

I patogeni emergenti in medicina trasfusionale



**The Globalisation of Culture means the
Globalisation of Disease**

Massimo Clementi, Università Vita-Salute San Raffaele, Milano

“No city on the earth is now more than 24 hours away from any other”. Economist 2003

“Annually, the world's airlines carry a staggering total approaching some two billion passengers. At any one moment, about half a million people world-wide are flying in commercial aircraft

“Committee on Science and Technology Fifth Report UK Parliament 2000

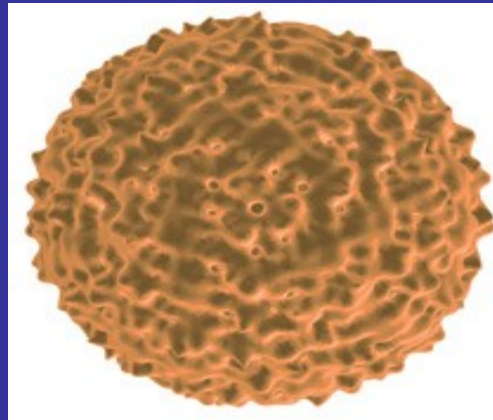
West Nile Disease Virus (WNDV)

Chikungunya

Dengue

West Nile Disease Virus (WNDV)

- *Flaviviridae (Flavivirus)*
- *virus simmetria icosaedrica e diametro di circa 50 nm, provvisto di envelope*
- *Gruppo antigenico YF e JE*
- *identificato per la prima volta dal sangue di una donna affetta da sindrome acuta febbrile in Uganda nel 1937*

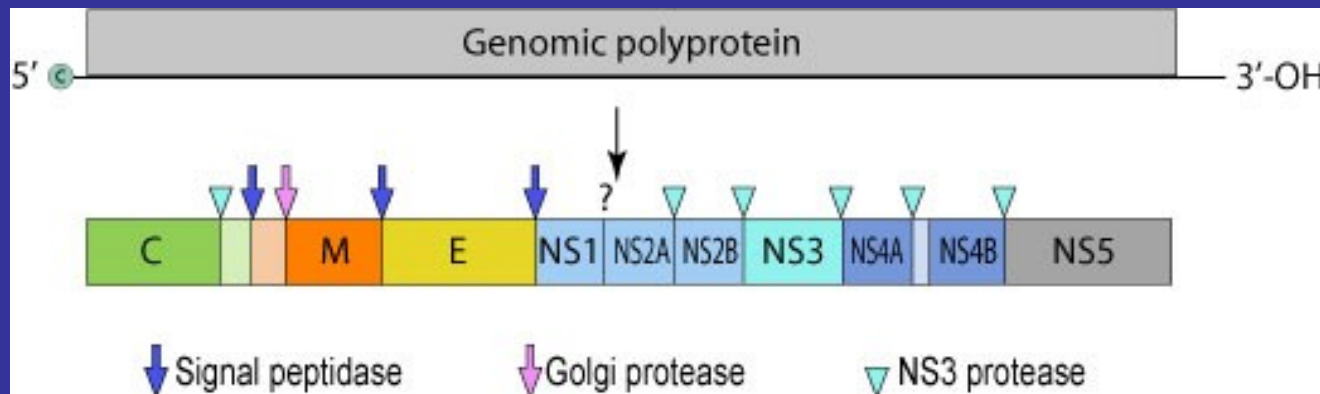


West Nile Disease Virus (WNDV)

RNA: genoma è costituito da una molecola a singolo filamento a polarità positiva, costituito da circa 11.000 paia di basi.

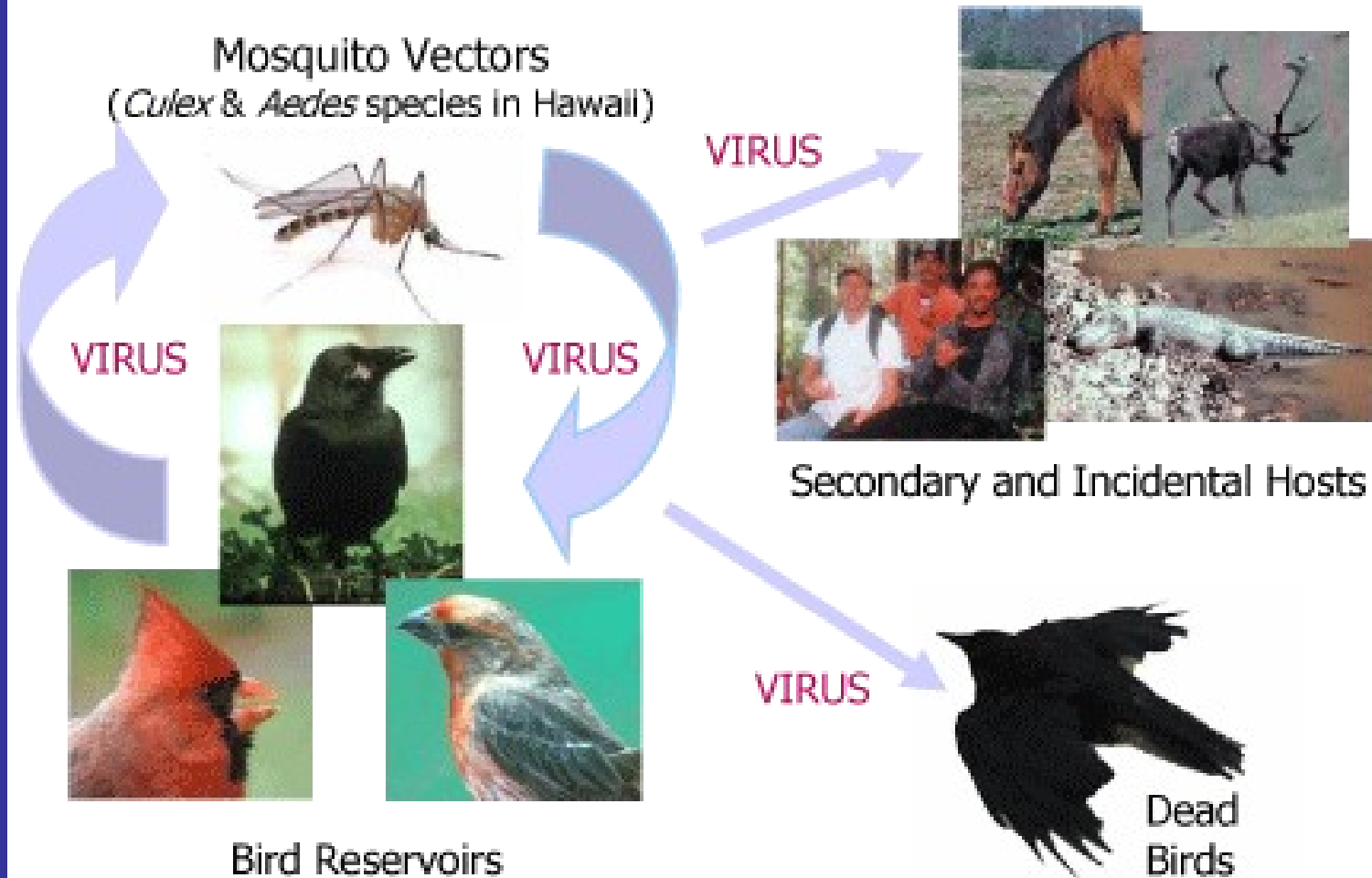
Regione 5' non tradotta (UTR), seguita da una singola lunga open reading frame (ORF) e da un'altra UTR all'estremo 3'

ORF codifica una poliproteina: 3 proteine strutturali (C, prM ed E) e 7 non strutturali (NS1, NS2A, NS2B, NS3, NS4A, NS4B e NS5)



West Nile Disease Virus (WNDV)

West Nile Virus Transmission Cycle

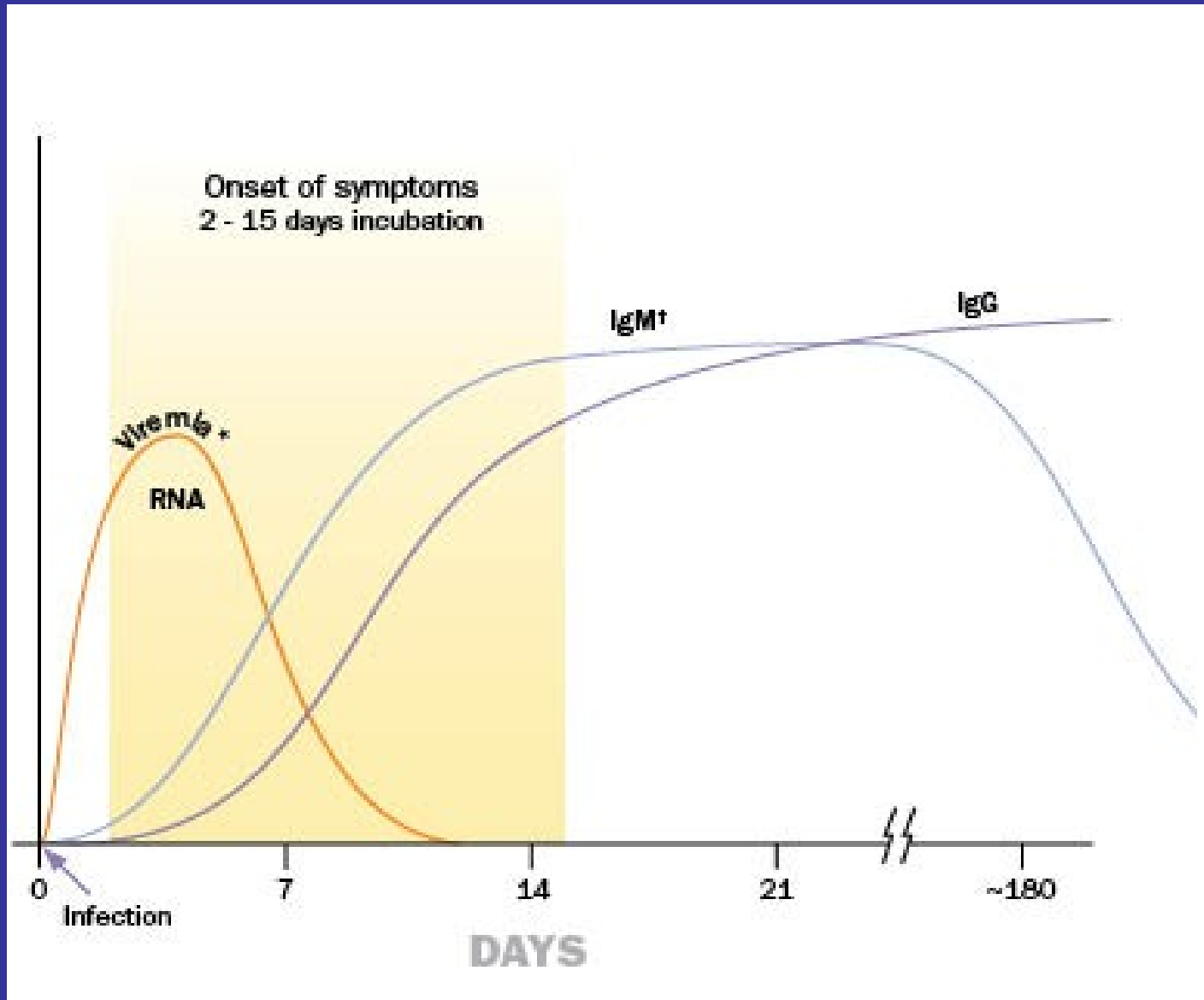


West Nile Disease Virus (WNV)

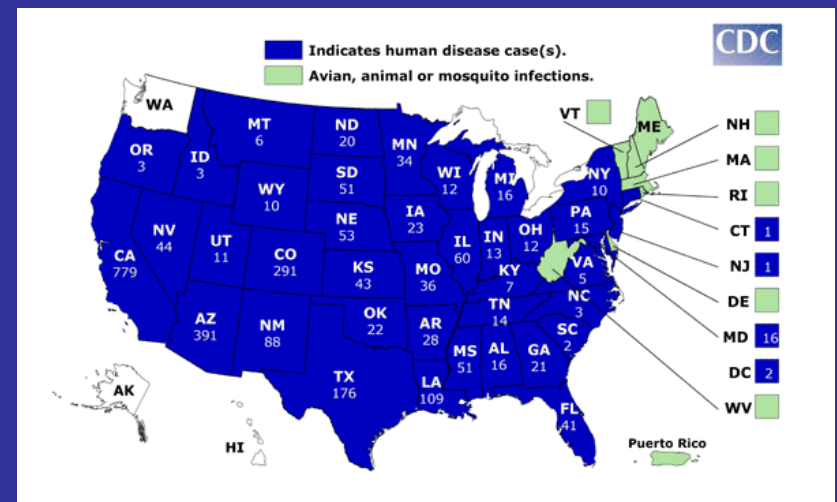
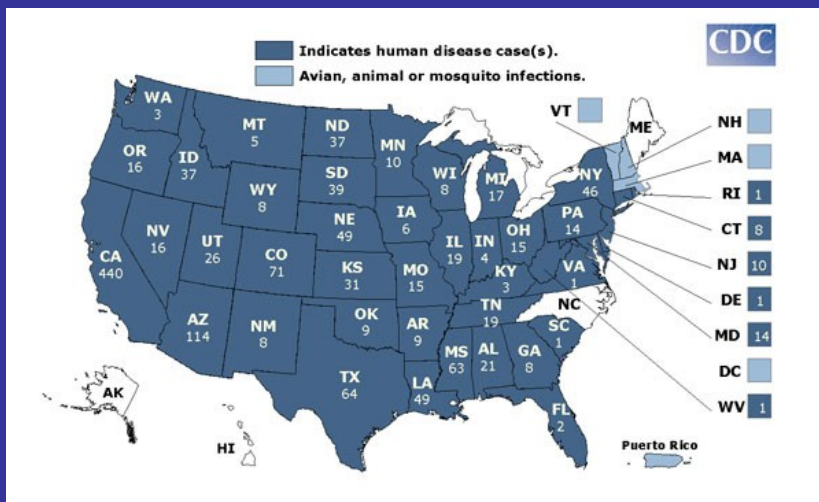
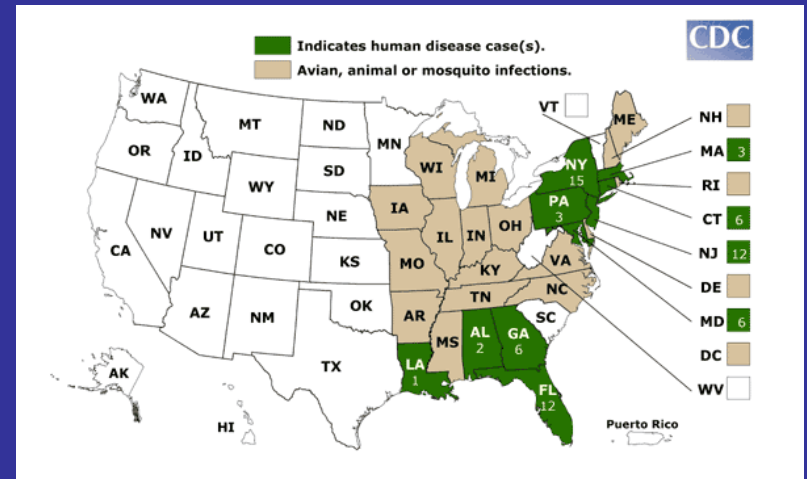
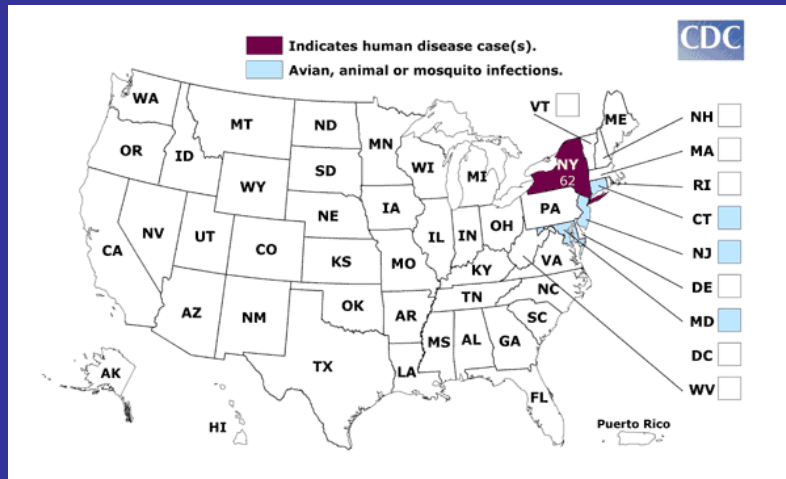
Symptoms of the infection in humans

