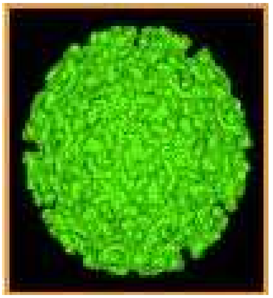
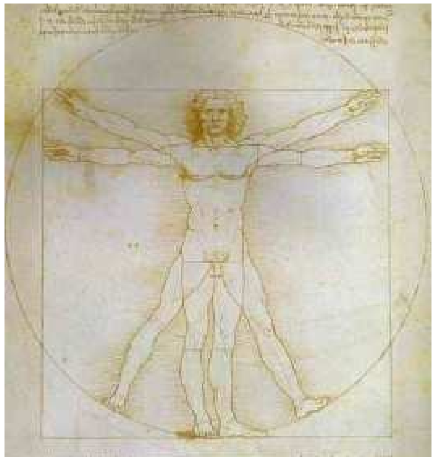
DENGUE

An epidemiological view by a malariologist

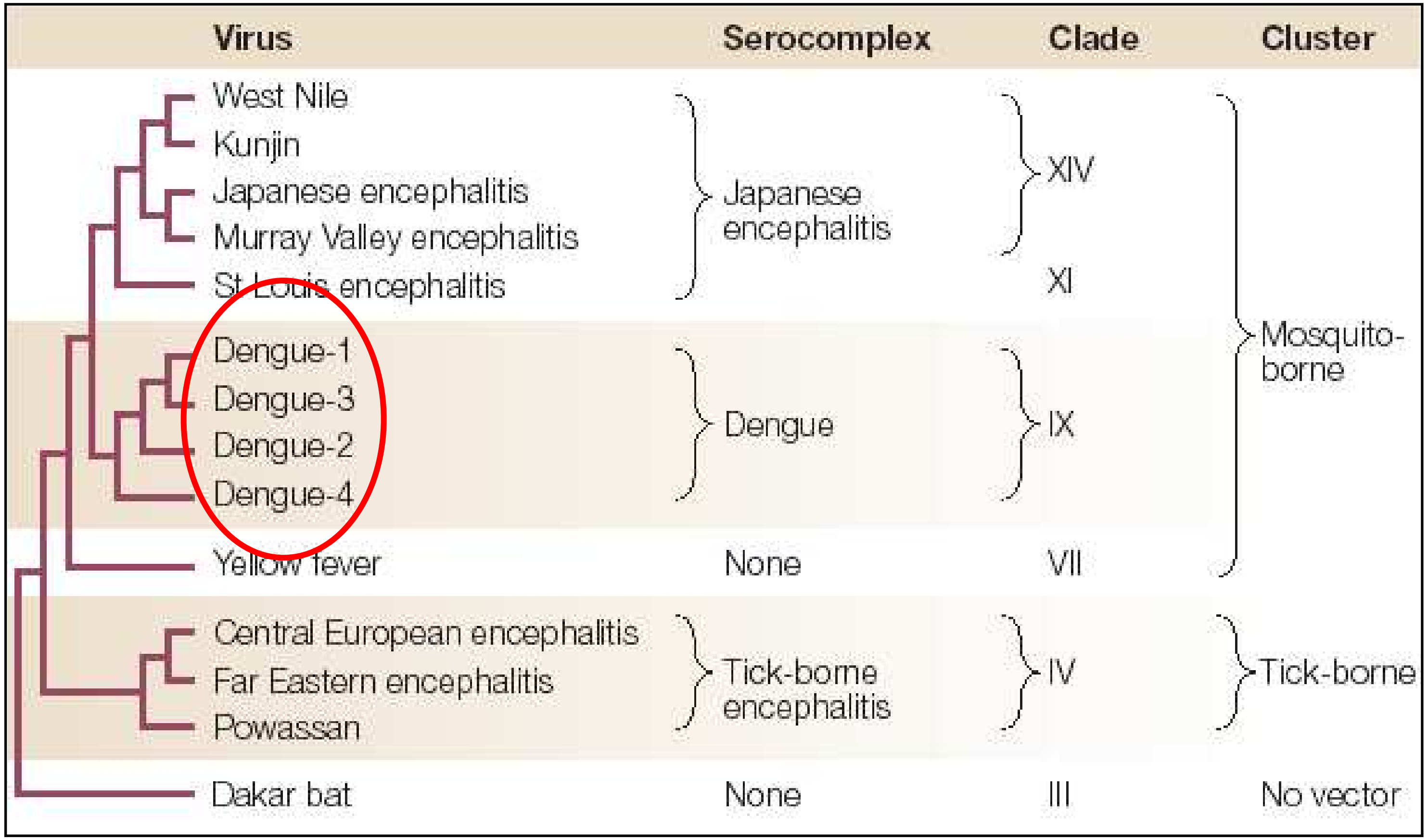




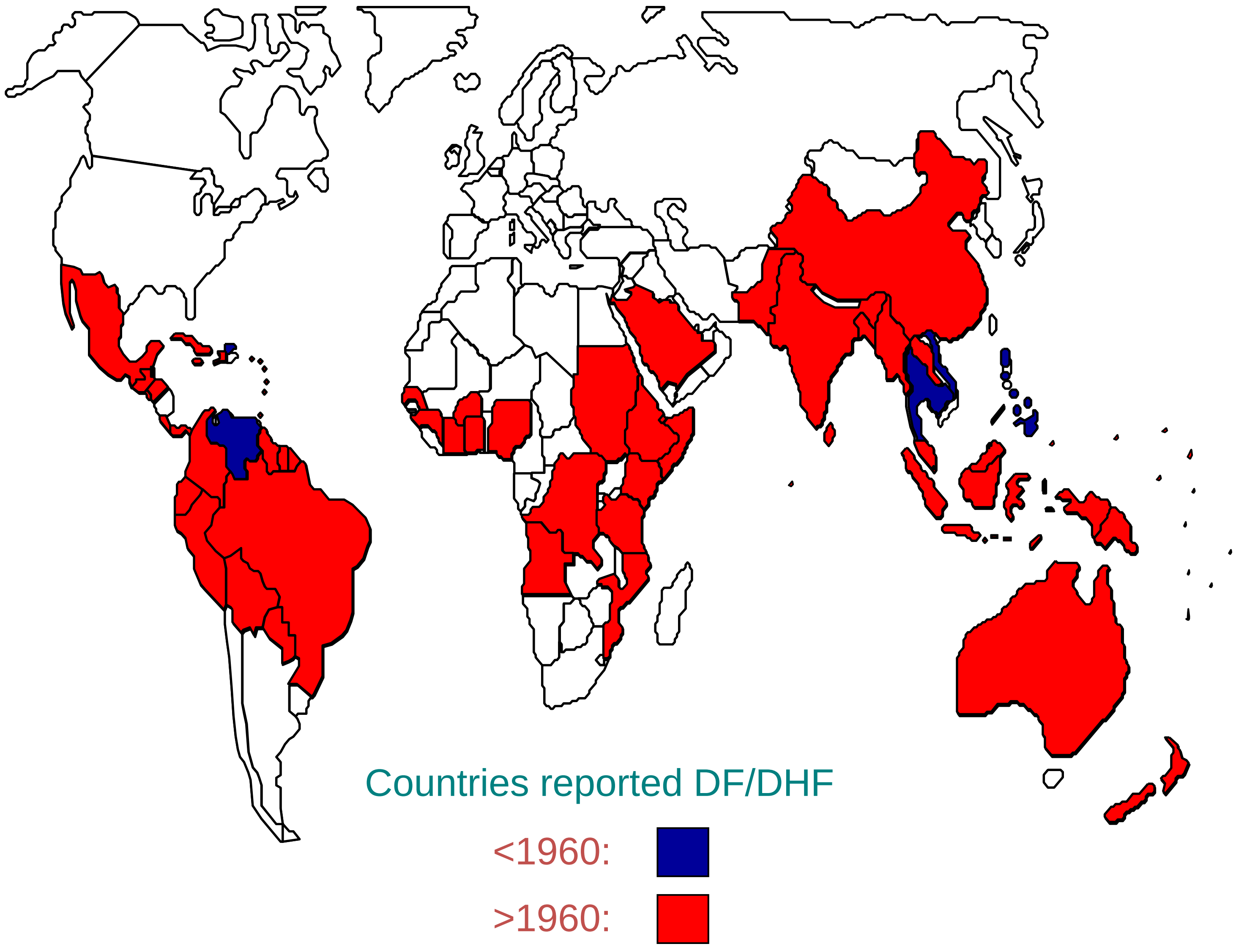
Rick Paul

Institut Pasteur

FLAVIVIRIDAE

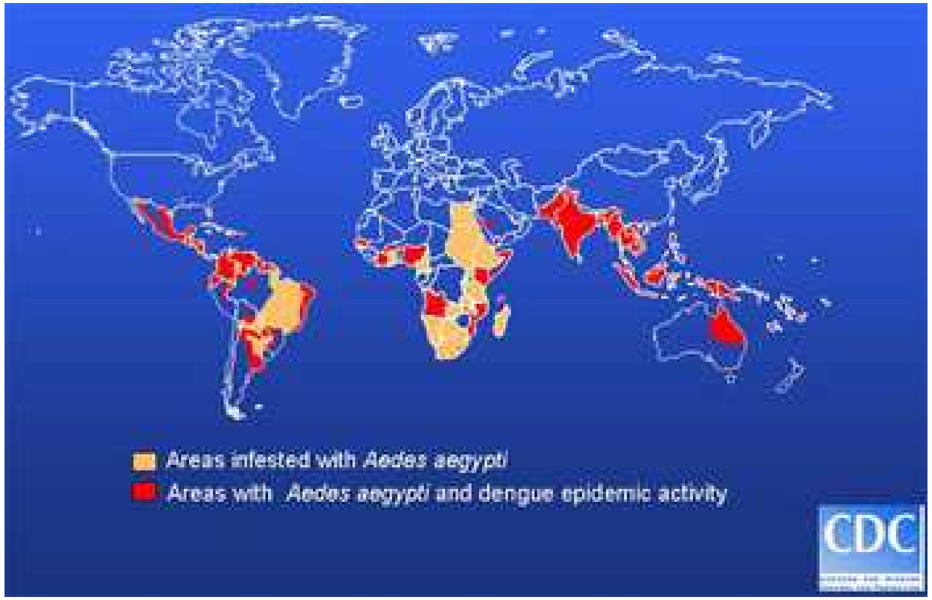


Emergence of Dengue Disease World-wide



Dengue – vectored by 2 mosquito spp.

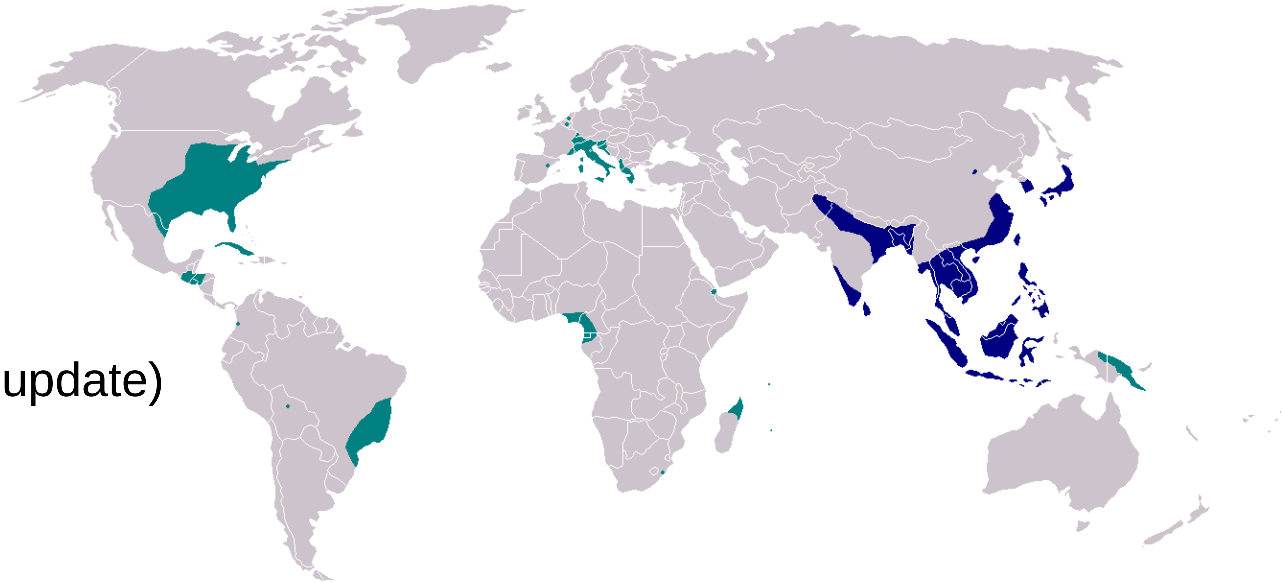
Aedes aegypti +/- dengue



Aedes aegypti & *Ae. albopictus*

Aedes albopictus

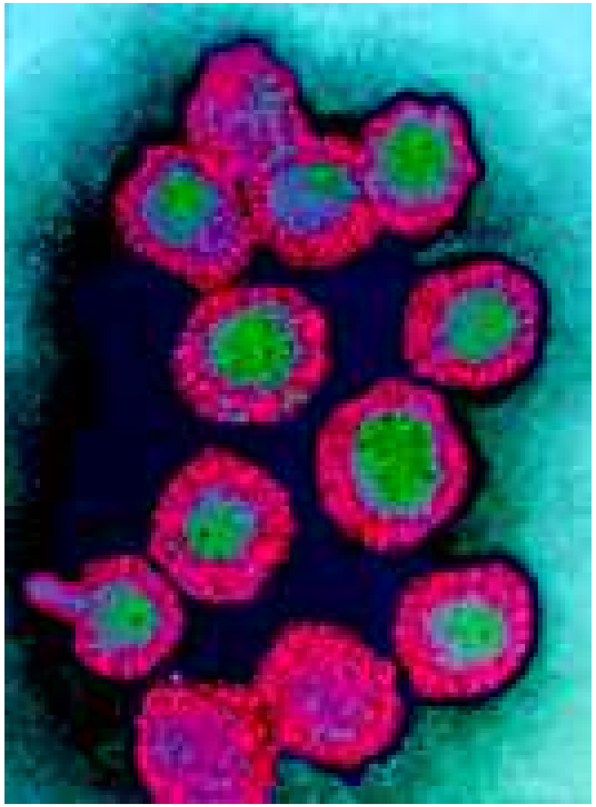
Blue –original distribution
Green – introduced (2007 update)



