Organizzazione

Istituto Zooprofilattico
Sperimentale
della Lombardia e dell'Emilia
Romagna

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EPIZONE

Istituto Zooprofilattico Sperimentale
della Lombardia e dell'Emilia Romagna

Istituto Zooprofilattico Sperimentale
delle Venezie

Workshop Epizone

ERG 2013

Mosquitos and sand fly—borne viruses across Europe and the Mediterranean sea

May 9—10, 2013 Brescia

Thursday May 9

9.00 Welcome
Prof. Cinotti/prof. Van der Poel/dr. Varisco/dr . Marangon

chairmen: dr. ssa Capelli / dr. Dottori (IZSVE-IZSLER)

9.30-10.30 a.m. First Session
Mosquito only Flaviviruses

9.30-10.00
dr. Calzolari (IZSLER)

10.00-10.30
round table discussion

10.30-11.00
coffee break

11.00 -12.00 Second Session
Phleboboviruses

11.00-11.30
prof. Remi N. Charrel

11.30-12.00
round table discussion

12.30-14.00
lunch (IZSLER canteen)

14.00-15.10 Third Session
Flaviviruses other than WNV/DENV

14.00-14.30
USUTUV: Italian situation - dr. Giovanni Savini (IZSAM)

14.30-15.00
Risk related to pathogenic flaviviruses (not only USUTUV , but also flavivirus at risk of introduction as JEV, RVF and others) - prof. Gerhard Dobbler

15.00-15.30
round table discussion

15.30-16.00
coffee break

16.00-17.30 Fourth Session
Are other Arboviruses circulating in European mosquitoes less relevant ?

16.00-16.45
Arboviruses circulating in Europe or at risk of introduction (THAV, BATV, SINV, other Bunyaviridae affecting livestock -except SBV) - prof. Zdenek Hubálek

16.45-17.30
round table discussion

Friday, May 10

9.00-10.30 Fifth Session
Role of entomological surveillance (mosquitoes) for risk assessment, research and future perspective

9:00-9:30
Dr. Romeo Bellini (Centro Agricoltura e Ambiente—Italy)

9.30-10.15
round table discussion

10.15-10.30
coffee break

10.30-12.00 Sixth Session
Future perspective

10.30-11.00
Research opportunities and financial resources: brief introduction by Dr.ssa Gioia Capelli (IZSVE) (braimstorming, discussion, ideas, input for future research and financial support)

11.00-12.00
round table discussion, concluding remarks and proposal

12.30
lunch and freedom

EPIZONE European Research Group

Network on Epizootic Diseases Diagnosis and Control

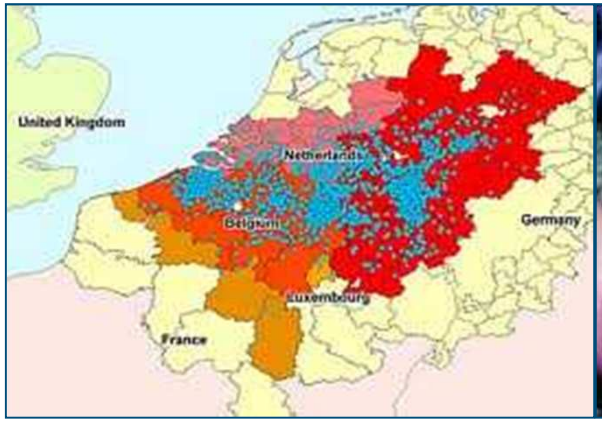
www.epizone-eu.net

Wim H. M. van der Poel
Coordinator

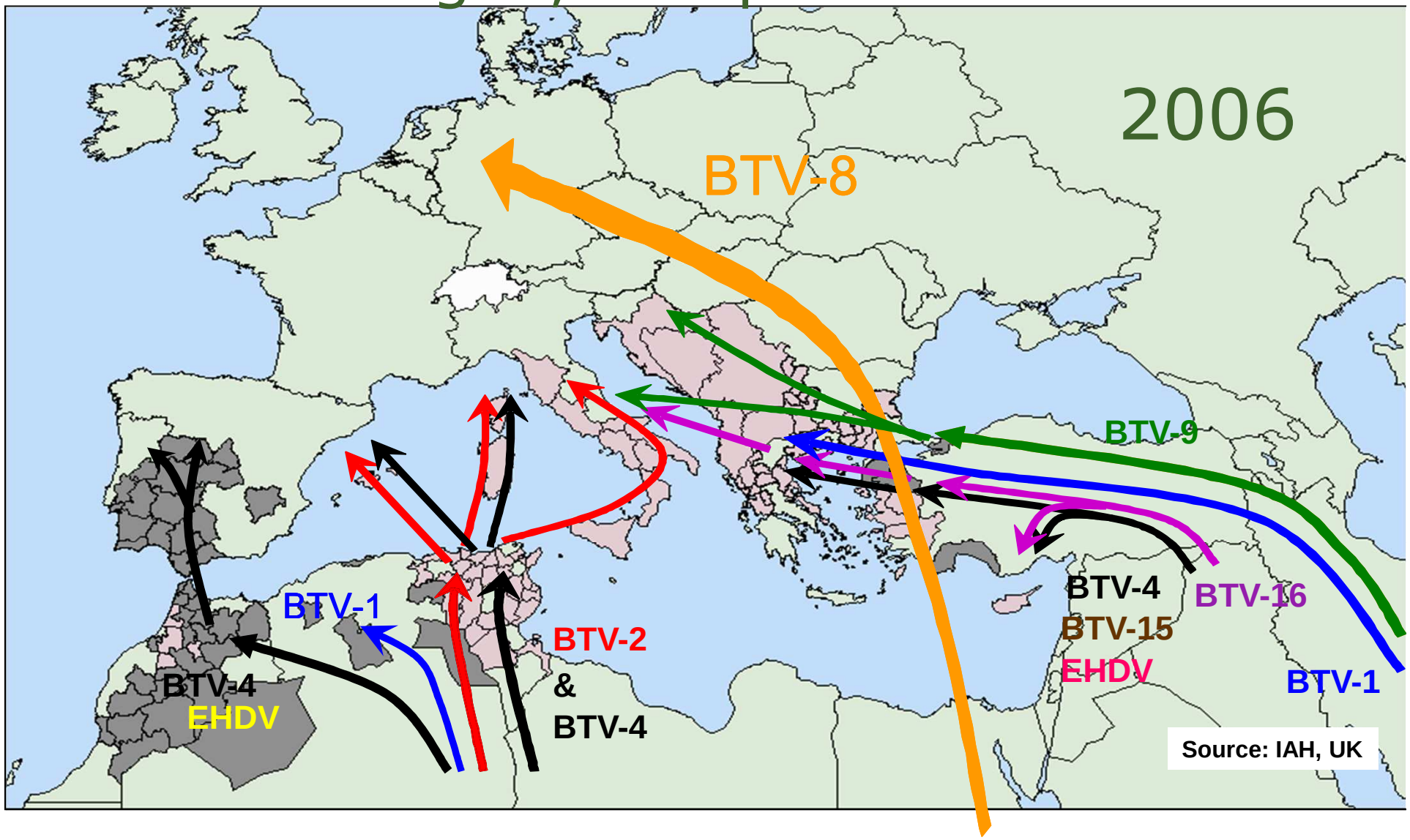
International Research Network on Epizootic Diseases Diagnosis and Control

Bluetongue virus type 8 outbreak, Europe

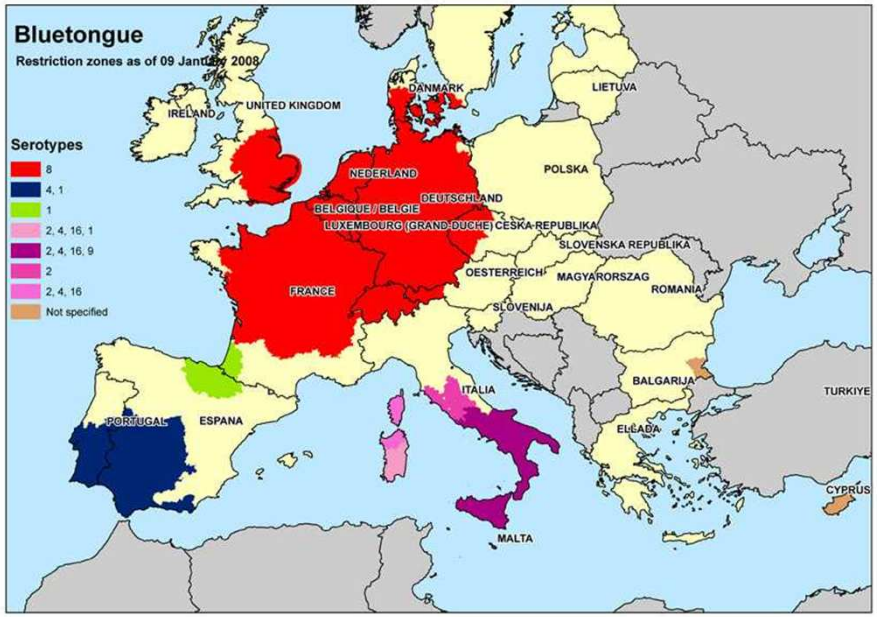
- Index case in province of Limburg, the Netherlands
- Bluetongue serotype 8
- First introduction in NW Europe



Bluetongue, Europe 1999– 2005



Bluetongue outbreak NW Europe 2006-2011



BTV restricted zones in Europe, 2009

2006: First notification BTV-8 (B, D, NL); Animal movement restrictions

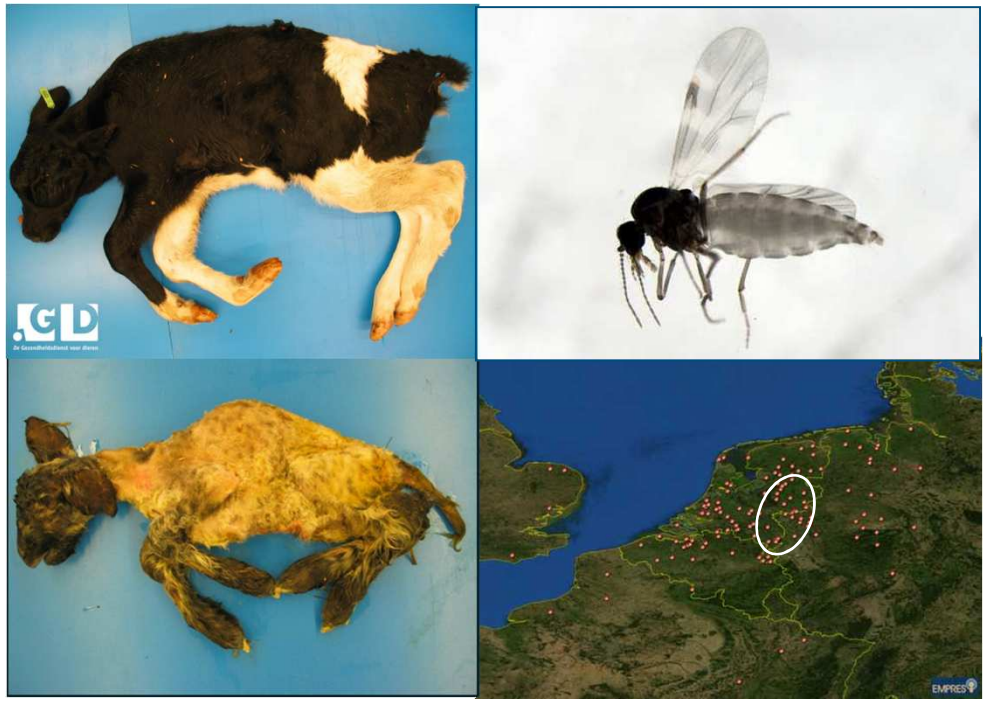
2007: Up to 60,000 holdings BTV-8 infected; Emergence in DK, CZ, CH, UK etc.

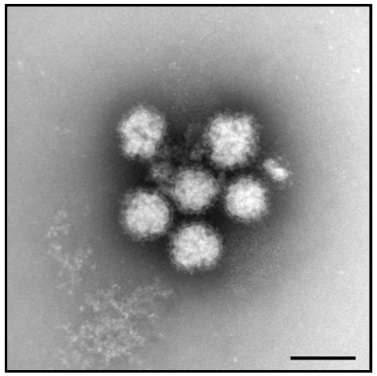
2008: BTV-6 and BTV-1 emergence; Inactivated vaccines available against BTV-1 and BTV-8; Vaccination programmes started

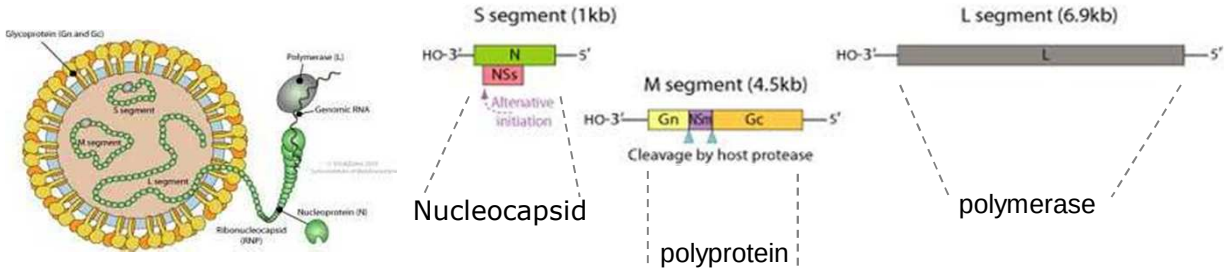
2011: Vaccination preferred control strategy

Schmallenberg virus

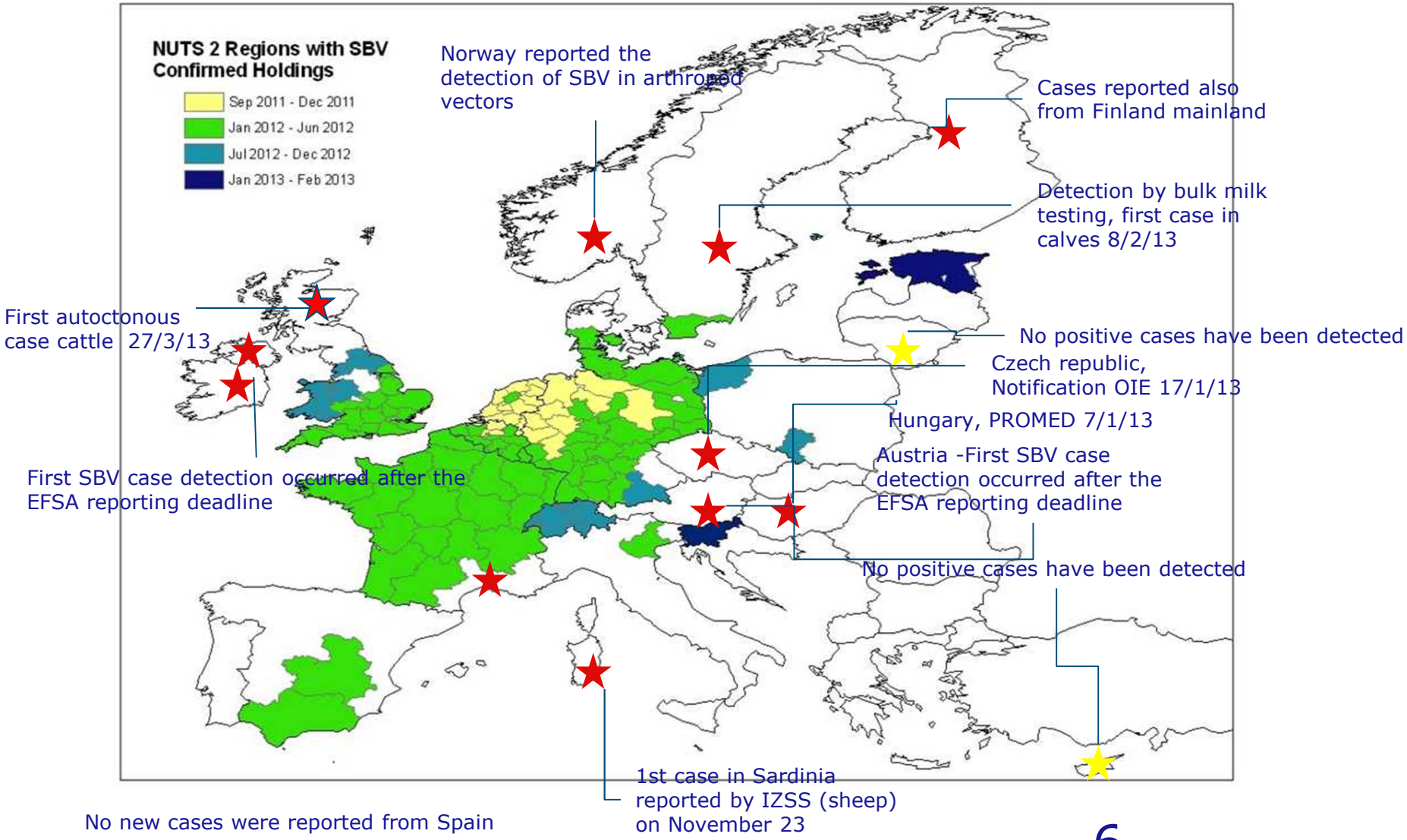
- Simbu serogroup virus
- Family Bunyaviridae
- Genus Orthobunyavirus
- ssRNA virus
- 3 RNA segments
- Culicoides vector







SBV geographic distribution



One World, One Health

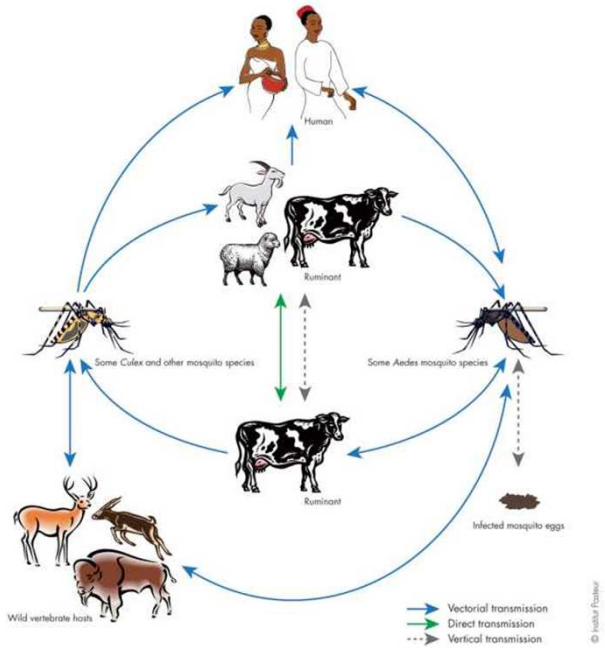
"The health of people, animals, and their environments are connected"

