

20-21 MAY 2015

IPFA/PEI 22ND INTERNATIONAL WORKSHOP ON
"Surveillance and Screening of Blood Borne Pathogens"

Arboviruses in the Southern European Region

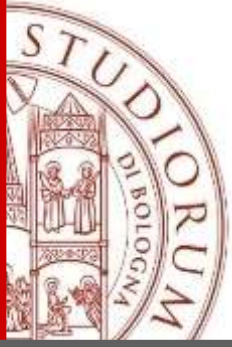
Prague – May 20th 2015

Vittorio Sambri

Alma Mater Studiorum - University of Bologna

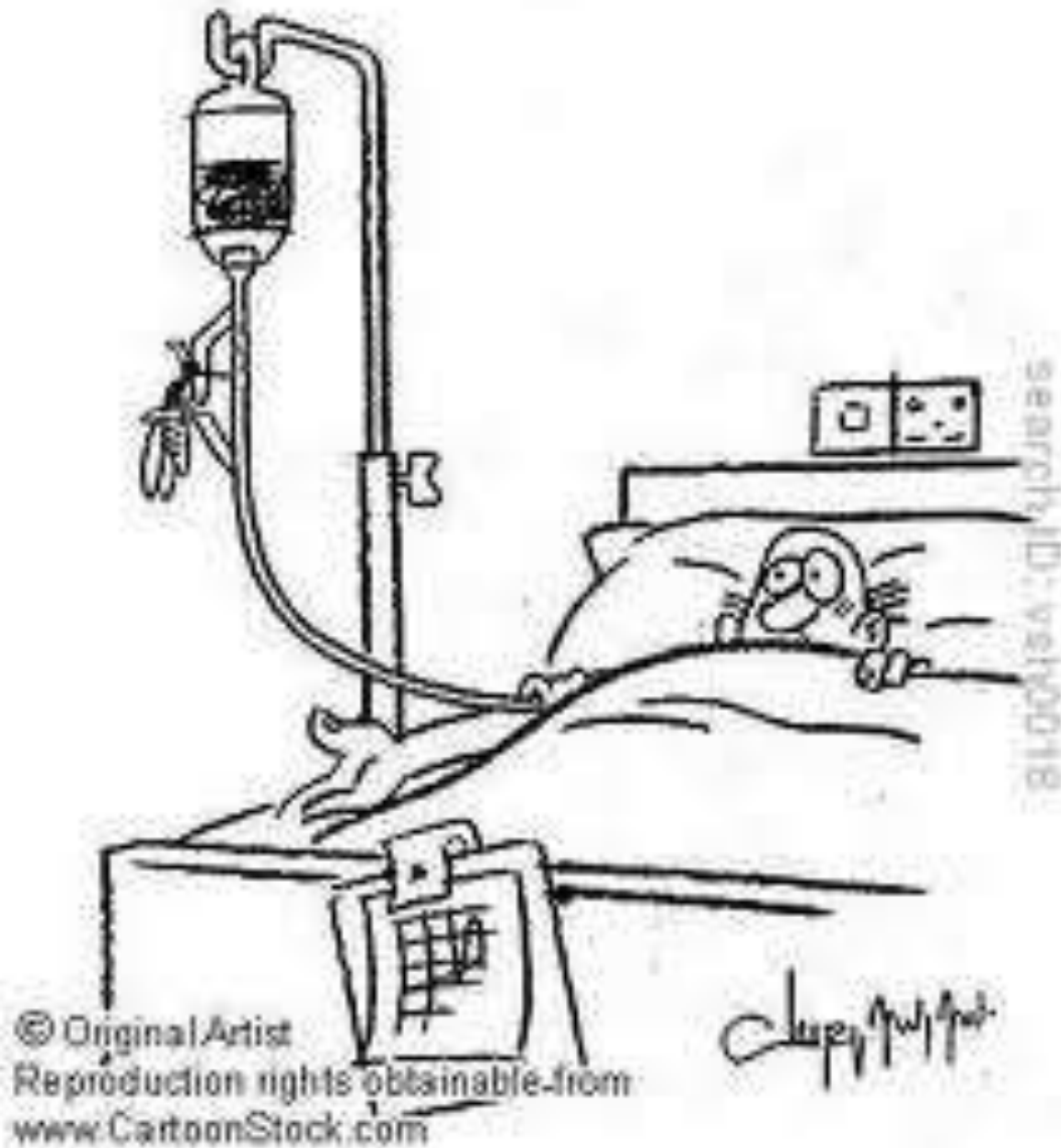
DIMES – Microbiology – The Greater Romagna Hub Laboratory





Potential Conflict of Interest disclosure

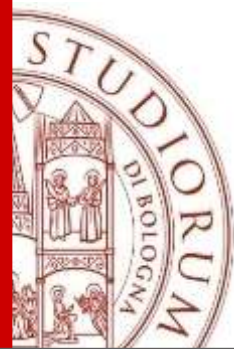
- Research grants from:
 - Roche Molecular Diagnostics
 - BioMerieux
 - Novartis Diagnostics
- Speaker fees from:
 - TerumoBCT
 - Roche Molecular Diagnostics
 - BioMerieux
 - Novartis Diagnostics - Grifols





ARBOviruses

- Heterogenous group of viruses
 - ARthropods (ticks – mosquitoes - sandflies)
 - BOrne
 - Flaviviruses
 - WNV
 - DENV
 - TBEV
 - USUV
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 - CHIKV
 - Bunyaviridae
 - CCHFV (Nairoviruses)
 - TOSV (Phleboviruses)



Flamingo Mosquito Meter



Hysterical



Horrible



Unpleasant



Bearable



Enjoyable



Mosquito Facts

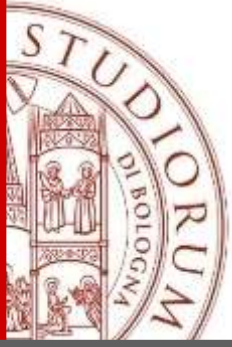
Flamingo's abundant mosquito is the Salt Marsh Mosquito (*Culiseta inornata*). Large swarms of mosquitoes can lay up to 10,000 eggs per square foot of salt in mangrove and coastal prairie habitats. They're generally most bothersome during the summer rainy season. University scientists and the U.S. military have selected Flamingo as one of the best places in the country to test mosquito repellents. The female is the blood sucker; she lays eggs on plant matter and feeds babies. Mosquitoes are eaten by dragonflies, birds, reptiles, and amphibians. In days gone by, when the "skunk" was bad, old timers in Flamingo would say... "you can swing a golf club in the air and get a quart of mosquitoes!" - A legend! If you're looking for a different mosquito, you have officially become part of the Flamingo bird world.