- Asymptomatic (80%)
- Fever, headache, muscle pain, cutaneous rash (15%)
- Neuro-invasive disease (meningo-encephalitis) (>1%)
- ~ 140-150 asymptomatic individuals/1 case of neuro-invasive disease

WNDV - Diagnosi di laboratorio



West Nile Disease Virus (WNV)



West Nile Disease Virus (WNV)

Epidemiology

Small epidemic episodes or large outbreaks in Africa, Asia and North-America

In Europe:

- Bucarest (1996): 350 human cases
- Italia (1998): 14 horses
- Francia (2000): 76 horses
(2003): 7 horses and 7 human cases
(2006): 6 horses
- 2008: human cases reported in Romania, Hungary and Italy
more than 300 horses infected in Italy

Rapid communications

DETECTION OF WEST NILE VIRUS INFECTION IN HORSES, ITALY, SEPTEMBER 2008

P Macini¹, G Squintani², A. C. Finarelli (afinarelli@regione.emilia-romagna.it)¹, P Angelini¹, E Martini², M Tamba³,
M Dottori¹, R Bellini⁴, A Santi², L Lolli Piccolomini², C Po¹

September 2008

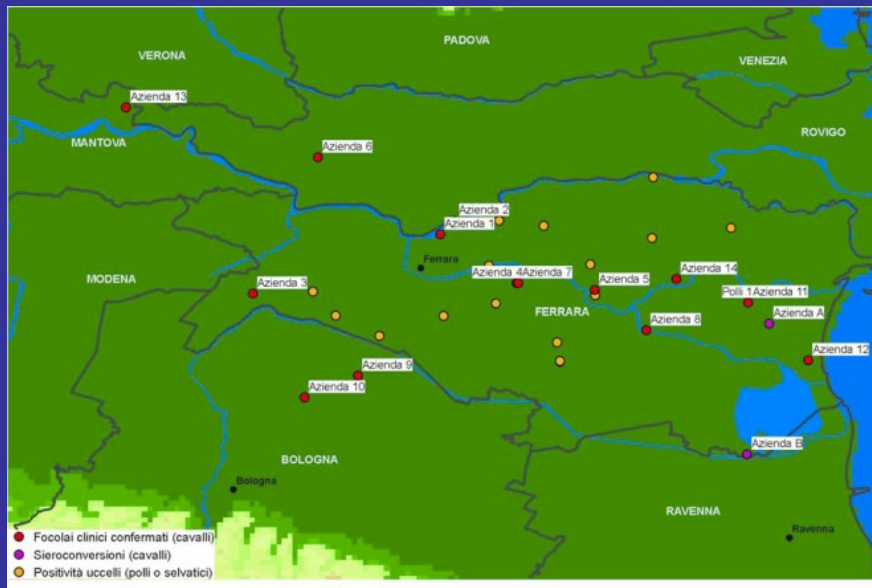
- 6 confirmed cases
- 5 suspected cases



Animal and human surveillance programme

Epidemiology at October 8th

Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise



✓ 20 symptomatic horses

✓ 14 farms in the provinces of
Ferrara (10), Bologna (2),
Mantova (1), Rovigo (1)

HUMAN CASE DEFINITION

- ≥ 15 years old,
- Fever $\geq 38.5^{\circ}\text{C}$
- Neurological symptoms: encephalitis, meningitis or Guillain-Barré syndrome or acute flaccid paralysis.

Cases are classified as:

- a) possible: clinical symptoms and clear CSF;
- b) probable: clinical symptoms and at least one of the following laboratory criteria: presence of IgM antibodies against WNV by ELISA; seroconversion by ELISA; fourfold increase of IgG antibodies against WNV in two consecutive samplings by ELISA;
- c) confirmed: clinical symptoms and at least one of the following laboratory criteria: isolation of WNV virus in blood or CSF; presence of IgM antibodies in CSF (by ELISA); detection of nucleic acid specific for WNV by RT PCR in blood or CSF; detection of increased levels of IgM and IgG antibodies against West Nile by ELISA confirmed by neutralization testing.

Rapid communications**FIRST HUMAN CASE OF WEST NILE VIRUS NEUROINVASIVE INFECTION IN ITALY, SEPTEMBER 2008 – CASE REPORT**

G Rossini¹, F Cavrini¹, A Pierro¹, P Macini², A. C. Finarelli², C Po², G Peroni³, A Di Caro⁴, M Capobianchi⁴, L Nicoletti⁵, M P Landini¹, V Sambri (vittorio.sambri@unibo.it)¹

WNDV human cases

1 st case	2 nd case	3 th case
Female	Male	Male
Ferrara/ Bologna	Ferrara	Ferrara
> 80 years old	> 60 years old	70 years old
Fever, vomiting, impaired consciousness, hallucinations	Fever, Symptoms of acute meningoencephalitis	Symptoms of acute meningoencephalitis
IgM and IgG positive	IgM and IgG positive RT-PCR positive	IgG positive

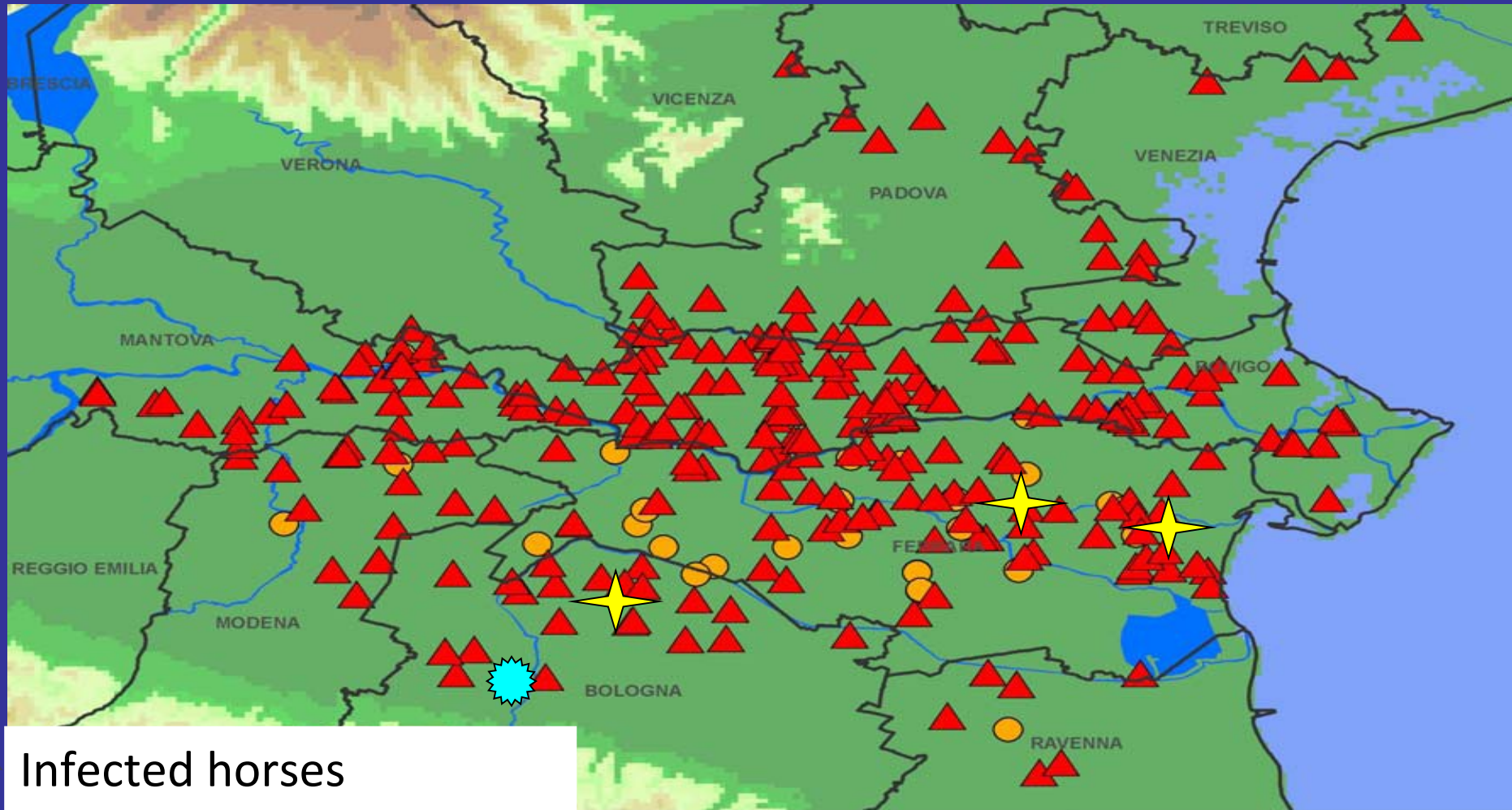
WNDV - Blood donation - 1 jan.-30 june 2007

	Blood donation #
Piacenza	7384
Parma	14224
Reggio Emilia	11524
Modena	17350
Bologna	31999
Ferrara	11000
Ravenna	14233
Fo-Ce RM	15474
Total	<u>123188</u>

WNV NAAT screening activity at CRREM

- CHIRON West Nile Virus (PROCLEIX TIGRIS)
 - Single testing
 - Oct 10th – Nov 30th
 - >6000 plasma samples
 - 185 organ and tissue donations
- No positive samples detected

WNV epidemiology in lower Po river valley



▲ Infected horses
● Infected birds

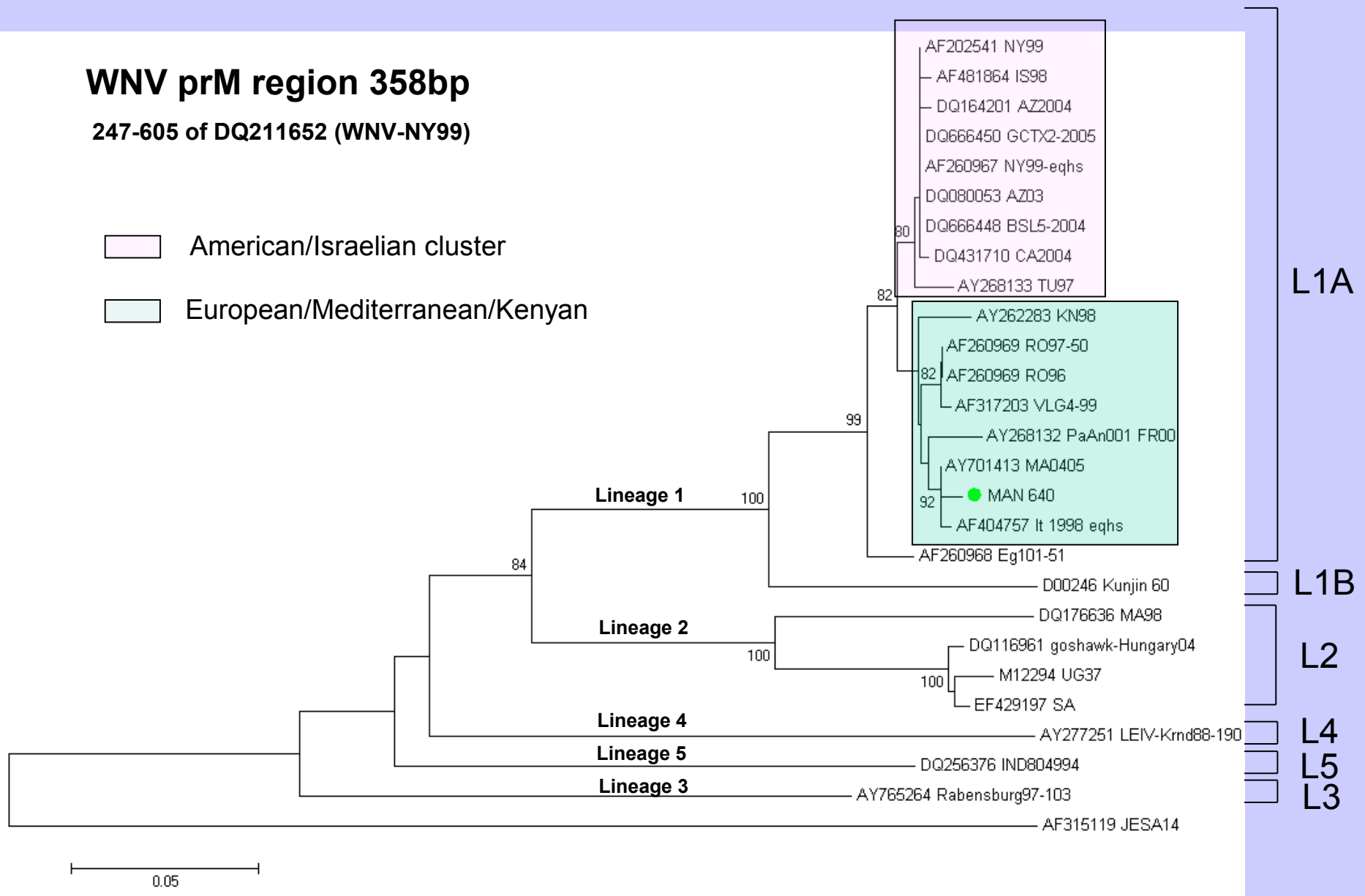
WNV NAAT screening activity at CRREM

- CHIRON West Nile Virus (PROCLEIX TIGRIS)
 - Single testing
 - Oct 10th – Nov 30th
 - >6000 plasma samples
 - 185 organ and tissue donations
- No positive samples detected

WNV prM region 358bp

247-605 of DQ211652 (WNV-NY99)

- American/Israeli cluster
- European/Mediterranean/Kenyan



WNDV seroprevalence investigation plan

- 10800 blood donations
- October 2008 – April 2009
- Province of Ferrara
- ELISA (IgG and IgM)
- Confirmation by IFA and IgG avidity
- “Geo-localization” of the positive BD

WNDV seroprevalence investigation results (March 12, 2009)

- 7521 blood donations tested
- 77 EIA IgG positive (0 IgM)
- 57 IFA IgG positive (confirmed)
- Low IgG avidity
- Geographically spread
- **Seroprevalence: 0.76%**



Chikungunya chase

New ways to monitor the viral disease that throws up new challenges

Tpurushottam Bhat: one of 70,493 cases of chikungunya fever in eight Indian states. His **24**-member family has **19** others who fell ill to the virus transmitted by mosquitoes.