Need for all inclusive collaborations between physicians, veterinarians, and other scientific-health related disciplines

- Environment-friendly production
- Public health protection
- Safe food
- Animal welfare

Future challenges

Global warming



Vector borne diseases

Reservoir hosts

New emerging diseases

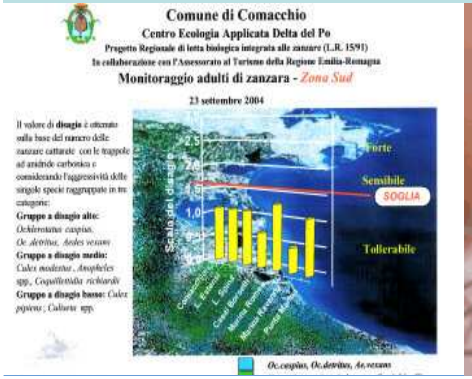
EPIZONE interactive opinion 2010: “Zoonotic Arboviruses expected to be more of a threat in 2020” (Kelly et al., Transboundary Emerg. Dis., 2012)

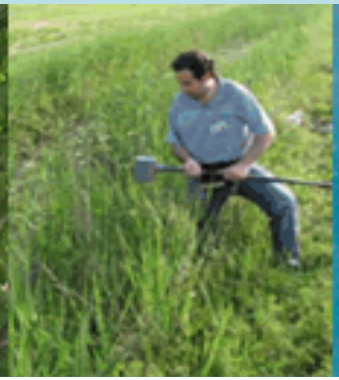
EPIZONE 7th annual meeting, Brussels 2013

Workshop EPIZONE 2013
Mosquito and sand fly borne viruses across Europe and the Mediterranean sea
IZLER Brescia, May 9-10, 2013

Entomological surveillance for mosquito borne diseases risk assessment

Romeo Bellini
Centro Agricoltura Ambiente "G.Nicoli", Crevalcore, Italy





EMILIA-ROMAGNA VBDs SYSTEM

- Health Regional Service Emilia-Romagna (Public Health Regional Service & Veterinary Regional Service)
- Local Health Units (PC, PR, RE, MO, BO, IM, FC, FE, RA, RN)
- Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna
- IZSLER Epidemiological Surveillance Emilia-Romagna
- Regional Reference Centre Microbiological Emergences
- Provinces (RN, RA, FC, FE, BO, MO, RE, PR, PC)
- Municipalities (348)
- Regional Agency for Environmental Protection

TWO CASES EXAMPLE

DENGUE-CHIK // *Aedes albopictus*

WEST NILE // *Culex pipiens &*
Culex modestus

Aedes albopictus

- **exotic species**
- **first detection in Europe:** 1978, Albania
- **larval habitat**: artificial containers, three holes
- **ethology**: female actives daily, very aggressive
- **hosts**: opportunistic
- **overwintering**: diapausing eggs

AEDES ALBOPICTUS VECTORIAL COMPETENCE

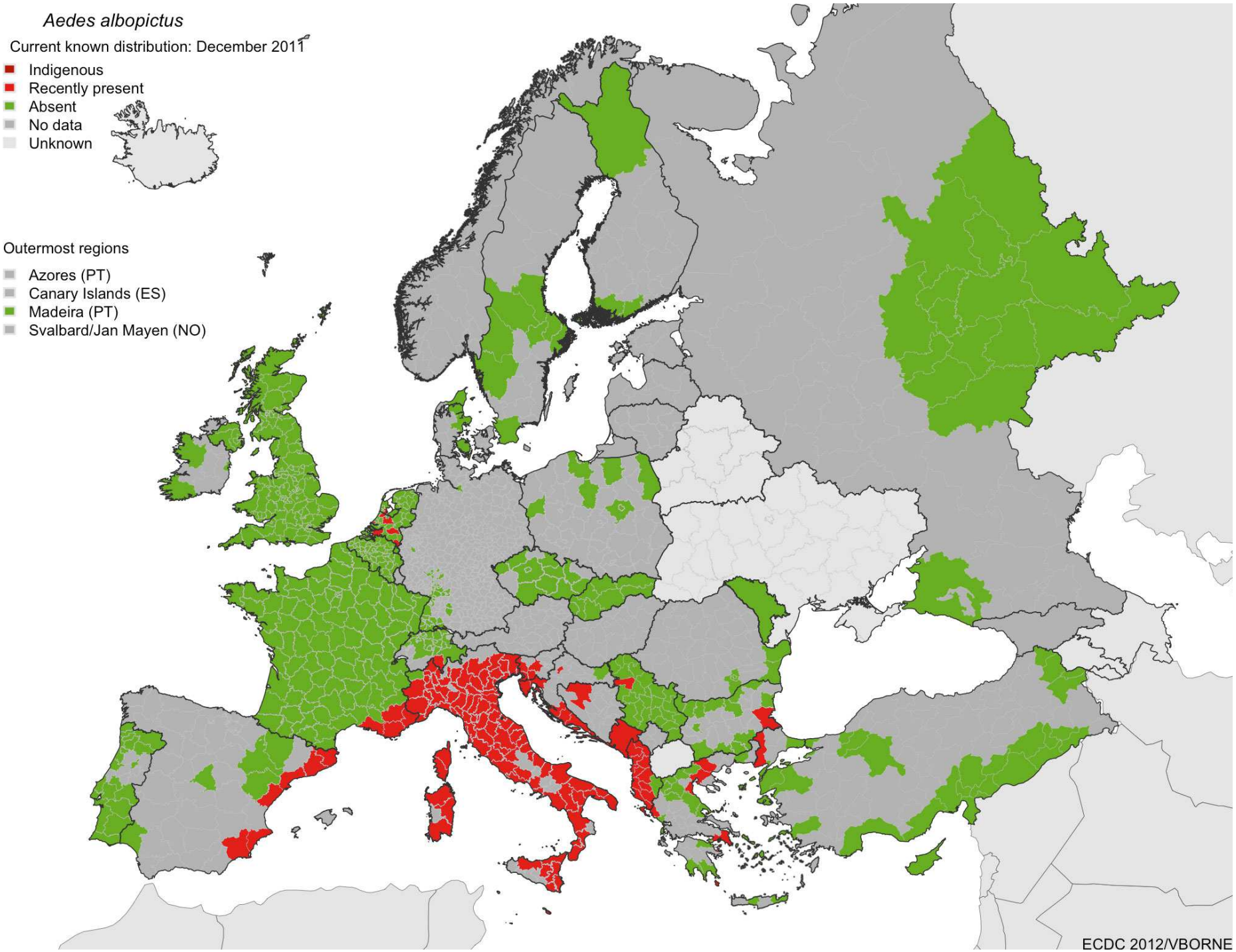
	infection	transmission	
FLAVIVIRIDAE			
Dengue 1,2,3,4	+++	+++	Mitchell et al., 1987
	+++	+++	cited in Mitchell, 1991
Yellow fever	++	++	cited in Mitchell, 1991
St. Louis E.	+	+	Savage et al., 1994
West Nile	+(?)	+(?)	cited in Shroyer, 1986
	+++	+++	Turrell et al., 2001
	+++	+++	Sardelis et al., 2002
TOGAVIRIDAE			
Eastern Equine E.	+++	++	Turell et al., 1994
Western Equine E.	+++	+++	cited in Mitchell, 1991
Venez. Equine E.	+++	++	Turell & Beaman, 1992
Ross River	++	++	Mitchell et al., 1987
Mayaro	++	++	cited in Mitchell, 1991
Chikungunya	++	++	Mangiafico, 1971
	+++	++	Turell et al., 1992
Sindbis	+(?)	+(?)	cited in Mitchell, 1994
	++	++	Dohm et al., 1995

+++ high; ++ moderate; + low; - negative; (?) not determined

AEDES ALBOPICTUS VECTORIAL COMPETENCE

	infection	transmission	
BUNYAVIRIDAE			
LaCrosse	+++	++	cited in Mitchell,1991
Jamest. Canyon	+++	+	cited in Mitchell, 1991
Keystone	+++	-	cited in Mitchell, 1991
Oropouche	+	-	cited in Mitchell, 1991
Potosi	+	+	cited in Mitchell, 1991
Rift Valley Fever	++	+	cited in Mitchell, 1991
Tahyna	+	(?)	Portolani et al., 2001
Trivittatus	+	-	cited in Mitchell, 1991
REOVIRIDAE			
Orungo	+(?)	+(?)	cited in Shroyer, 1986

+++high; ++ moderate; + low; - negative; (?) not determined


Aedes albopictus
Current known distribution: December 2011
Indigenous
Recently present
Absent
No data
Unknown
Outermost regions
Azores (PT)
Canary Islands (ES)
Madeira (PT)
Svalbard/Jan Mayen (NO)
ECDC 2012/VBORNE

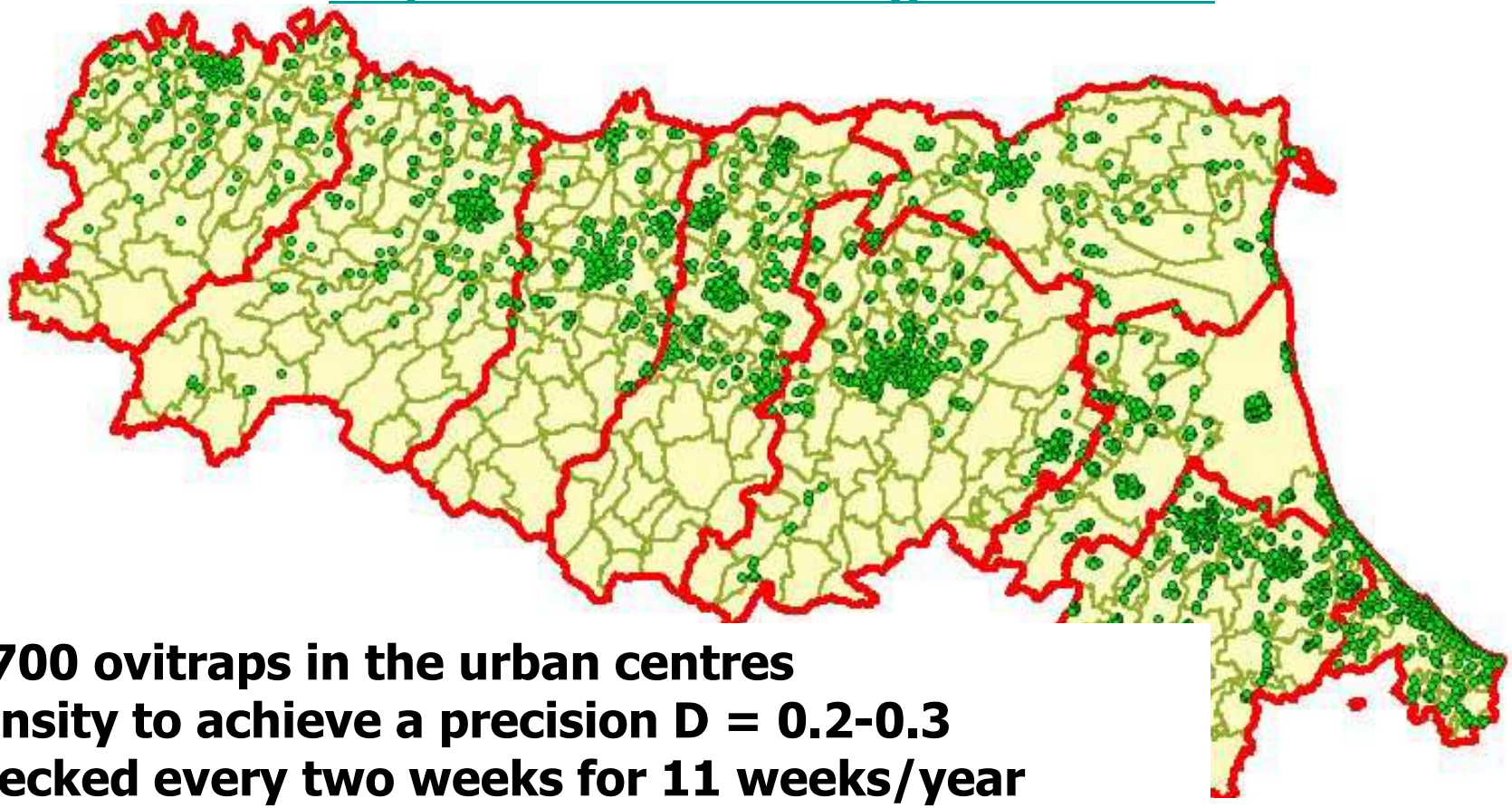
Number of probable/confirmed cases Chikungunya outbreaks Emilia-Romagna 2007

Index case lab confirmed **1**
Lab confirmed **217**
Probable, drawing denied **30**
Lab negative **89**

In total, **248 (247+1 case index)** probable/confirmed cases, from four provinces (Ravenna, Forlì-Cesena, Rimini, Bologna)

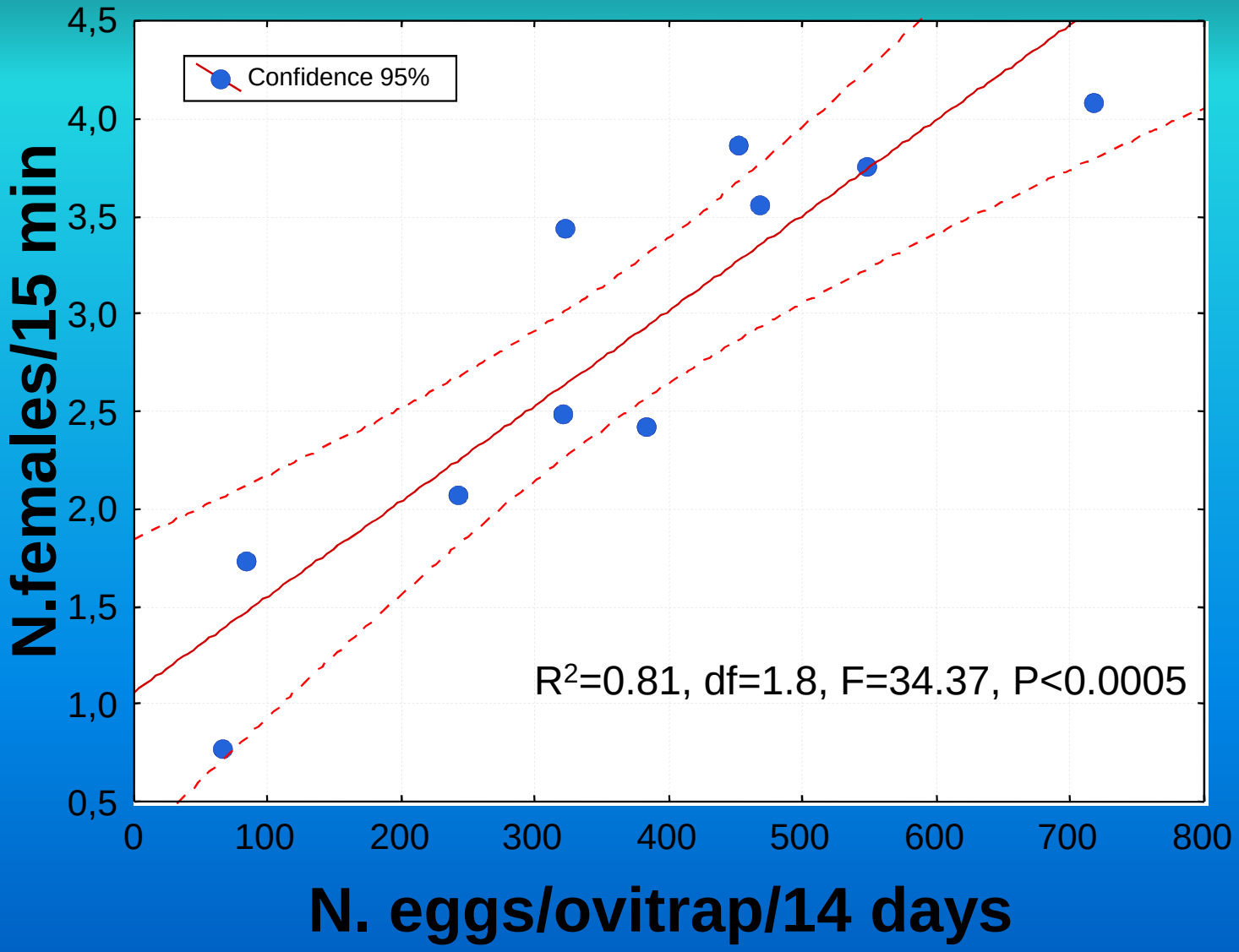
Quantitative monitoring of *Aedes albopictus*
Emilia-Romagna (4.4 ML inhabitants)

http://www.zanzaratigreonline.it/



-2,700 ovitraps in the urban centres
-density to achieve a precision D = 0.2-0.3
-checked every two weeks for 11 weeks/year
-cost: 250,000 €/year (~ 5% of the total plan cost)