Flamingo Nat'l Park, FL.
2010



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West Nile virus in Europe: emergence, epidemiology, diagnosis, treatment, and prevention

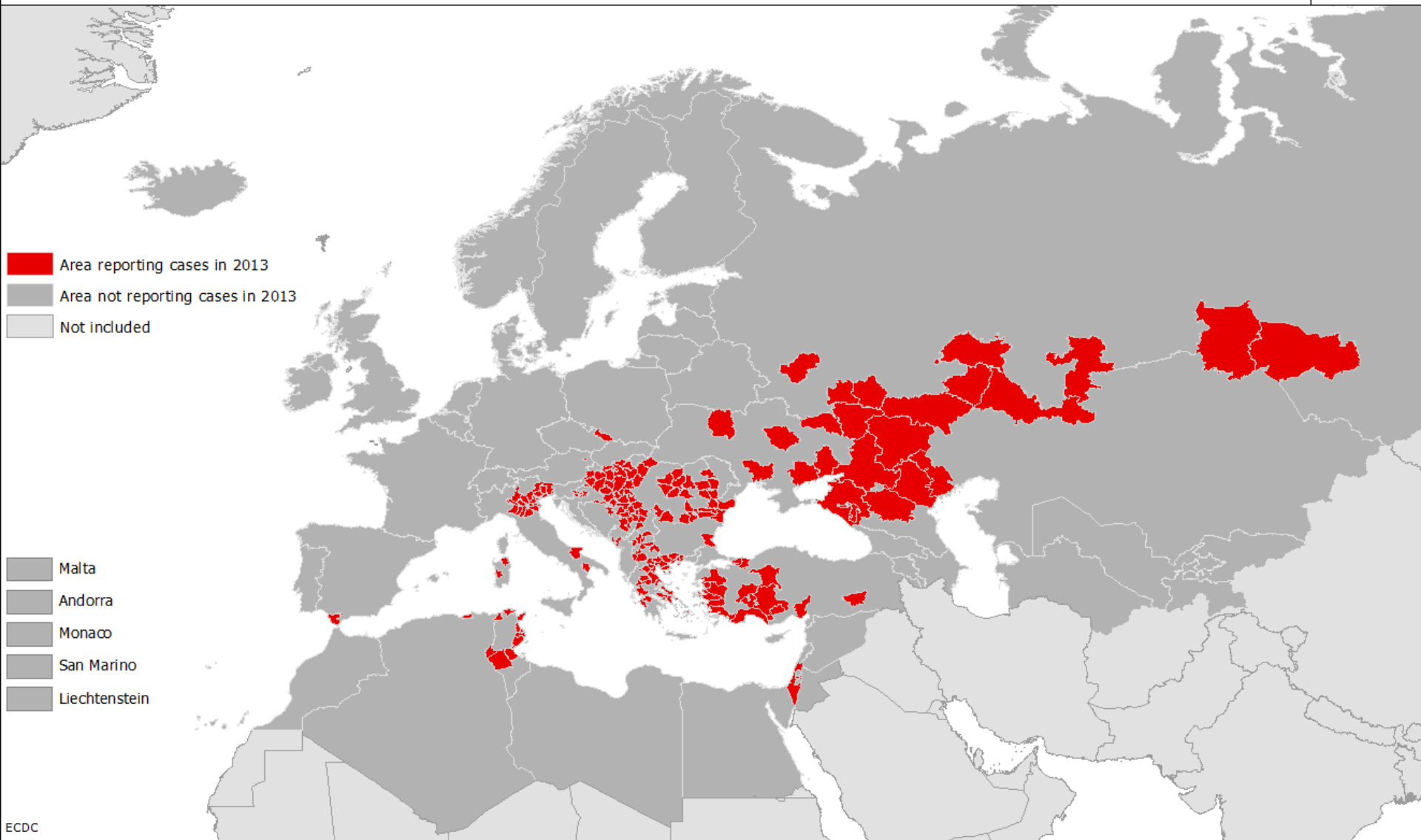
V. Sambri^{1,2}, M. Capobianchi³, R. Charrel^{4,5}, M. Fyodorova⁶, P. Gaibani⁷, E. Gould^{4,5,8}, M. Niedrig⁹, A. Papa¹⁰, A. Pierro⁷, G. Rossini⁷, S. Varani¹, C. Vocale⁷ and M. P. Landini^{1,7}

West Nile virus (WNV), a mosquito-borne flavivirus in the Japanese encephalitis antigenic group, has caused sporadic outbreaks in humans, horses and birds throughout many of the warmer regions of Europe for at least 20 years. Occasional cases of West Nile encephalitis have also been associated with infected blood transfusions and organ donations. Currently, WNV appears to be expanding its geographical range in Europe and causing increasing numbers of epidemics/outbreaks associated with human morbidity and mortality. This brief review reports on the current epidemic situation regarding WNV in Europe, highlighting the clinical, diagnostic and preventive measures available for controlling this apparently emerging human pathogen.

Clinical Microbiology and Infection, Volume 19 Number 8, August 2013

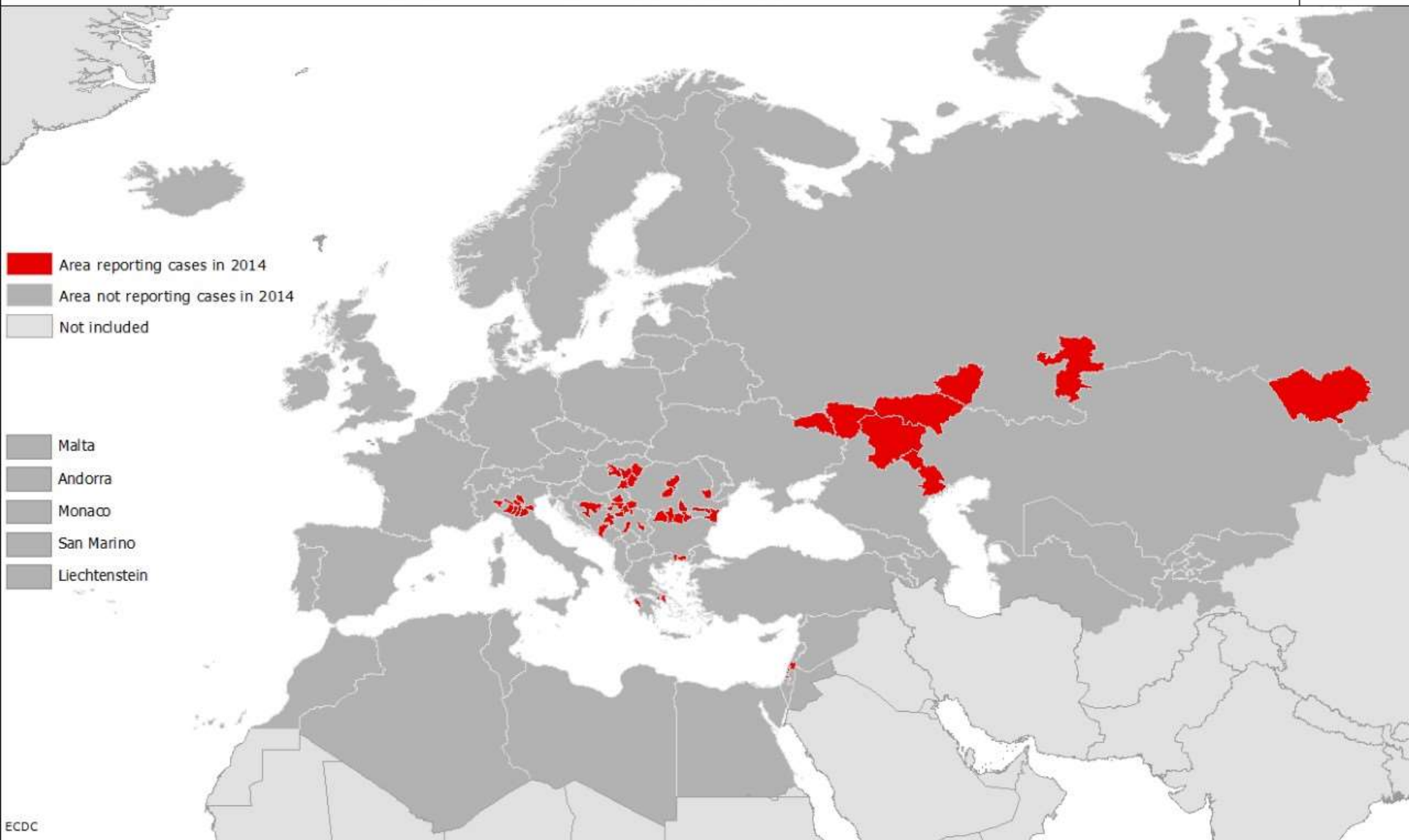
Distribution of West Nile fever cases by affected areas, European region/Mediterranean basin