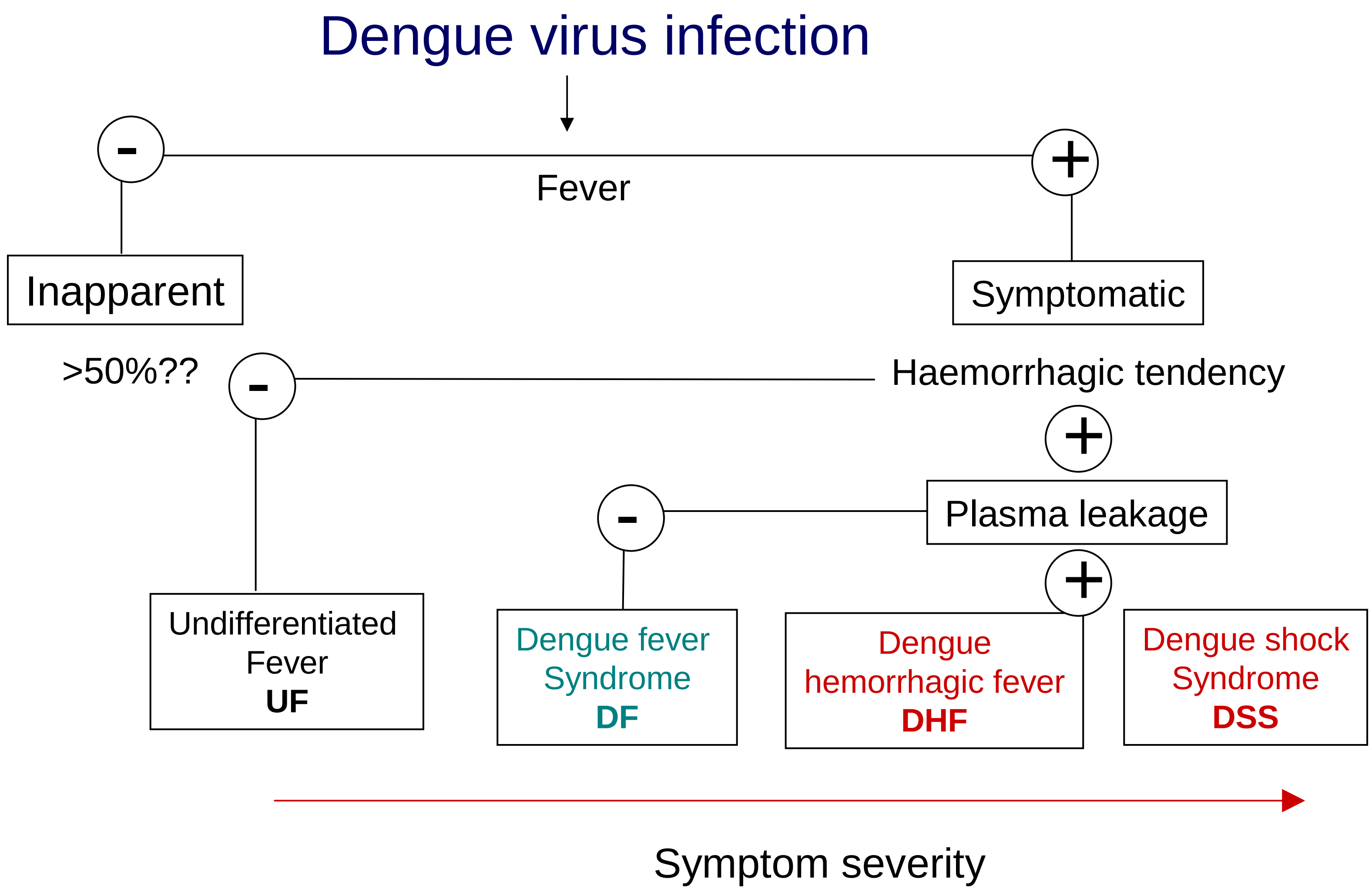
Morbidity & Mortality

~100 million cases » ~25,000 deaths

- Fever develops in only a small proportion (<1-20%?) of exposed individuals.
- Disease progresses to life threatening levels in approximately 200,000 to 500,000 annually.
- Currently it is unclear what factors lead to the development of severe disease.
  - Antibody-dependent enhancement
  - Cytokine/T-cell activation
  - Genetic susceptibility/resistance
  - Viral genotype

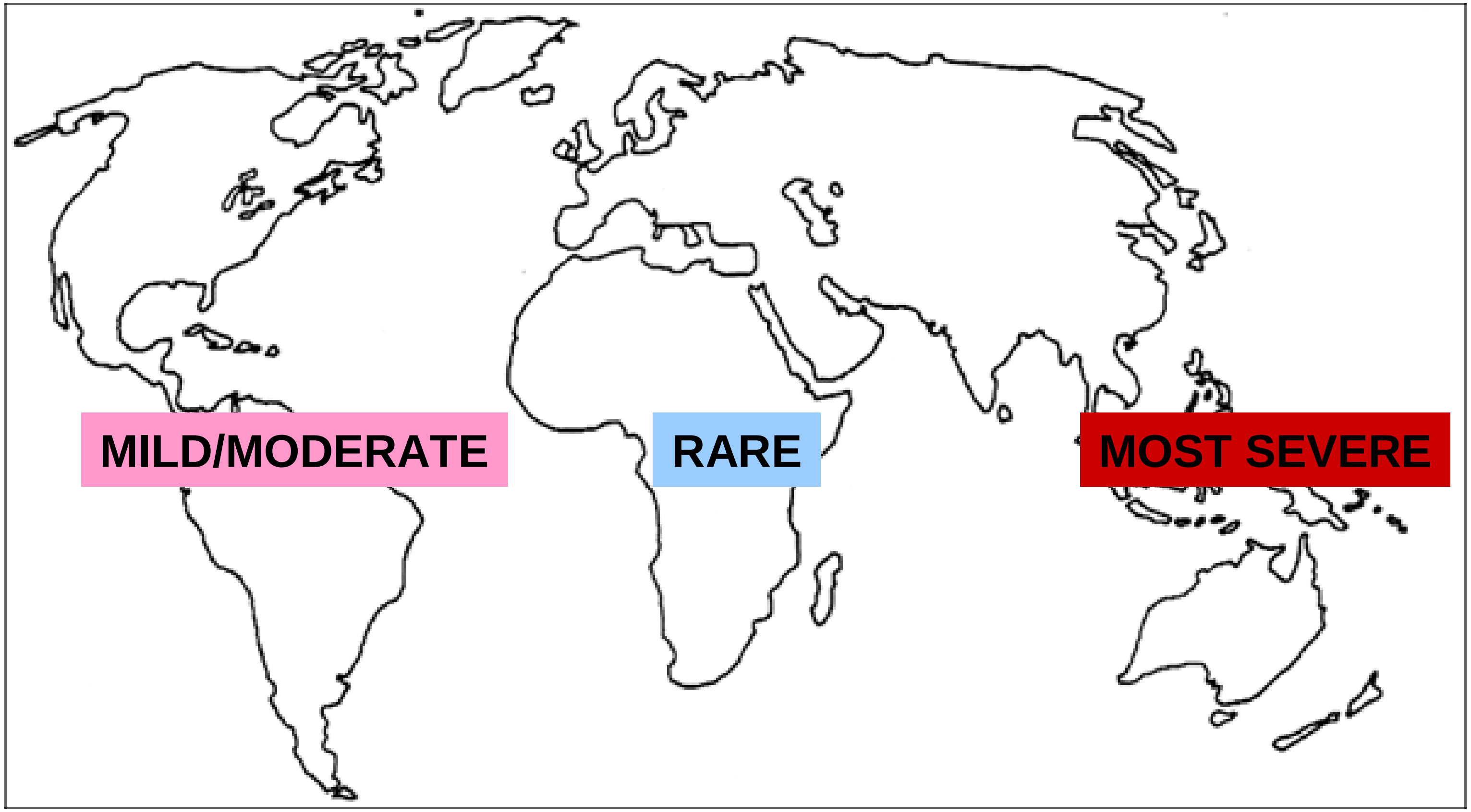


Manifestations of the dengue syndrome



Rare manifestations:Encephalitis, Hepatitis

World wide dengue disease severity difference



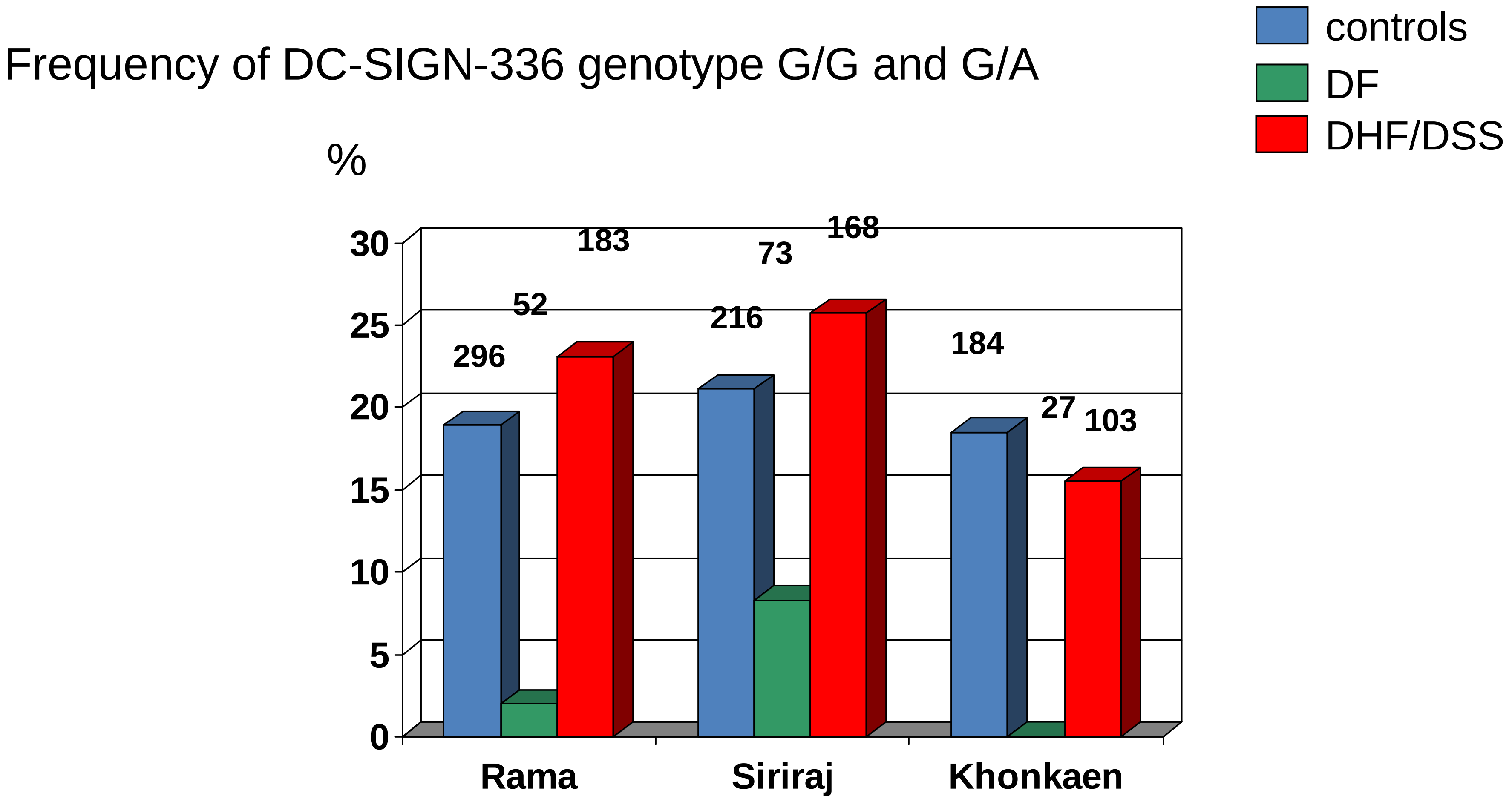
Human genetics ? But becoming more severe in Latin America……….