Chikungunya

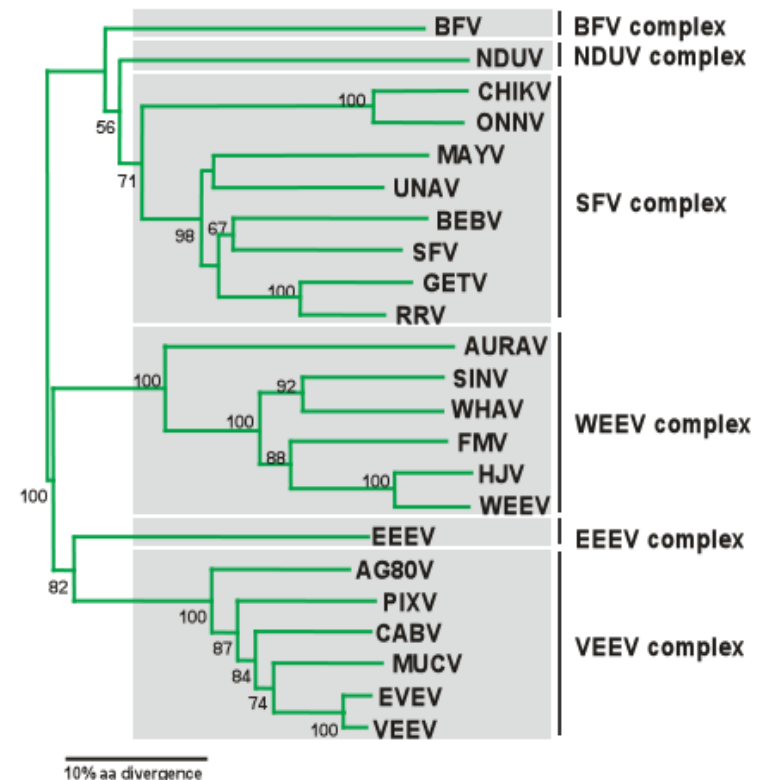
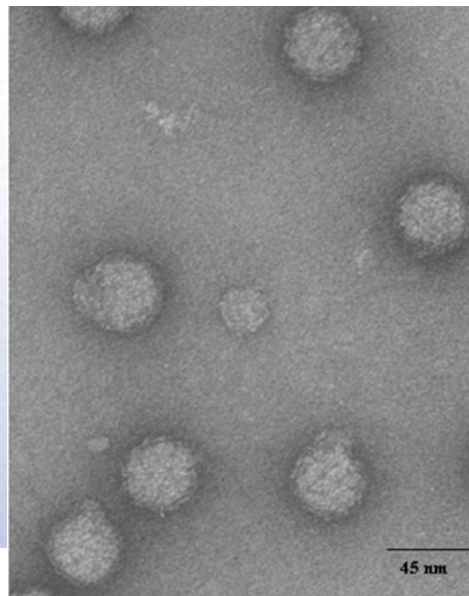
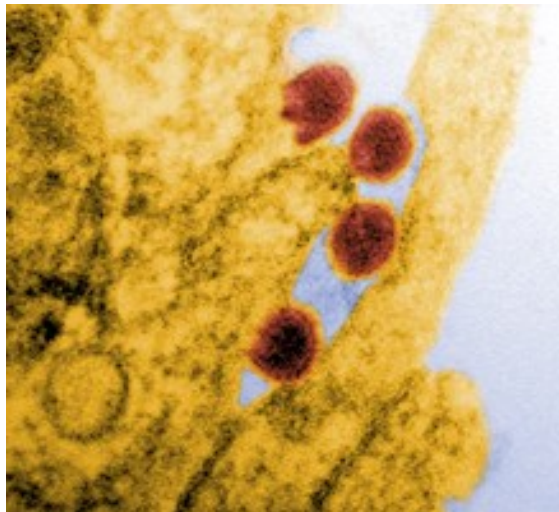
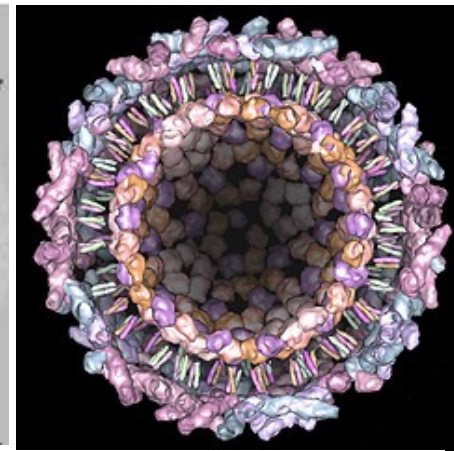
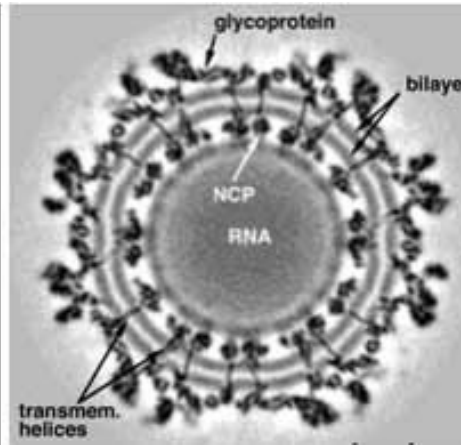
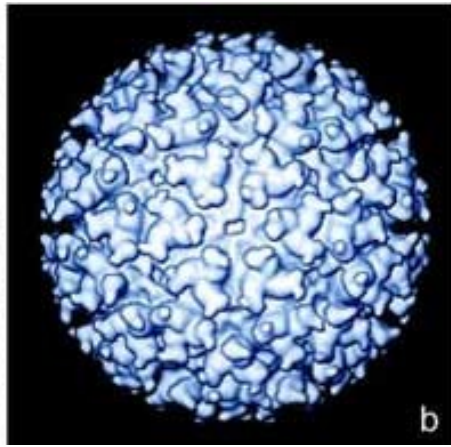
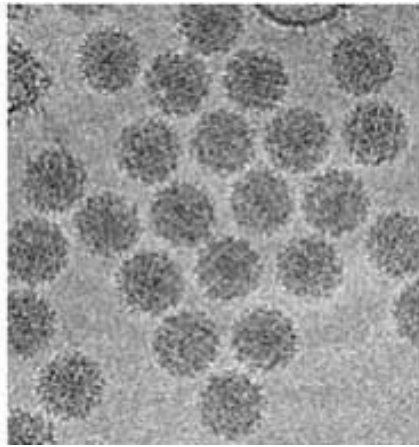
La Chikungunya è una malattia di origine virale, causata da un virus della famiglia delle Togaviridae, trasmessa tramite le punture della Zanzara Tigre.

Bacino endemico della malattia sono diverse zone tropicali dell'Asia e dell'Africa.

Nelle ultime settimane di agosto 2007, la Chikungunya è stata notificata anche in alcune frazioni dell'Emilia-Romagna, in particolare nella provincia di Ravenna.

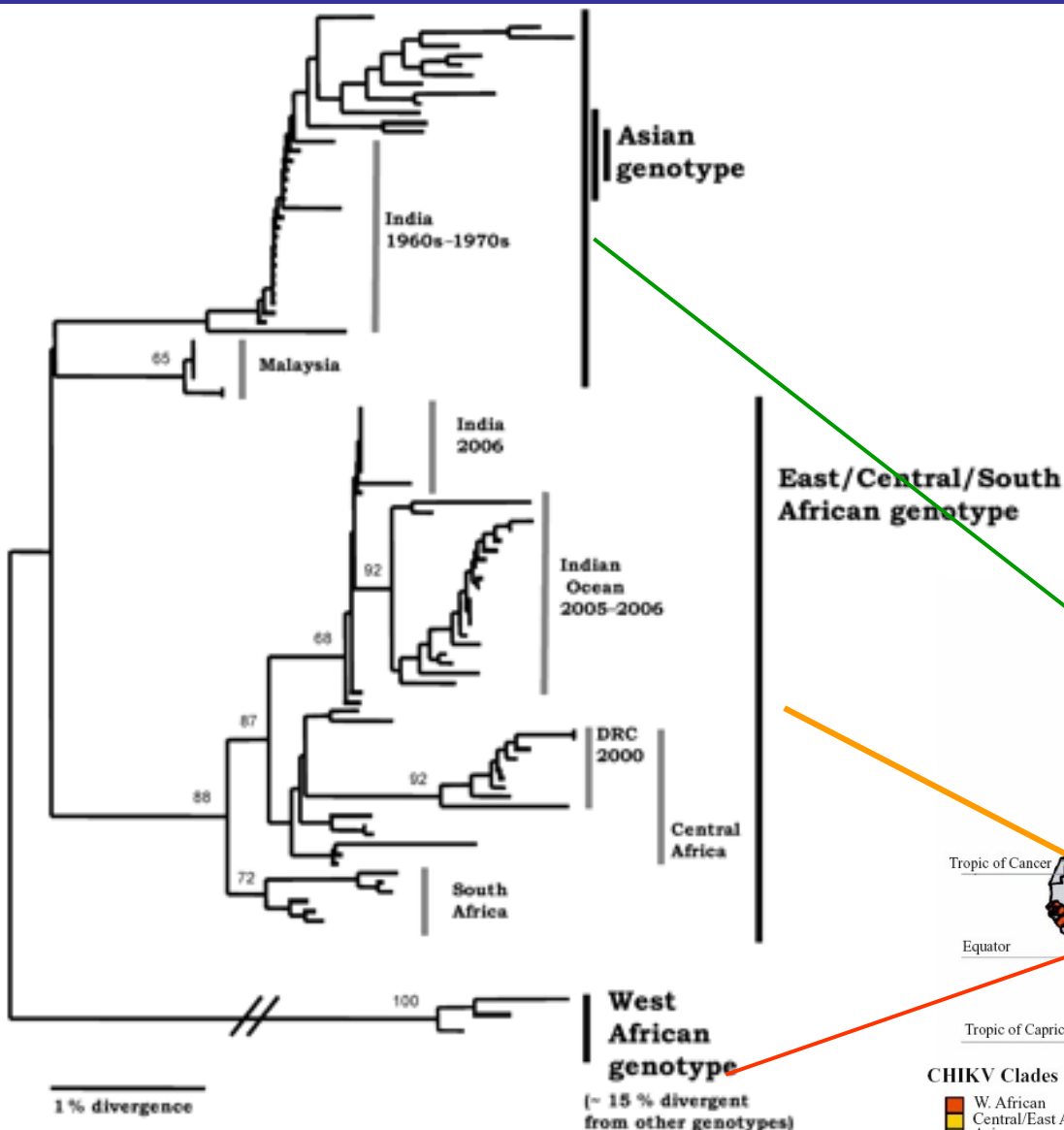
Chikungunya virus

Family: Togaviridae, genus: alphavirus

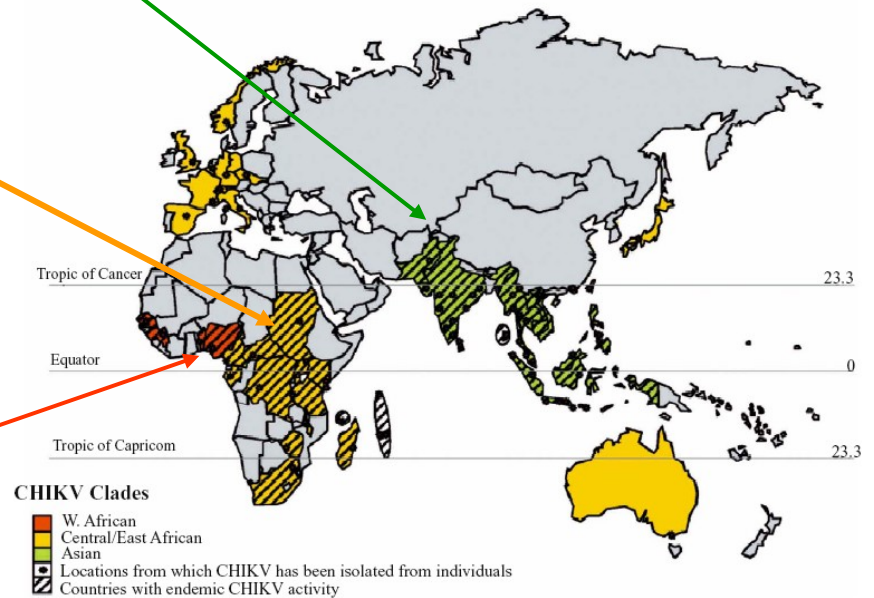


**Spherical shape with a diameter of 42 nm
composed of a 25–30 nm core**

Phylogram of 99 CHIKV E1 sequences demonstrating the main genotypes and close relationships among the lineages from each genotype based upon geography and time of the outbreak.