Transmission season 2013 (latest update 05/06/2014)



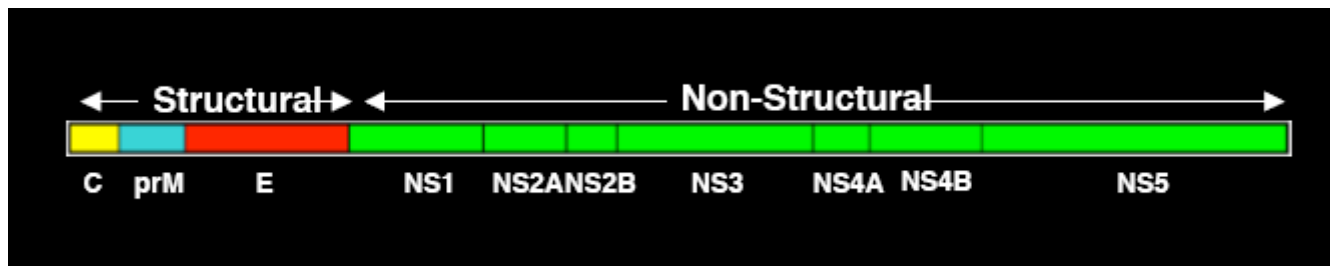
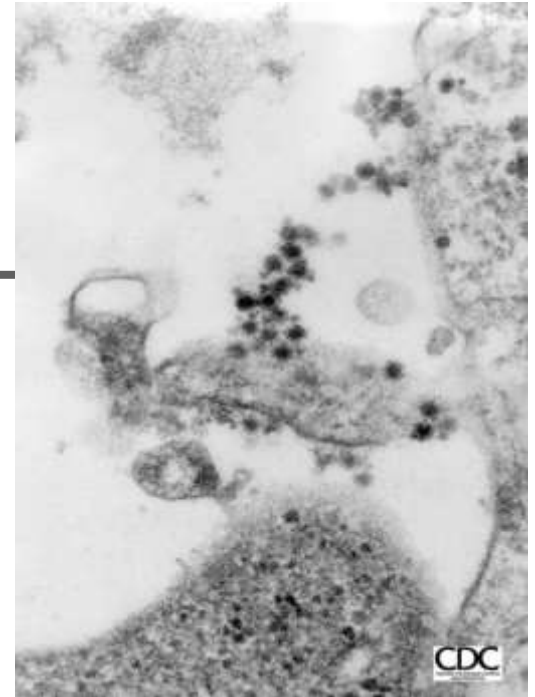
Distribution of West Nile fever cases by affected areas, European region/Mediterranean basin

Transmission season 2014; latest update 25/09/2014

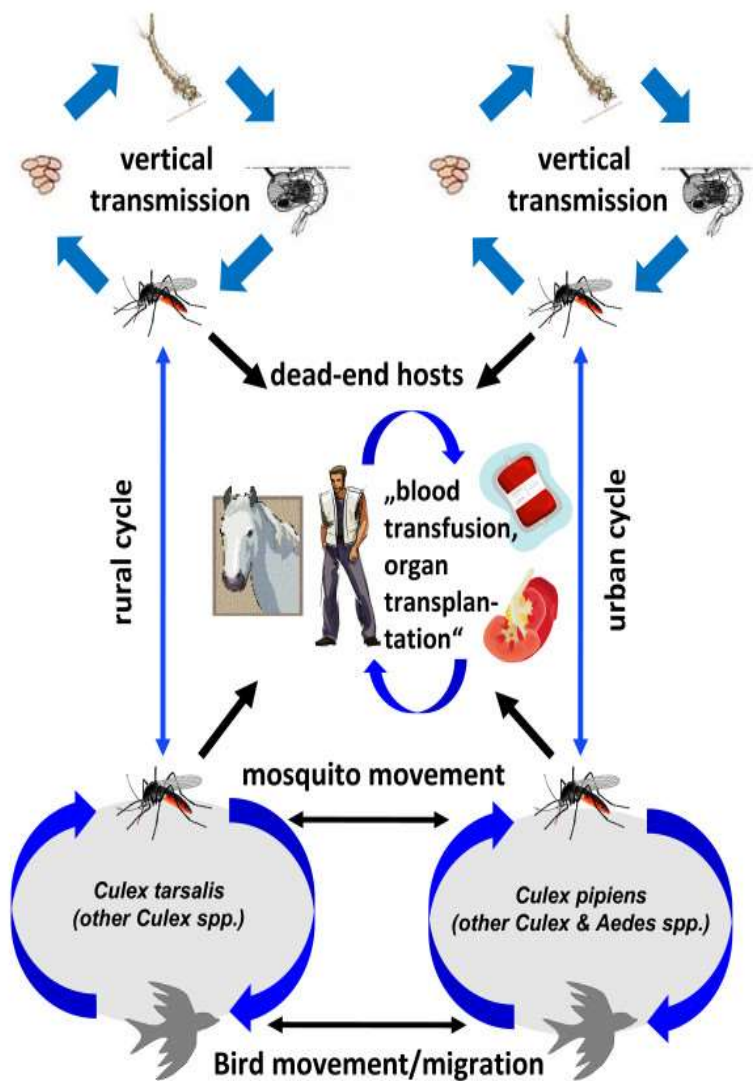


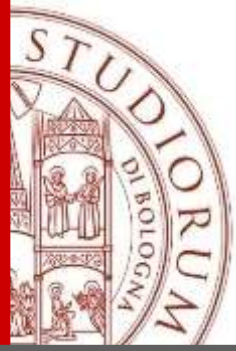
West Nile Virus (WNV)

- *Flaviviridae* (*Flavivirus*)
- ss (+) RNA
- Virion (spherical shape), 40-50 nm
- *envelope* (proteins **E** and **prM**)
- Capsid (protein **C** 20-30 nm)