Correlation between HLC and N. eggs



Epidemiological equation for VBD

N bites/human/day

Vectorial competence

Length extrinsic cycle

$$R_0 = \frac{ma^2 * V * P^n}{-\log_e P}$$

Daily survival

Log daily survival

Epidemiological equation for VBD

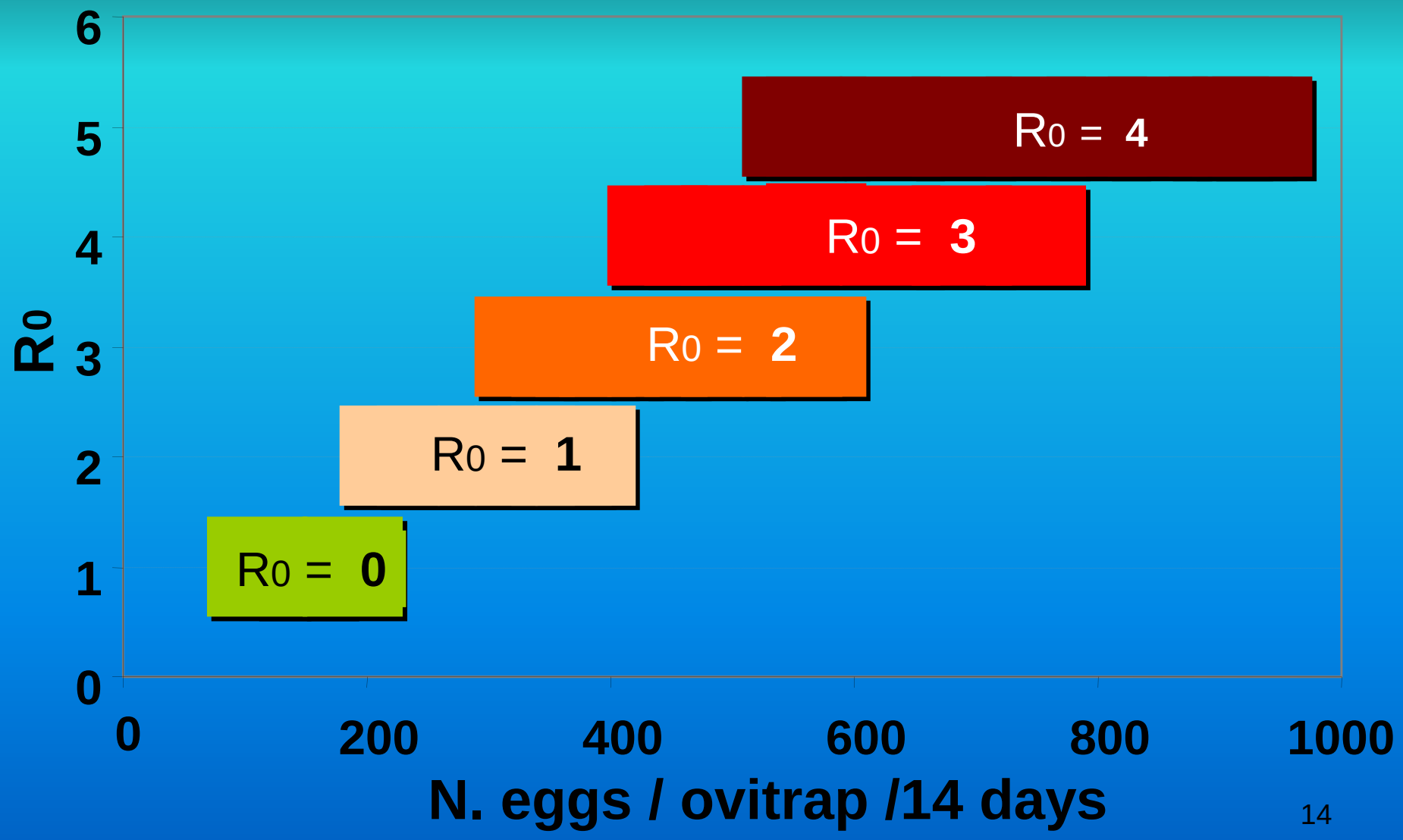
N eggs/ovitrap

$$R_0 = \frac{ma^2 * V * P^n}{-\log_e P}$$

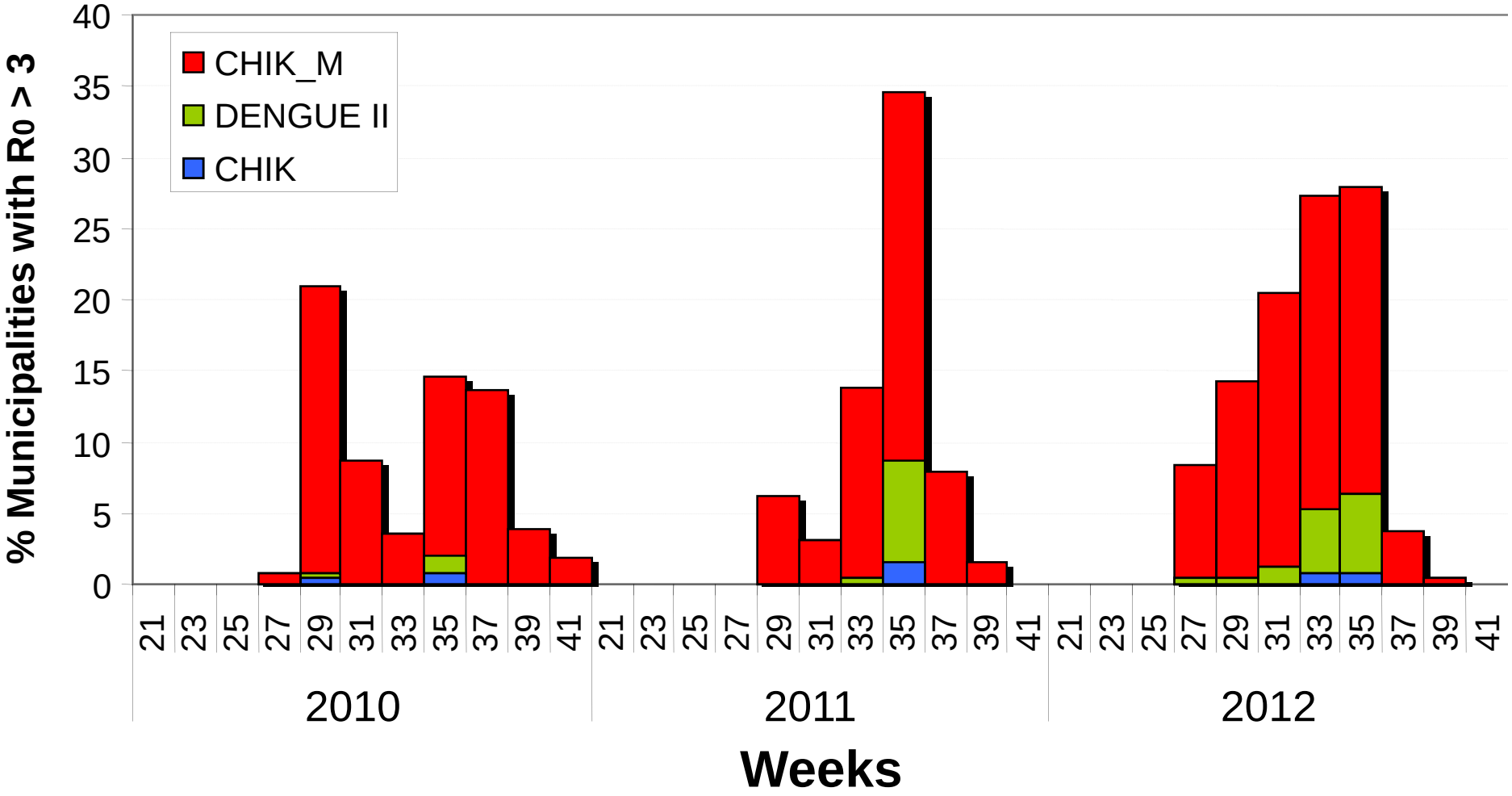
Parameters in the R_0 equation

Parameter	Label	Value	Reference
Multifeeding/gonotrophic cycle	*mF*	1.20	Hawley 1988
Host Feeding Pattern	*AI*	0.86-0.96	Valerio et al. 2010
Gonotrophic cycle	*GC*	4 - 11 days	Calculated in function of temperature by means the model of Focks et al. 1993.
Vector competence	*Sm*	Chik.: 24 – 80%	Vazeille et al. 2007 Talbalaghi et al. 2010 Mitchell 1991
Viremia	*V*	6 days	Peters and Dalrymple 1990 Boelle et al. 2008
Females daily survival rate	*p*	0.90	Hawley 1988 Willis and Nasci 1994 Almeida et al. 2005
Extrinsic incubation period	*i*	EIP=0.71GC	Dubrulle et al. 2009 Hawley 1988
Population susceptibility to Dengue and CHIKV	*Sv*	1	Moro et al. 2010
Vectorial capacity correction factor	X_V	0.101	Calculated
Bites per Egg Rate	*B*	PDS: 0.033 ± 0.015 HLC: 0.042 ± 0.021 NBC: 0.027 ± 0.028	Calculated

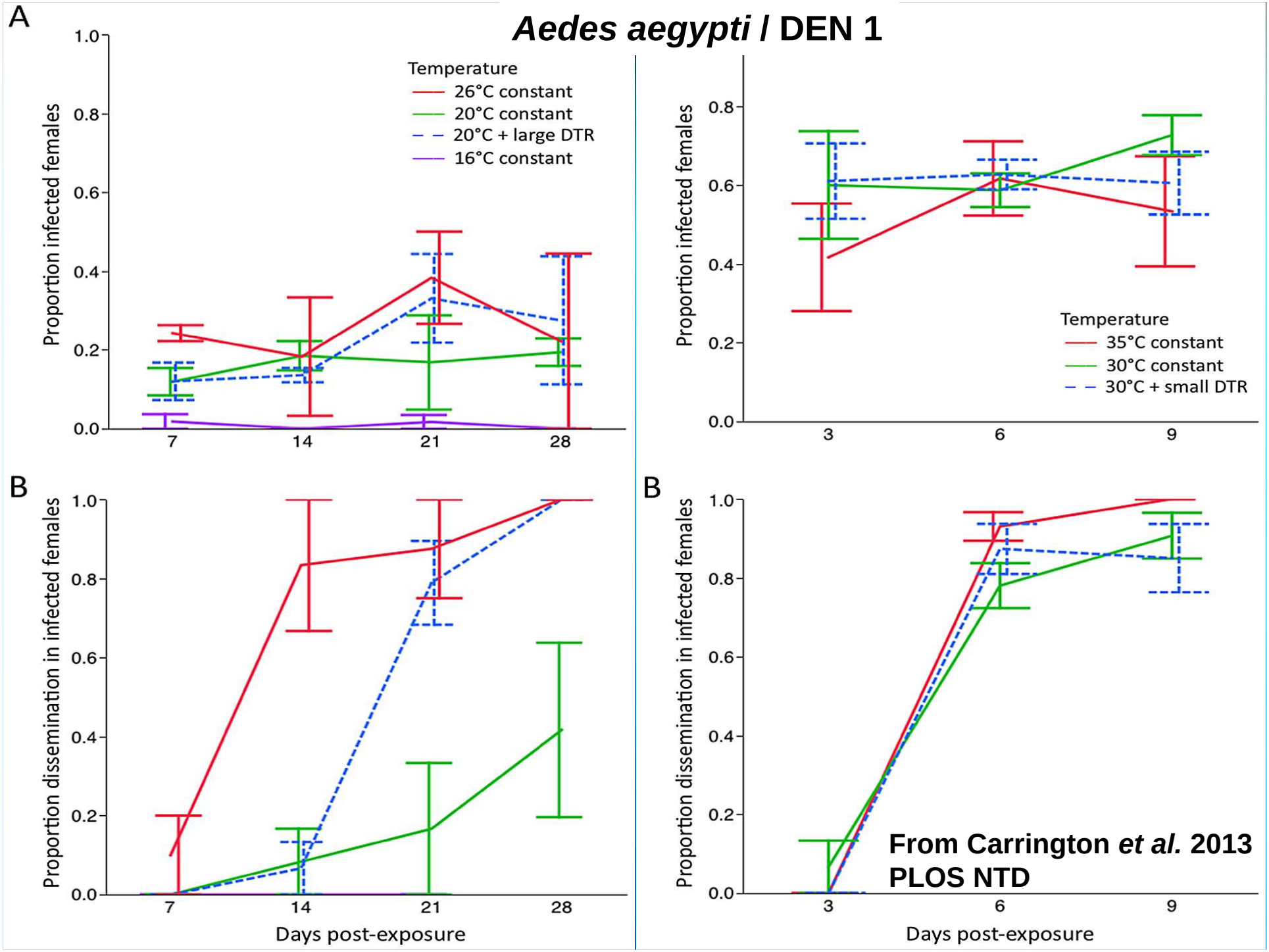
Relation between egg densities and R_0



% Municipalities with average number of eggs supportive $R_0>3$



Aedes aegypti / DEN 1



AEDES ALBOPICTUS SURVEILLANCE

PRO

- stimulating positive competition between Municipalities in doing vector control
- allowing quantitative risk evaluation
- allowing population density comparison and new species detection
- fed models

CONS

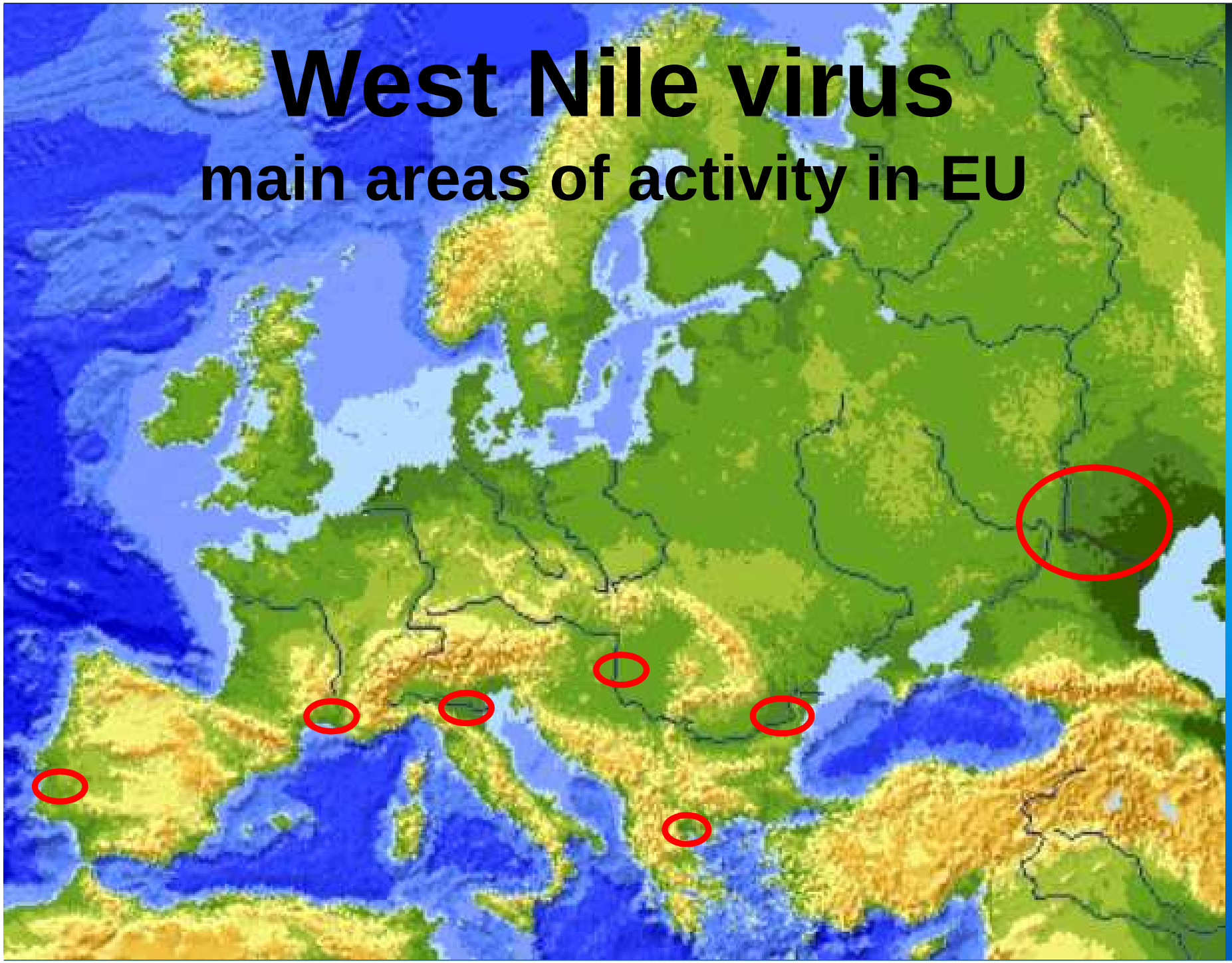
- field data manipulation
- possible negative impact on media
- stimulate adulticides treatments

WEST NILE VIRUS // *CULEX*

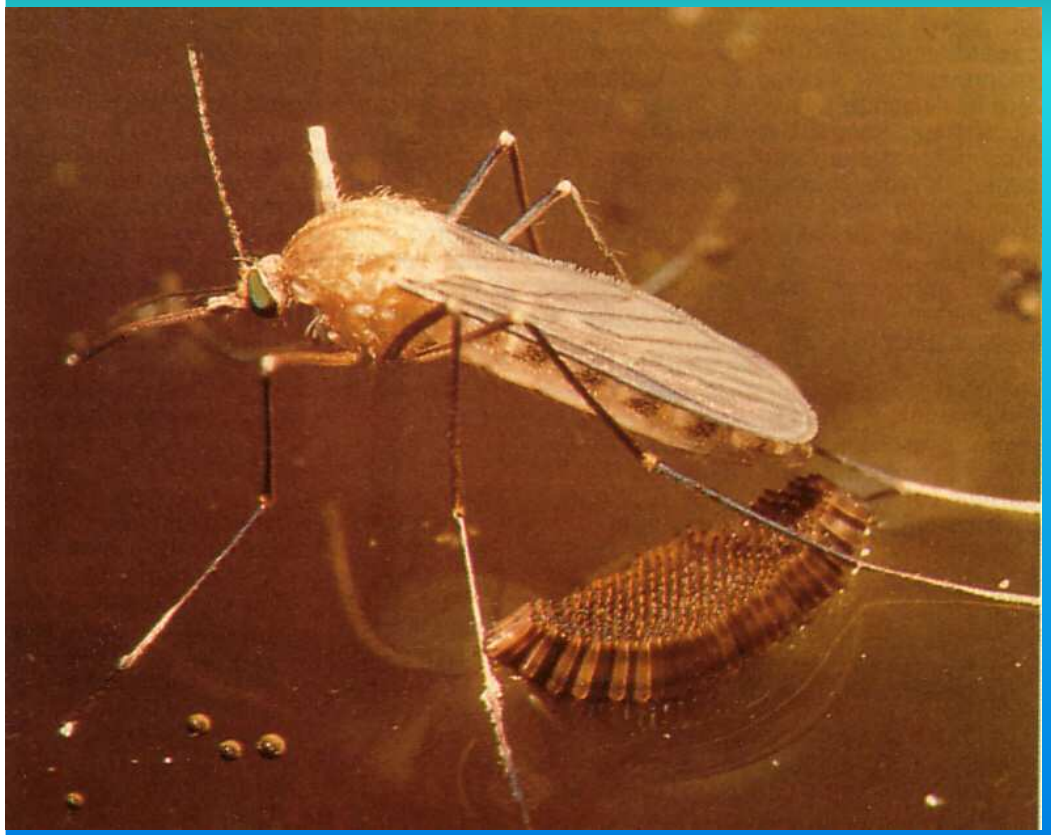
- Family Flaviviridae, genus Flavivirus
- The most widespread arbovirus in the world
- Epidemiology & vectors differ between regions