Geographical Distribution of Chikungunya Virus



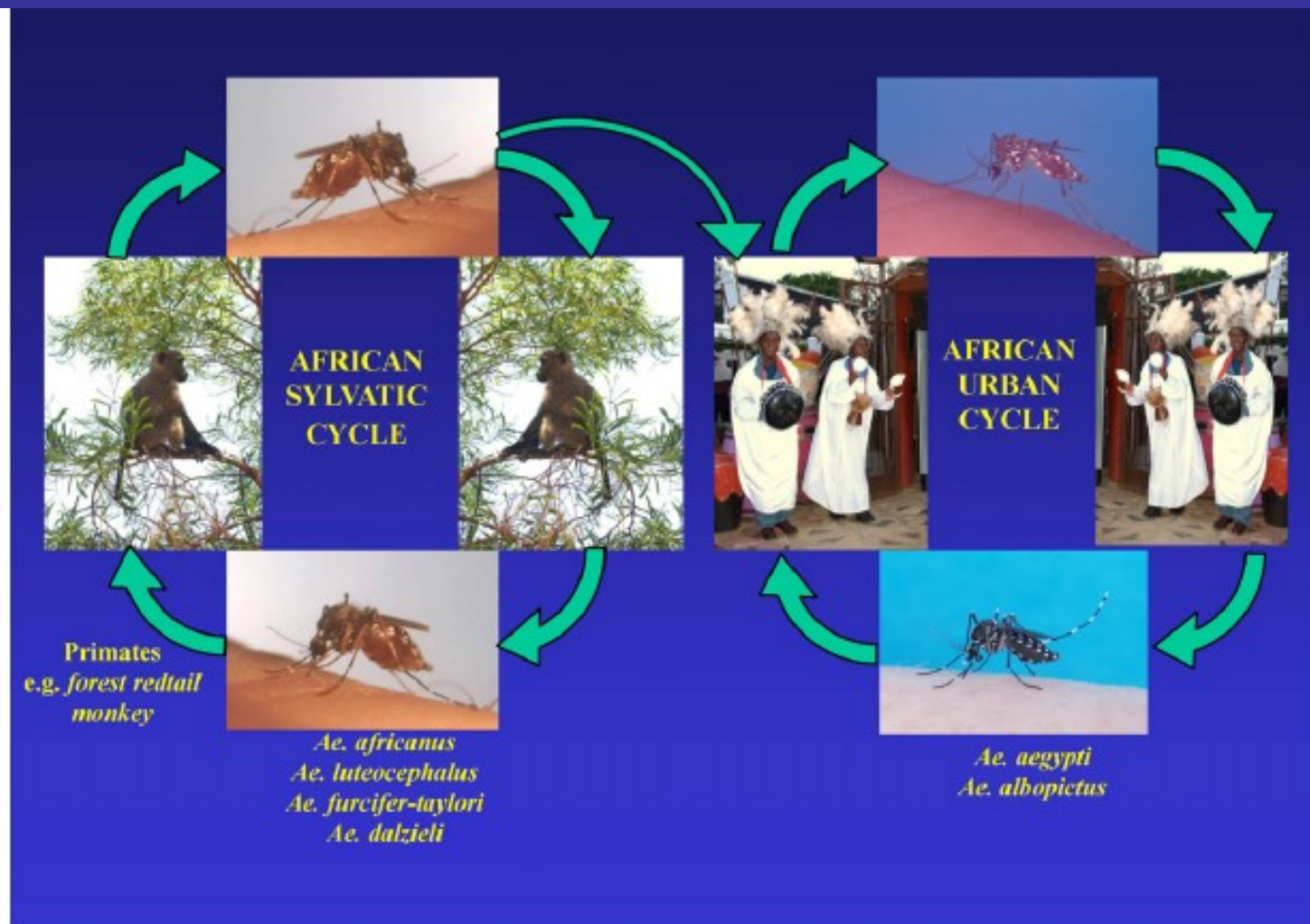


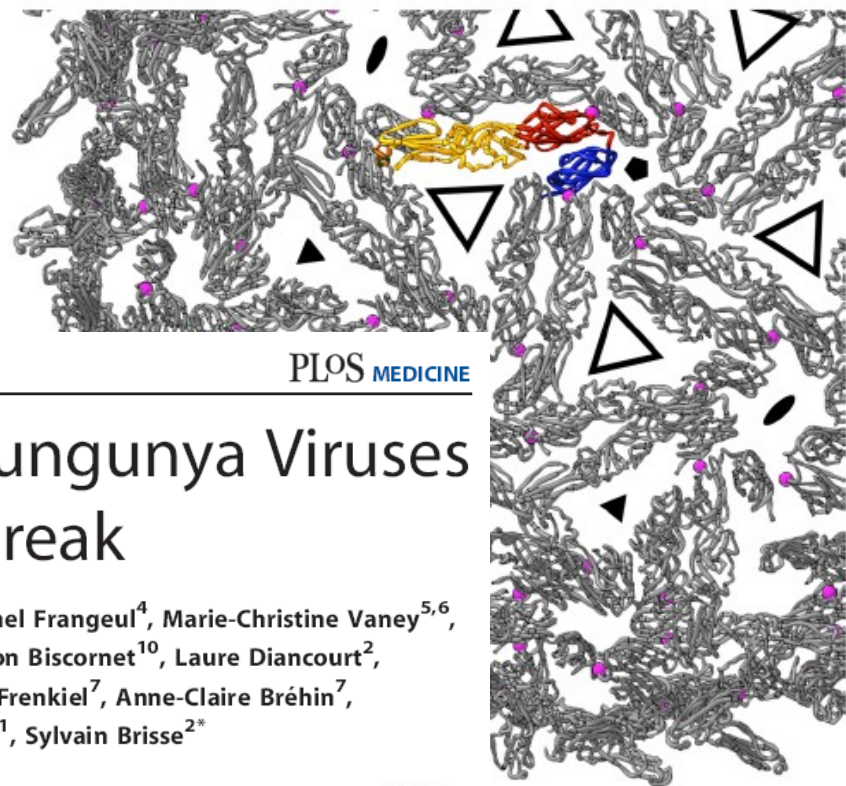
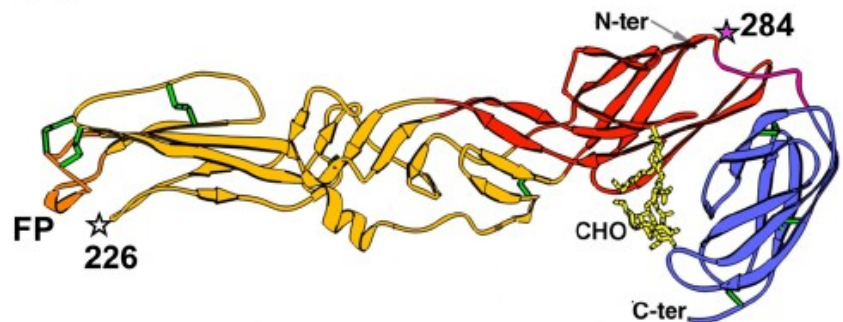
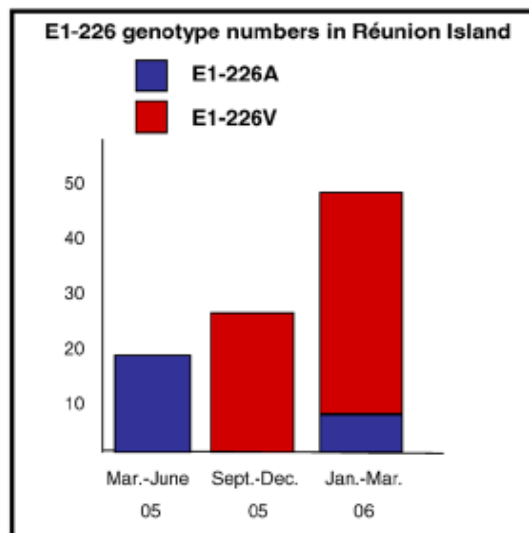
Figure 1 Representation of chikungunya virus life cycle in Africa.

Chikungunya virus exists in Africa in a forest cycle involving baboons and other primates and forest species of mosquitoes. It can also be transmitted in a human-mosquito-human cycle by *Aedes aegypti*.

Gould EA, Higgs S. *Trans R Soc Trop Med Hyg* (2008),

Chikungunya Fever: why this huge outbreak?

- Virus changes: the strain causing the outbreak in Indian Ocean contains a mutation at residue 226 of the membrane fusion glycoprotein E1 (E1-A226 V). It can cause better adaptation to the vector and make strain more virulent for humans;
- Vector changes: *Aedes albopictus* involved in La Reunion (in previous outbreaks *Aedes aegypti* involved), that is adapted to urban and semi-urban ecosystems;
- Environmental and human-induced changes: modification due to agriculture, climate changes, introduction of *Aedes* in new areas with trade and travel



OPEN ACCESS Freely available online

PLOS MEDICINE

Genome Microevolution of Chikungunya Viruses Causing the Indian Ocean Outbreak

Isabelle Schuffenecker^{1*}, Isabelle Itman², Alain Michault³, Séverine Murri¹, Lionel Frangeul⁴, Marie-Christine Vaney^{5,6}, Rachel Lavenir², Nathalie Pardigon⁷, Jean-Marc Reynes⁸, François Pettinelli⁹, Leon Biscornet¹⁰, Laure Diancourt², Stéphanie Michel¹, Stéphane Duquerroy^{5,6,11}, Ghislaine Guigon², Marie-Pascale Frenkiel⁷, Anne-Claire Bréhin⁷, Nadège Cubito¹, Philippe Desprès⁷, Frank Kunst¹², Félix A. Rey^{5,13}, Hervé Zeller¹, Sylvain Brisse^{2*}

July 2006 | Volume 3 | Issue 7 | e263

Figure 1. Localization of the E1 Changes on the 3D Structure Modelled from the Crystal Structure of SFV E1

Short report

Open Access

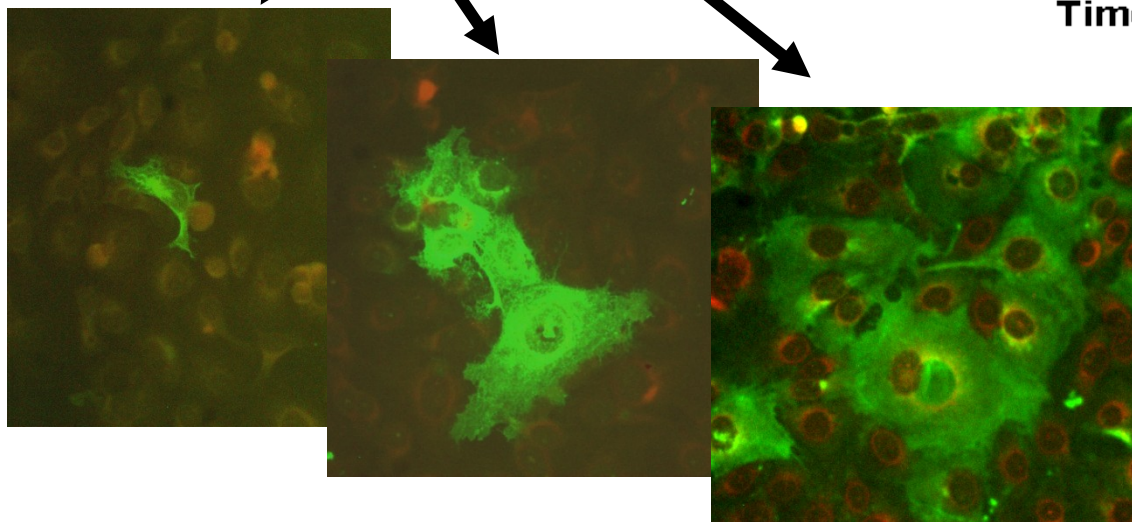
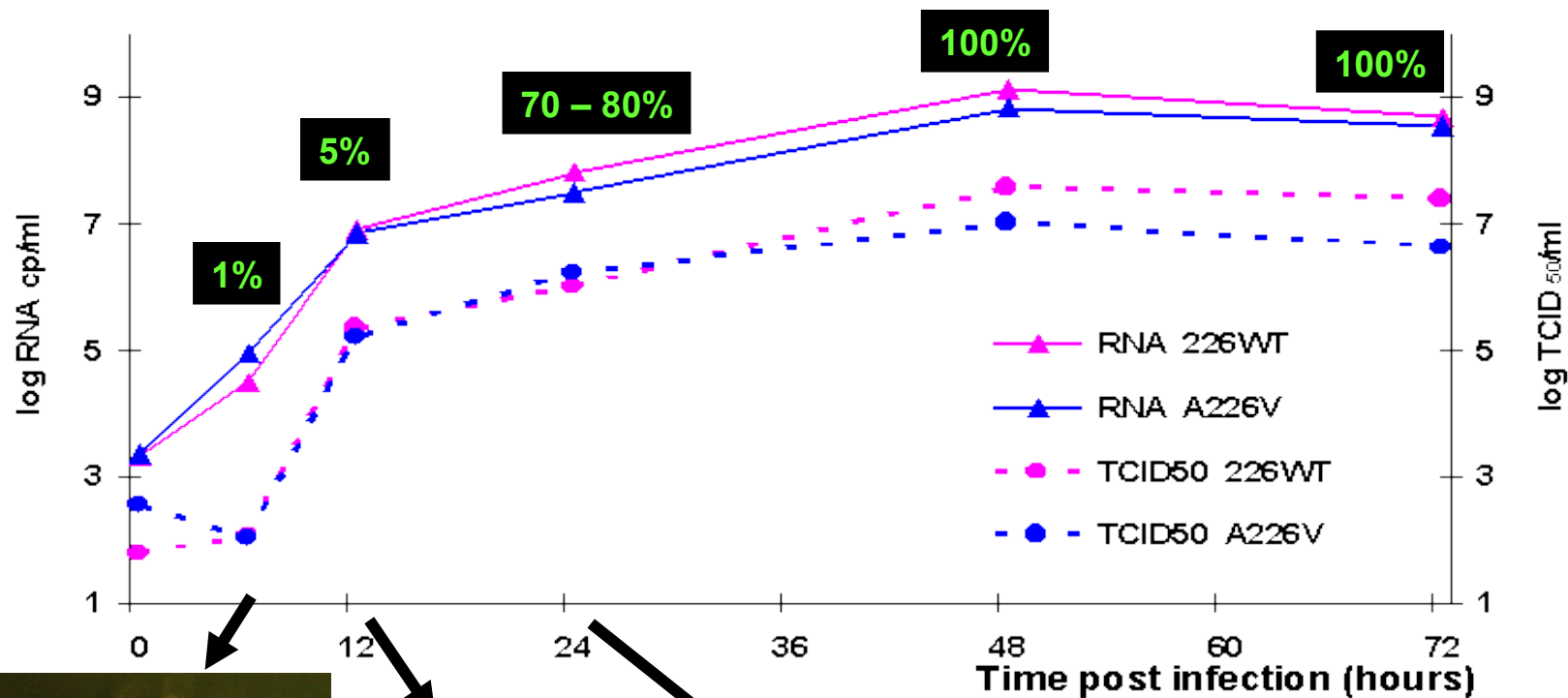
Chikungunya virus adapts to tiger mosquito *via* evolutionary convergence: a sign of things to come?