West Nile Cycle





Human Transmission

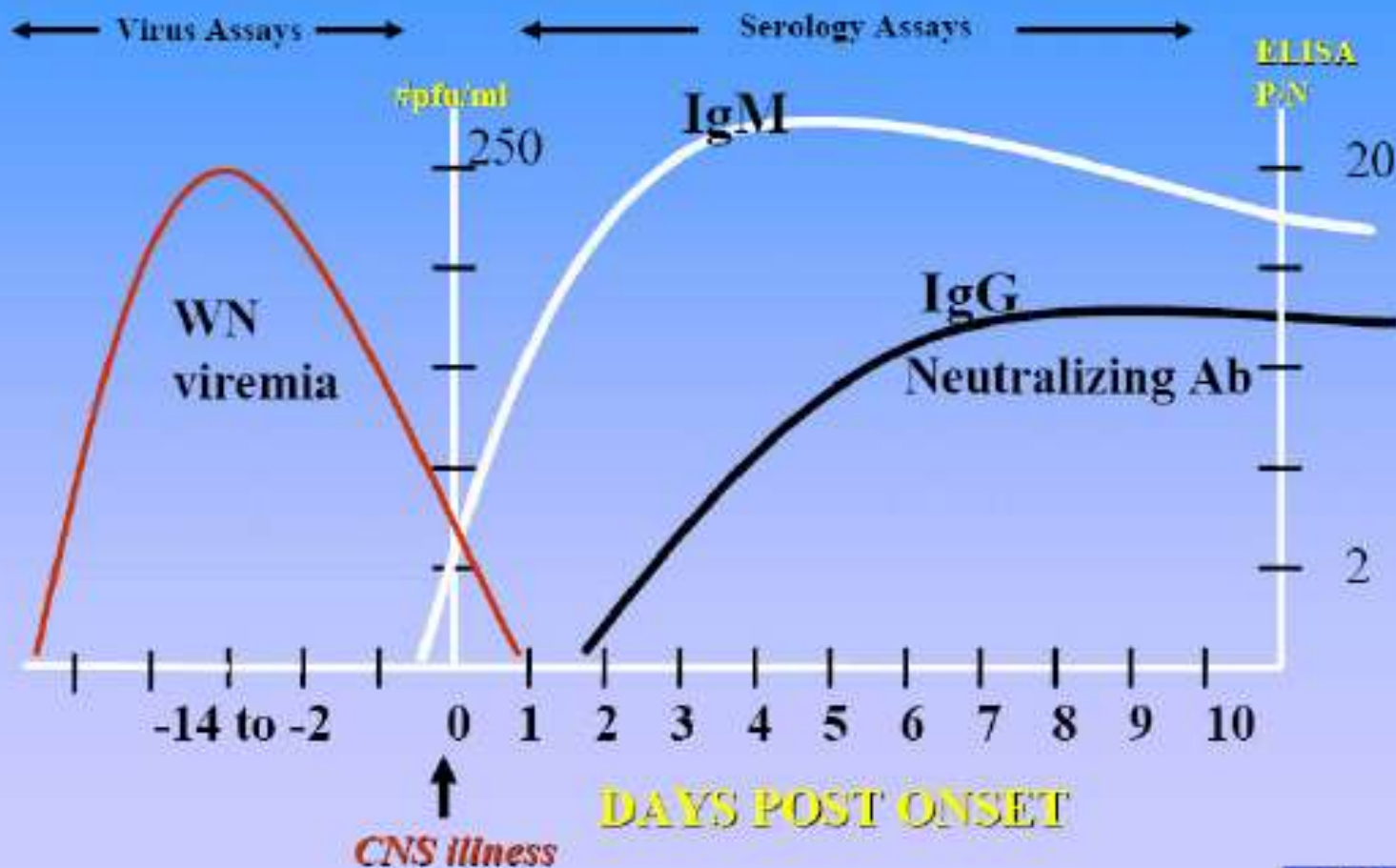
- **Mosquito**

- Laboratory acquired

- **Blood transfusions and SOT**

- Transplacental transmission
- Breast feeding

Theoretical Depiction of WNV Human Viremia & Immune Response



Clinical Epidemiology of WNV infection in humans

WNV Human Infection “Iceberg”

1 CNS disease case
=
~150 total infections

<1%
CNS
disease

~20%
“West Nile Fever”

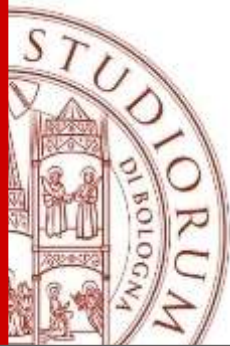
Very crude
estimates

~80%
Asymptomatic

Incubation period: 3-14 days

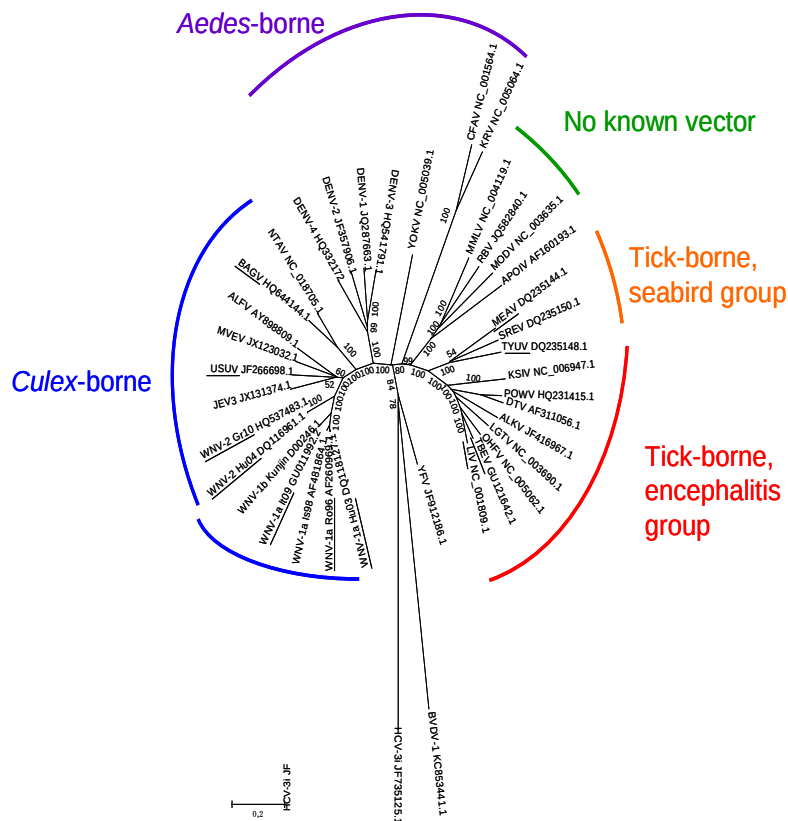
Encephalitis
(meningoencephalitis)

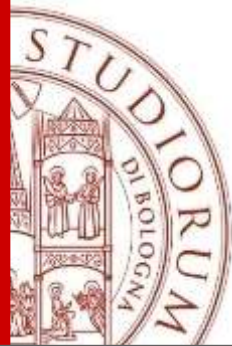
fever, weakness,
headache, myalgia,
nausea, vomiting
(3 – 6 gg.)