Human genetic factors in severity of dengue disease

Dengue viruses cause clinical manifestations in only a small percentage of infected individuals

- Caucasian> African/ Chinese > Malaysian
- HLA-A and B association study
- Others studies
  - FCGR IIA
  - TNFα
  - MIC A & B

DC-SIGN-336 association study

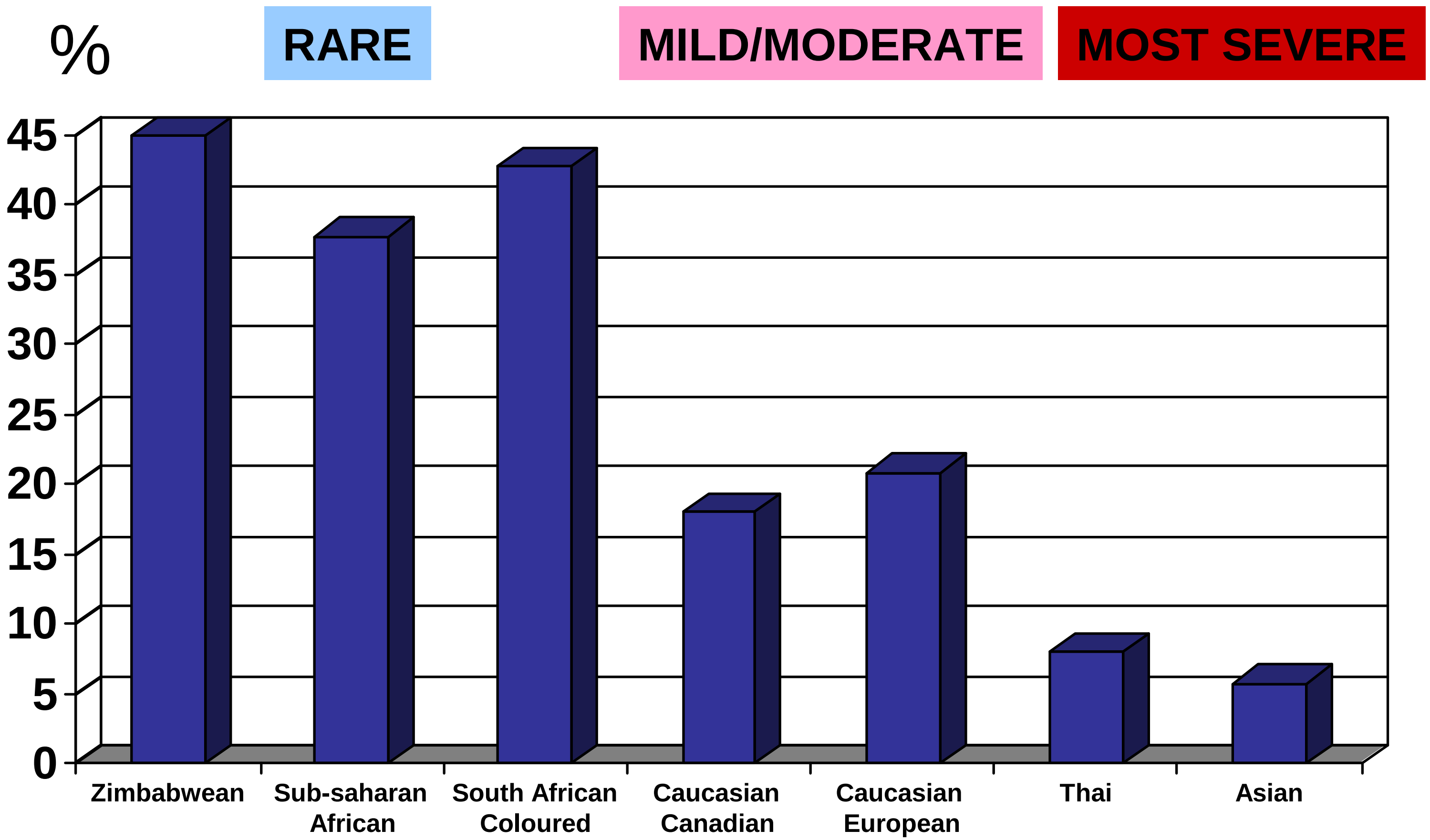


DF vs DHF/DSS

	Rama	Siriraj	Khonkaen
OR (95% CI)	14.31 (3.34-61.23)	3.79 (1.62-8.87)	99.75 (12.70-783.54)
P- value	**2.3×10^{-4}**	**0.0024**	**0.037**

Sakuntabhai, et al.
Nat Genet 2005

Allelic distribution of DC-SIGN-336G in different populations



DC-SIGN-336G -DF protective allele

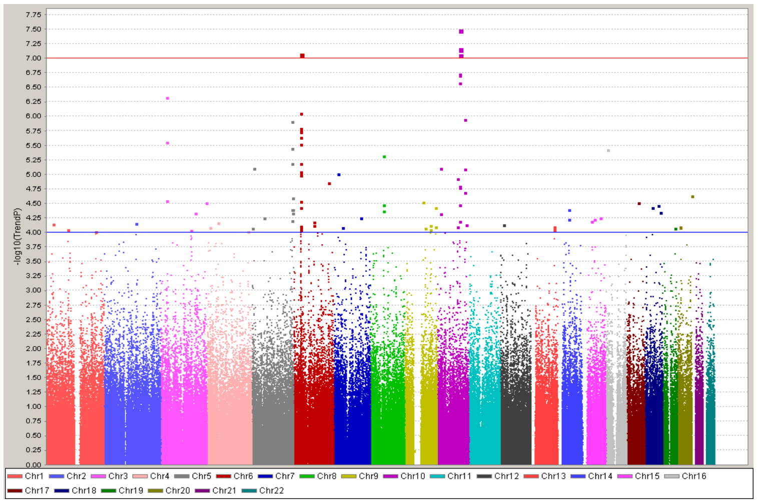
Boily-Larouche et al. 2007

GWAS OF DENGUE SHOCK SYNDROME

Genome-wide association study identifies susceptibility loci for Dengue shock syndrome at *MICB* and *PLCE1*.

Lead SNP *P* = 3.29 x 10-8

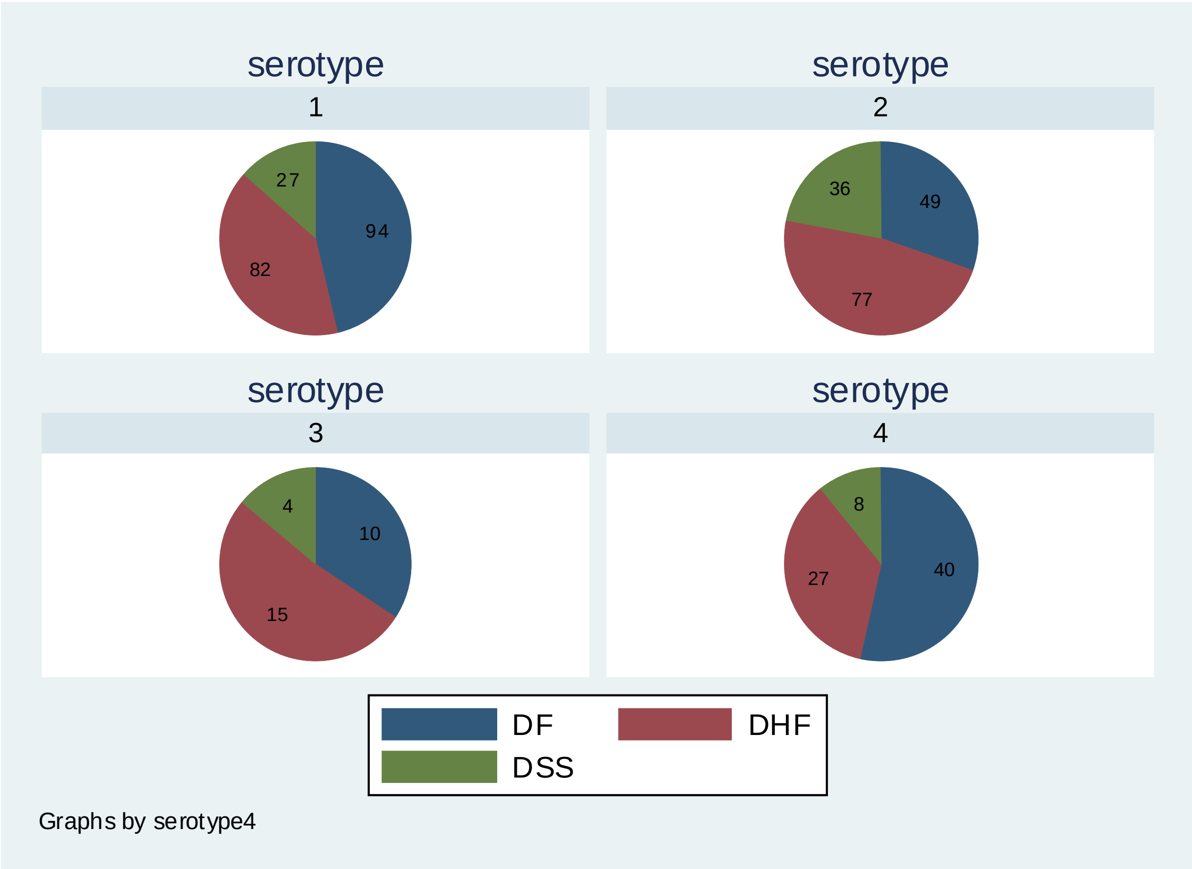
Lead SNP *P* = 8.50 x 10-8



Khor et al., *Nat Genet* 2011

The data suggests that **MICB** is an important determinant in **early NK and $CD8^+$ T cell mediated immune control of dengue virus infection** and **PLCE1** a factor in **vascular endothelial dysfunction and circulatory hypovolemia**.

Severity by serotype?

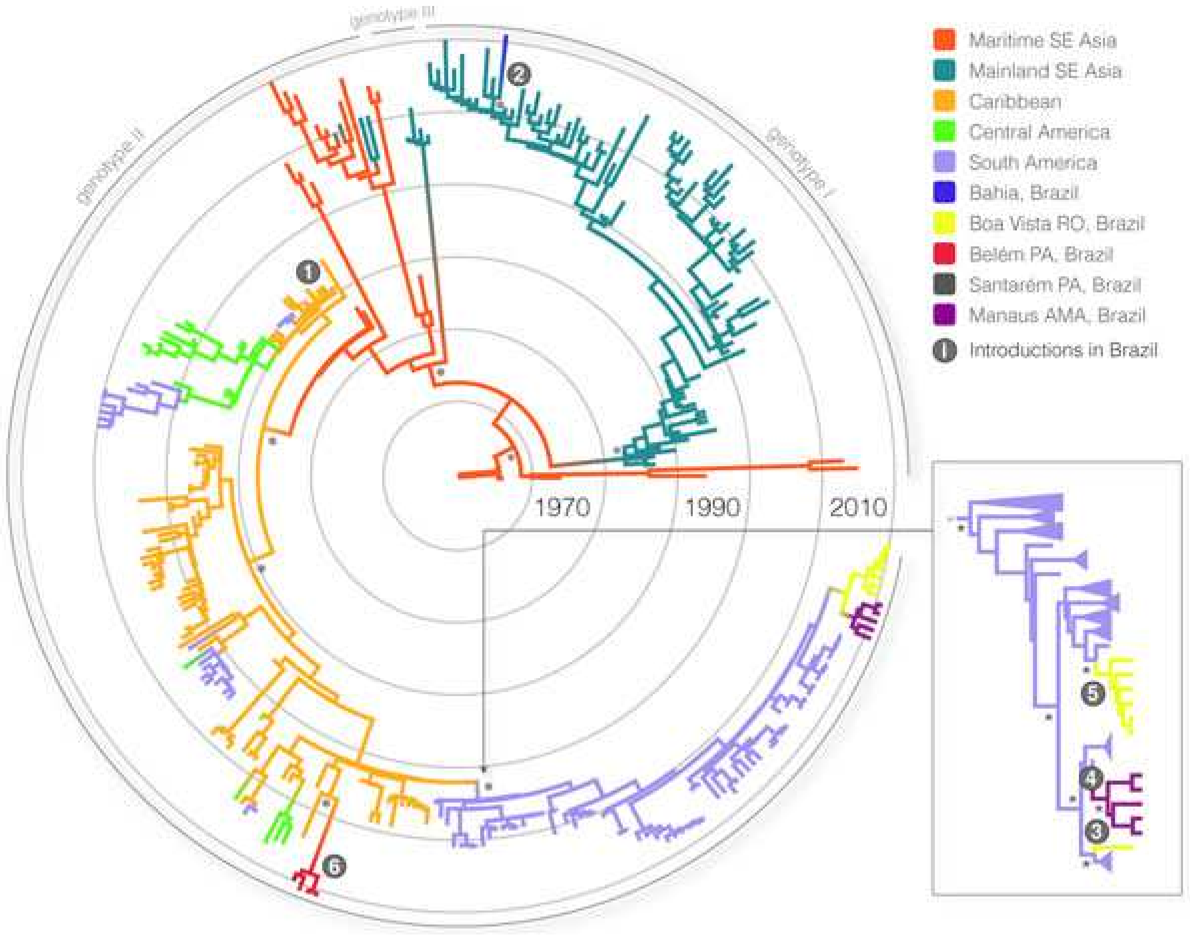


Established second infection sequences leading to DHF

- **2 – 1 Thailand; Indonesia**
- **3 – 1 Thailand**
- **1 – 2 Cuba, 1981; Cuba 1997; Thailand**
- **3 – 2 Thailand**
- **4 – 2 Thailand**
- **1 – 3 Cuba, 2001; Thailand; Indonesia**
- **2 – 3 Thailand, DF in Cuba**
- **1 – 4 Thailand**
- **2 – 4 Indonesia**
- **3 – 4 Thailand**
- **4 – 1**
- **4 – 3**

Source: On-line ppt SB Halstead

Importance of genotypes within 4 serotypes?