Xavier de Lamballerie^{*1}, Eric Leroy², Rémi N Charrel¹,
Konstantin Ttsetsarkin³, Stephen Higgs³ and Ernest A Gould¹

Analysis of fulllength viral sequences reveals three independent events of virus exposure to *Ae. Albopictus*, each followed by the acquisition of a single adaptive mutation providing selective advantage for transmission by this mosquito.

This disconcerting and current unique example of "evolutionary convergence" occurring in nature illustrates rapid pathogen adaptation to ecological perturbation, driven directly as a consequence of human activities.

Influence of A226V on CHIK replication in primate cells



MOI 00.1

Parola et al. EID 2006



Bone scintigraphy of the wrists and hands showing an intense focus of technetium-99m-labeled methylene diphosphonate tracer uptake



Fig. 4. Clinical manifestations of CHIK infection. (A) Edematous exanthema of the face (acute stage). (B) Raynaud's phenomenon at the third month after disease onset (chronic stage). (C) Polyarthrititis in hands and hypertrophic tenosynovitis in wrists at the third month after disease onset (chronic stage). (D) Bursitis of dorsal side of the hand (chronic stage). (E) Chronic swelling and stiffness of the fingers with loss of grip strength (chronic stage).

Manifestazioni cliniche

- ☺ Incubazione 3-12 giorni (generalmente 3-7)

Malattia generalmente autolimitante

con andamento bifasico:

- ☺ I^a fase (6-10gg):

febbre, cefalea, importanti artralgie che possono persistere a lungo.

- ☺ II^a fase (2-3gg):

esantema maculo-papulare pruriginoso, ricomparsa della febbre



Down To Earth



Ishwar Bhat, Karnataka. People in Mani village in Bantwal taluk of Dakshin Kannada district complain their phones are not working. The reason: three of the five BSNL linemen have contracted chikungunya. Ishwar Bhat, 48, senior telephone operator assistant, is one of them. The place has overhead lines and it was impossible for the linemen to climb up the poles to correct the faults. On the evening of May 30, Bhat's wrists started hurting. When he woke up the next day, he could barely open his fingers. For two days, he moved around the house on all fours. He had to tie a rope in the toilet for support. Fever and rashes were nothing compared to the pain.

That's the chikungunya peculiarity: its lingering debilitation takes away the victim's productivity and hits family incomes for weeks or months.

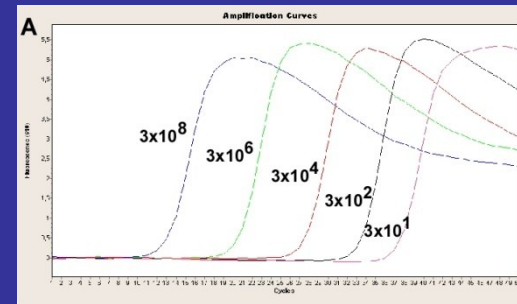
Short Report: Rapid Detection and Quantification of Chikungunya Virus by a One-Step Reverse Transcription–Polymerase Chain Reaction Real-Time Assay

Fabrizio Carletti, Licia Bordi, Roberta Chiappini, Giuseppe Ippolito, Maria R. Sciarrone, Maria R. Capobianchi, Antonino Di Caro,* and Concetta Castilletti

National Institute for Infectious Diseases L. Spallanzani, Rome, Italy

CHIKUNGUNYA diagnosis

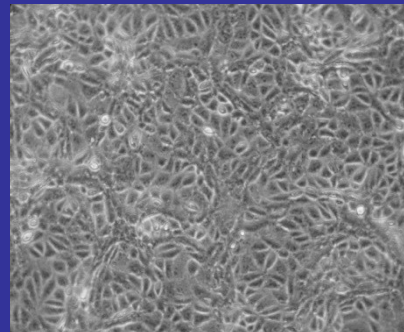
Clin. Samp. Isolates



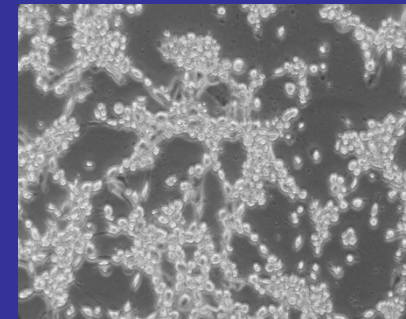
1) Molecular tests:
RT-PCR targeting *NsP1* and *E1*

Real-Time PCR targeting *NsP1*
(Carletti AmJTropMed Hyg 2007)

2) Viral isolation:
(C6/36, Vero E6)

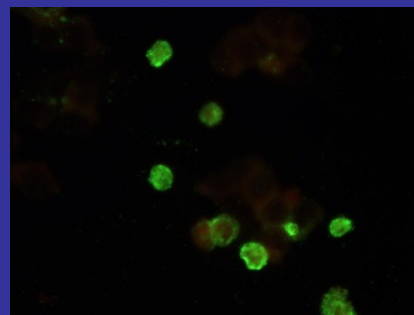


Vero E6 Mock

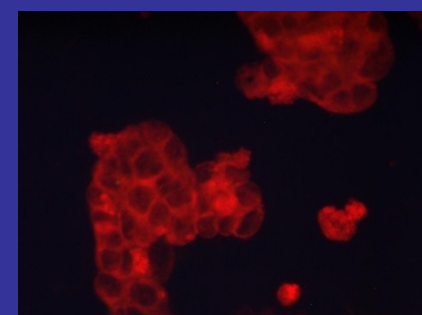


Vero E6 CPE

3) IFA (infected Vero E6)
Neutralization (Vero E6)



Positive serum



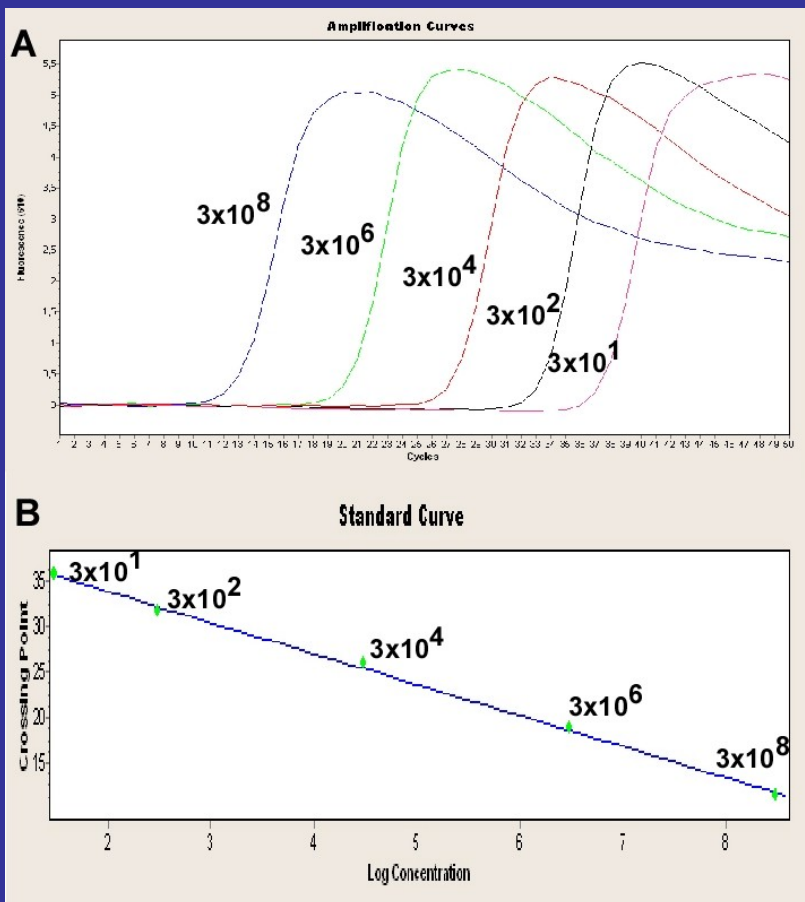
Negative control

Short Report: Rapid Detection and Quantification of Chikungunya Virus by a One-Step Reverse Transcription–Polymerase Chain Reaction Real-Time Assay

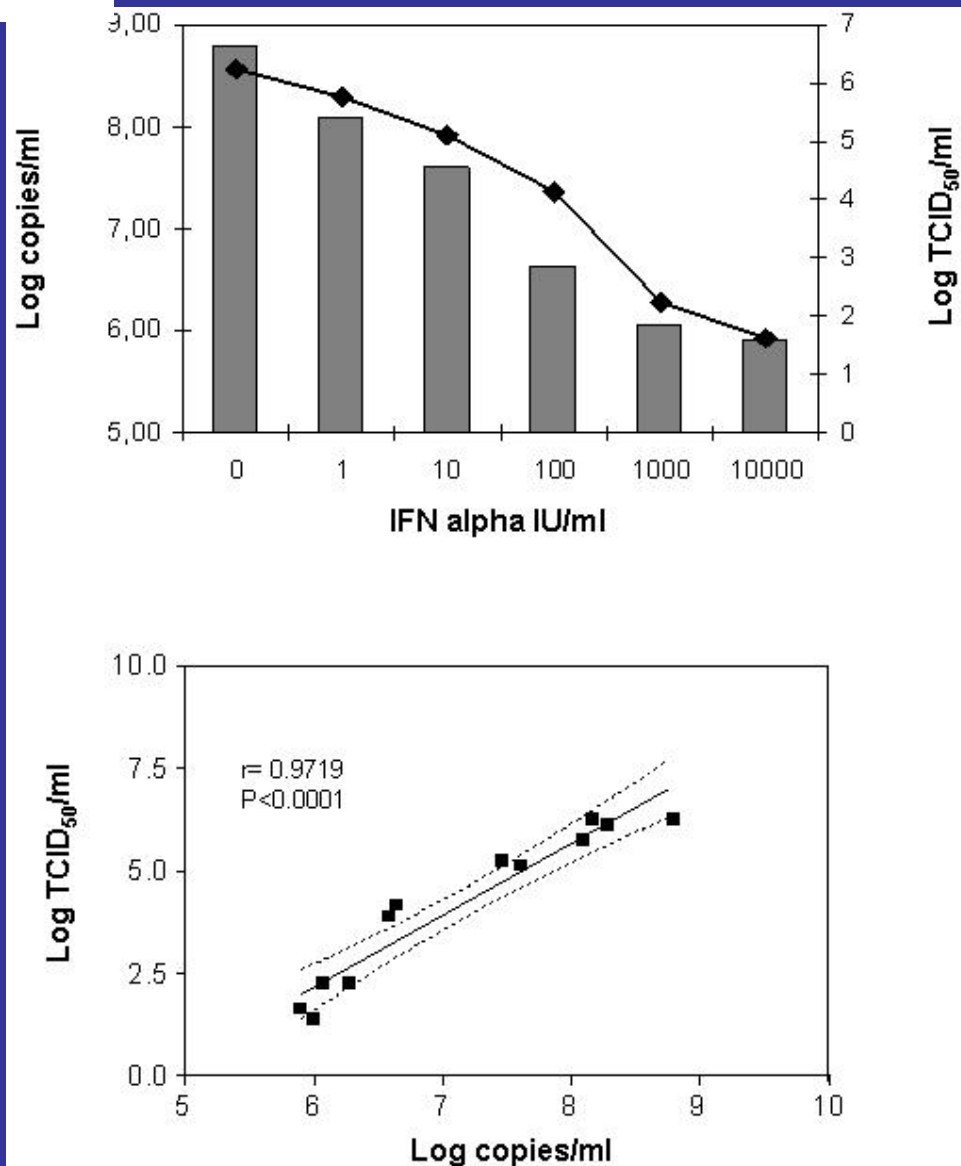
Fabrizio Carletti, Licia Bordin, Roberta Chiappini, Giuseppe Ippolito, Maria R. Sciarrone, Maria R. Capobianchi, Antonino Di Caro,* and Concetta Castilletti

National Institute for Infectious Diseases L. Spallanzani, Rome, Italy

Establishment of new quantitative real time PCR targeting Nsp1 of Chikungunya virus



Validation of qPCR by measuring inhibition of viral replication by IFN and correlating viral infectivity to viral RNA



Application of CHIKV qPCR to clinical samples

viremia was detected during the acute phase; viral load ranged from 1.3×10^5 to 5.9×10^8 cp/ml

Patient ID, gender , age	Travel history, beginning of symptoms	Sampling date	Ab IFA IgG (titers)	Ab IFA IgM	RT-PCR (nsP1 /E1)	qPCR (cp/ml)	Viral Isolation (strain designation)
CG L, M, 48	Mauritius 27/03/06	31/03/06	Neg***	Pos§	Pos	1.3×10^5	Neg
		28/04/06	$\geq 1:320$	Pos	nd*	Neg**	nd
TAM, M, 45	Mauritius 03/04/06	05/04/06	Neg	Neg***	Pos	1.3×10^8	Pos (CHKV ITA1 TAM)
		27/04/06	$\geq 1:320$	Pos	Neg	Neg	nd
RS, M, 56	Seychelles 30/05/06	06/06/06	Neg	Neg	Pos	2.2×10^7	nd
		10/10/06	1:320	Neg	nd	Neg	nd
MR, F, 35	India 07/09/06	09/09/06	Neg	Neg	Pos	5.9×10^8	Pos (CHKV ITA3 MR)
		29/09/06	1:320	Pos	nd	nd	nd

