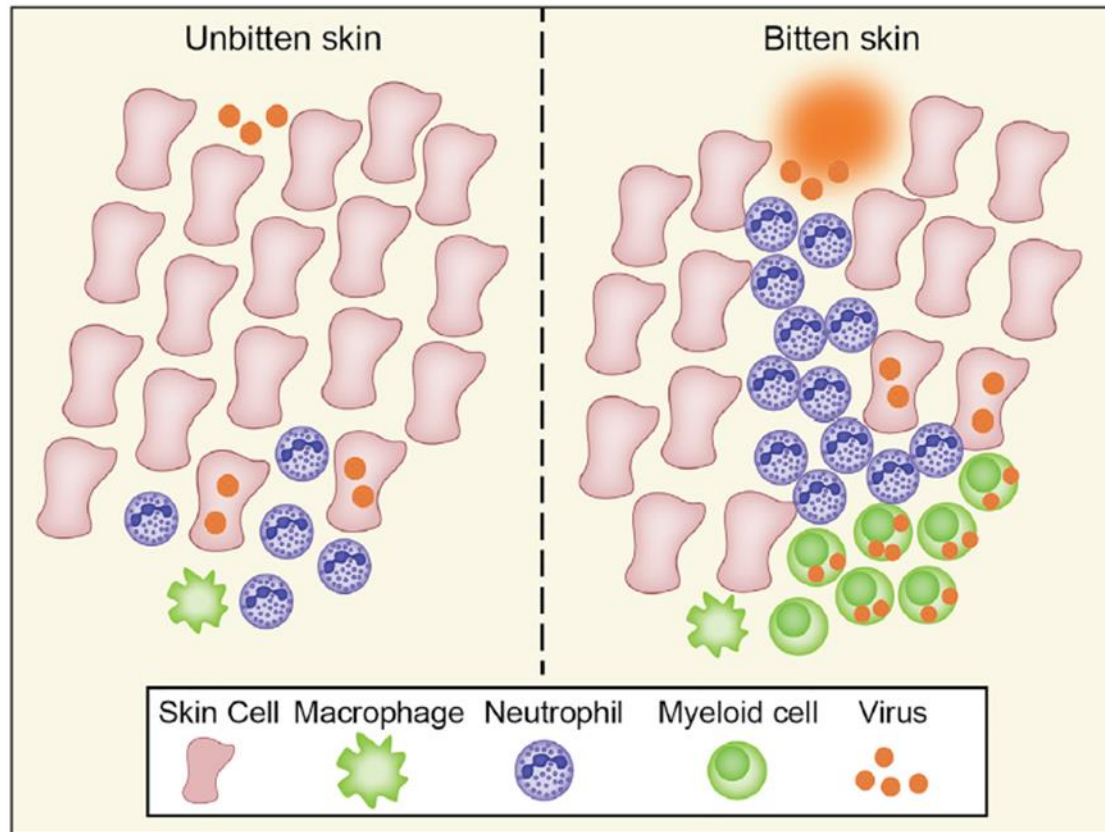


Figure 1. Insect Bites Encourage Viral Spread

Virus particles in an unbiten inoculation site do not infect a large number of macrophages, resulting in minimal viral spread (left). When virus is inoculated into a bite site, a larger number of neutrophils are attracted to the site by the bite. These neutrophils secrete cytokines, which in turn attract myeloid cells to the bite site. These myeloid cells become infected with the virus, enter the blood stream, and spread the pathogen throughout the body.