Flaviviruses in Europe: Complex Circulation Patterns and Their Consequences for the Diagnosis and Control of West Nile Disease

Int. J. Environ. Res. Public Health **2013**, *10*, 6049–6083;





Phylogenetic Analysis of West Nile Virus Isolates, Italy, 2008–2009

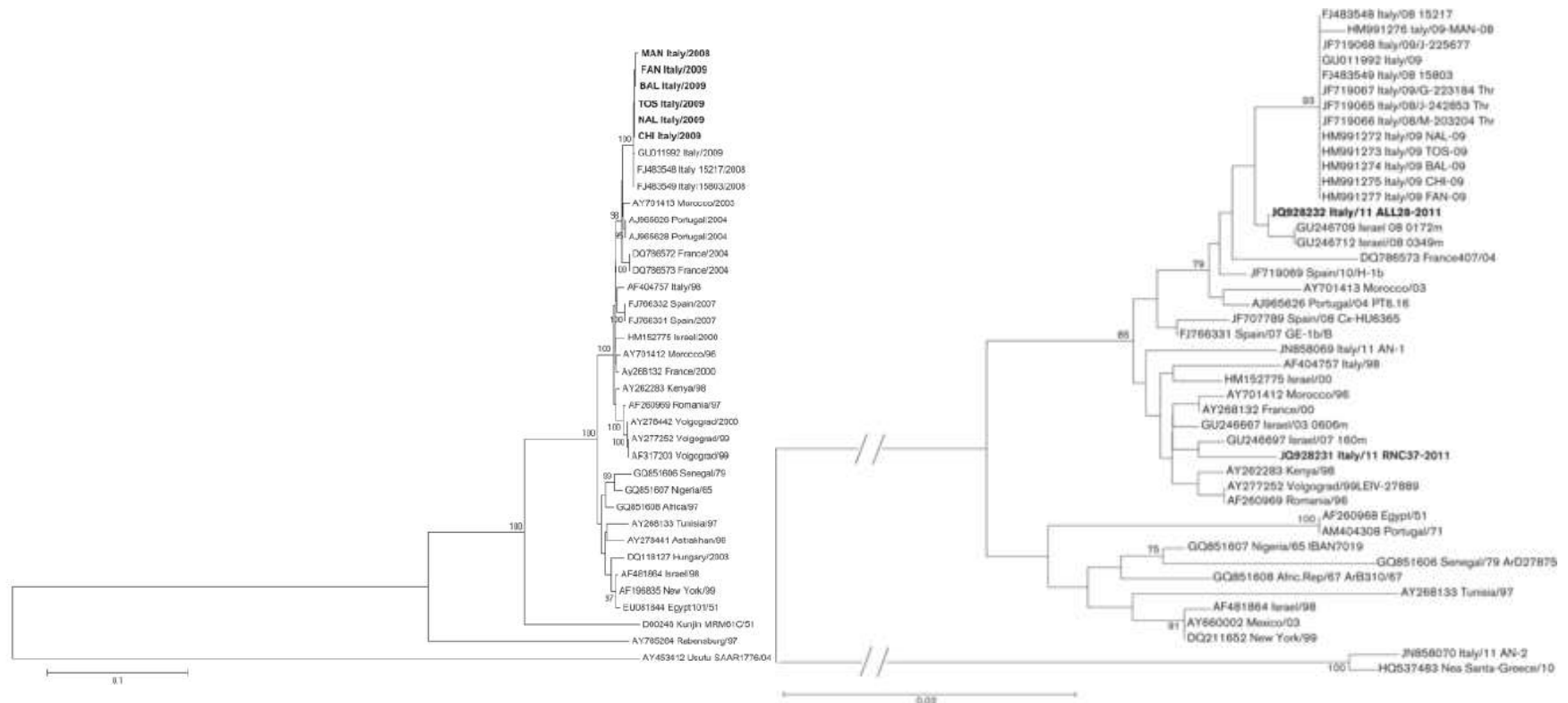
Giada Rossini,¹ Fabrizio Carletti,¹ Licia Bordini,¹ Francesca Cavrini, Paolo Gaibani, Maria P. Landini, Anna Pierro, Maria R. Capobianchi, Antonino Di Caro, and Vittorio Sambri

Heterogeneity of West Nile virus genotype 1a in Italy, 2011

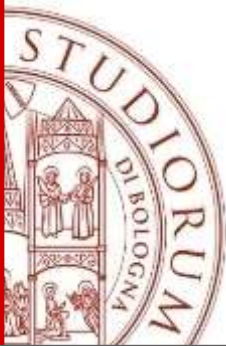
Giada Rossini, Fabrizio Carletti, Roberto Rigoli, Sandro Piga, Patrizia Bagnarelli, Paolo Gaibani, Anna Pierro, Alessandro Nanni Costa, Paolo Grossi, Giuseppe Ippolito, Maria Paola Landini, Antonino Di Caro, Maria Rosaria Capobianchi and Vittorio Sambri

Journal of General Virology (2013)

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 17, No. 5, May 2011



Emergence of WNV Lineage 2



VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 10, Number 7, 2010
© Mary Ann Liebert, Inc.
DOI: 10.1089/vbz.2009.0230

West Nile Virus Lineage 2 as a Cause of Zoonotic Neurological Disease in Humans and Horses in Southern Africa

Marjetta Venter^{1,2} and Robert Swanepoel³

Detection of West Nile virus lineage 2 in mosquitoes during a human outbreak in Greece

A. Papa¹, K. Xanthopoulou¹, S. Gewehr² and S. Mourelatos²

1) Department of Microbiology, Medical School, Aristotle University of Thessaloniki, Thessaloniki and 2) Eco-Development, S.A., Thessaloniki, Greece

Clinical Microbiology and Infection, Volume 17 Number 8, August 2011

Circulation of West Nile virus lineage 1 and 2 during an outbreak in Italy

F. Magurano¹, M. E. Remoli¹, M. Baggieri¹, C. Fortuna¹, A. Marchi¹, C. Fiorentini¹, P. Bucci¹, E. Benedetti¹, M. G. Ciufolini¹, C. Rizzo², S. Piga³, P. Salcuni⁴, G. Rezza¹ and L. Nicoletti¹

Clinical Microbiology and Infection ©2012

Novel West Nile virus lineage 1a full genome sequences from human cases of infection in north-eastern Italy, 2011

L. Barzon^{1,2}, M. Pacenti², E. Franchin^{1,2}, L. Squarzon¹, E. Lavezzo¹, S. Toppo¹, T. Martello², M. Cattai², R. Cusinato² and G. Palù^{1,2}

Clinical Microbiology and Infection ©2012

Human case of autochthonous West Nile virus lineage 2 infection in Italy, September 2011

P. Bagnarelli (p.bagnarelli@univpm.it)¹, K. Marinelli¹, D. Trotta¹, A. Monachetti¹, M. Tavio², R. Del Gobbo², M. R. Capobianchi¹, S. Menzo³, L. Nicoletti⁴, F. Magurano⁴, P. E. Varaldo¹

West Nile virus in Europe: emergence, epidemiology, diagnosis, treatment, prevention.