Focus has been on dengue pathogenesis and epidemiology of severe dengue

………and somewhat anecdotal

Need to focus on dengue epidemiology

- The key determinant of incidence and prevalence of infection is the basic reproductive number $\mathbf{R_o}$.

- $\mathbf{R_o}$ measures the average number of secondary cases generated by one primary case in a susceptible population

- Many factors determine its magnitude, including those that influence the typical course of infection in the patient and those that determine transmission between people.

Basic (Ross-MacDonald) model for malaria (and dengue)

$$R_0 = \frac{ma^2bc}{\mu\gamma}\exp(-\mu\tau)$$

m = density of mosquitoes per human

a = biting rate

b = probab. successful infections in human from an infectious bite

c = probab. successful infections in mosquito after biting infected human

μ= mortality rate of mosquitoes

γ = recovery rate in human

τ = external incubation period (time for mosquito to become infectious) ~10days

But see Massad & Coutinho (2012) Mem Inst Oswaldo Cruz for refinement

Mosquito parameters

Biting rate: classically a female mosquito will bite twice after eclosion prior to first egg batch and then every 2-3 days (gonotrophic cycle)

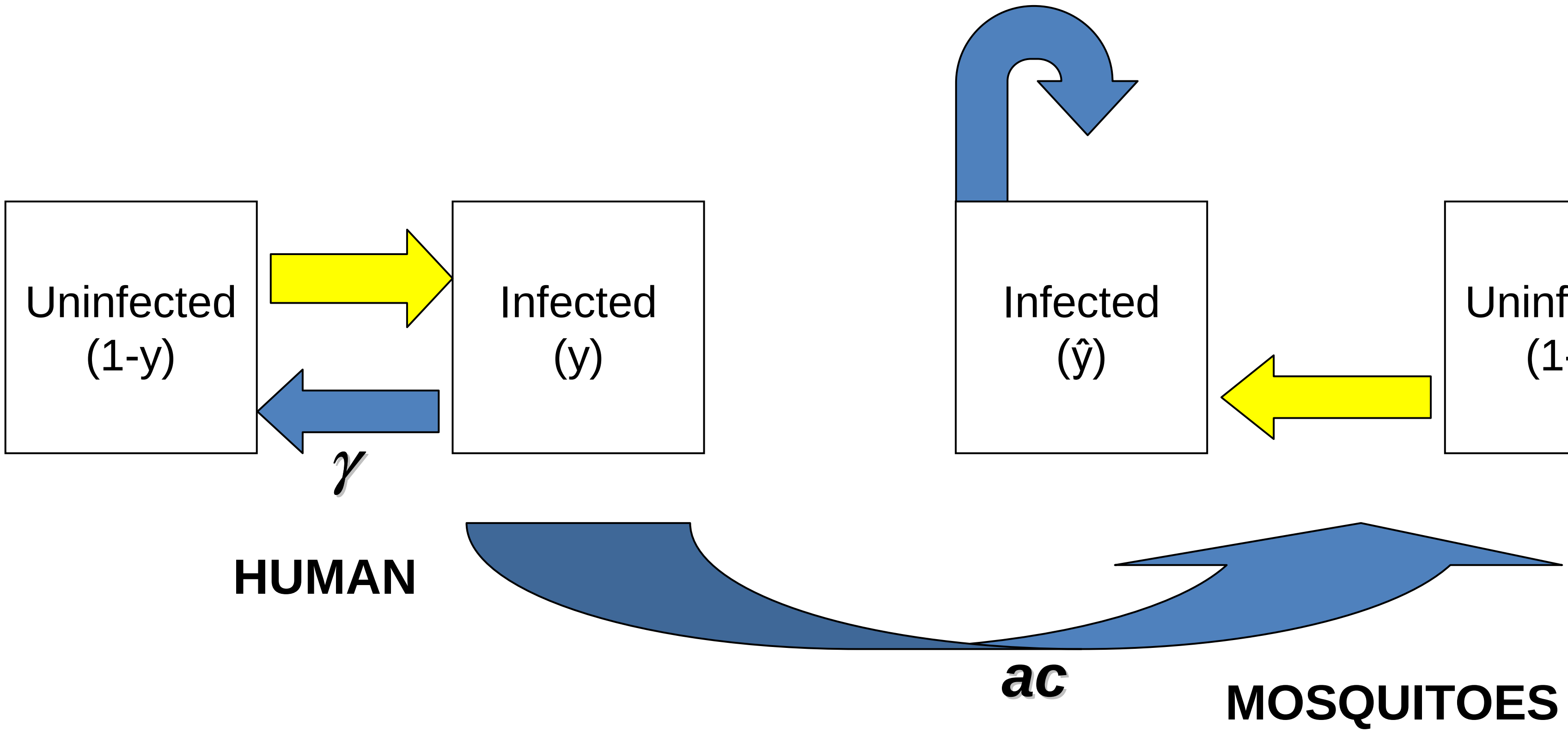
Aedes aegypti feeds more frequently

Mortality: classically considered linear but not so.
Calculated using marking or parity (stretch marks)

Dispersal: Low (<100m) but documented up to 500m
Likely variable under rural/urban conditions
Likely under natural selection

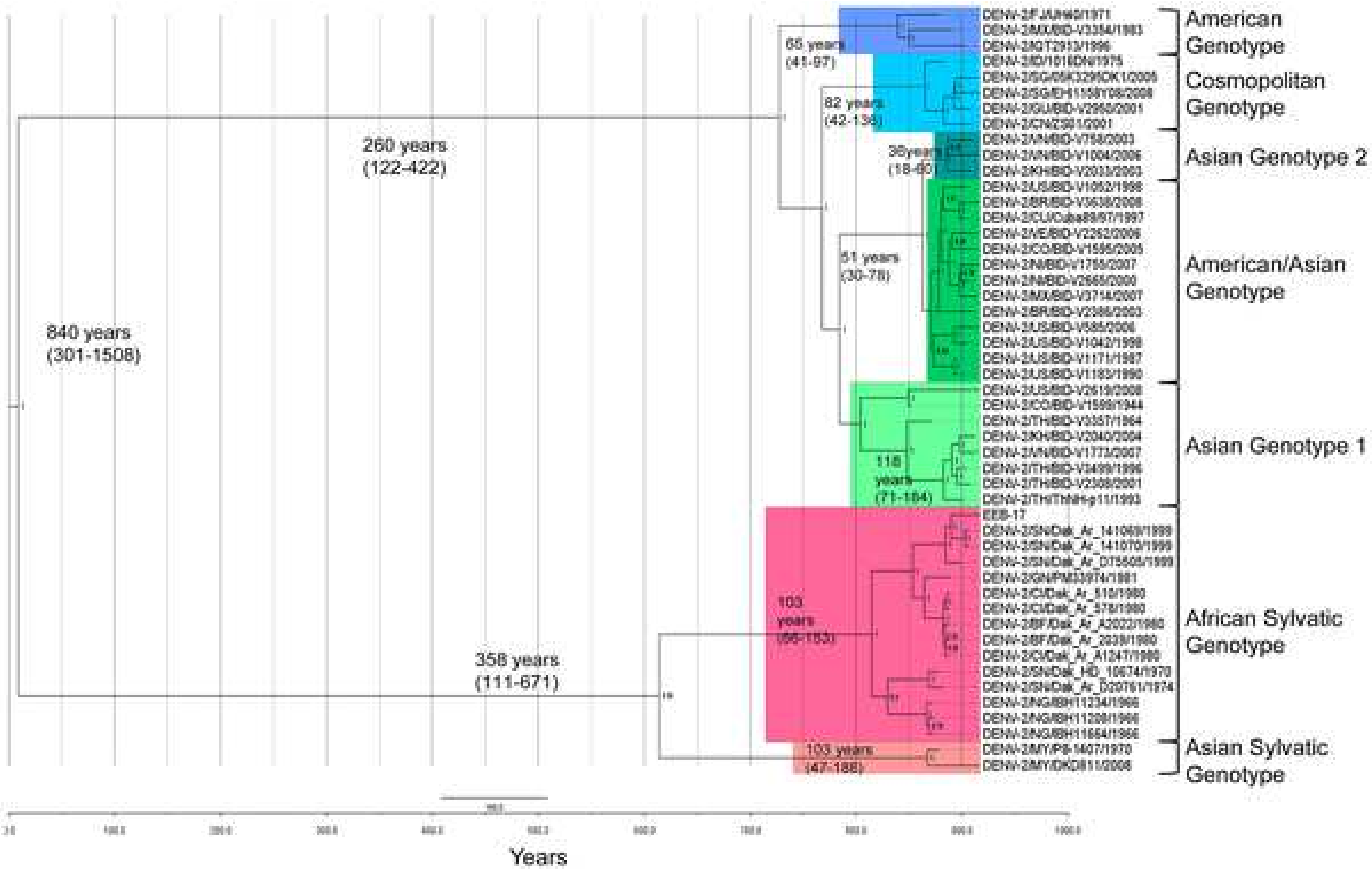
Source of infection in mosquitoes - Vertical transmission

(of uncertain importance)

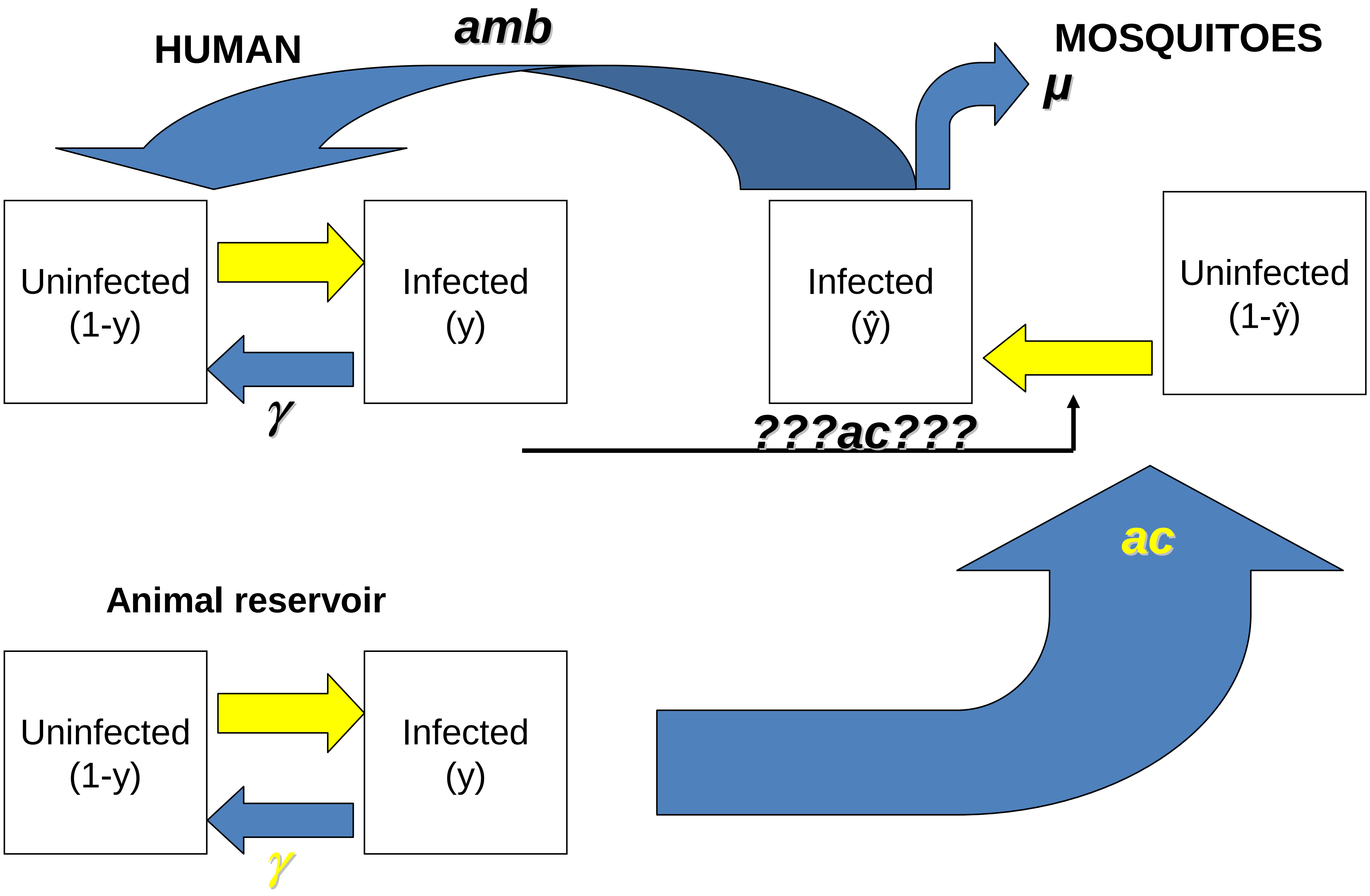


Rosen L. Am J Trop Med Hyg. 1988

Anthropozoonoses – what of Sylvatic Dengue?



Franco L et al. (2011) First Report of Sylvatic DENV-2-Associated Dengue Hemorrhagic Fever in West Africa. PLoS Negl Trop Dis


HUMAN
amb
MOSQUITOES
μ
Uninfected
(1-y)
Infected
(y)
Infected
(ŷ)
Uninfected
(1-ŷ)
γ
???ac???
ac
Animal reservoir
Uninfected
(1-y)
Infected
(y)
γ

Impact of multiple mosquito vector species

(& populations within a species)

Mosquito density, m, biting rate, a and mortality, μ differ

- among species
- among populations of same species (degree of anthropophagy, exophily etc)

Calculate the mean a1/ μ1 + a2/ μ2 weighted by their respective m.

Pertinent for Dengue mosquito vectors?