*nd: not done; ** neg:< 4×10^3 cp/m; *** neg:<1:20; § pos:>1:20 (titre if available)

Casi in Europa di Chikungunya in viaggiatori di ritorno da aree endemiche riportati dall' ECDC a giugno 2006:

☀ 307 casi in Francia importati + 1 caso autoctono (incidente)

☀ 17 casi in Germania

☀ 12 casi in Belgio

☀ 9 casi nel Regno Unito

☀ 1 caso in Norvegia

☀ 1 caso nella Repubblica Ceca

☀ 11 casi in Italia



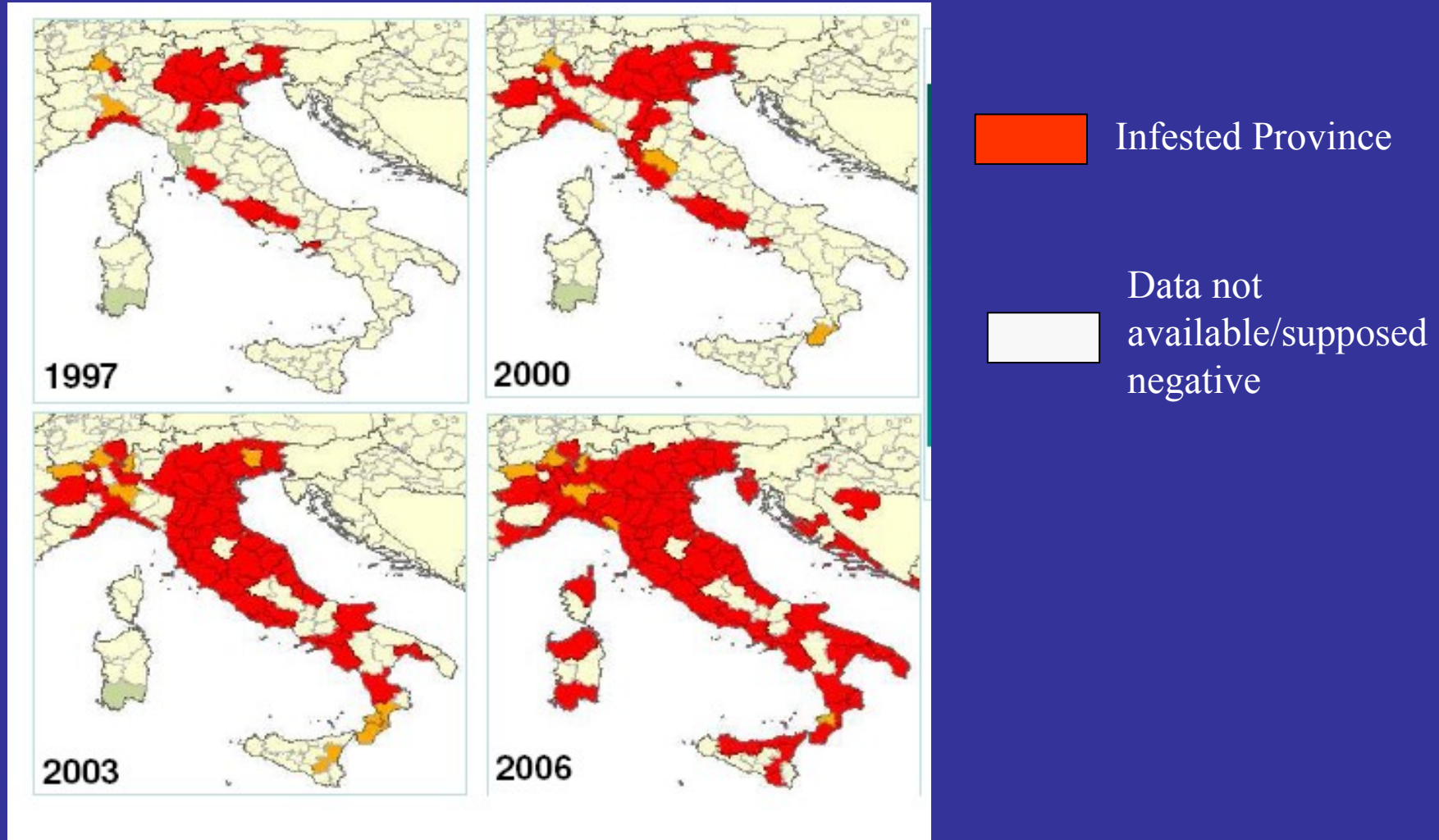
Concreta possibilità di casi autoctoni and possibilità di instaurazione di una catena di trasmissione locale per la presenza del vettore (Ae. Albopictus)



PCAS FEHD HK SARG

Chikungunya Fever: Reasons for Concern for Italy

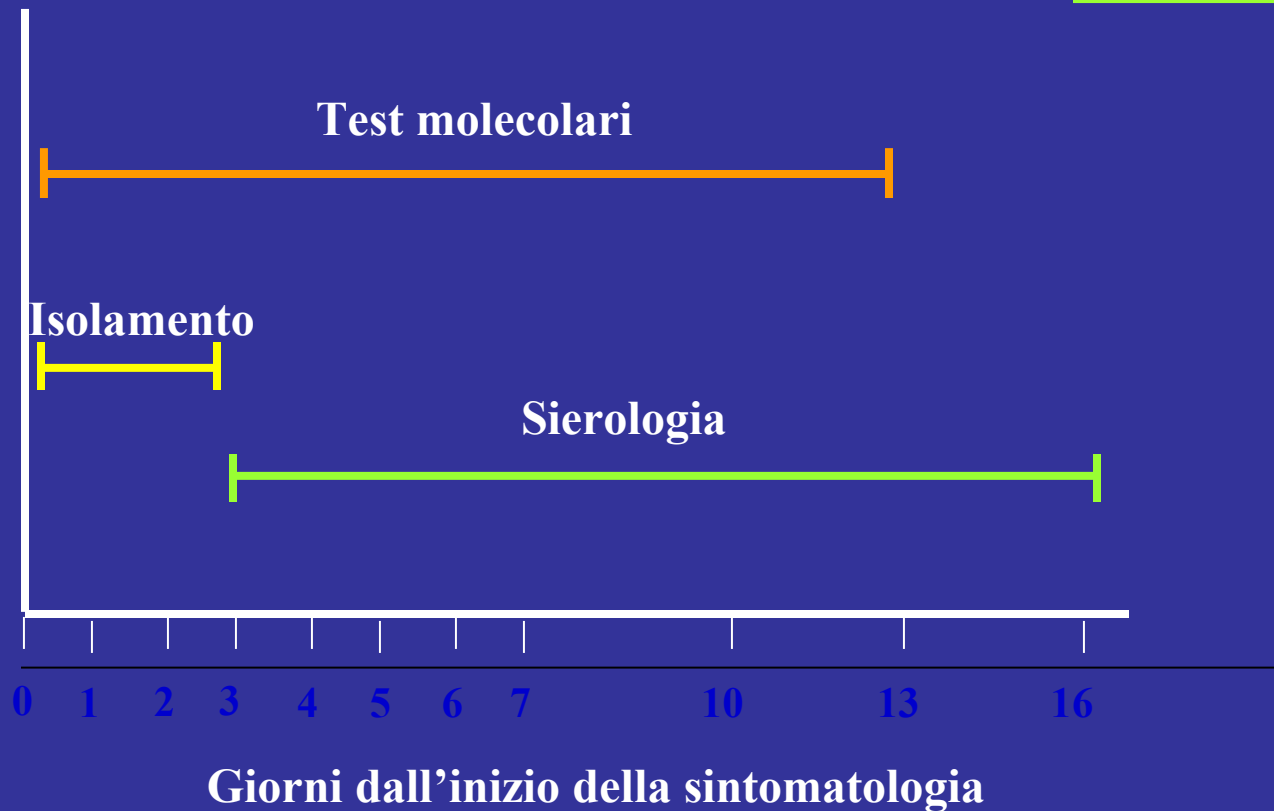
Aedes Albopictus in Italy



Source: Istituto Superiore di Sanità – ISS

National Reference Centre for *Ae. Albopictus* Surveillance

Chikungunya: Approccio Diagnostico



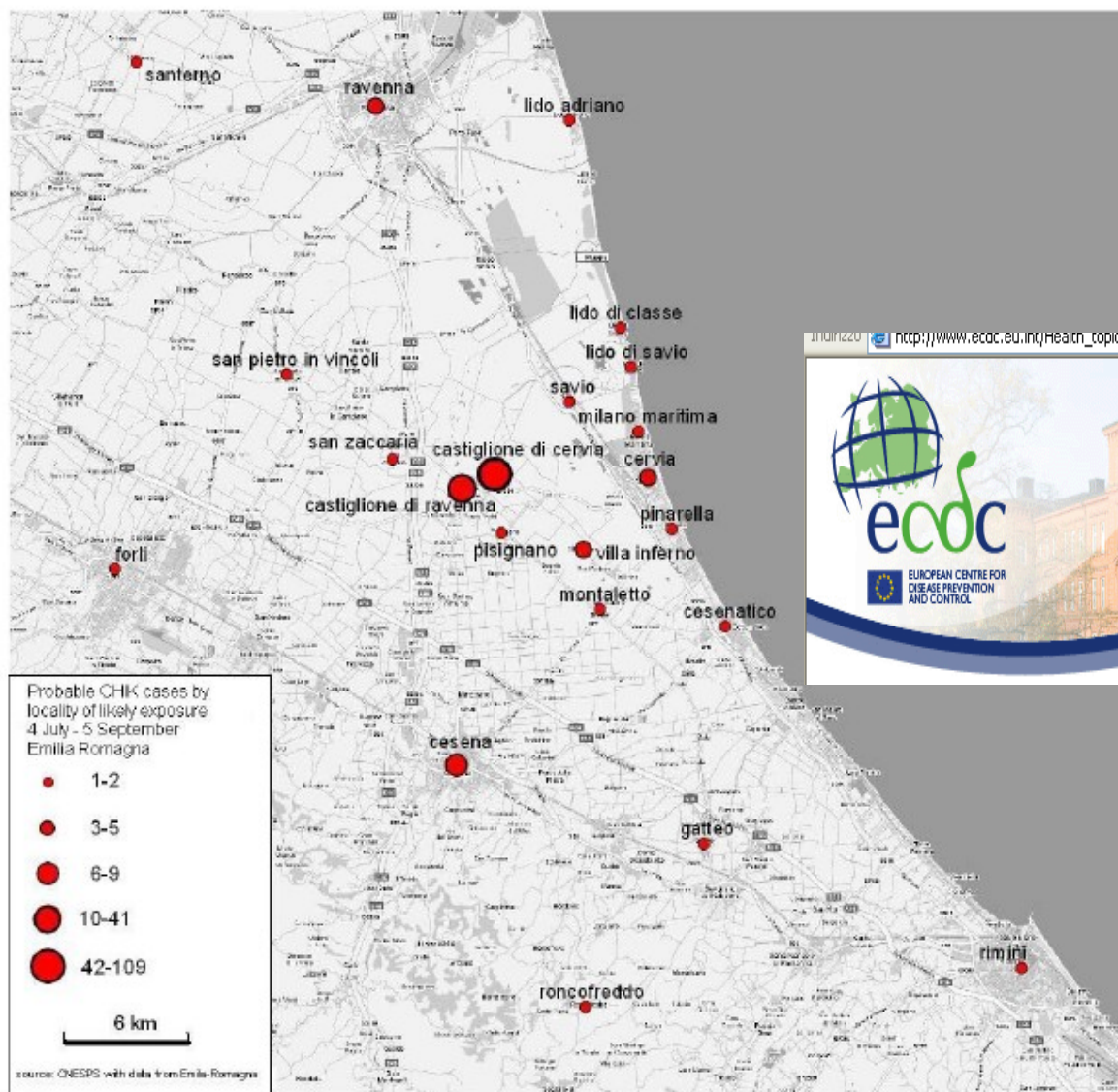
Ravenna IT

23 giugno 2007

Kerala India

Image NASA
Image © 2008 TerraMetrics



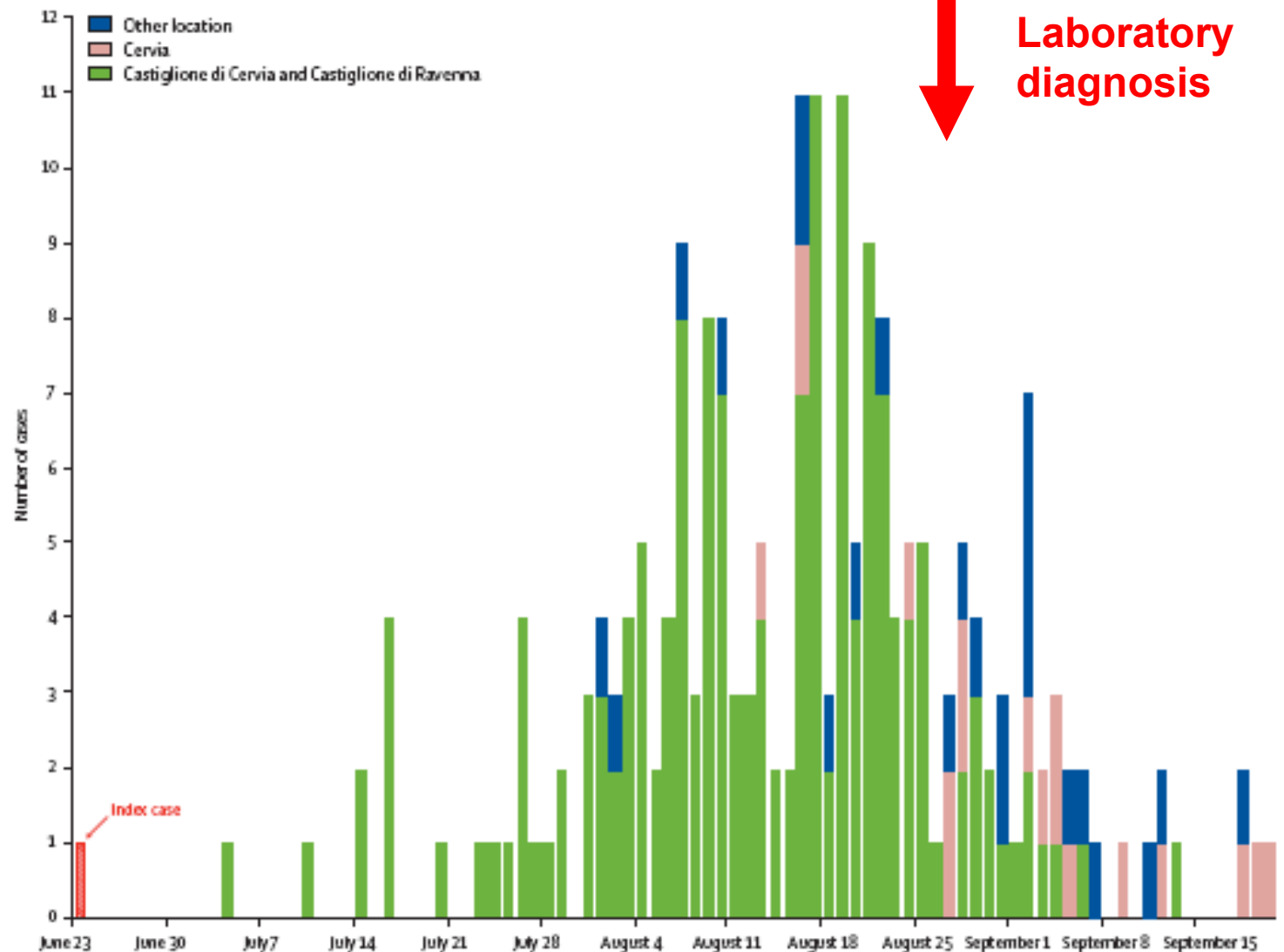


http://www.ecdc.eu.int/Health_topics/Chikungunya_Fever/italy/070904_11A_chikungunya.nm1