West Nile virus

main areas of activity in EU



Culex pipiens molestus



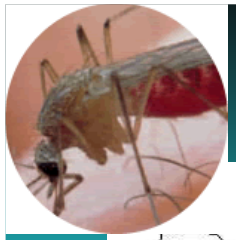
- **larval habitat**: ditches, catch basins, gardening containers, underground waters
- **ethology**: female active at night
- **hosts**: birds & mammals
- **overwintering**: mated female
- **autogeny, stenogamy, endophily**

COST-BENEFIT ANALYSIS IN WNV SURVEILLANCE

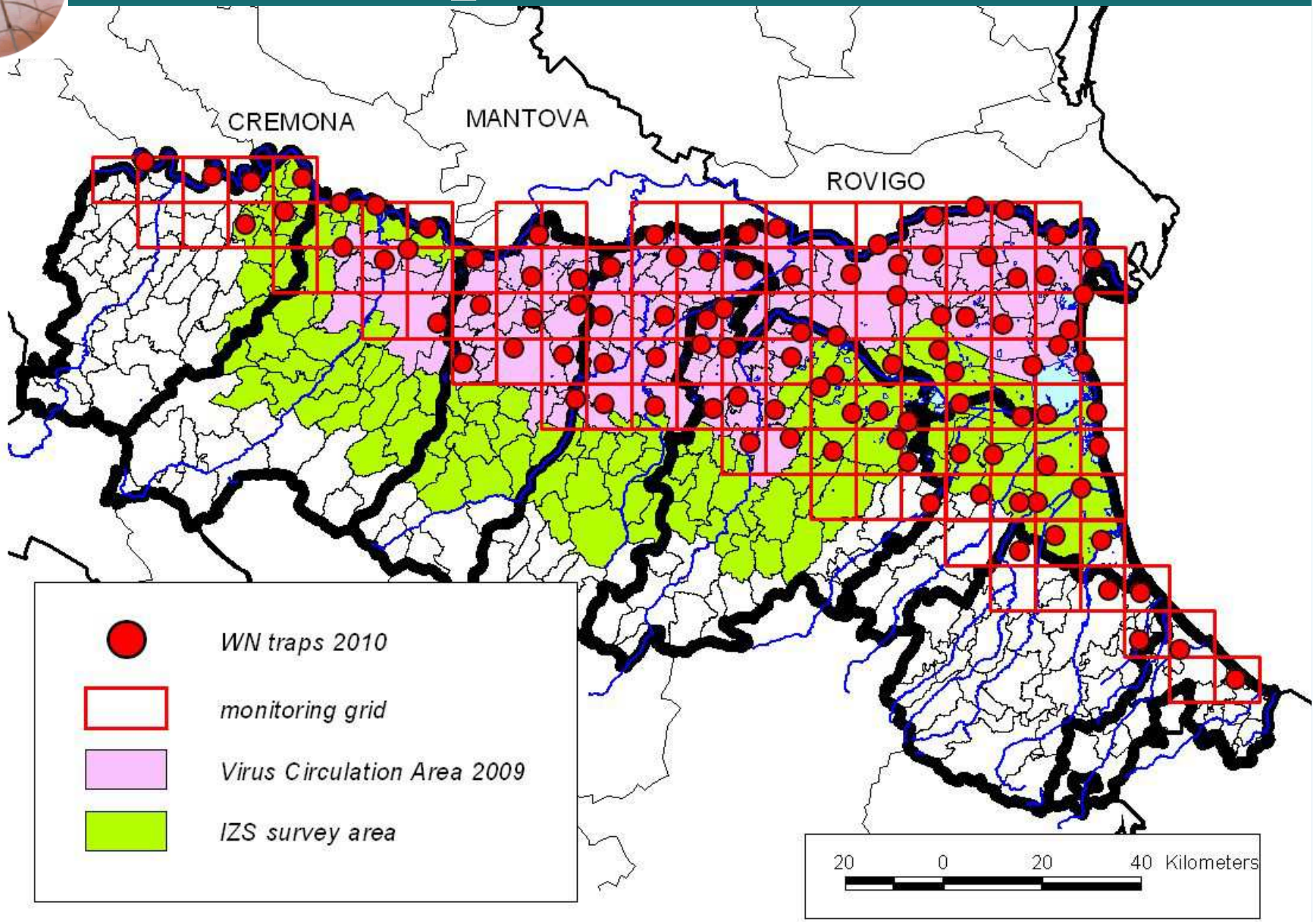
- Sensitivity
- Early detection capacity
- Usefulness

WEST NILE VIRUS INTEGRATED SURVEILLANCE

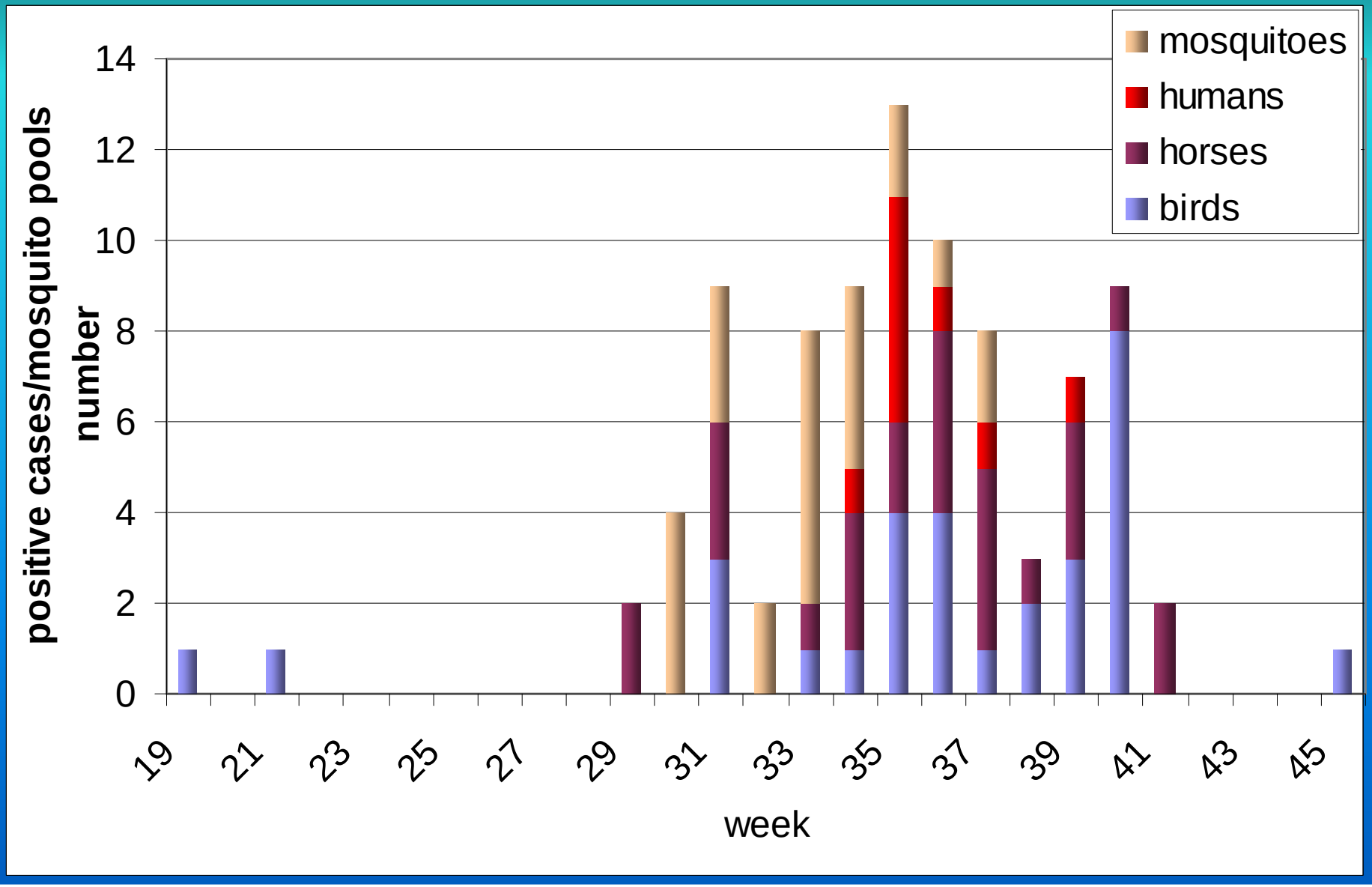
- Mosquitoes
- Birds
- Horses
- Humans



CO_2 traps position



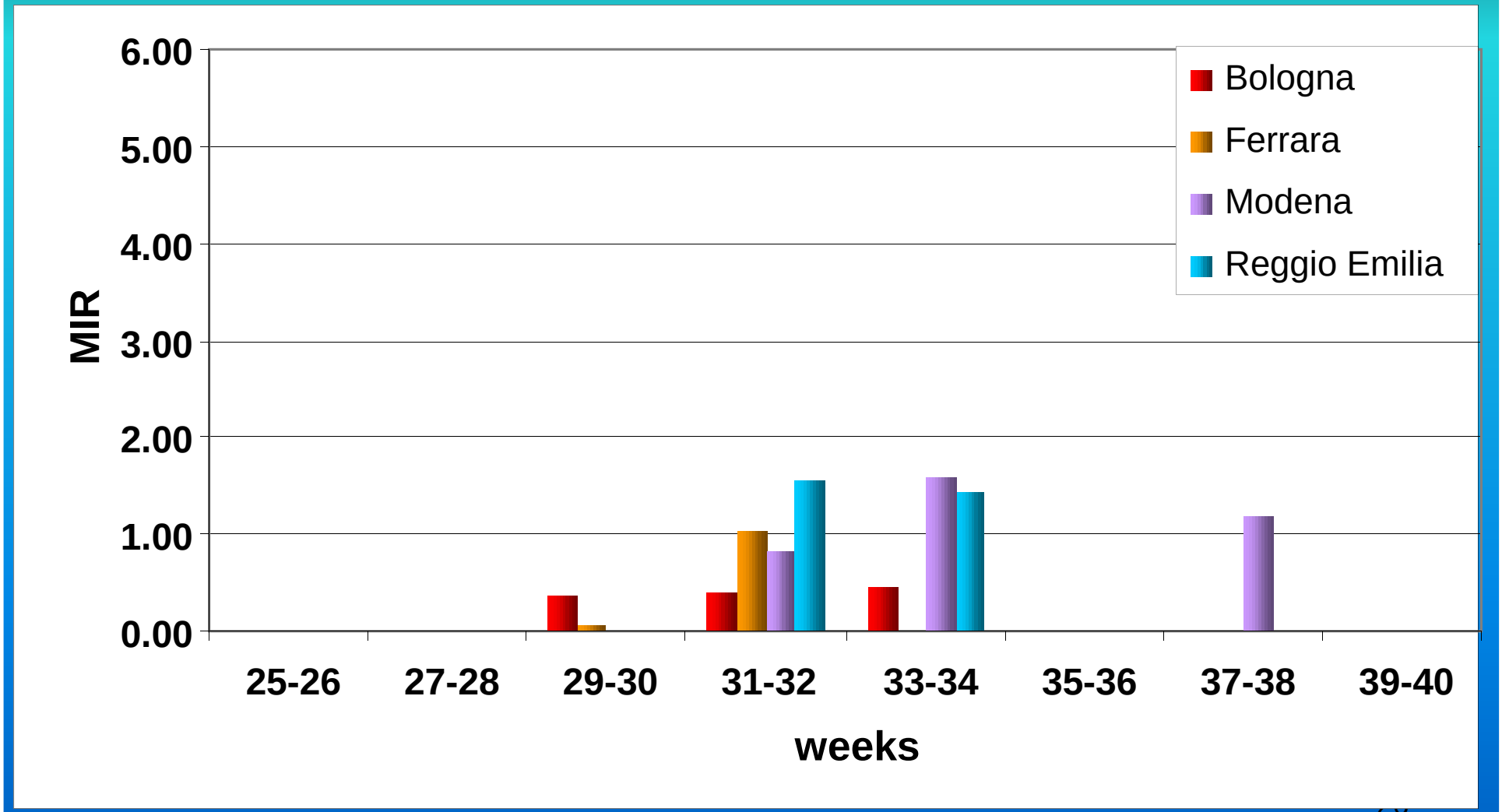
WNV seasonal evidences in E-R - 2009



Mosquitoes collected by CO_2 traps and PCR analyzed Emilia-Romagna June-October 2009

	N.	N.pools	WN RT-PCR +
Cx.pipiens	155,053	1,259	27
Ae.caspius	29,283	314	--
Ae.albopictus	1,227	108	--
Cx.modestus	246	26	--
Ae.vexans	4,597	60	--
An.maculipennis	82	14	--
Ae.detritus	5	2	--
Cs.annulata	2	2	--
TOTAL	190,414	1,785	

MIR (*Culex pipiens*) – 2009



E-R WN SURVEILLANCE DATA 1/2

	2009	2010
MOSQ(pools)	27/1789(July 21)	3/2533(August 23)
HOR(sentinel)	18/114(August)	1/147(September)
BIRDS	44/1218(July 30)	2/1039(August 1st)
HUMANS	9(end of August)	0

E-R WN SURVEILLANCE DATA 2/2

	2011	2012
MOSQ(pools)	0/1824	0/1806
HOR(sentinel)	//	//
BIRDS	0/1361	0/1310
HUMANS	0	0

IN CASE OF WN ACTIVITY DETECTION ?

Depending from the seasonal period and intensity of virus circulation, modulation of:

- Blood system screening;
- Information on self protection measures;
- Vector control ?

Risk area	Risk level	Probability of human outbreak	Description	Recommended response
Predisposed	**1**	Unknown	▪ Ecological condition suitable to WN circulation but no historical circulation of WNV	▪ Consider drafting WNV preparedness plan
Imperilled	**2**	Unknown	▪ Ecological condition suitable to West Nile virus circulation ▪ AND past evidences of West Nile virus circulation	▪ Develop West Nile virus preparedness plan, including surveillance activities and an integrated vector control plan ▪ Allocate resources necessary to enable emergency response ▪ Implement larval control as part of the integrated vector contrc in case of West Nile virus circulation in previous year
Imperilled	**3a**	Low	▪ Current surveillance findings (i.e. mosquito or birds screening) indicating West Nile virus epizootic activity in the area, in the **second** part of the season (August-September-October)	▪ As in risk level 2 ▪ AND Implement public education programs focused on risk potential, personal protection, and emphasizing residential source reduction ▪ Vector control focuses on larval control
Imperilled	**3b**	Low to moderate	▪ Current surveillance findings (i.e. mosquito or birds screening) indicating WNV epizootic activity in the area, in the **first** part of the season (May-June-July)	▪ As in risk level 3a ▪ AND increase entomological and bird surveillance ▪ AND increase effort for public information on personal protection and continued source reduction ▪ AND If surveillance indicates virus circulation is increasing initiate ground adult control in areas at high risk for humans or in hot spot sites (if known)
Imperilled	**4**	High	▪ WNV specific IgM detected in local non vaccinated horse(s) or WNV isolated from local horse.	▪ As in risk level 3b ▪ If surveillance indicates virus circulation is increasing initiate ground adult control in areas at high risk for humans or in hot spot sites (if known)
Affected	**5**	ongoing outbreak, uncertainty about size	▪ at least one human cases detected (i.e. probable or confirmed human case according to EU case definition)	▪ Response as in level 4 ▪ AND intensify ground adult mosquito control with multiple applications in areas of high risk of human cases ▪ AND enhance risk communication ▪ AND monitor efficacy of spraying on target mosquito populations. ▪ AND In case a large area is involved coordinate the program by an emergency unit with all authorities involved

DRAFT

Prospects

- **The entomological surveillance shows good usefulness for WNV early detection;**
- **With the increasing understanding and collection of field based evidences it might be possible to reduce the surveillance management costs;**
- **Possible measures to prevent/control WNV outbreaks will benefit of surveillance activities;**
- **The entomological surveillance may evidence other arboviruses activity (e.g. Usutu Virus);**
- **Longitudinal data sets will support the development of predictive models useful in risk assessment.**

ISTITUTO G. CAPORALE
TERAMO

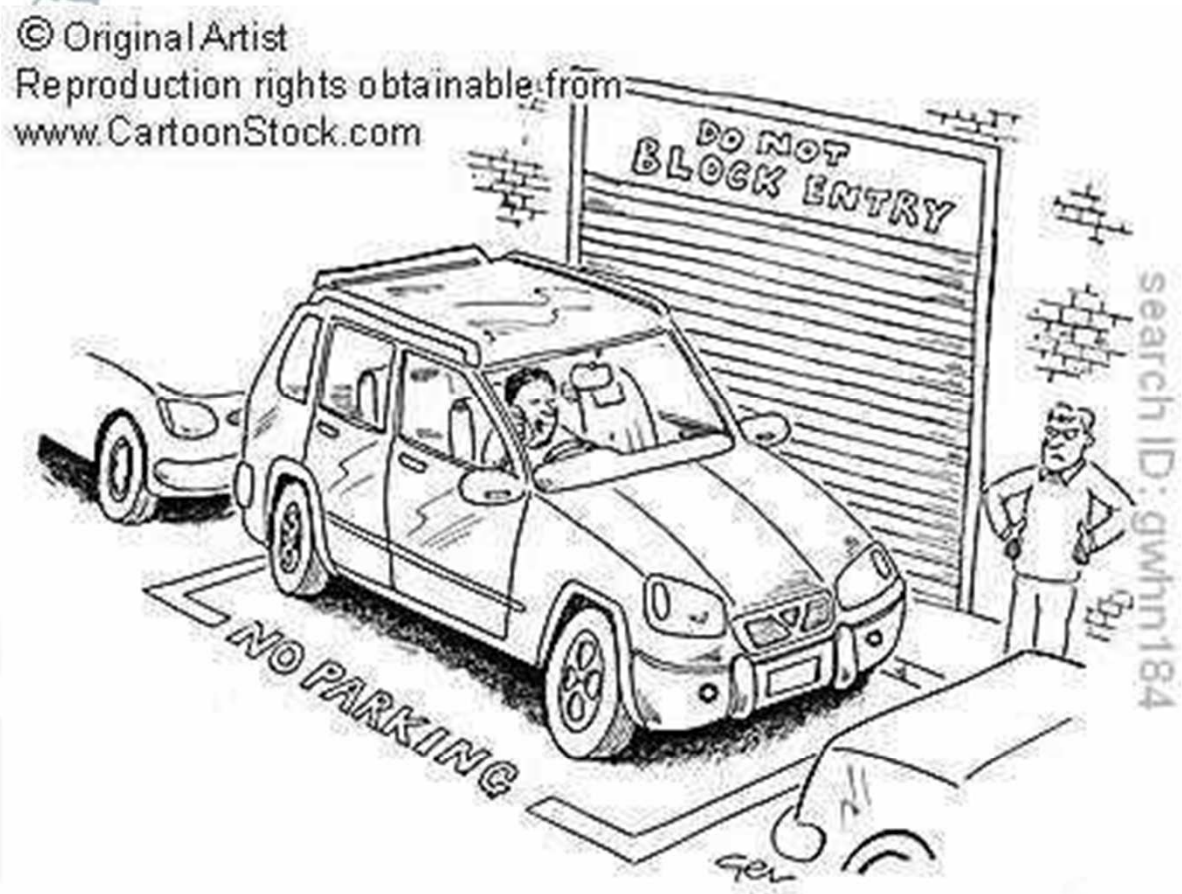
USUTU VIRUS: ITALIAN SITUATION

Virology Department
Istituto G. Caporale Teramo (Italy)
Giovanni Savini

Brescia, 9 May 2013

ISTITUTO G. CAPORALE
TERAMO

What's USUV?