V. Sambri et al. *CMI* 2013, in press



Int. J. Environ. Res. Public Health **2013**, *10*, 4669–4689;





Seroprevalence of West Nile Virus–Specific Antibodies in a Cohort of Blood Donors in Northeastern Italy

A. Pierro et al.

- IgG and IgM levels against West Nile virus (WNV) were measured in 20,033 serum samples that were obtained between October 2008 to September 2009 from 9913 blood donors in the district of Ferrara, northeastern Italy.
- As confirmatory test, a microneutralization assay was used to detect the presence of neutralizing antibodies against WNV.
- Sixty-eight subjects (0.69%) were positive for anti-WNV by immunofluorescence assay.
- Large differences in the prevalence of antibodies to WNV were noted between towns in the area evaluated.

Vector-Borne and Zoonotic Diseases. December 2011, 11(12): 1605-1607. doi:10.1089/vbz.2011.0616.



Persistence of Anti-West Nile Virus-Specific Antibodies Among Asymptomatic Blood Donors in Northeastern Italy

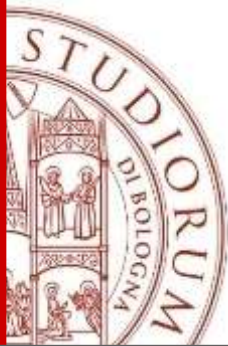
A. Pierro et al. VBZD 2013

- The development and persistence of anti-West Nile Virus (WNV) immunoglobulin G (IgG)- and IgM-specific antibodies were investigated in 68 asymptomatic blood donors (BDs) previously tested as positive between October, 2008, and September, 2009, and living in northeastern Italy.
- Our study showed that WNV-specific IgG titers became negative (41%) or decreased (33%), while they increased in a smaller percentage (10%); 16% of BDs showed no titer variation.
- ***Reversion to seronegative status within a short time frame suggests that WNV surveillance should be maintained year after year.***



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Dengue in Europe

Several imported cases (travellers)
2010-2013: autochthonous transmission in
Southern France, Croatia, Madeira

Dengue virus infection in a traveller returning from Croatia to Germany

J Schmidt-Chanasit (jonassi@gmx.de)¹, M Haditsch², I Schöneberg³, S Günther¹, K Stark¹, C Frank²

1. Bernhard Nocht Institute for Tropical Medicine, Department of Virology, Hamburg, Germany

2. Labor Hannover MVZ GmbH, Hannover, Germany

3. Robert Koch Institute, Department for Infectious Disease Epidemiology, Berlin, Germany

Euro Surveill. 2010;15(40):pii=19677.

**First cases of autochthonous dengue fever and chikungunya fever in
France: from bad dream to reality!**

E. A. Gould^{1,2}, P. Gallian³, X. de Lamballerie^{2,4} and R. N. Charrel^{2,4}

CMI, 16, 1702–1704

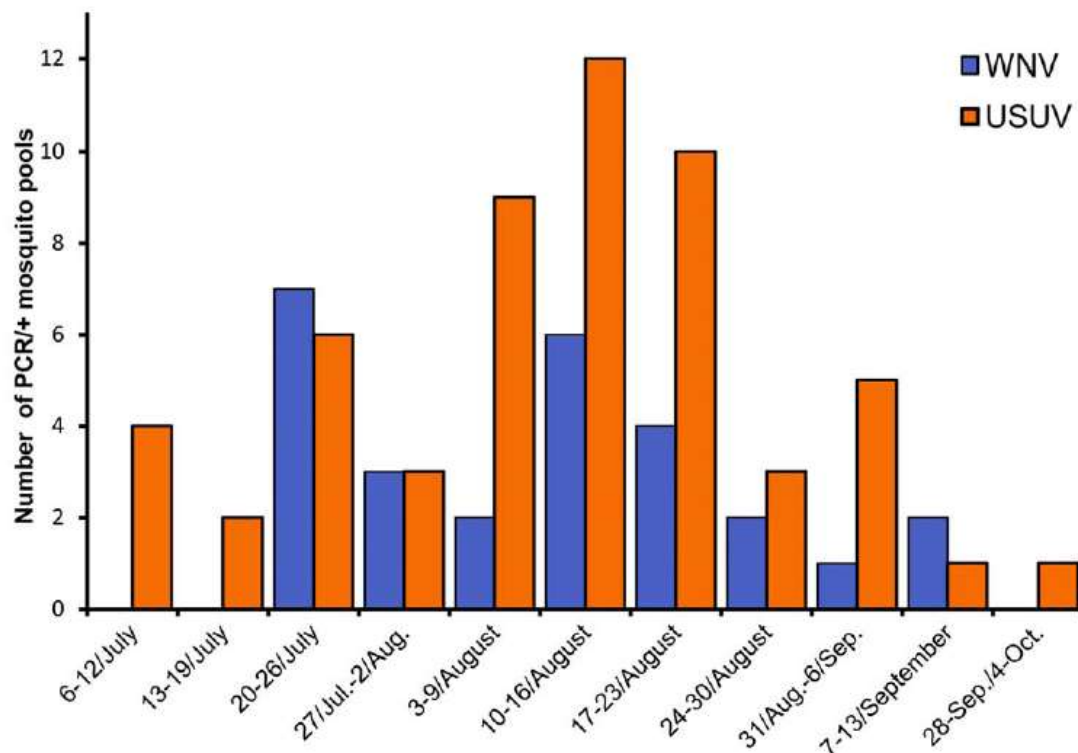


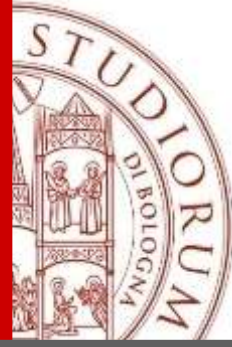
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 - **TOSV** (Phleboviruses)

Detection of Usutu Virus Within a West Nile Virus Surveillance Program in Northern Italy

Marco Tamba,¹ Paolo Bonilauri,¹ Romeo Bellini,² Mattia Calzolari,¹ Alessandro Albieri,²
Vittorio Sambri,³ Michele Dottori,¹ and Paola Angelini⁴





Rapid communications

FIRST HUMAN CASE OF USUTU VIRUS NEUROINVASIVE INFECTION, ITALY, AUGUST-SEPTEMBER 2009

M Pecorari¹ (pecorari.monica@policlinico.mo.it)¹, G Longo², W Gennari¹, A Grottole¹, A MT Sabbatini¹, S Tagliacucchi¹, G Savini¹, F Monaco³, M L Simone⁴, R Lelli¹, F Rumpianesi¹

1. Department of Diagnostic and Laboratory Services and Legal Medicine, University of Modena and Reggio Emilia, Azienda Ospedaliera Policlinico, Modena, Italy

2. Department of Oncology and Hematology, University of Modena and Reggio Emilia, Azienda Ospedaliera Policlinico, Modena, Italy

3. Department of Virology, National Reference Centre for West Nile and Usutu disease, DUE Reference Laboratory for Bluetongue, Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise 'G. Caporale', Teramo, Italy