*Dati CNESPS, REMI e
Regione Emilia Romagna*

Distribution of dates of onset of symptoms for CHIKV cases by presumed place of infection- Italy 2007



[Indicod-Ecr e GS1](#)

[Economia e Marketing](#)

[EPC/Rfid e Tecnologie](#)

[Supply chain](#)

[Dossier](#)

[Opinioni](#)

[Homepage](#) > [Notizie del giorno](#)

VIRUS CHIKUNGUNYA, MINISTERO SALUTE: SORVEGLIANZA IN TUTTA ITALIA



Chikungunya Fever Outbreak in Italy:

from 15/06/2007 until 17/10/2007

Laboratory confirmed cases *	200
Probable cases	45
Excluded cases	81

**according to the case definition agreed with ECDC*

A total of **245** probable/confirmed cases
from three provinces (Ravenna, Forlì/Cesena, Rimini)

Sources: Regione Emilia Romagna – CNESPS - Ministry of Health

Courtesy of L. Fellucci – M.G. Pompa, Italian MoH



Chikungunya.net

Chikungunya

Albopictus

FAQ

Presse

Forums

Témoignages

AVEC

SIRIUS

Adresse

videos

TELECHARGEZ
l'album PEDAGOGHIK

☒ site ☐ Web

OK

Dernières nouveautés et actualités

1. Point mensuel : 22 suspectés, aucun confirmé
2. Sida, le patient zéro : haïtien ou canadien ?
3. Evaluation des risques du chik en Europe
4. Evaluation des pesticides utilisés à La Réunion
5. Point mensuel : 3 chik et 2 denque suspectés

Vous avez eu le Chikungunya en 2006
COMMENT ALLEZ-VOUS EN 2007
Participez à notre enquête express

Bulletin épidémique hebdomadaire

Revue de presse

Forums

Chik dans le monde

Chik en Italy

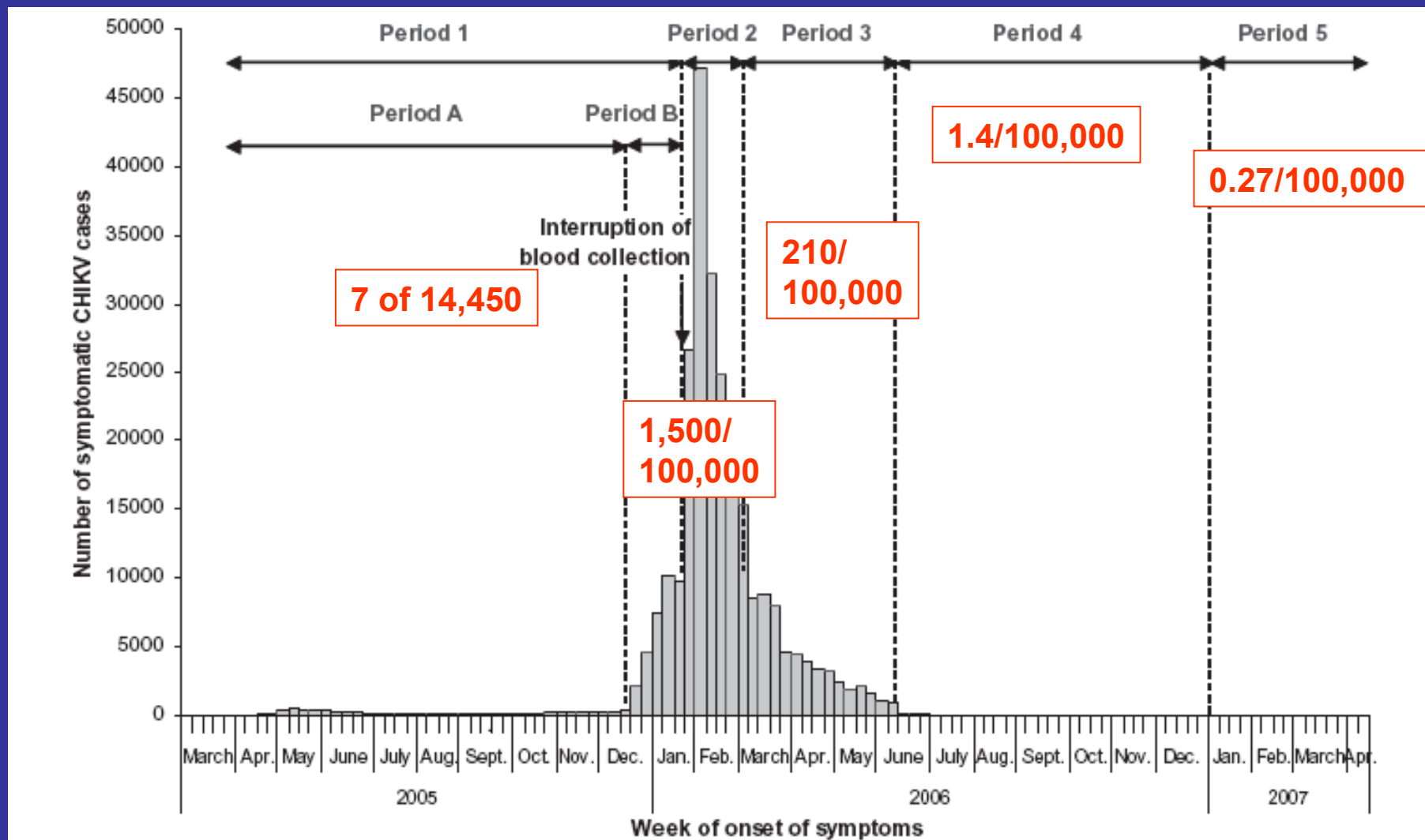
Grippe Aviair



Dichiarazione di cessazione dell'epidemia

- **In data 20 novembre 2007 il Ministero della Salute dichiara l'estinzione dell'epidemia,**
- ***Il 22 novembre teleconferenza Ministero della Salute/ISS/Regione Emilia-Romagna/ECDC/OMS per formalizzare la chiusura dell'evento.***

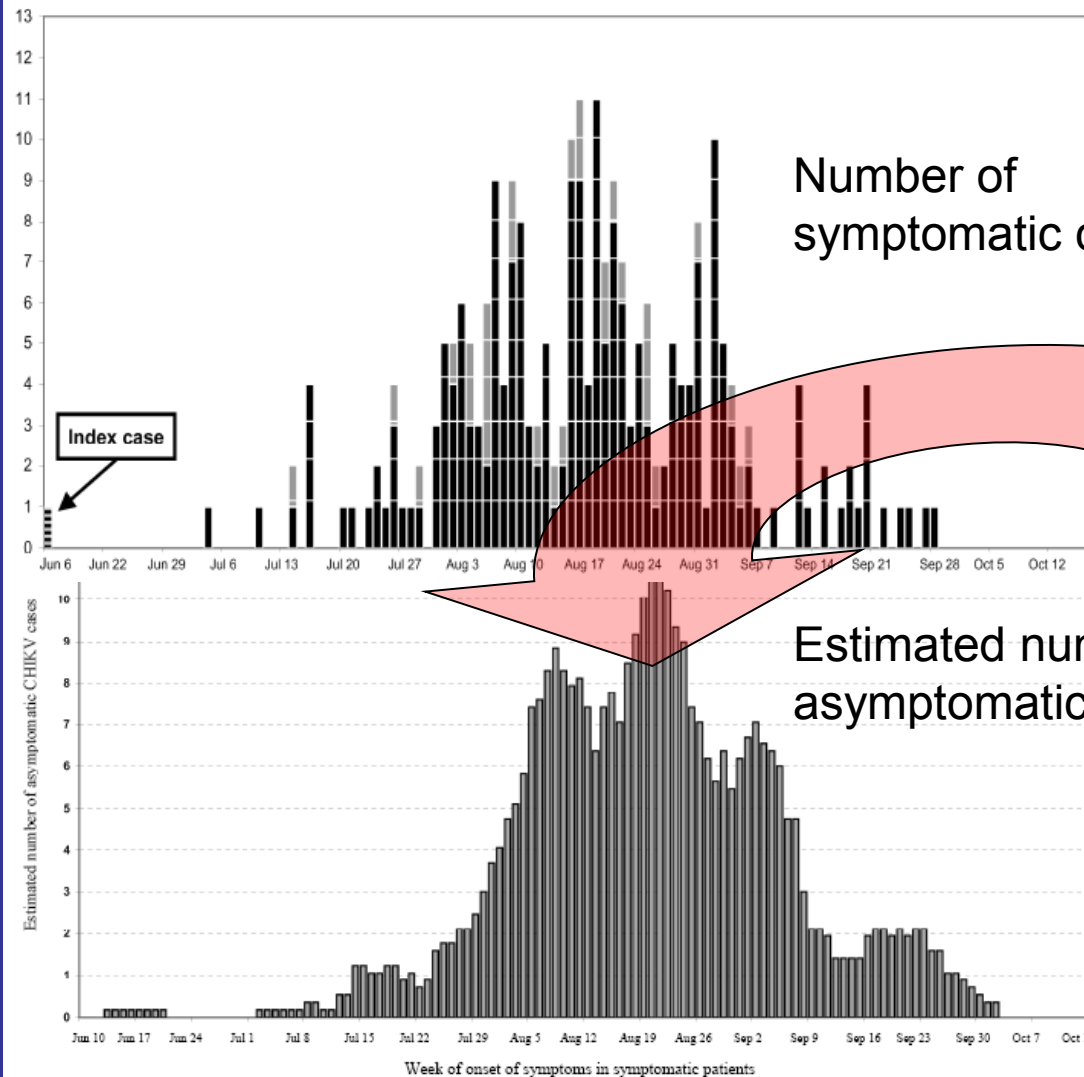
Assumed 7.5 days for the mean duration of viremia in asymptomatic individuals



Réunion (March 2005-April 2007): potentially **47 of 35,750 blood donations** might have been viremic if blood collection had not been interrupted (total infected estimated: 312,538)

The collection of blood donations on the island start again from June 14, 2007.

Mathematical model to assess the risk of blood donations from asymptomatic donors



**Number of
blood
donations
necessary to
yield an
infected blood
unit:
~1:100,000**

Total distribution of estimated number of asymptomatic cases of CHIKV infection per week, in the Region of Emilia-Romagna, Italy, June 10th, 2007, through October 14th, 2007

The Chikungunya epidemic in Italy and its repercussion on the blood system

Giancarlo Maria Liunbruno^{1,2}, Deanna Calteri^{1,6}, Kyriakoula Petropulacos³, Andrea Mattivi⁴, Claudio Po⁴, Pierluigi Macini⁴, Ivana Tomasini⁵, Paolo Zucchelli⁶, Anna Rita Silvestri⁶, Vittorio Sambri⁷, Simonetta Pupella¹, Liviana Catalano¹, Vanessa Piccinini¹, Gabriele Calizzani¹, Giuliano Grazzini¹

	September 2007																														October 2007															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
Ravenna	39 days																																													
Cervia	44 days																																													
Cesena		38 days																																												
Cesenatico		12 days																																												
Rimini																					21 days																									

Figure 4 - Periods of suspension of blood donations in the blood transfusion centres in the areas affected by the Chikungunya epidemic

The precautionary measures adopted - **justified by the unavailability of a laboratory test for routine biological qualification of blood components** - produced a considerable impact on the blood supply of Emilia-Romagna that changed its role from being an exporter of blood components to being an importer.

Table II - Impact on regional blood supply of precautionary measures adopted in the Region of Emilia-Romagna as from 1st September, 2007

	RBC (units)	FFP [°] (litres)
August	725*	613.73*
September	3748 [§]	1809.08 [§]
October	657 [#]	448.37 [#]
Total	5130	2871.18

°: includes apheresis and whole blood fresh-frozen plasma (FFP).

*: eliminated by blood transfusion centres (BTC) or plasma manufacturer.

§: uncollected by all the regional BTC (compared to blood collection data of the same month of 2006).

#: uncollected by the BTC involved in the epidemic (compared to blood collection data of the same month of 2006).

Several factors seem to have contributed to the establishment of local transmission in continental Europe:

- The presence in high density of *Aedes albopictus* in an area of the Emilia-Romagna region where it had appeared relatively recently and was therefore not yet covered by the vector monitoring system;
- An ecological situation favourable for the development of the vector and for virus transmission, considering the dense local vegetation and domestic backyards with plant pots and potential water containers in the two villages;
- The introduction of the virus by a visitor returning from a chikungunya high endemic area;
- Sufficient (human) population density

The outbreak of chikungunya fever in North-Eastern Italy is the first documented local vectorborne transmission of Chikungunya virus within the European mainland.

- The importance of this event should not be underestimated and its comprehensive documentation is crucial to ensure a maximal benefit to control its spread and for future preparedness in case of similar occurrences of mosquito-borne disease transmission, in Italy and elsewhere.
- It cannot be excluded that, in case of a particularly mild winter, local vector activity may persist during winter, especially in urban settings, potentially resulting in few sporadic cases which would, however, maintain mosquito-to-human transmission cycles until the spring.
- The virus could also be reintroduced through international travel.
- It is important that the winter period is used to **prepare as much as possible for the re-emergence of the virus**, in all areas in Europe where present.