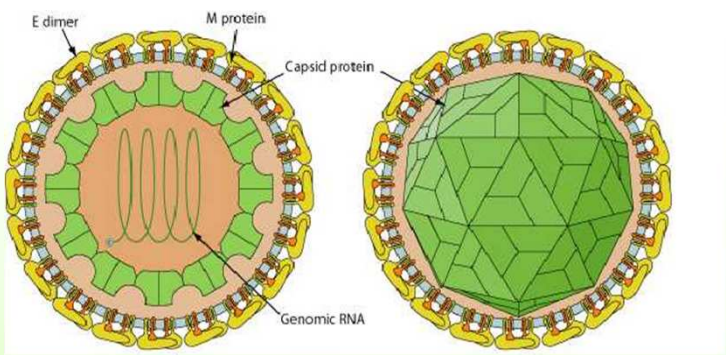


TYPICAL 'FAUX PAS FOUR'

ISTITUTO G. CAPORALE
TERAMO

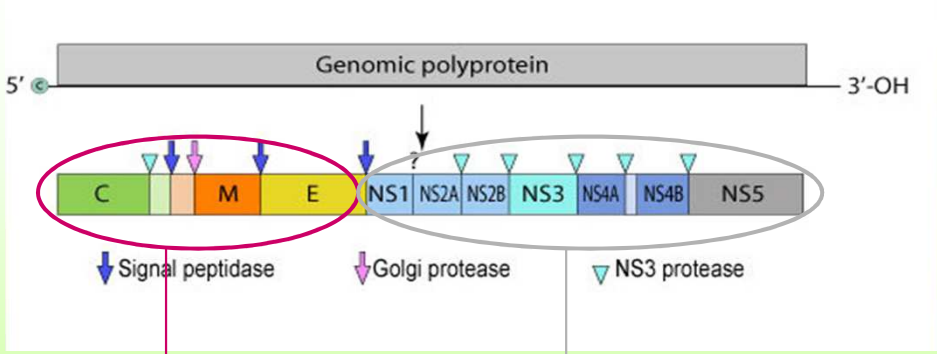
Usutu virus: *Family*: **Flaviridae**

Genus: Flavivirus



Spheric virion (50nm in diameter)
Isometric capsid
Envelope (matrix protein M, envelope E)
Linear ssRNA positive polarity (11kb)

RNA is infectious and serves as both the genome and viral messenger RNA. It is translated as a single polyprotein that is then cleaved into three structural proteins and seven non-structural proteins.



3 Structural proteins

7 Non Structural proteins

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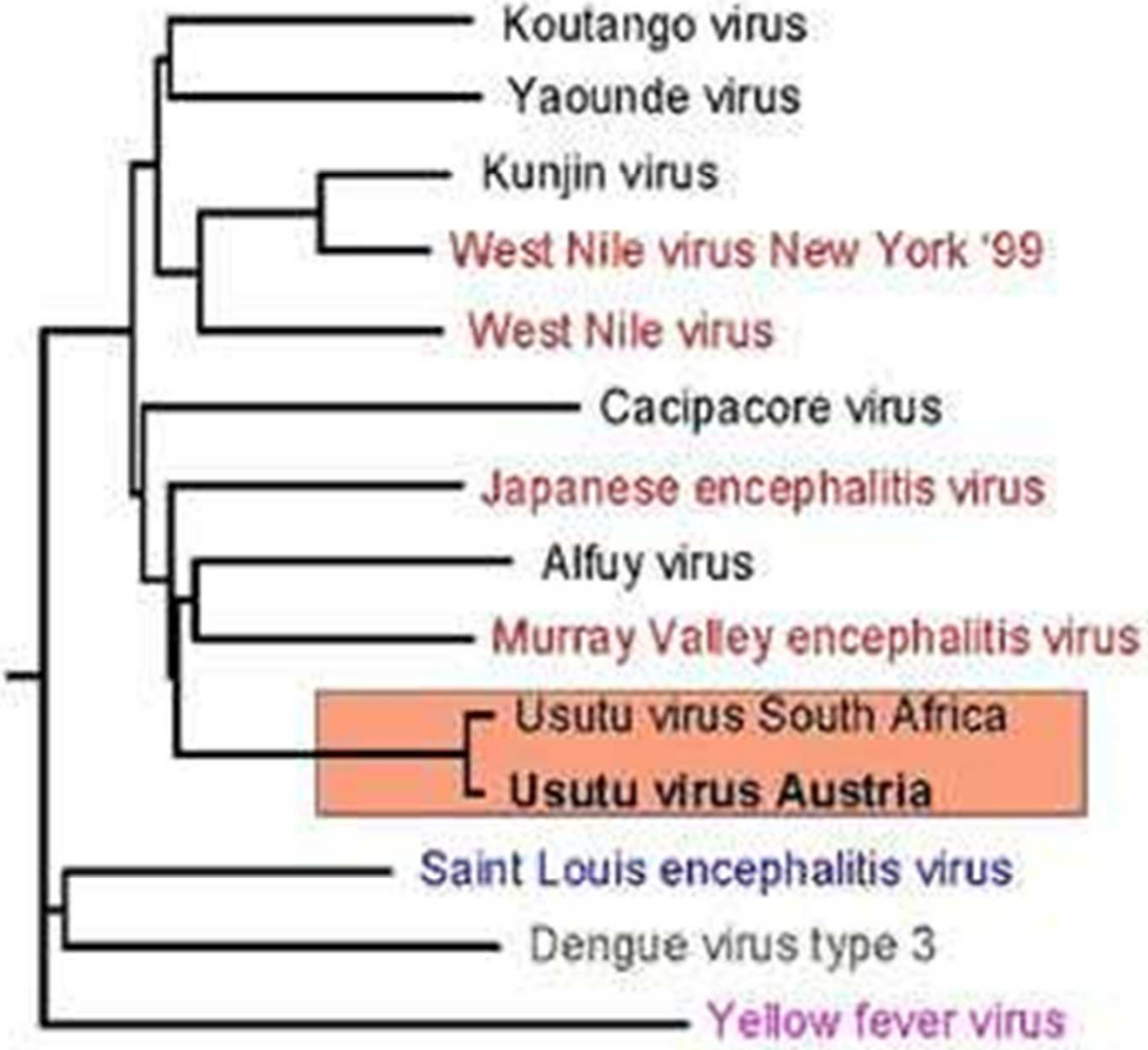
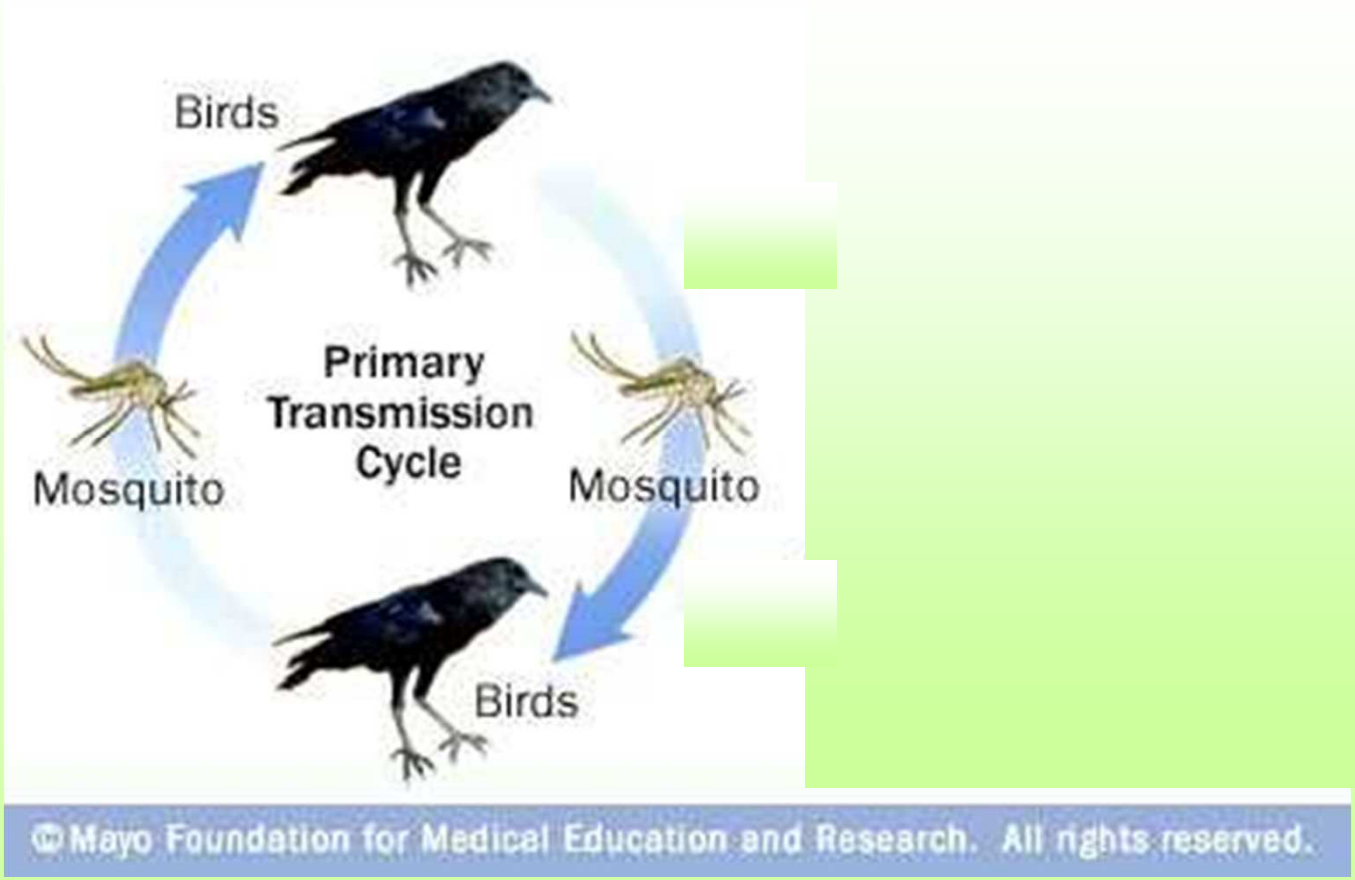
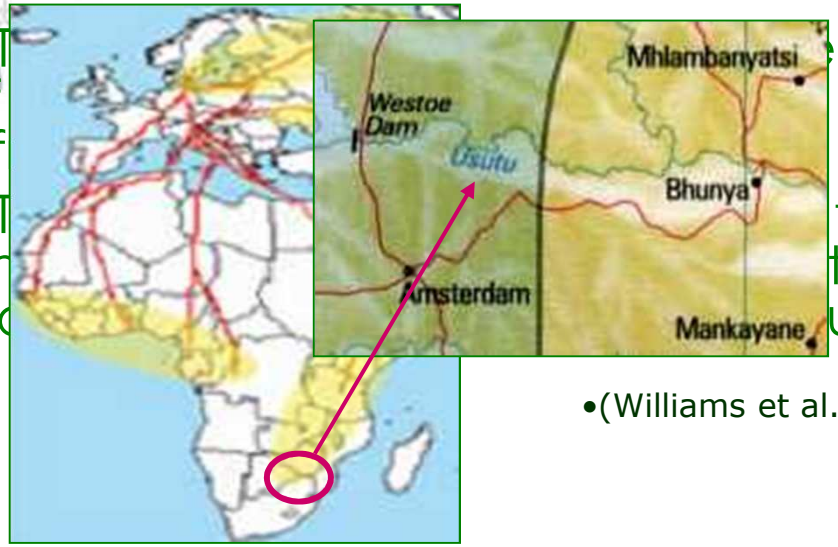


Usutu virus life cycle



USUTU: background



✓T ed in 1959 from a mosquito pool of *neavei)* in Swaziland (South Af

✓T the reference number SAAr1776 an tu according to a river located cl uitoes were captured.

•(Williams et al., 1964; Theiler and Downs 1973; Karabatsos 1985)

✓Since that moment it has been reported in several other African countries: Senegal, Central African Republic, Nigeria, Uganda, Burkina Faso, Cote d'Ivoire and Marocco.

In addition to mosquitoes, USUV was also isolated in different species of birds and…

(Nikolay et al., 2011)

USUTU: human infection in Africa

- In 1959 USUTU virus was first isolated in Swaziland (South Africa) from a woman exhibiting a fever and skin rash.

(Adams F, Institut Pasteur de Dakar)

- In 1981 a case occurred in the Central African Republic in a patient with fever and rash

(Nikolai B et al., 2011)

- In 2004 in Burkina Faso, another case of a 10 year old patient with fever and jaundice was reported.

(Nikolai B et al., 2011)

USUTU in Africa

- Even if grouped with important human pathogens, USUV was considered for many years as an exotic virus of minor importance confined to a small area of sub-Saharan Africa and characterised by a very low pathogenicity for humans and animals.

USUTU in Europe

- In 2001 USUV appeared in Europe (for the first time outside of Africa) and coinciding with this occurrence in Austria (Vienna 2001 strain), the virus appears to have changed its behaviour: fatal cases linked to the USUV infection were reported in wild birds but not in other animals and humans. (Weissenbock et al., 2002)

- In the following years, the virus has been detected in dead birds and/or mosquitoes in Hungary in 2005 (Budapest 2005 strain) and in several other European countries (Spain, Italy, Switzerland) and the USUTU infection has also been demonstrated serologically in wild bird hosts in Czech Republic, England, Germany, Poland and again in Italy, Spain, Switzerland. (Vazques et al., et al., 2011)