Aedes aegypti – originating from Africa
has spread globally
« domesticated », anthropophilic, urban
major vector of dengue

Aedes albopictus – originating from SE Asia forests
spreading globally, highly invasive
increasingly domesticated but rural/sub-urban
cold resistant eggs permits greater altitude/longitude range
secondary vector of dengue

Competition between species → competitive exclusion

But they do co-exist: e.g. Brazil, Central Africa

……..and question mark over Sylvatic dengue

R_0 , basic SIR

Force of infection, λ = (abm).ŷ

Mean age of first infection, A $\approx 1/\lambda$

$x_{(age,a)} = e^{-\lambda a}$ (probability stay uninfected at age, *a*, if transmission homogenous)

$$R_0 \approx 1/X \approx \lambda L \approx L/A$$ **(L is human lifespan)**

………….but only applicable when
first infection induces a sterilising immunity

Is immunity lifelong following infection by a serotype?

Classical answer is YES. (my) Current limited literature search has found from Sabin 1952 – Research on Dengue during World War II. AmJTropMedHyg:

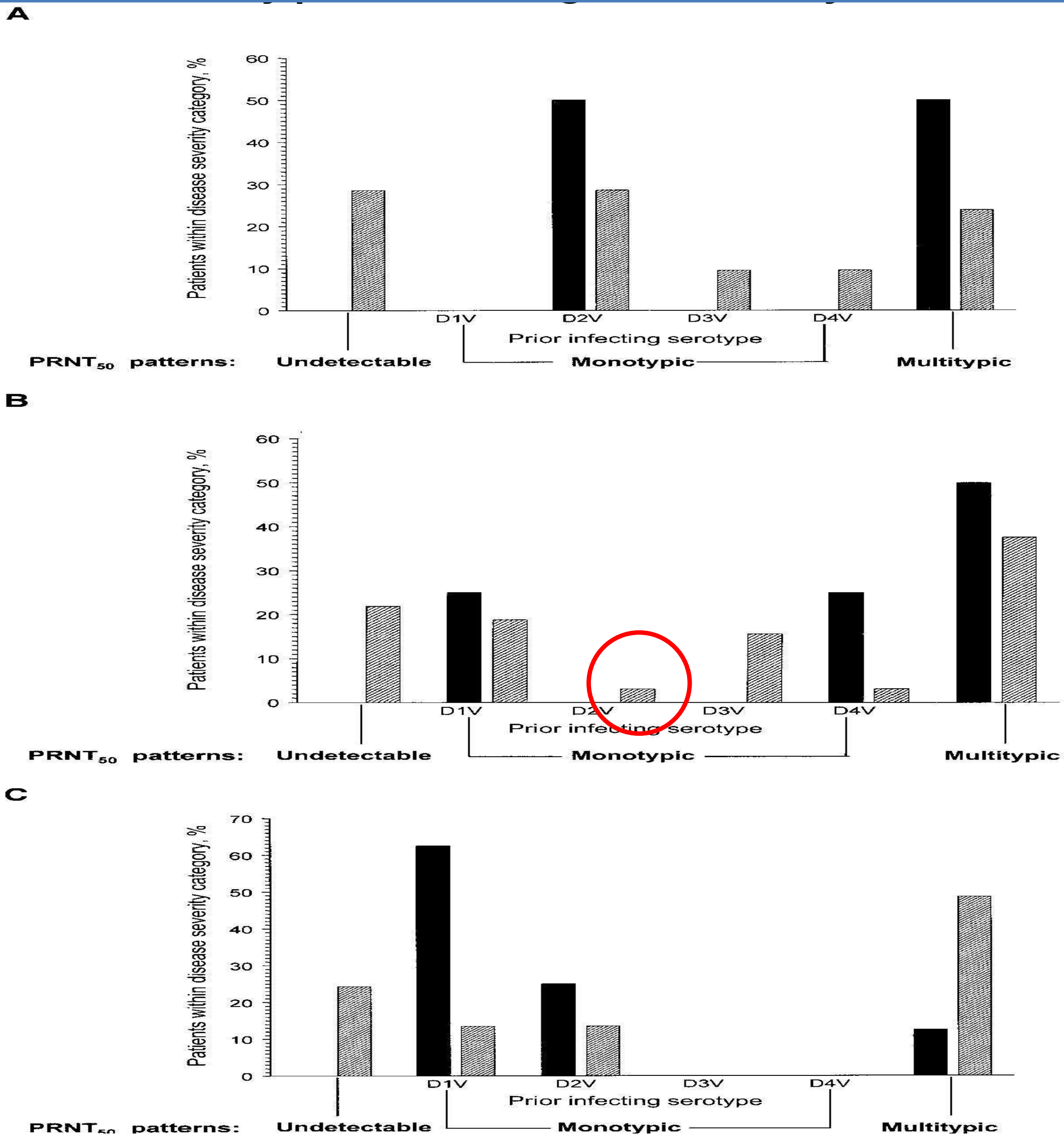
1. Simmons, St. John and Reynolds (Philippine J. Sci. 44: 1-247, 1931) established…. …..(c) the persistence of immunity to the homologous strain of virus for 13 months in human volunteers residing in an endemic region,

2. Human volunteers reinoculated with the same strain of virus proved to be completely immune for as long as 18 months after a single infection

3. The results of reinoculation with a heterologous strain were found to depend on the interval after the original attack. Active immunity to heterologous strains was, as a rule, demonstrable during the first 2 months after an attack.

4. Reinfection with a different immunologic type of dengue virus approximately 2 to 3 months after a primary attack had been found to give rise to malaise and slight fever for less than 24 hours, and mosquitoes which fed on such patients acquired the capacity to transmit the unmodified disease. Group immunity was still evident for as long as 9 months after the primary attack, since volunteers who were then shown to be resistant to the homologous type reacted with a rash-free, febrile illness of 2 to 3 days‘ duration upon inoculation with a heterologous type of dengue virus.

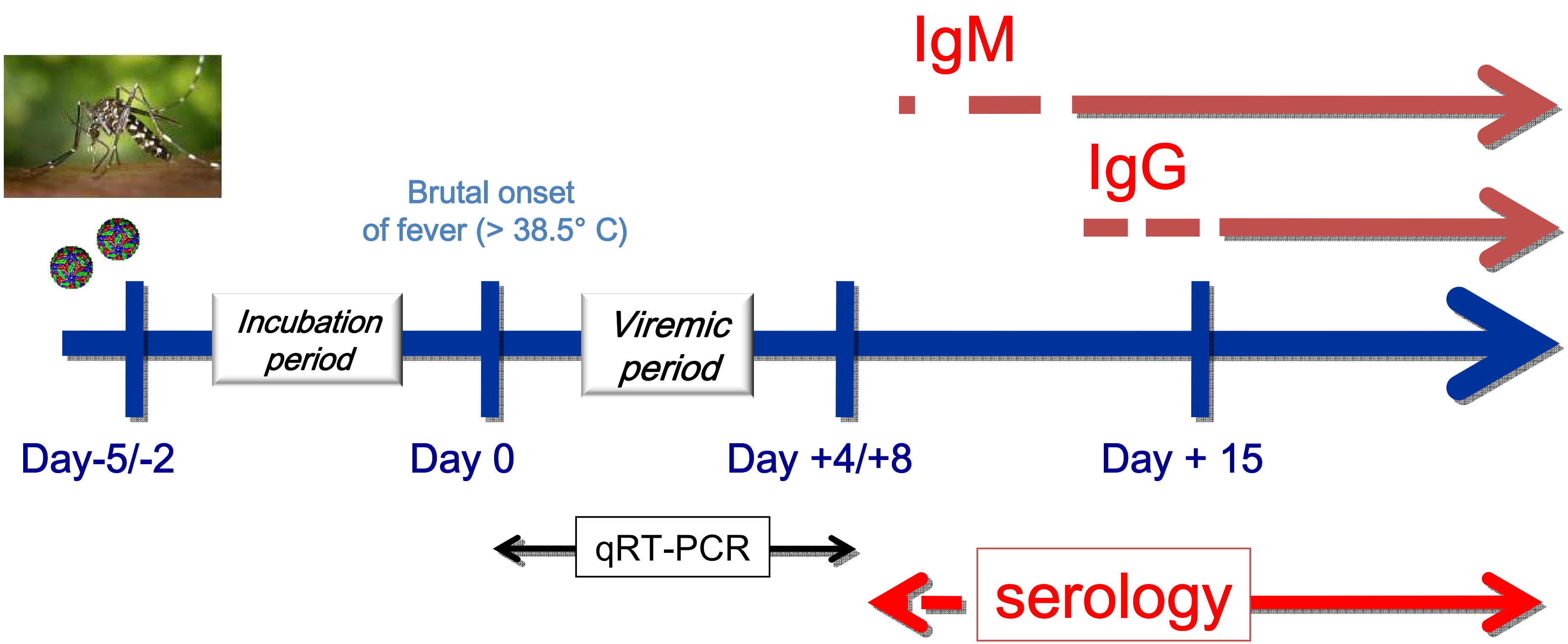
Serotypic lifelong immunity?



DF in child who had prior D2V neut antibody

Endy et al. 2004 JID

Laboratory serodiagnosis of arbovirus infection



Confirmed infection:

- positive qRT-PCR in acute serum sample
- isolation of arbovirus on cultured cell lines
- arbovirus-neutralizing antibodies by plaque reduction neutralisation tests

Presumptive infection:

- anti-arbovirus IgM
- anti-arbovirus IgG

Issues for Herd Immunity

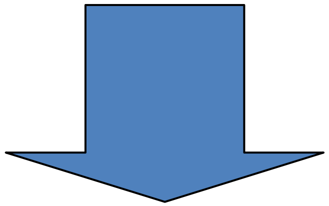
- The impact of the fraction immune in the community on the per capita rate of transmission of an infectious agent.
- The level of herd immunity can be measured by reference to the magnitude of reduction in the value of R_o.
- What if immunity not sterilising or life-long?

Neutralisation tests – do they tell the true tale of antibody immunity?
(enhancing or protective)

1. Interpretation requires high skill - there is always cross-reactivity

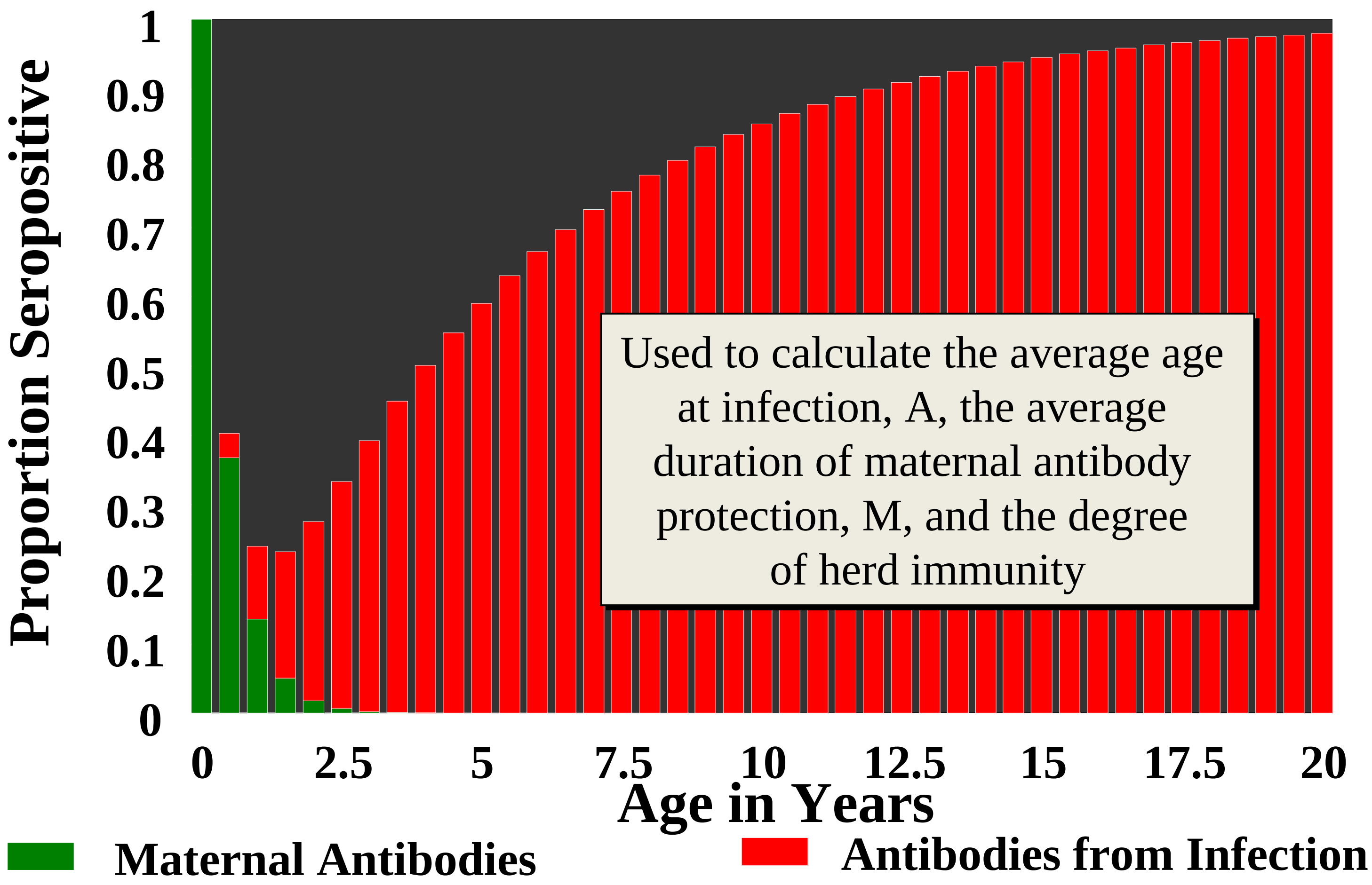
2. Cell dependent – Gold standard uses kidney-derived cell-line, but monocytes more biologically realistic?