Apart from chikungunya, *Aedes albopictus* is also a vector including Dengue virus.
Mission Report | Chikungunya in Italy, 17-21.09.2007
Joint ECDC/WHO visit for European risk assessment



Chikungunya in Europe: what's next?

Panel: Factors that might have contributed to epidemics of chikungunya fever (2004-07)⁴

Biological and genetic

Non-immune human populations

Genetic adaption in virus to *A albopictus* during epidemic⁵

Ecological

East Africa drought that promoted standing water and disease transmission⁶

Warm European summer with high abundance of vector³

Physical environment

Artificial vector-breeding sites (household water-stores, manholes, used tyres)

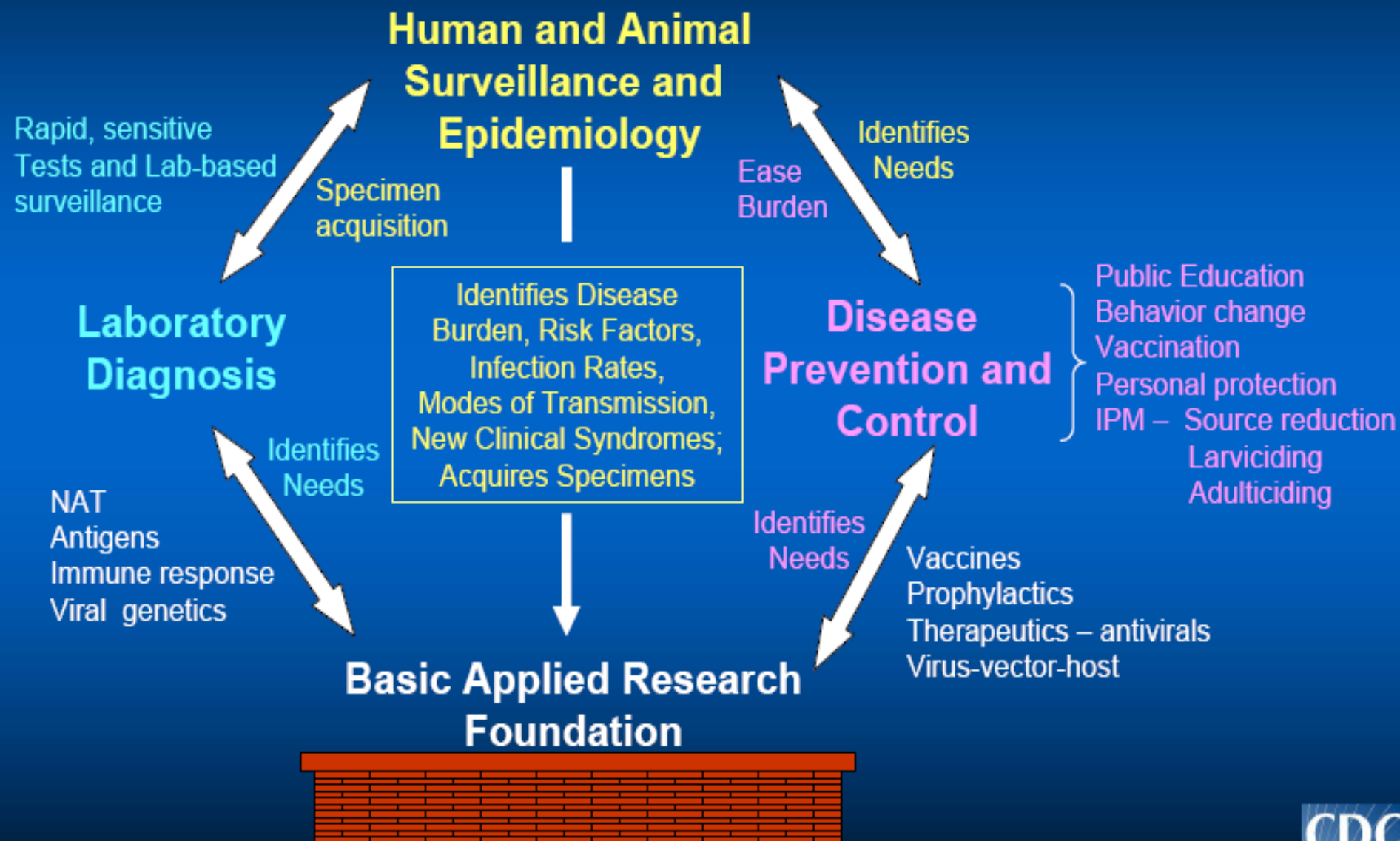
Social, political, and economic

International and domestic travel

Delayed identification and control of initial outbreaks

Previous introduction of exotic *A albopictus* to Indian Ocean islands and Italy

Vector-borne Viral Disease - An Integrated Approach



DENGUE

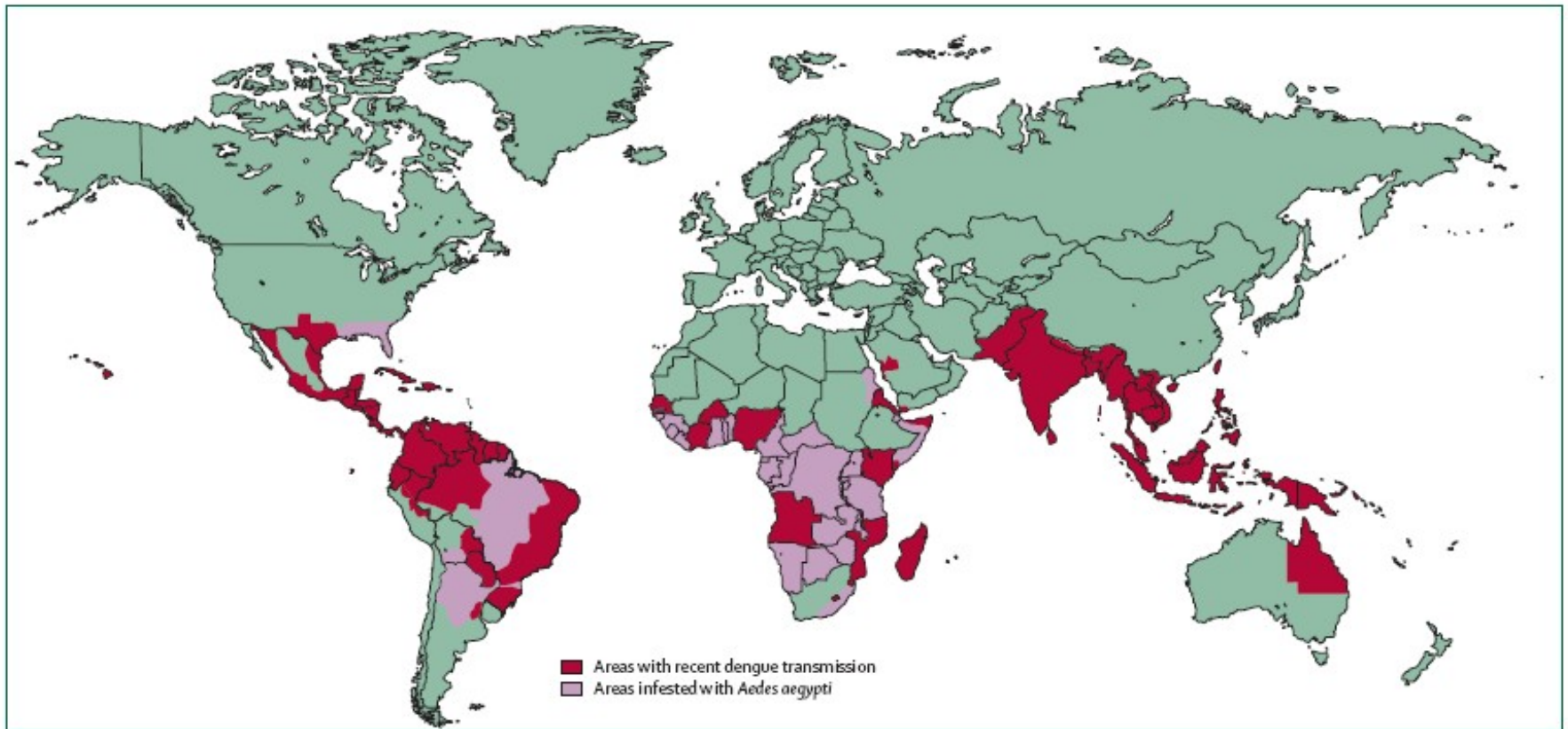


Figure 1: Approximate global distribution of dengue and *Aedes aegypti* in 2005
Reprinted with permission of the US Centers for Disease Control and Prevention.

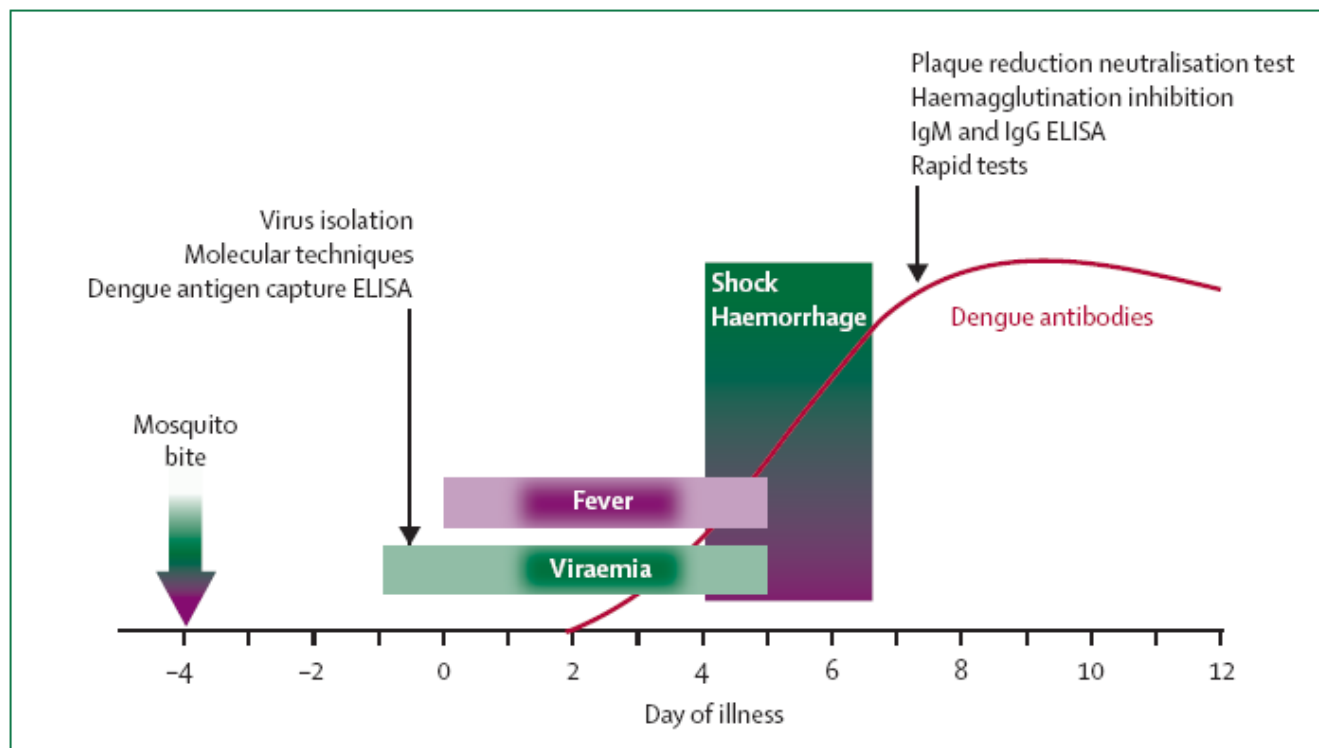


Figure 3: Course of dengue infection and timings of diagnosis

Published with permission of Timothy Endy, Syracuse University, Syracuse, NY, USA.

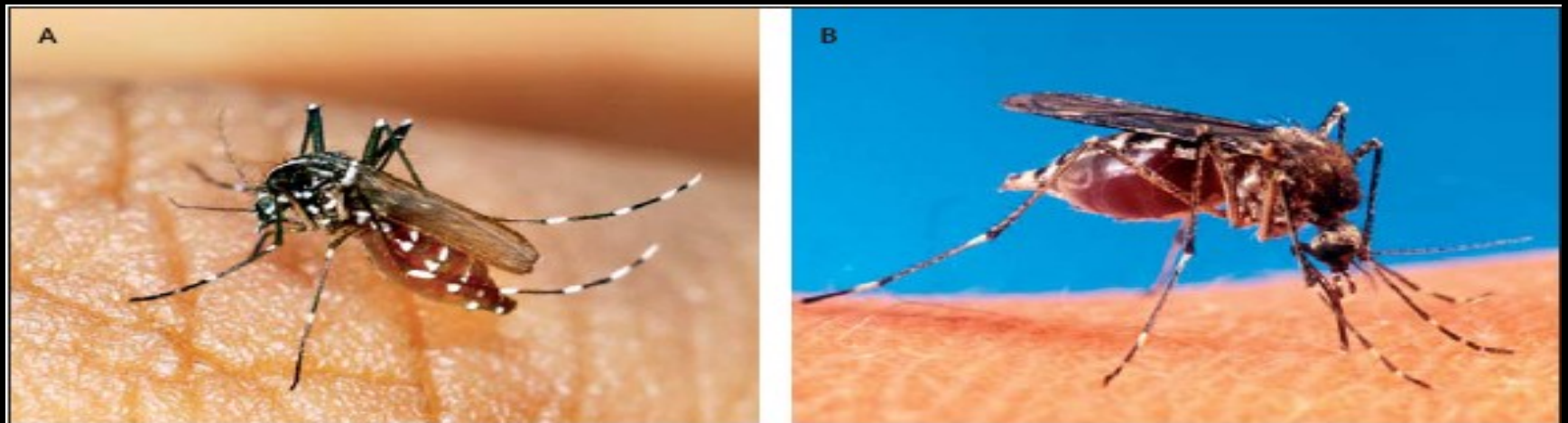


Figure 1: Mosquito vectors of chikungunya virus

(A) Blood-gorged *A albopictus* female feeding on a human host. *A albopictus* is the primary chikungunya virus vector in the current Indian Ocean outbreak. (B) *A aegypti* mosquito. *A aegypti* is the primary chikungunya virus vector in Asian chikungunya outbreaks. Images from United States Department of Agriculture.