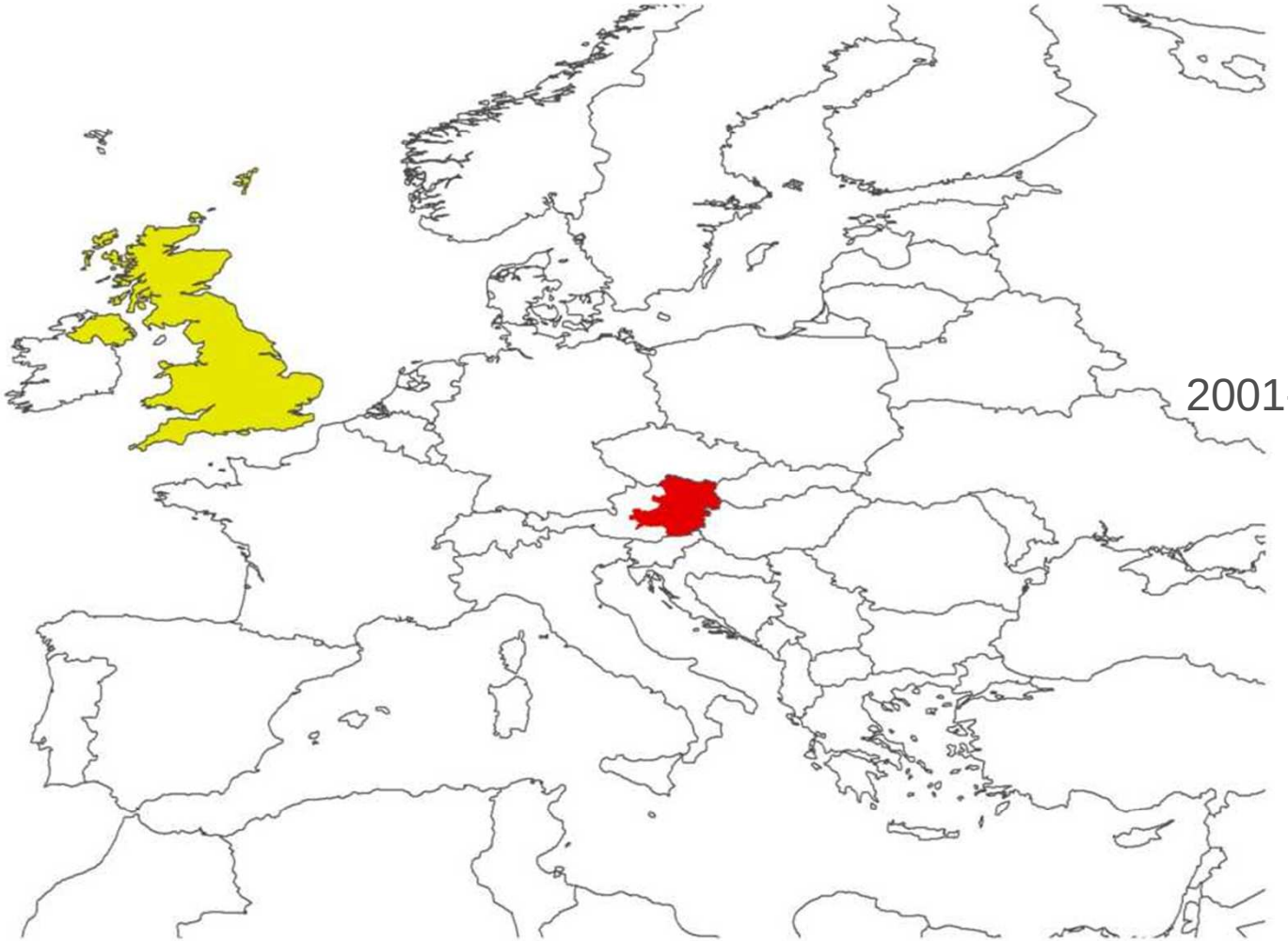
USUTU IN EUROPE

2001

USUTU IN EUROPE

2001-2002

USUTU IN EUROPE

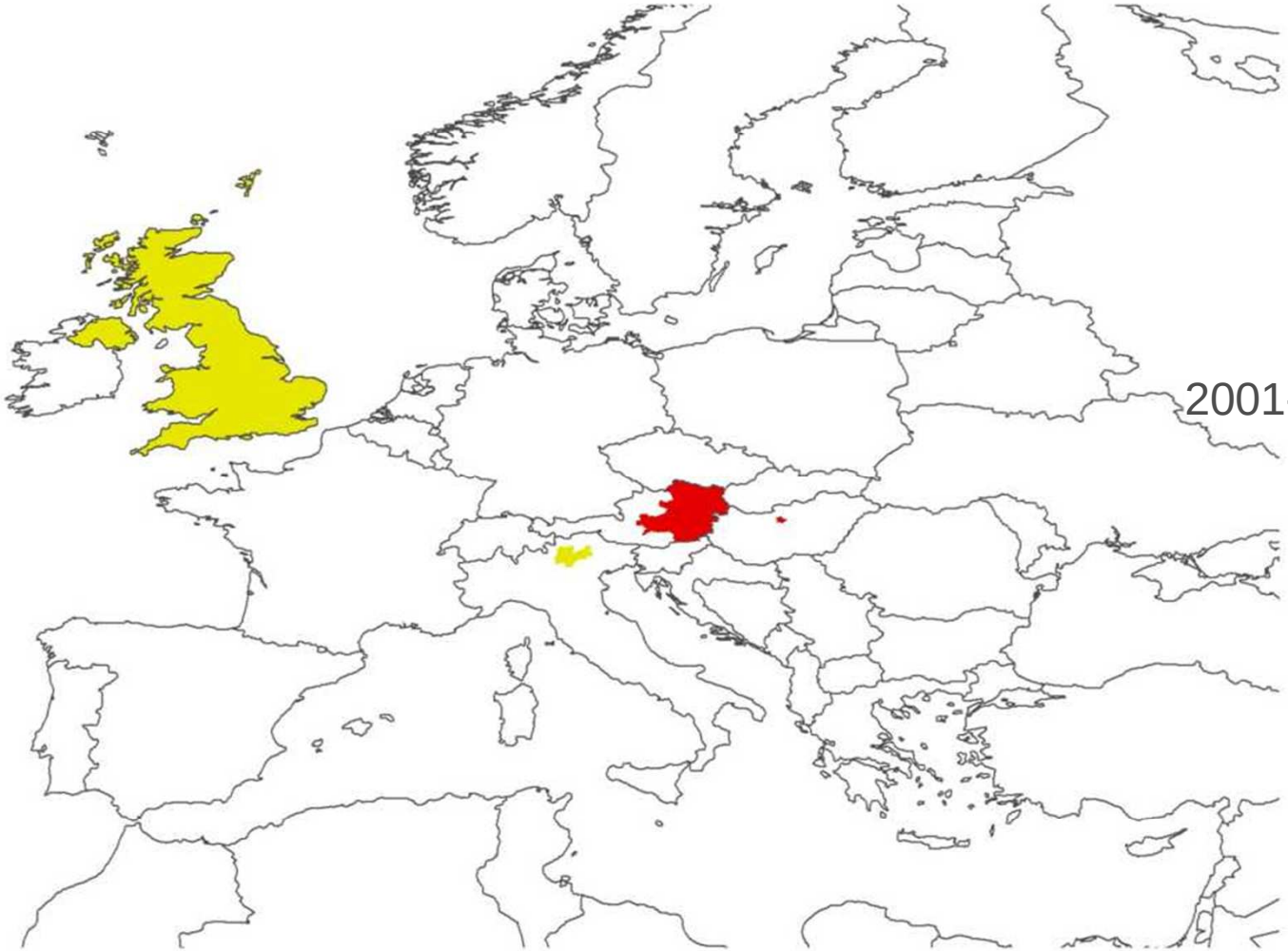


2001-2003

rosso: isolamento virale

giallo: test sierologici

USUTU IN EUROPE

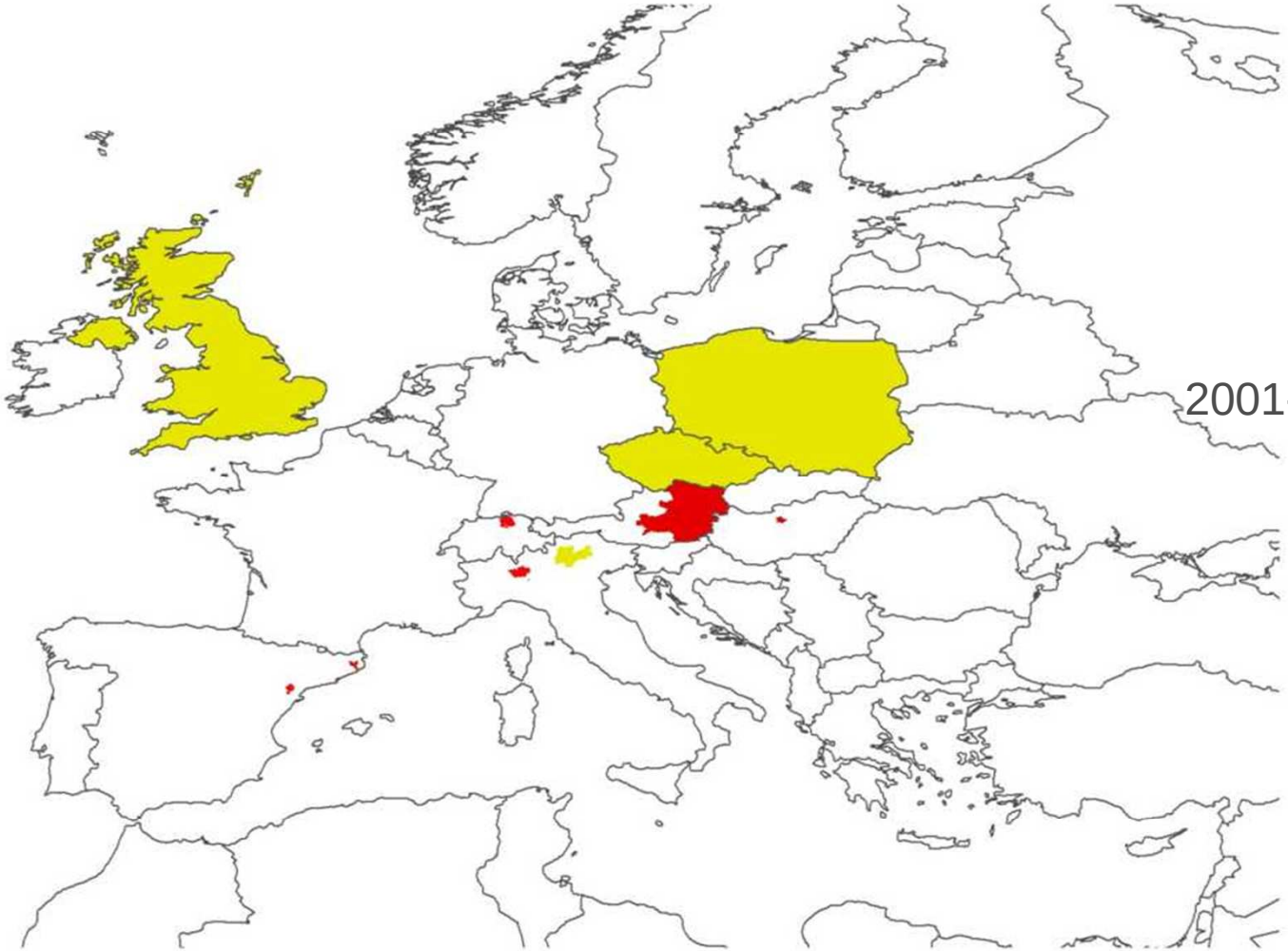


2001-2005

rosso: isolamento virale

giallo: test sierologici

USUTU IN EUROPE

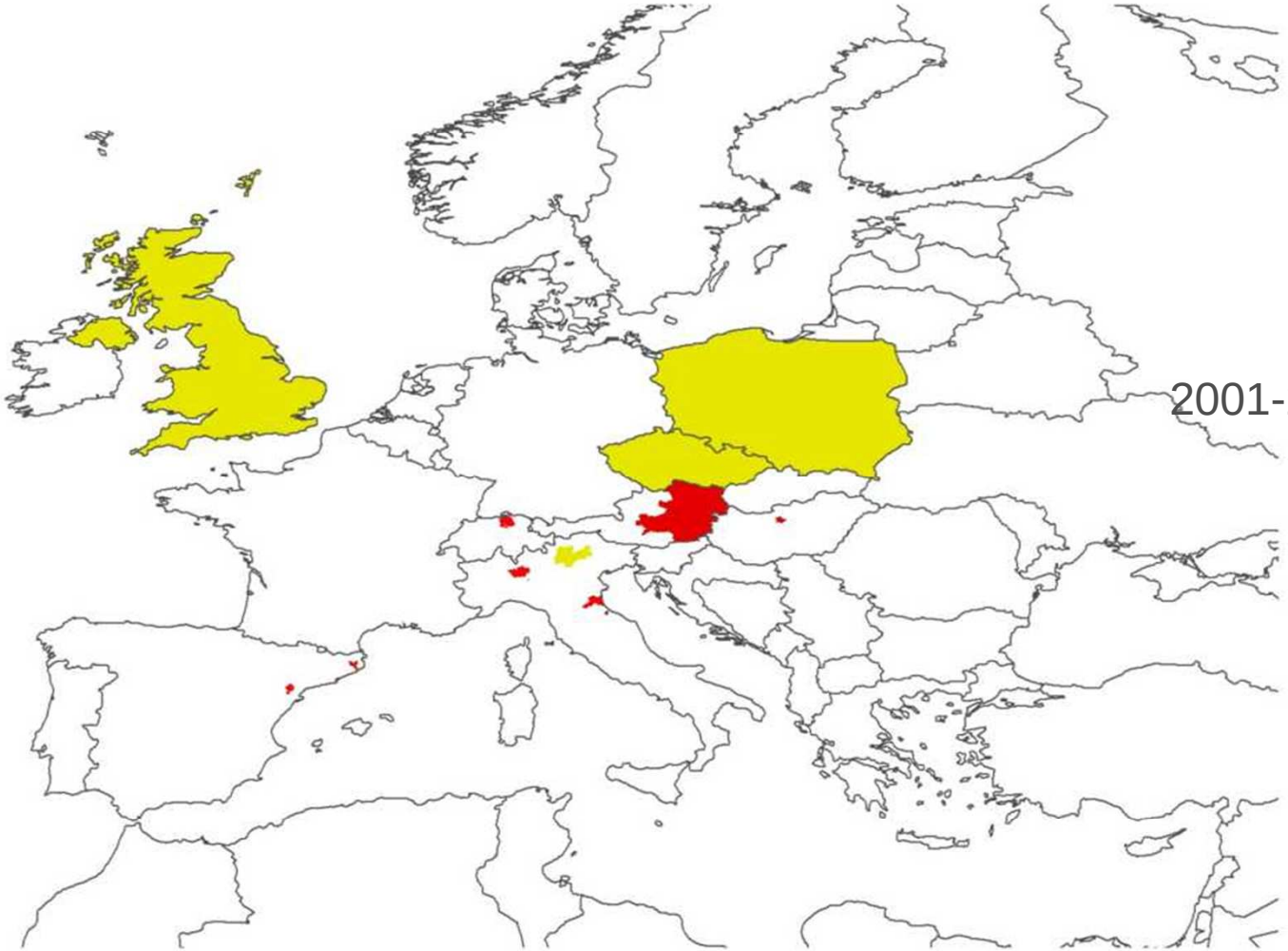


2001-2006

rosso: isolamento virale

giallo: test sierologici

USUTU IN EUROPE

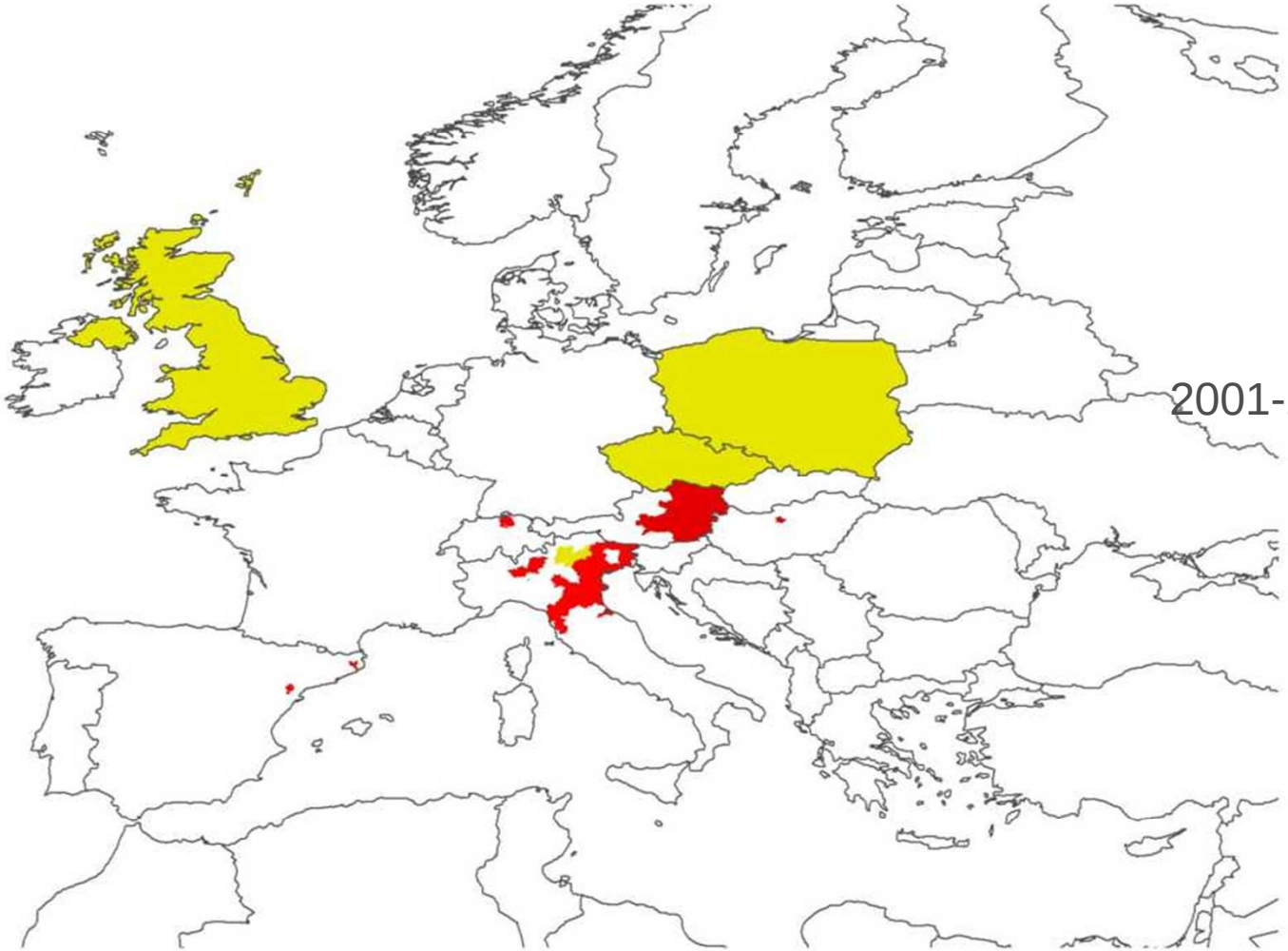


2001-2007

rosso: isolamento virale