3. Absence of correlation in recent vaccine trial



Serious problems of reliable serology

Sero-prevalence – cornerstone of understanding epidemiology



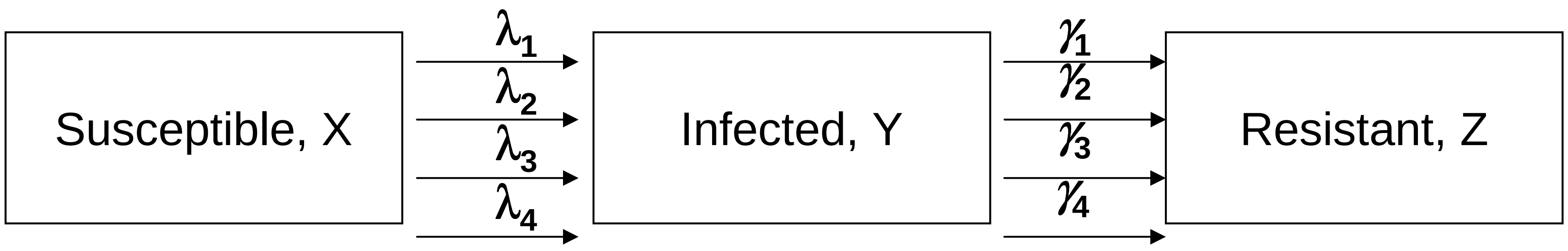
If the proportion seropositive at age **a** is **p(a)**, then the average force or rate of infection **λ** over the interval 0 to **a** years of age is given by:

$\lambda(a)=p(a)/[L(1-e(-a/L))]$

Fig. courtesy of Roy Anderson Delhi lecture

Cross-immunity to Dengue viruses

4 serotypes, several genotypes and many clades



Observed R_0 is sum of R_0 of each serotype/genotype/clade?

$A = L/ \Sigma R^i_0$

Dengue epidemiology in urban vs. rural zones

Expect contrasts because of differences in population densities

Urban – combination of

complex demography,
herd immunity,
poor case reporting,
inapparent infections

Rural – human density too low to maintain pathogen,

Look to Yellow Fever dynamics

Transmission to mosquitoes

Direct feeding on viremic patients

– almost no data

- attenuated vaccine trials (eg Bancroft et al. 1982 AmJTropMedHyg)

Indirect feeding (viremic blood from patients fed indirectly)

- ???

Membrane feeding

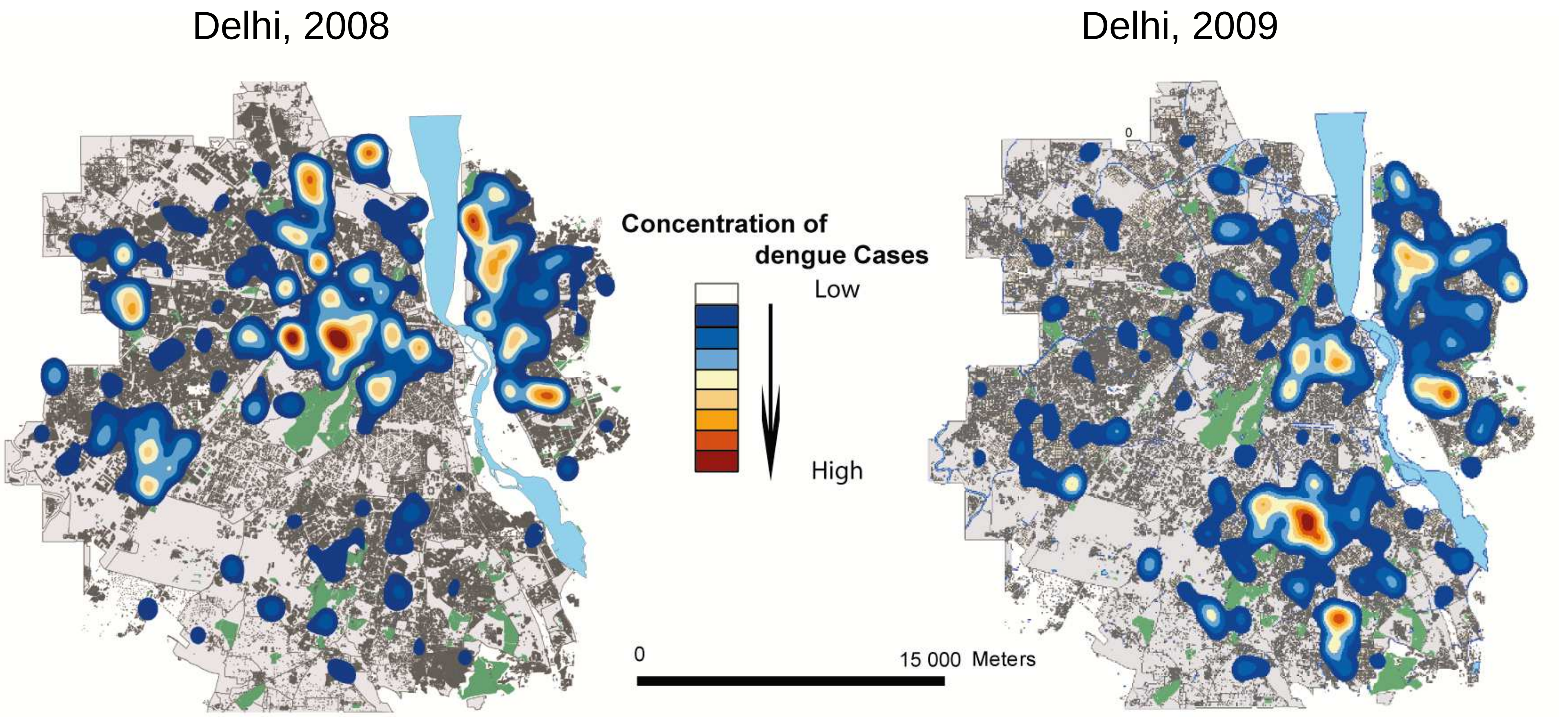
- for exerimental purposes of vector competence, Wolbachia etc

Conclusion

No real idea of relationship between viremia and infectiousness

Inherent spatiality in dengue epidemiology – forest fires

Courtesy of Olivier Telle, Univ. Rouen & Institut Pasteur



Collabs: Municipal Corporation of Delhi, Centre de Sciences Humaines,
National Institute of Malaria Research

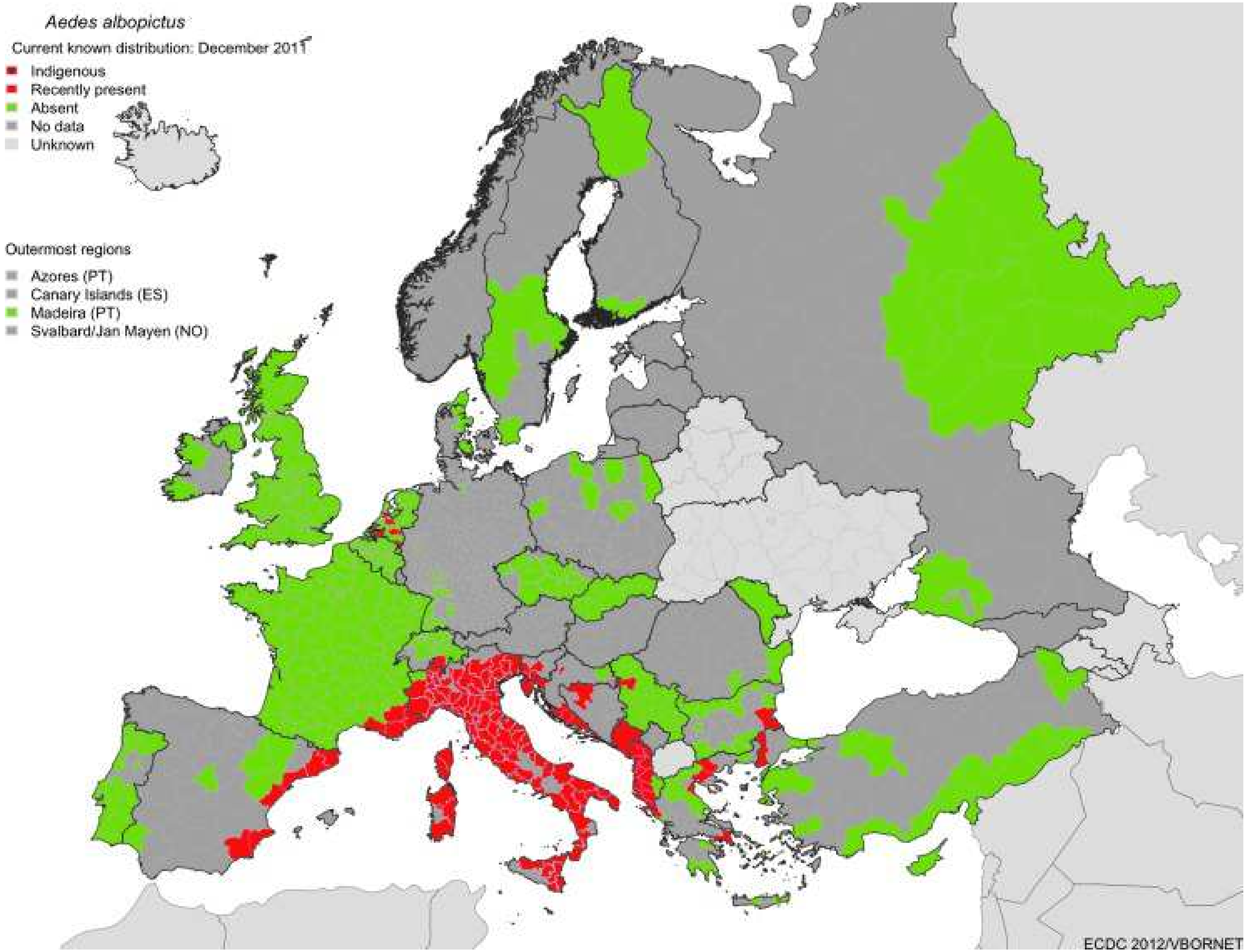
DENGUE RESEARCH FRAMEWORK
FOR RESISTING EPIDEMICS IN EUROPE

DENGUE TRANSMISSION IN EUROPE

Un premier cas non importé de dengue en métropole
LEMONDE.FR avec AFP | 13.09.10 | 14h56

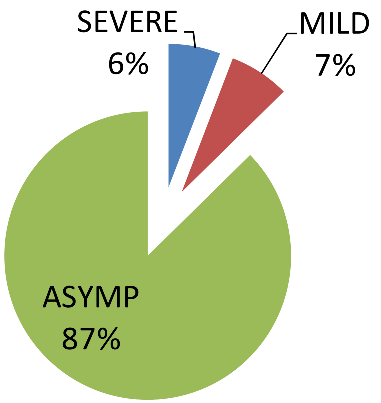
Un deuxième cas autochtone de dengue signalé à Nice
LEMONDE.FR | 18.09.10 | 18h19

Distribution of *Aedes albopictus* in Europe (2011)



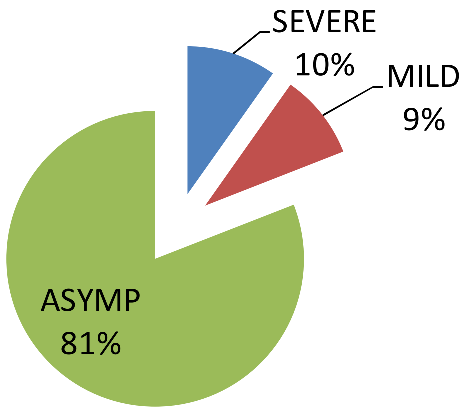
Medlock et al. Vector Borne Zoonotic Dis. 2012 Jun;12(6):435-47