4. Department of Biomedical Sciences, University of Modena and Reggio Emilia, Modena, Italy

Rapid communications

USUTU VIRUS INFECTION IN A PATIENT WHO UNDERWENT ORTHOTROPIC LIVER TRANSPLANTATION, ITALY, AUGUST-SEPTEMBER 2009

F Cavrini^{1,2}, P Gaibani^{1,2}, G Longo¹, A M Pierro¹, G Rossini¹, P Bonfauri¹, G E Gerundi¹, F Di Benedetto³, A Pasetto⁶, M Girardi⁴, M Dottori⁴, M P Landini¹, V Sambri¹ (vittorio.sambri@unibo.it)¹

1. Clinical Microbiology Unit, Regional Reference Centre for Microbiological Emergencies - CRREM, St. Orsola-Malpighi University Hospital, University of Bologna, Bologna, Italy

2. These Authors contributed equally to this paper and are listed in alphabetical order

3. Oncology and Haematology Unit, Modena University Hospital, Modena, Italy

4. Experimental Institute for Animal Health and Protection of Lombardia and Emilia-Romagna, Brescia, Italy

5. Liver and Multivisceral Transplant Center, University of Modena and Reggio Emilia, Modena, Italy

6. Anaesthesiology and Intensive Care Unit 1, Modena University Hospital, Modena, Italy

Comparative Genomic and Phylogenetic Analysis of the First Usutu Virus Isolate from a Human Patient Presenting with Neurological Symptoms

Paolo Gaibani^{1*}, Francesca Cavrini¹, Ernest A. Gould², Giada Rossini¹, Anna Pierro¹, Maria Paola Landini¹, Vittorio Sambri³

First evidence of simultaneous occurrence of West Nile virus and Usutu virus neuroinvasive disease in humans in Croatia during the 2013 outbreak

T. Vilibic-Cavlek · B. Kaic · L. Barbic · I. Pem-Novosel · V. Slavic-Vrzic · V. Lesnikar · S. Kurecic-Filipovic · A. Babic-Erceg · E. Listes · V. Stevanovic · I. Gjenero-Margan · G. Savini



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Chikungunya

- Spherical virions, about 70 nm diameter with envelope
- Nucleocapsid icosaedric (40nm, nucleoprotein C).
- Envelope: spikes (glycoproteins gpE1 e gpE2)
- ssRNA (+), fully sequenced

Chikungunya: epidemiology



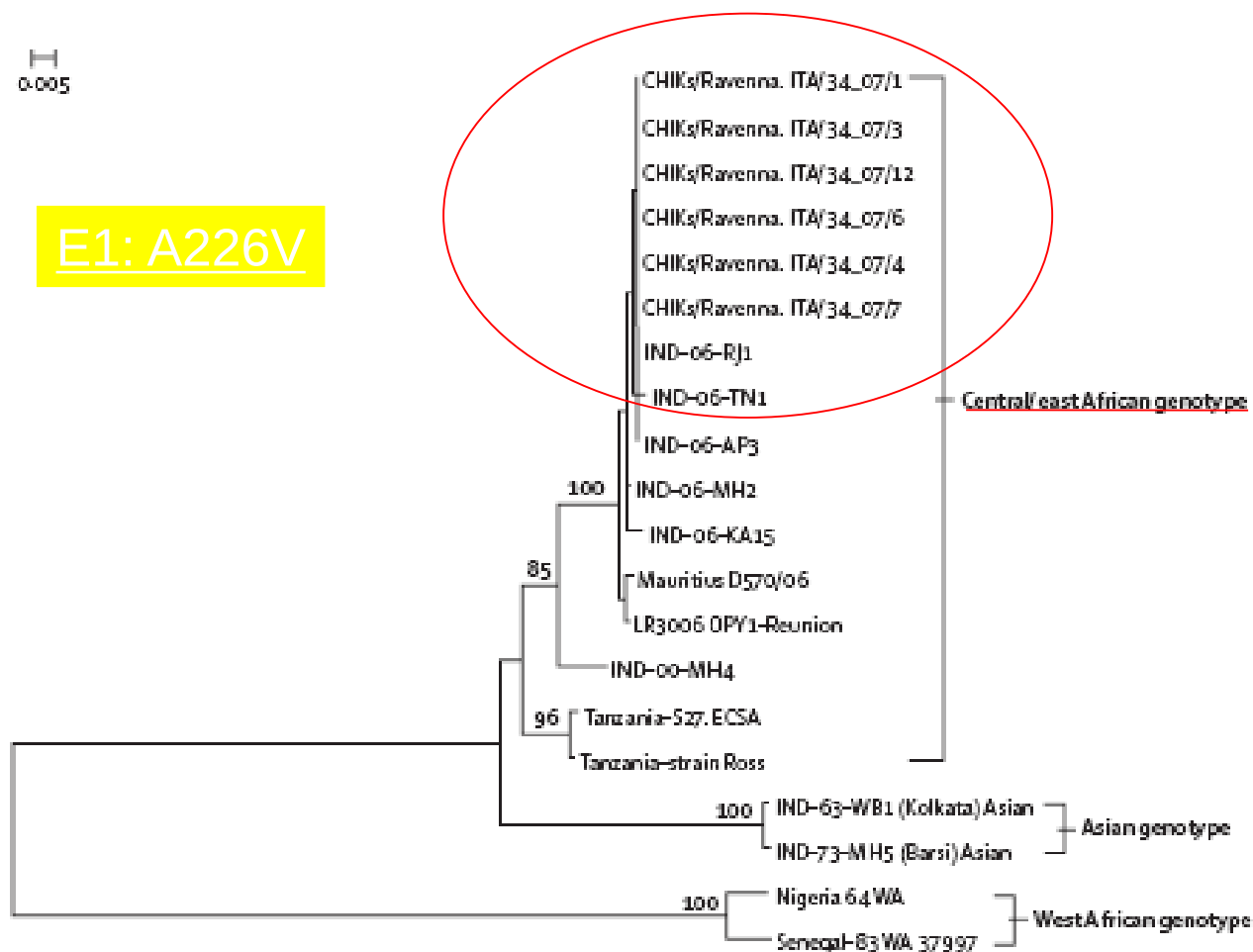
www.chikungunya.net





H
0.005

E1: A226V



Bordi et al., Clin Infect Dis 2008.