giallo: test sierologici

USUTU IN EUROPE

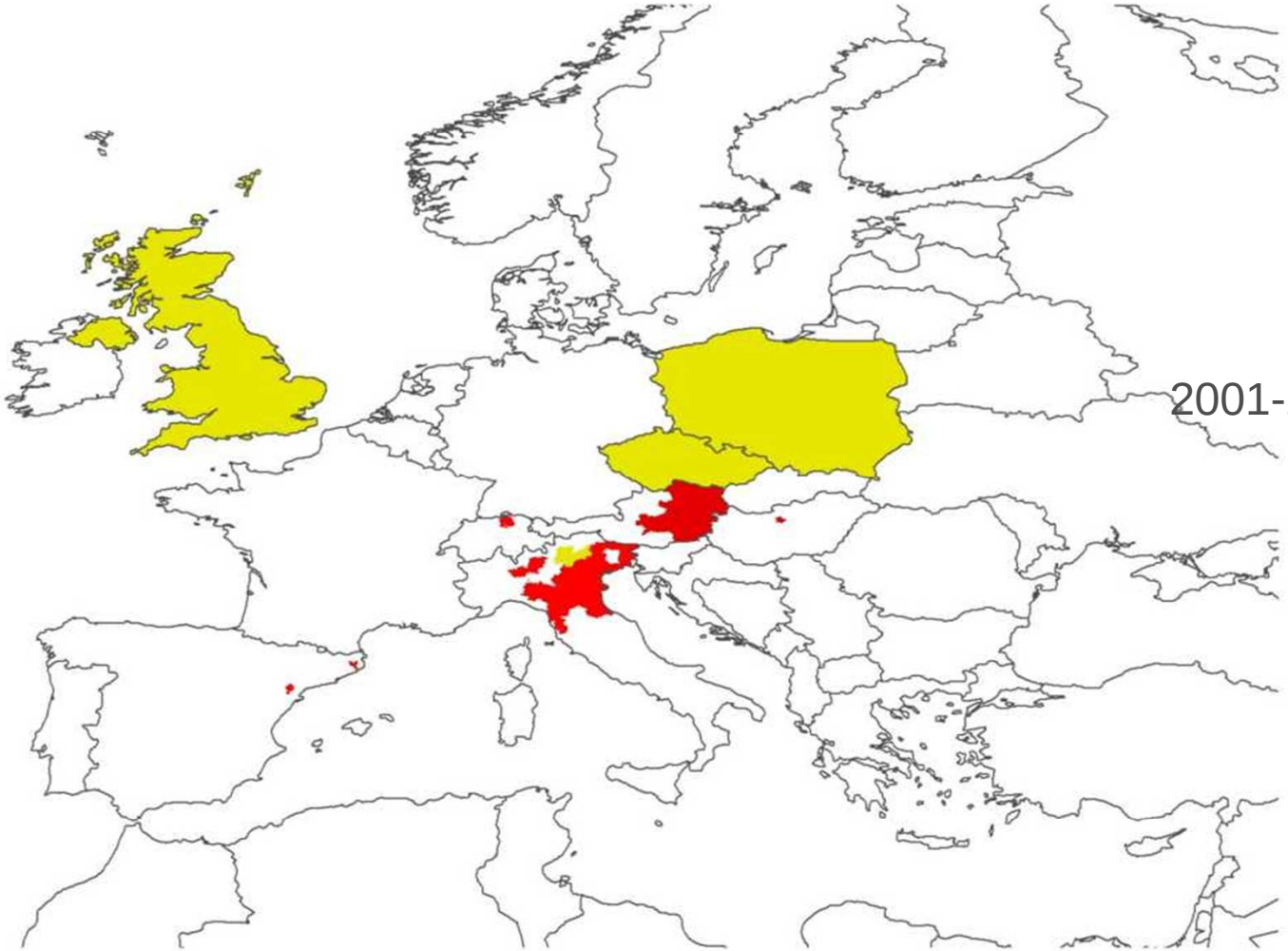


2001-2008

rosso: isolamento virale

giallo: test sierologici

USUTU IN EUROPE

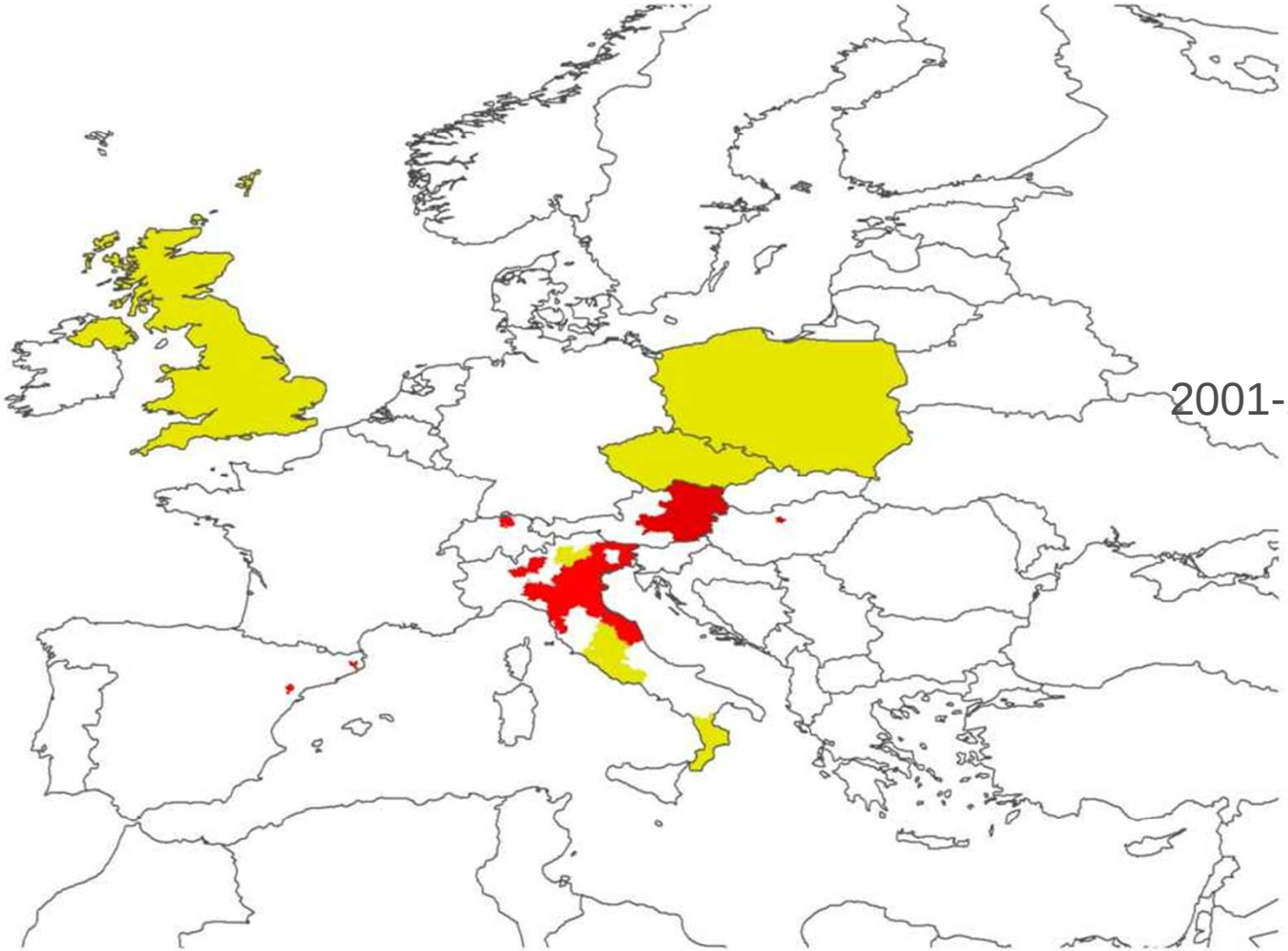


2001-2009

rosso: isolamento virale

giallo: test sierologici

USUTU IN EUROPE

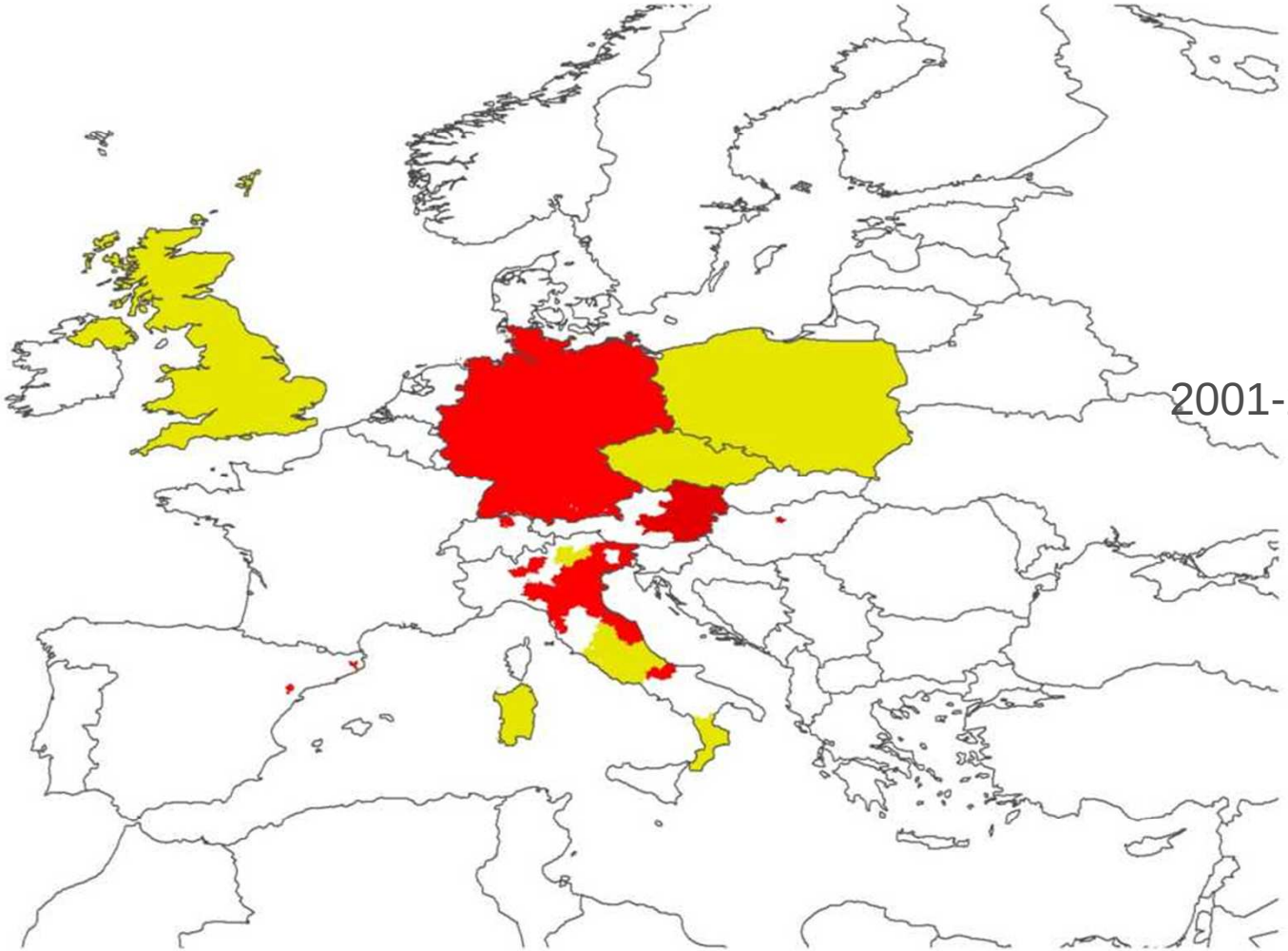


2001-2010

rosso: isolamento virale

giallo: test sierologici

USUTU IN EUROPE



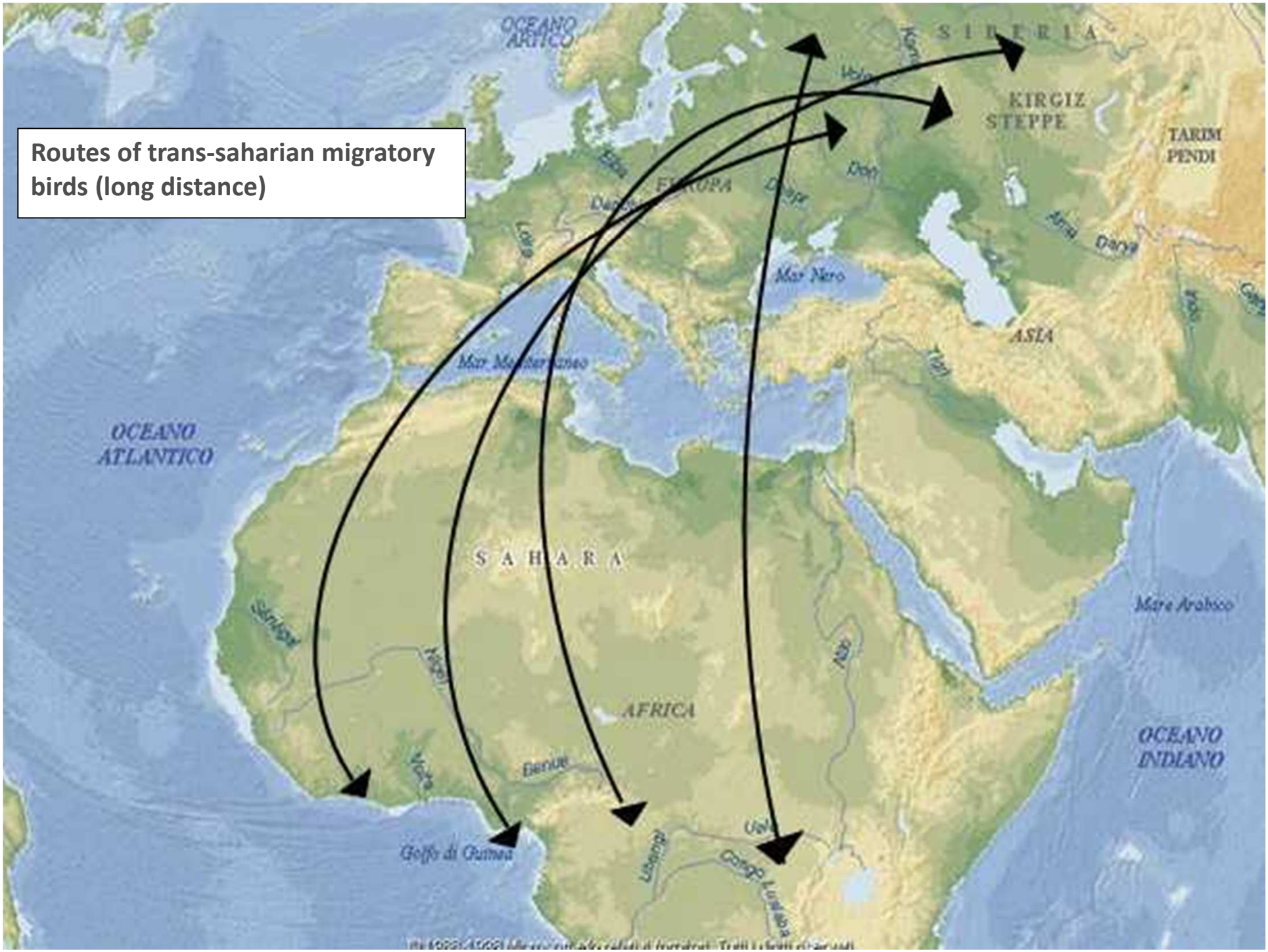
2001-2011

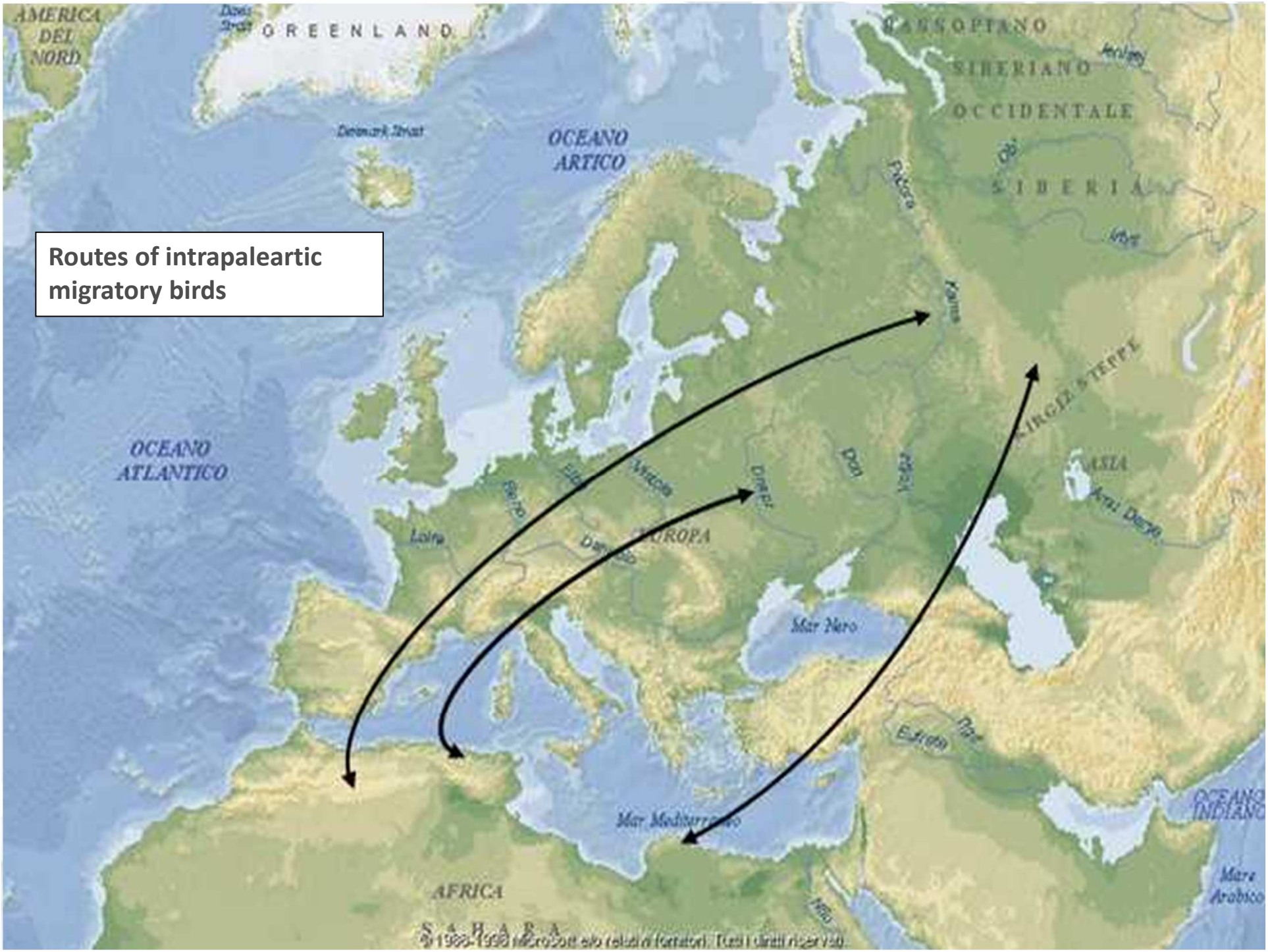
rosso: isolamento virale

giallo: test sierologici

…. and in Italy?