INAPPARENT DENV INFECTION

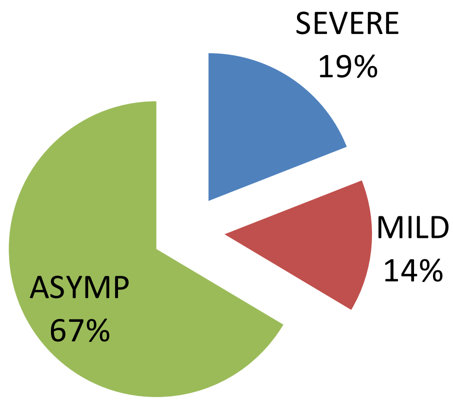


Bangkok, Thailand 80-81: DENV-1 and 2

Burke et al. ,Am J Trop Med Hyg 1988

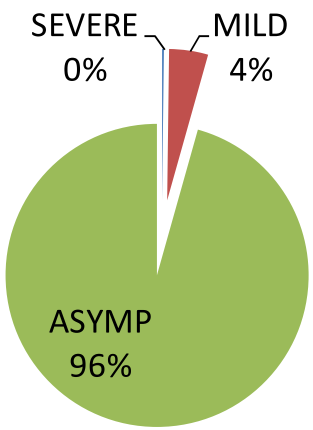


Vietnam 04: DENV-2



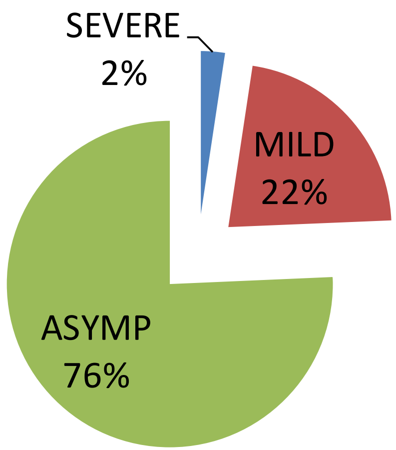
Vietnam 07: DENV-1

Trans R Soc Trop Med Hyg, 2010



Singapore 04: DENV-1

Yew et al., Ann Acad Med Singapore 2009



West Java, Indonesia 00-02: DENV-2

Porter et al., Am J Trop Med Hyg, 2005

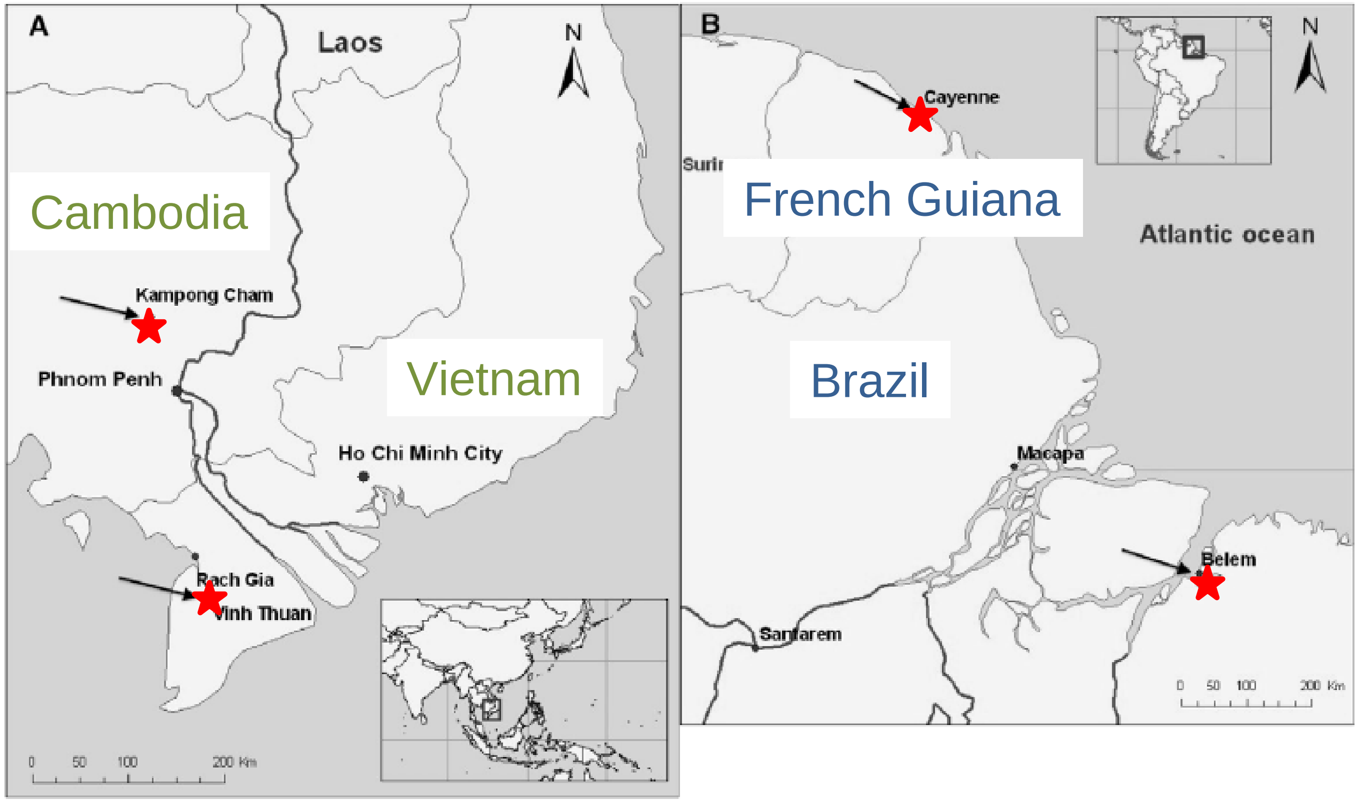
RESEARCH QUESTIONS ON INAPPARENT INFECTIONS

- Lower viremia in asymptomatic DENV infection?
- Duration of viremia?
- Different viral strains or quasi-species?
- Type of immune responses?
- Transmit the viruses to mosquito vectors?
- If yes, their contribution to DENV endemics, epidemics and spreading to uninfected areas?
- If they play an important role, how can we detect them and prevent them from transmitting the virus?

How can we detect inapparent viremic individuals?

DENFRAME- FP6 EU program

Coordinator: Laurence Baril, Philippe Despres, Nathalie Pardigon



South East Asia | Latin America

OPEN ACCESS Freely available online
PLoS NEGLECTED TROPICAL DISEASES

Clinical and Virological Study of Dengue Cases and the Members of Their Households: The Multinational DENFRAME Project

Philippe Dussart[1]*, Laurence Baril[2,3], Laure Petit[2], Lydie Beniguel[2], Luong Chan Quang[4], Sowath Ly[5], Raimunda do Socorro Silva Azevedo[6], Jean-Baptiste Meynard[7], Sirenda Vong[5], Loïc Chartier[2], Aba Diop[3], Ong Sivuth[8], Veasna Duong[8], Cao Minh Thang[9], Michael Jacobs[10], Anavaj Sakuntabhai[11], Marcio Roberto Teixeira Nunes[6], Vu Ti Que Huong[9], Philippe Buchy[8], Pedro Fernando da Costa Vasconcelos[6]

Household investigation study design

DENGUE INDEX CASE (DIC)

Inclusion criteria:
Clinical symptoms plus
viral culture +ve
or viral genome +ve
or DENV NS1 +ve

HOUSEHOLD MEMBERS (HHM)

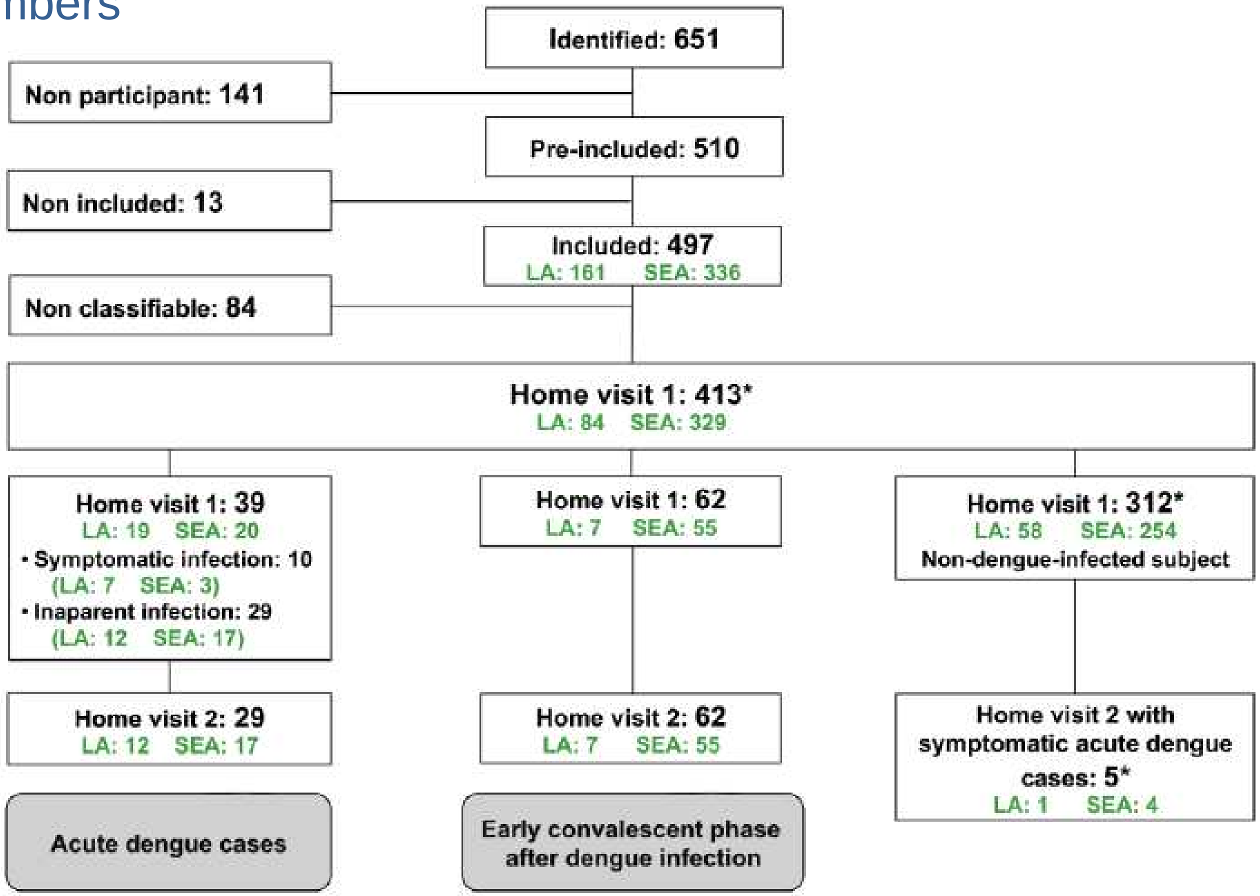
viral culture
viral genome
DENV NS1
anti-DENV IgM
anti-DENV IgG

14 days monitoring

DENGUE INDEX CASES & HOUSEHOLD MEMBERS

Dengue Index Case
443
LA 254 SEA 189

Household members



LA: 23%(14%) SEA: 6%(5%) LA: 8% SEA: 17%

Main Different Characteristics of Uninfected/Inapparent/Symptomatic Dengue

Table 3. Main characteristics of subjects with inapparent dengue infections compared to non-dengue-infected subjects among Household members.

	Non-dengue-infected n = 307 (%)	Inapparent dengue infection n = 29 (%)	Crude OR	95% CI	P*	Adjusted OR	95% CI	P
Neutrophils ($\times 10^9$/L)								
>2	288 (93.8)	18 (62.1)	1			1		
≤2	18 (5.9)	11 (37.9)	9.8	[4–23.8]	<0.0001	7.75	[2.5–24]	**<0.0001**
Missing data	1 (0.3)	-						
Monocytes ($\times 10^9$/L)								
>0.2	298 (97.1)	23 (79.3)	1			1		
≤0.2	8 (2.6)	6 (20.7)	9.72	[3.1–30]	<0.0001	9.1	[1.8–44]	**0.006**
Missing data	1 (0.3)	-						

Table 4. Main characteristics of subjects with inapparent dengue infections compared to symptomatic dengue-infected subjects.

	Symptomatic dengue-infected n = 192 (%)	Inapparent dengue infection n = 29 (%)	Crude OR	95% CI	P*	Adjusted OR	95% CI	P
Lymphocytes ($\times 10^9$/L)								
>2	16 (8.3)	15 (51.7)	1			1		
≤2	176 (91.7)	14 (48.3)	0.08	[0.03–0.2]	<0.0001	0.09	[0.02–0.4]	**0.001**
NS1 antigen								
Negative	21 (10.9)	23 (79.3)	1			1		
Positive	171 (89.1)	6 (20.7)	0.03	[0.01–0.1]	<0.0001	0.05	[0.01–0.2]	**<0.0001**