Number of cases by lab results Emilia-Romagna 4th July 2007 – 28th September 2007

Index case lab confirmed **1**

Lab confirmed **247**

In total, **248 (247+1 case index)** persons affected, from four provinces (Ravenna, Forlì/Cesena, Rimini, Bologna)

Cluster of CHIKV Infection Emilia Romagna August – November 2007





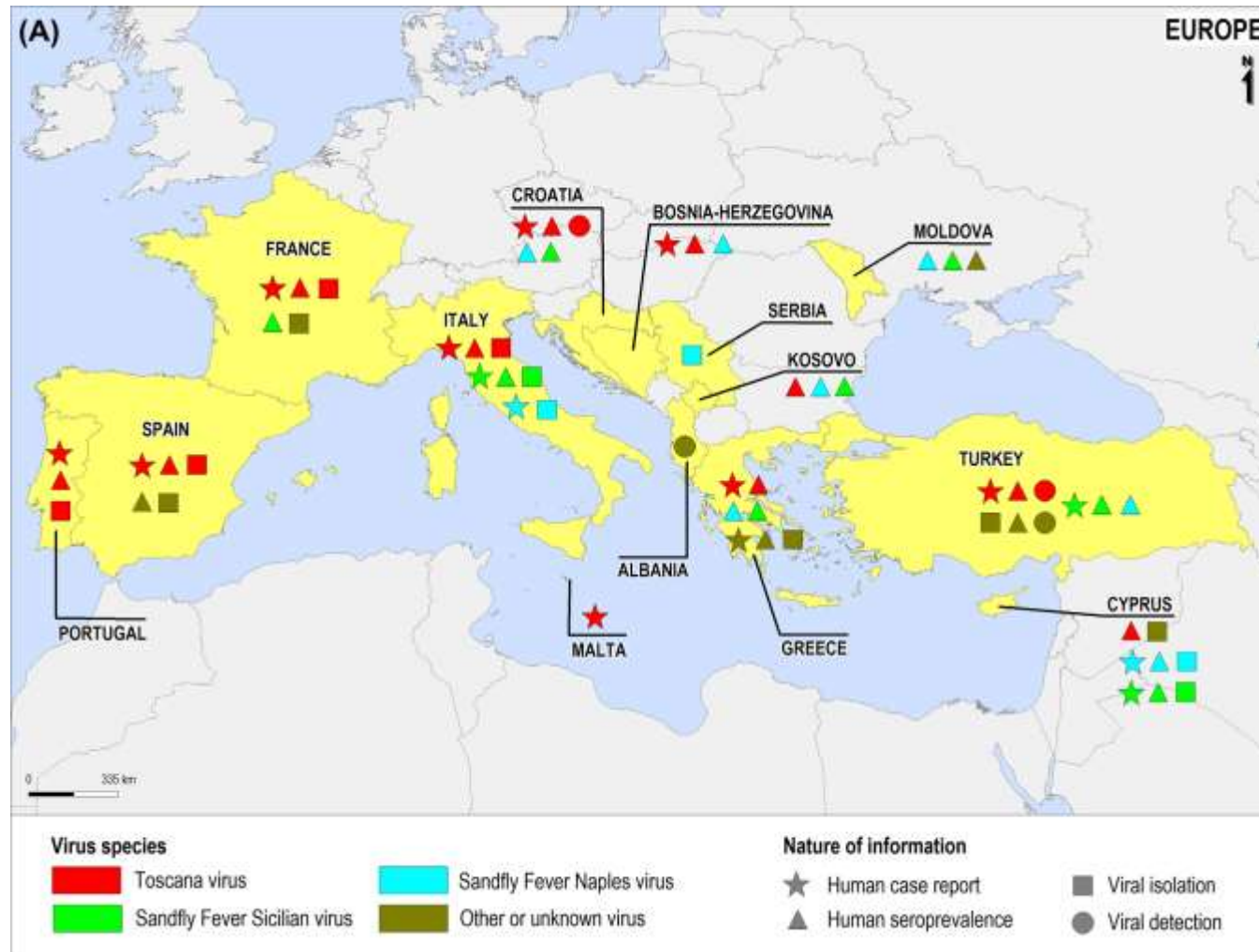
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Sandfly-borne phleboviruses of Eurasia and Africa: Epidemiology, genetic diversity, geographic range, control measures

Cigdem Alkan^a, Laurence Bichaud^a, Xavier de Lamballerie^{a,b}, Bulent Alten^c, Ernest A. Gould^{a,b}, Rémi N. Charrel^{a,b,*}

Antiviral Research 100 (2013) 54–74



Toscana virus infections: A case series from France

J. Dupouey^{a,b,c}, L. Bichaud^{a,b,c}, L. Ninove^{a,b}, C. Zandotti^{a,b},
L. Thirion-Perrier^{a,b}, X. de Lamballerie^{a,b}, R.N. Charrel^{a,b,*}

Journal of Infection (2014) 68, 290–295

Table 1 Epidemiological, clinical and biological data of the TOSV infection cases.

Patient	Sex	Age	Onset	Time	Clinical data	CSF analysis	Acute phase			Convalescent phase		OMS criteria	Comments
							IF	MN	PCR	IF	MN		
Confirmed infection													
P1	H	47	Aug 2009	5 days	AM, severe headache and vomiting	230/93%	G+M+	—	POS	—	NEG	C1	HIV infection
P2	H	39	Aug 2009	3 days	AM	178/70%	G+M+	—	POS	—	—	C1	
P3	H	17	Jun 2010	5 days	AM with fever	840/70%	G+M+	NEG	POS	—	—	C1	
P4	H	38	Aug 2008	—	AM and hepatomegaly	120/90%	G+M+	—	POS	G+M+	1/40	C1+C3	Returned from Tuscany
P5	F	20	Jul 2008	—	AM	65/50%	G+M+	NEG	POS	—	—	C1	
P6	H	49	Aug 2011	—	Encephalitis with seizures	106/70%	G—M+	—	NEG	G+M+	1/160	C2+C3	
P7	F	50	Jul 2008	—	Fever with general asthenia and left lower limb fascitis	—	G—M+	NEG	—	G+M+	—	C2	Returned from Croatia
P8	H	7	Aug 2007	—	Fever, headache and stiff neck	—	G—M—	NEG	—	G+M+	—	C2	
P9	F	4	Sep 2009	7 days	Encephalitis	54/59%	G—M—	—	NEG	G+M+	NEG	C2	
P10	F	40	Jul 2008	—	Fever, myalgia, headache and stiff neck	<5	G—M—	—	—	G+M—	NEG	C2	
P11	F	33	Nov 2009	13 days	Encephalitis with fever and drowsiness	150/90%	G+M+	—	NEG	G+M+	1/20	C3	Returned from Croatia
P12	H	61	Jul 2010	—	Fever with myositis	—	G+M+	1/80	NEG*	G+M—	1/160	C3	
P13	H	43	Jul 2004	—	AM	28/95%	G—M+	—	—	—	1/40	C3	
P14	H	76	Jul 2007	—	Left upper limb hemiparesis with headache and confusion	—	G—M+	1/40	—	G+M+	1/10	C2+C3	
P15	H	64	Jun 2009	1 day	Encephalitis, seizures with fever and stiff neck	46/0%	G+M+	1/160	—	G+M+	1/80	C3	
Probable infection													
P16	F	42	Mar 2010	1 day	Fever with cervical lymphadenopathy	—	G+M+	—	—	G+M—	NEG	C5	
P17	H	30	Apr 2007	—	Fever with headaches and sudden right facial paralysis	<5	G—M+	NEG	—	G+M—	—	C5	

AM: Aseptic meningitis.

CSF analysis: Leukocytes (/mm³) – Proportion of lymphocytes (%), in cerebral spinal fluid (CSF).

PCR: Polymerase chain reaction targeting Toscana virus genome in CSF (* in blood).

—: Not available.