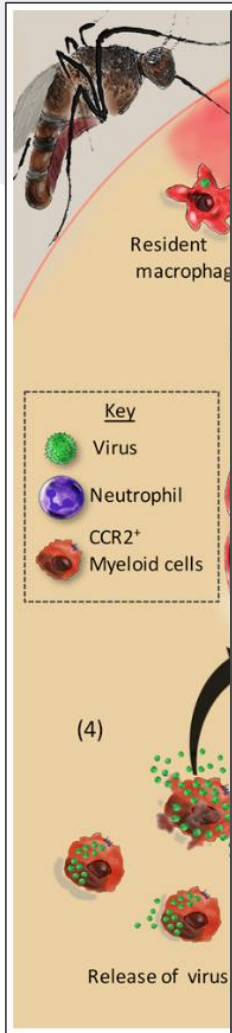
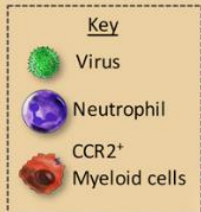
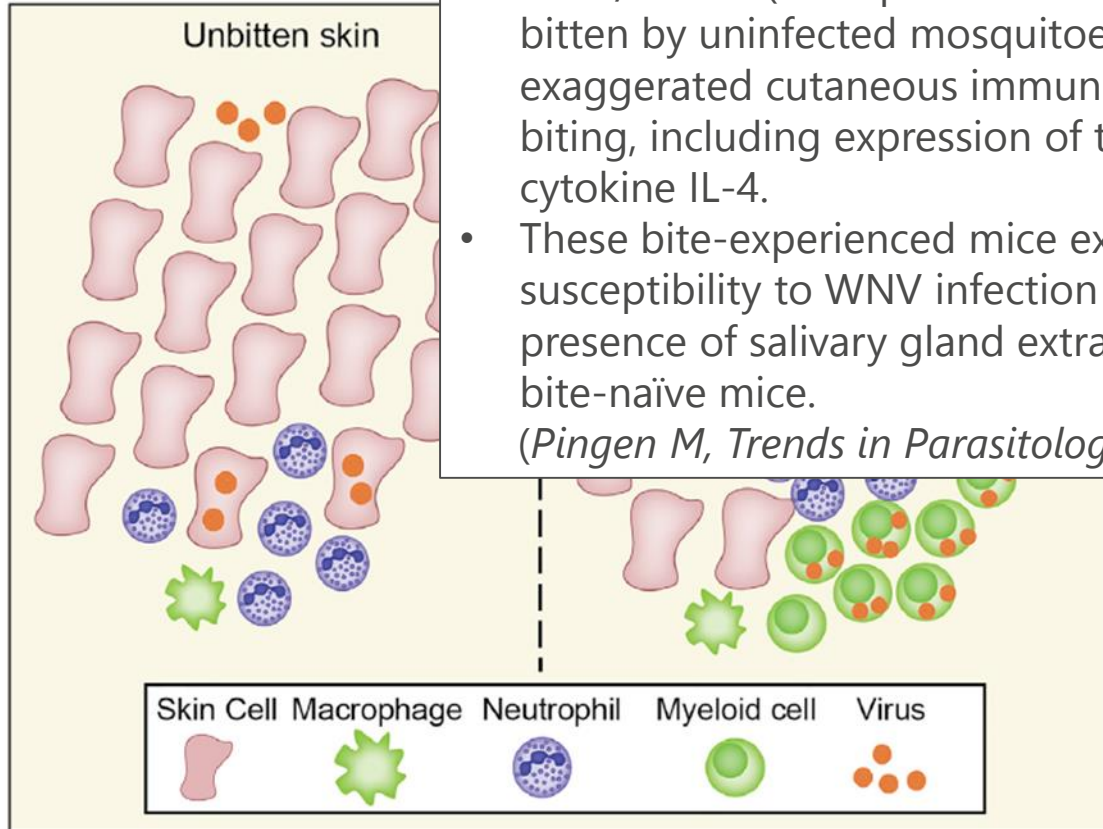
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Pingen M et al, *Immunity*, 2016



Effect of pre-existing immunity to vector saliva

- BALB/c mice (susceptible to allergy) when repeatedly bitten by uninfected mosquitoes, demonstrated exaggerated cutaneous immune responses to further biting, including expression of the Th2-associated cytokine IL-4.
- These bite-experienced mice exhibit increased susceptibility to WNV infection when inoculated in the presence of salivary gland extract (SGE) as compared to bite-naïve mice.

(Pingen M, *Trends in Parasitology*, 2017)

leukocyte recruitment to the bite site enhances viral infection

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Virus particles in an unbidden inoculation site do not infect a large number of macrophages, resulting in minimal viral spread (left). When virus is inoculated into a bite site, a larger number of neutrophils are attracted to the site by the bite. These neutrophils secrete cytokines, which in turn attract myeloid cells to the bite site. These myeloid cells become infected with the virus, enter the blood stream, and spread the pathogen throughout the body.

Schneider DS, *Immunity*, 2016

Next steps:

- Pathogenicity (vs infectivity) of blood product-driven arbovirus transmission
- Direct comparison of mosquito bite-driven vs i.v. blood product – driven arbovirus infection and pathogenicity

Next steps:

- Pathogenicity (vs infectivity) of blood product-driven arbovirus

Overall,

- Arboviruses can result in transfusion-transmitted (TT) infection and disease
- Severity of TT arboviral infection is variable
 - From important (WNV infection in immunosuppressed patients) to perhaps close to null (Chikungunya?);
 - Important unknowns persists, including most probably unknown unknowns.
- Apparently, no direct and proportionate relation between circulating arbovirus in the donor and disease in the recipient
- Understanding TT arbovirus infectivity and pathogenesis is crucial with regard to transfusion risk mitigation measures
 - to guide research and development
 - to inform decisions-making
 - to assist implementation
 - to assess efficacy and cost-effectiveness

Next steps:

- Pathogenicity (vs. non-pathogenicity)
- Overall,
 - Arboviruses cause disease
 - Severity of TT
 - From important to perhaps
 - Important unknowns
- Apparently, no arbovirus in the blood supply
- Understanding with regard to
 - to guide research
 - to inform policy
 - to assist in decision-making
 - to assess risk

Methodologies to assess transfusion-transmission risk

Busch et al, Blood, 2019; Lanteri et al Transfusion 2016