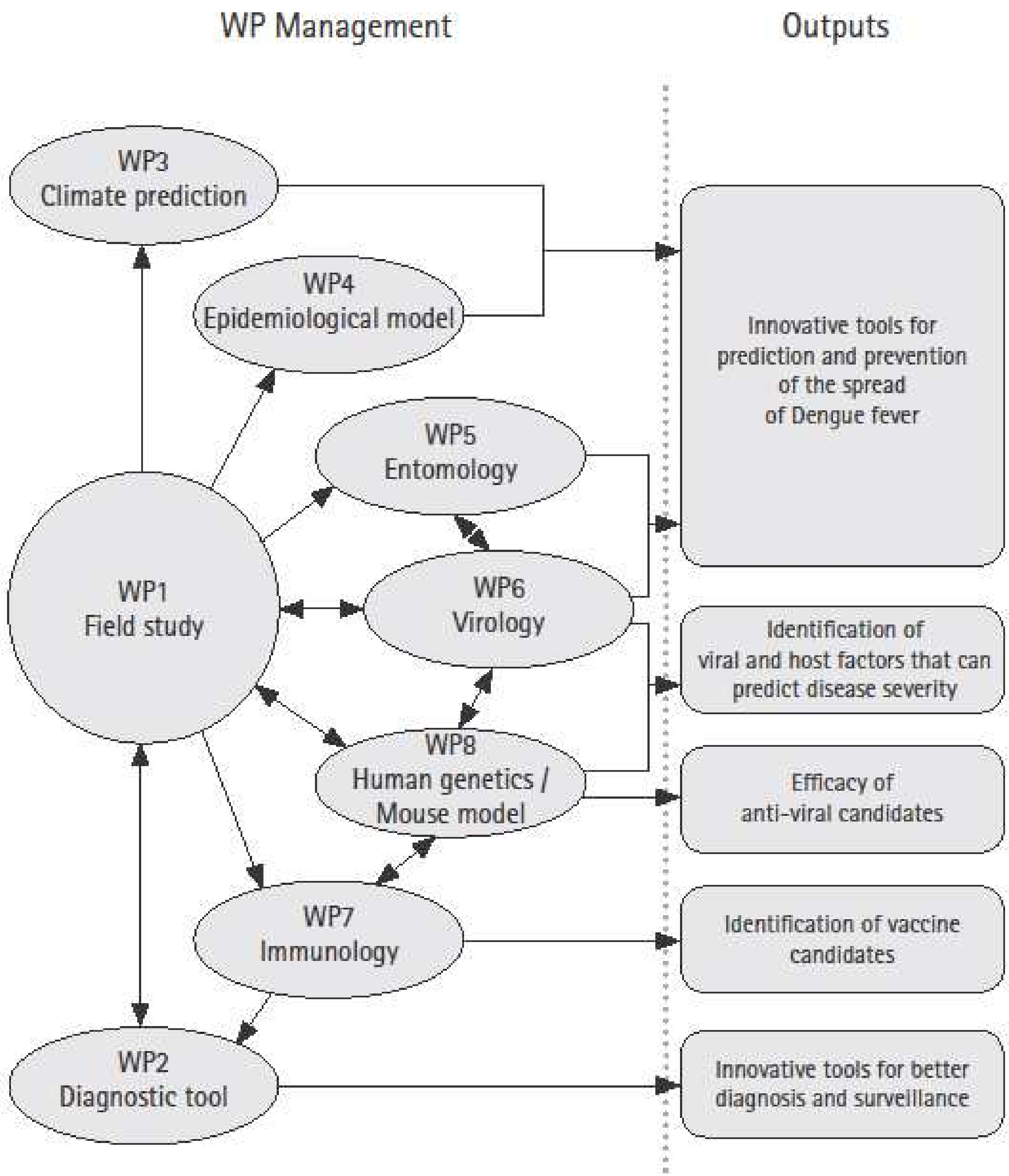
DENGUE RESEARCH FRAMEWORK FOR RESISTING EPIDEMICS IN EUROPE

Coordinator: A. Sakuntabhai,
Institut Pasteur Paris

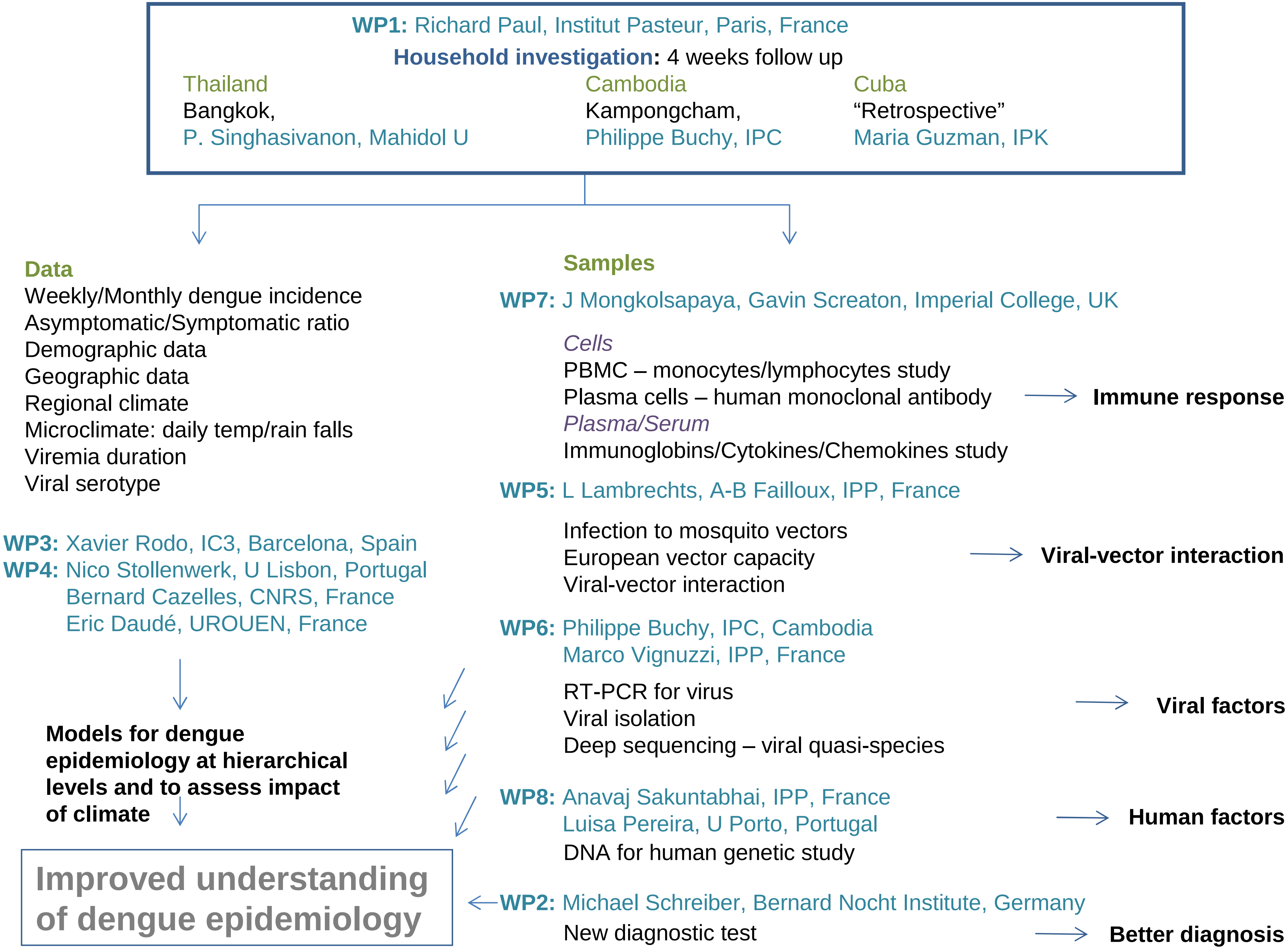
14 partners

R Paul, M Vignuzzi, L Lambrechts, A-B Failloux, F Rey	Institut Pasteur Paris, France
G Screaton, J Mongkolsapaya	Imperial College, UK
M Schreiber	Bernard Nocht Institute, Germany
P Singhasivanon	Mahidol University, Thailand
P Buchy, V Deubel	Institut Pasteur Cambodia, Cambodia
X Rodo	Fundacio Institut Catala De Ciencies Del Clima , Spain
E Daudé, A Vaguet	University Of Rouen, France
B Cazelles	CNRS, France
N Stollenwerk, M Aguiera	Cmaf, Fundacao Da Faculdade De Ciencias Da Universidade De Lisboa, Portugal
L Pereira	Instituto De Patologia E Imunologia Molecular Da Universidade Do Porto, Portugal
T Kanninen	Biocomputing Platforms Ltd Oy, Finland
G Krubb	AmpTec GmbH, Germany
M Thursz	RioTech Pharmaceticals Ltd, UK
M Guzman	Institute of Tropical Medicine “Pedro Kouri”, Cuba

WORK PLAN



DENFREE
DENGUE RESEARCH FRAMEWORK FOR RESISTING EPIDEMICS IN EUROPE


WP1: Richard Paul, Institut Pasteur, Paris, France
Household investigation: 4 weeks follow up
Thailand
Bangkok,
P. Singhasivanon, Mahidol U
Cambodia
Kampongcham,
Philippe Buchy, IPC
Cuba
"Retrospective"
Maria Guzman, IPK
Data
Weekly/Monthly dengue incidence
Asymptomatic/Symptomatic ratio
Demographic data
Geographic data
Regional climate
Microclimate: daily temp/rain falls
Viremia duration
Viral serotype
WP3: Xavier Rodo, IC3, Barcelona, Spain
WP4: Nico Stollenwerk, U Lisbon, Portugal
Bernard Cazelles, CNRS, France
Eric Daudé, UROUEN, France
Models for dengue epidemiology at hierarchical levels and to assess impact of climate
Improved understanding of dengue epidemiology
Samples
WP7: J Mongkolsapaya, Gavin Screaton, Imperial College, UK
Cells
PBMC – monocytes/lymphocytes study
Plasma cells – human monoclonal antibody
Plasma/Serum
Immunoglobins/Cytokines/Chemokines study
Immune response
WP5: L Lambrechts, A-B Failloux, IPP, France
Infection to mosquito vectors
European vector capacity
Viral-vector interaction
Viral-vector interaction
WP6: Philippe Buchy, IPC, Cambodia
Marco Vignuzzi, IPP, France
RT-PCR for virus
Viral isolation
Deep sequencing – viral quasi-species
Viral factors
WP8: Anavaj Sakuntabhai, IPP, France
Luisa Pereira, U Porto, Portugal
DNA for human genetic study
Human factors
WP2: Michael Schreiber, Bernard Nocht Institute, Germany
New diagnostic test
Better diagnosis

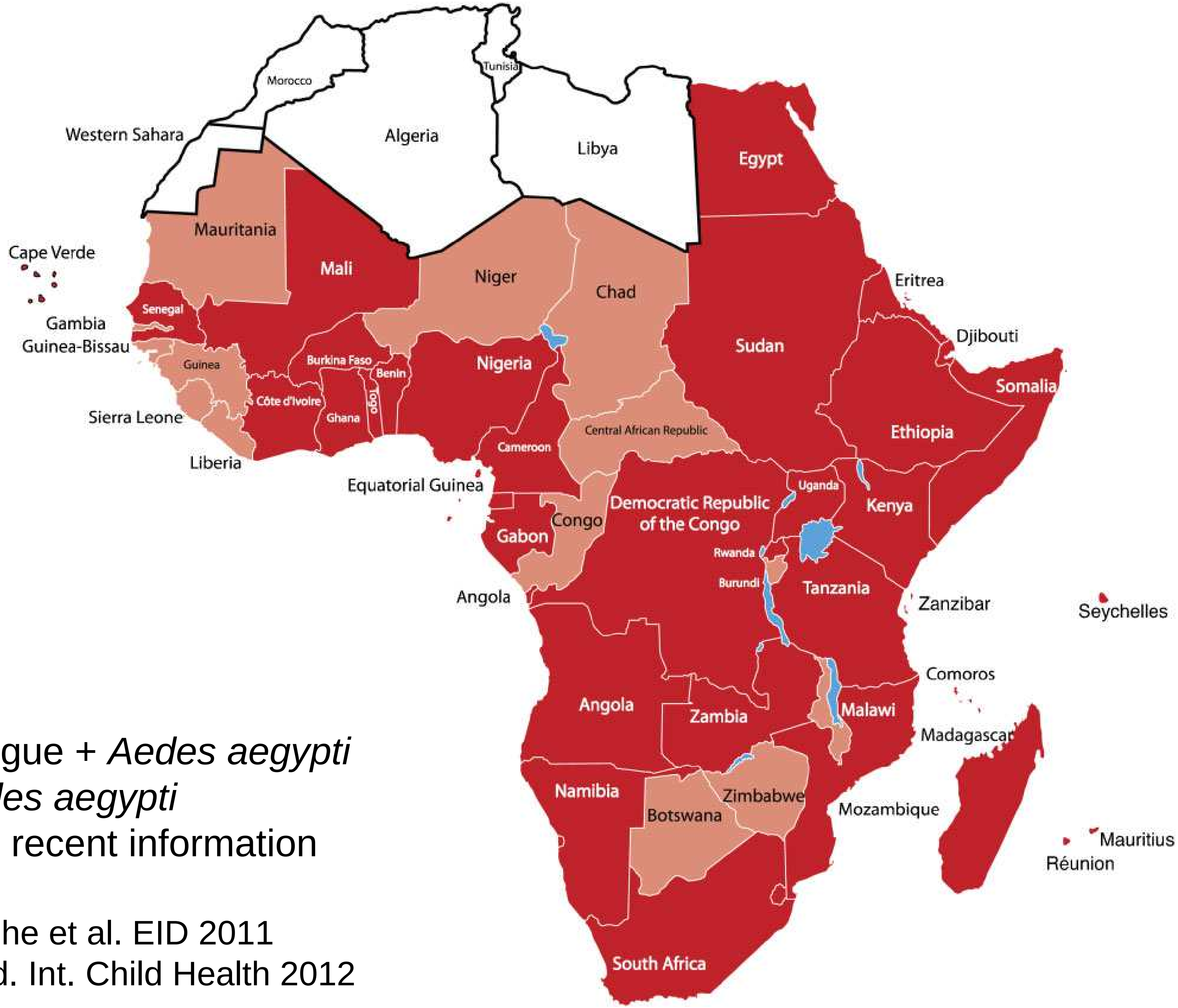
3D initiatives

DengueTools

DENFREE

The Global Dengue Risk Map Project

Dengue reports in Africa



Red: Dengue + *Aedes aegypti*
Pink: *Aedes aegypti*
White: no recent information

Amarasinghe et al. EID 2011
Were Paed. Int. Child Health 2012

VBORNET
European Network for Arthropod Vector Surveillance for Human Public Health

http://www.vbornet.eu/

The objective of VBORNET is to establish a European Network of entomological and public health specialists in order to assist ECDC in its preparedness activities on vector borne diseases (VBD). This will be achieved in three steps:

1.Establishment of the VBORNET consortium who will develop the VBORNET network and the VBORNET inventory.

2.Establishment of a VBORNET network of contributing members who are representative of the wide range of vector-borne disease related research and public health (PH) activities currently ongoing in Europe. One of its main tasks in year one will be to set the basis for Pan-European administrative unit distribution maps of the major arthropod vectors of diseases. Subscription is on a voluntary basis;

3.Establishment of a VBORNET inventory which aims at making an exhaustive catalog of VBD and related public health (PH) activities (and expertise) in Europe.

Thank you for your time and attention