…. because of its particular geographical location and favourable climate, Italy has always played an important role in the epidemiology of vector borne diseases


Routes of trans-saharian migratory birds (long distance)
OCEANO ARTICO
SIBERIA
KIRGIZ STEPPE
TARIM PENDI
EUROPA
Mar Nero
ASIA
Mar Mediterraneo
OCEANO ATLANTICO
SAHARA
Mare Arabico
AFRICA
OCEANO INDIANO
Golfo di Guinea


Routes of intrapaleartic migratory birds
AMERICA DEL NORD
GREENLAND
OCEANO ARTICO
OCEANO ATLANTICO
SIBERIANO OCCIDENTALE
SIBERIA
EUROPA
ASIA
Mar Nero
Mar Mediterraneo
AFRICA
SAHARA
OCEANO INDIANO
Mare Arabico

USUTU in Italy

- In **2005** the presence of USUV was linked to serological positives in sentinel chickens from the **Trentino region**. (Rizzoli et al., 2007)

Between **2006** and **2008** a few fatal cases of USUV were also described in owls and blackbirds near **Milano** in the **Lombardia region** and serological positives in sentinel horses in Ferrara (**E. Romagna region**).

(Lelli et al. 2008, Manarolla et al., 2010

Hypotheses on origin of USUV in Italy

1998-2008

1996-2007

Milan, 2006;
Ravenna, 2007

Tuscany, 1996

MORTALITA' DI MERLI (*Turdus merula*) IN TOSCANA

P. MANI[1], G. ROSSI[1], S. PERRUCCI[1], S. BERTINI[2]
[1] Dipartimento di Patolog a Animale Profilassi ed Igiene degli Alimenti, Università di Pisa
[2] Istituto di Clinica Medica Veterinaria, Università di Pisa

Parole chiave: *Turdus merula*, mortalità, parassitosi, inquinamento, ambiente

P. Mani, G. Rossi, S. Perrucci, S. Bertini
Mortality of *Turdus merula* in Tuscany

La Selezione Veterinaria 1998, 8-9; 749-753

Key words: *Turdus merula*, mortality, parasitological infection, pollution, environment

Abstract: Results of anatomo-histopathological, microbiological, parasitological and toxicological investigations performed on numerous blackbirds and some wild avian species found dead during the months of August and September in an extensive geographical area spanning the provinces of Florence and Pistoia are described in this study. The episode involves a densely populated area which is highly industrialised and where there is an extensive use of pesticides in the agricultural areas. In almost all the birds examined the presence of severe lesions closely related to parasitic infections are described. In addition, the positivity of one subject to toxicological examination for the presence of an inhibitor of cholinesterases drug is also reported. The role of sensitive bioindicator of environmental problems played by this permanent avian species can be noted.

Correspondence: P. Mani - Dipartimento di Patologia Animale Profilassi ed Igiene degli Alimenti - Facoltà di Medicina Veterinaria - Università degli Studi - Viale delle Piagge, 2 56124 Pisa

Zoonoses and Public Health

ORIGINAL ARTICLE

Serological Evidence of USUTU Virus Occurrence in North-Eastern Italy

R. Lelli[1], G. Savini[1], L. Teodori[1], G. Filipponi[1], A. Di Gennaro[1], A. Leone[1], L. Di Gialleonardo[1], L. Venturi[2] and V. Caporale[1]

[1] Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise 'G. Caporale', Via Campo Boario, Teramo, Italy
[2] Azienda Unità Sanitaria Locale di Ravenna, Ravenna, Italy

ISTITUTO G. CAPORALE
TERAMO

Hypotheses on origin of USUV in Italy

Usutu Virus, Italy, 1996

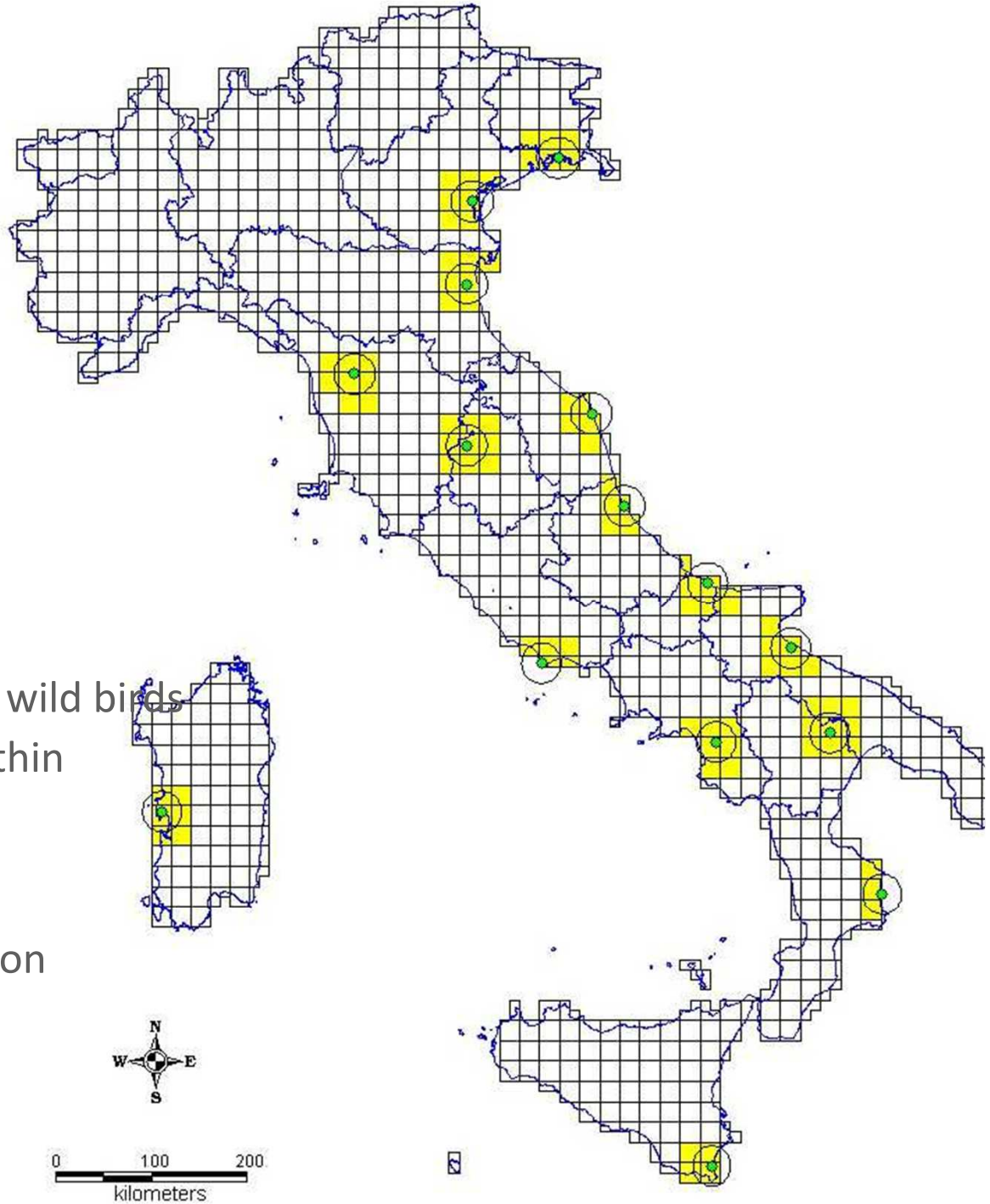
Herbert Weissenböck, Tamás Bakonyi, Giacomo Rossi, Paolo Mani, and Norbert Nowotny

Retrospective analysis of archived tissue samples from bird deaths in the Tuscany region of Italy in 1996 identified Usutu virus. Partial sequencing confirmed identity with the 2001 Vienna strain and provided evidence for a much earlier introduction of this virus into Europe than previously assumed.

Emerging Infectious Diseases Vol. 19, No. 2, February 2013

ISTITUTO G. CAPORALE
TERAMO

- **Surveillance on equids**
  - ✓ Sentinel horses
- **Surveillance on birds**
  - ✓ Sentinel chicken
  - ✓ Screening of carcases of wild birds found death or killed within control programs
- **Entomological surveillance**
  - ✓ Catching and identification



USUV infection in Italy

- Cavalli

2007

- Uccelli

2007

USUV infection in Italy

- Cavalli

2008

- Uccelli

2008

USUV infection in Italy

- Cavalli

2009

- Uccelli

2009

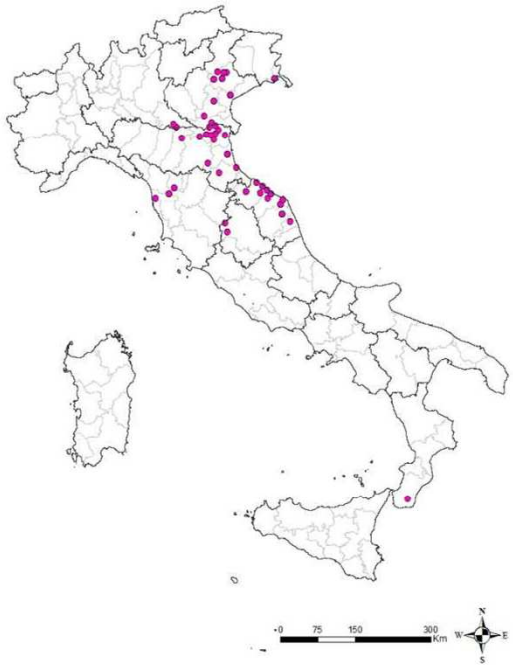
USUV infection in Italy

- Cavalli

2010

- Uccelli

2010



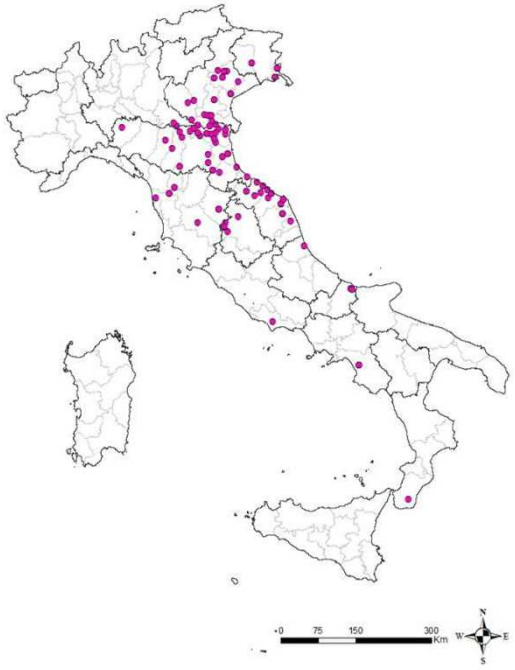
USUV infection in Italy

- Cavalli

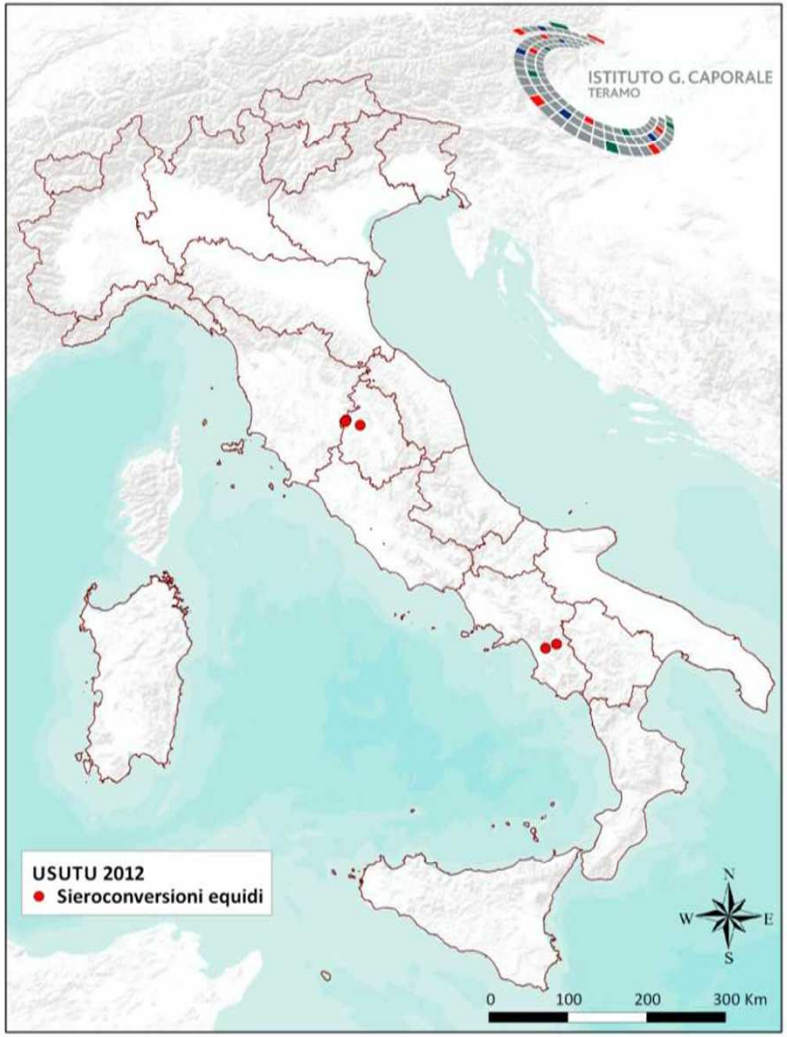
2011

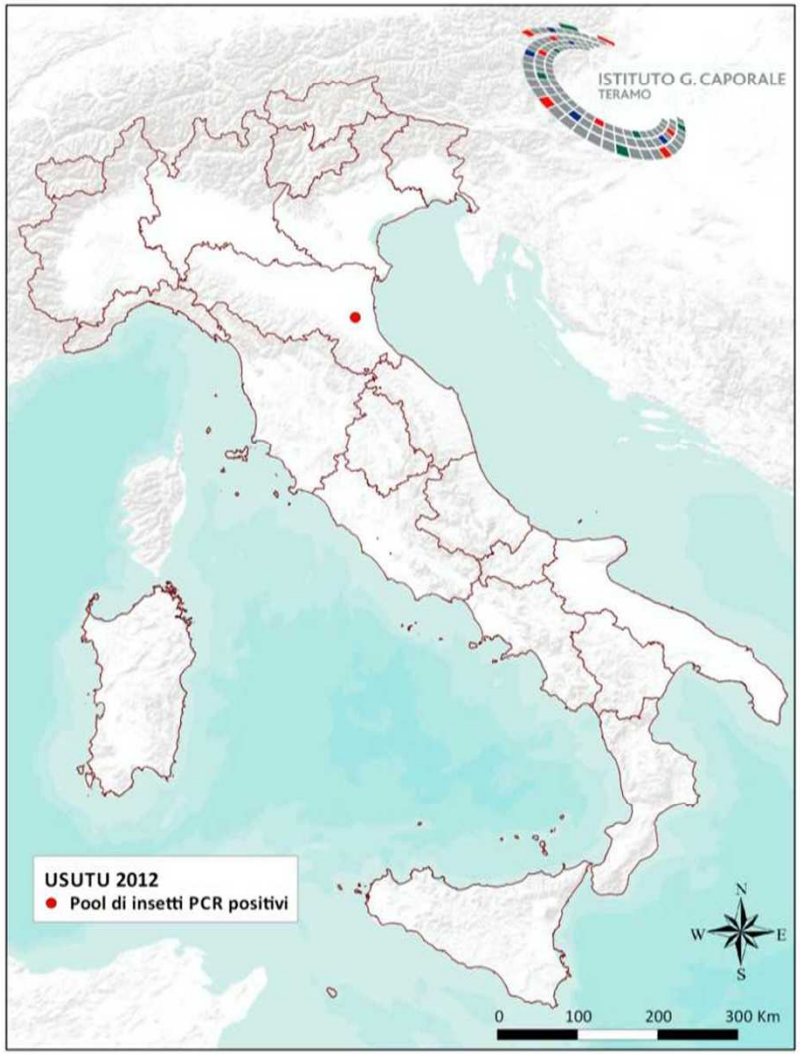
- Uccelli

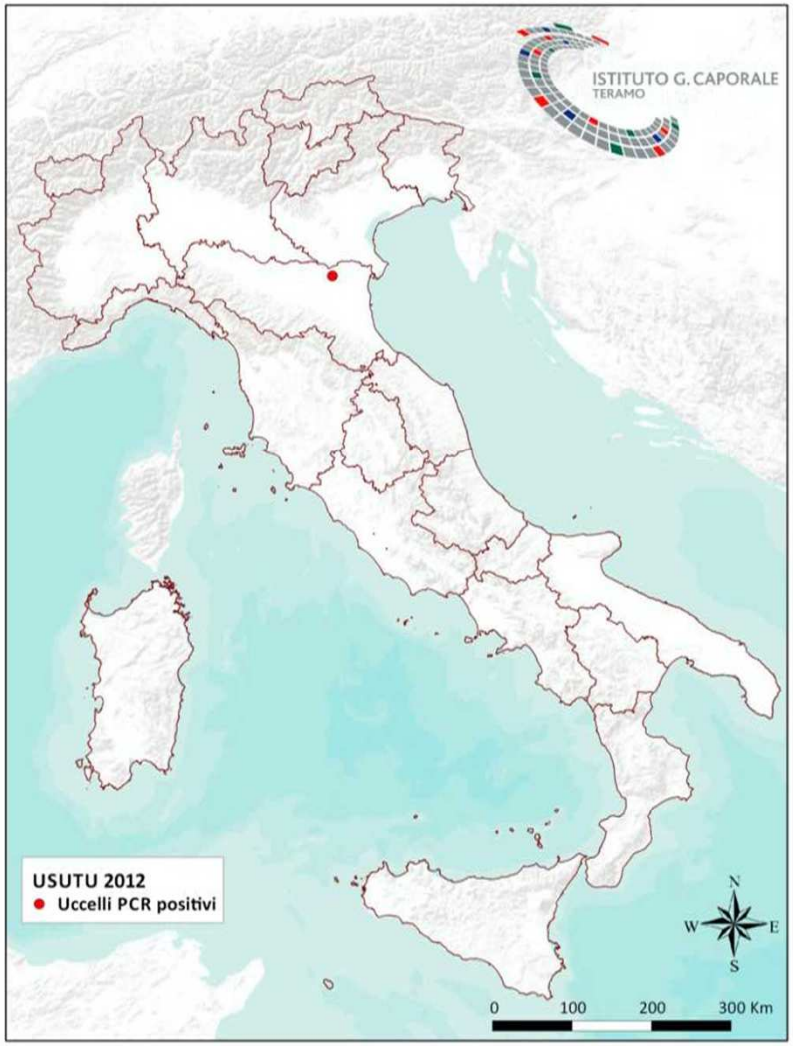
2011



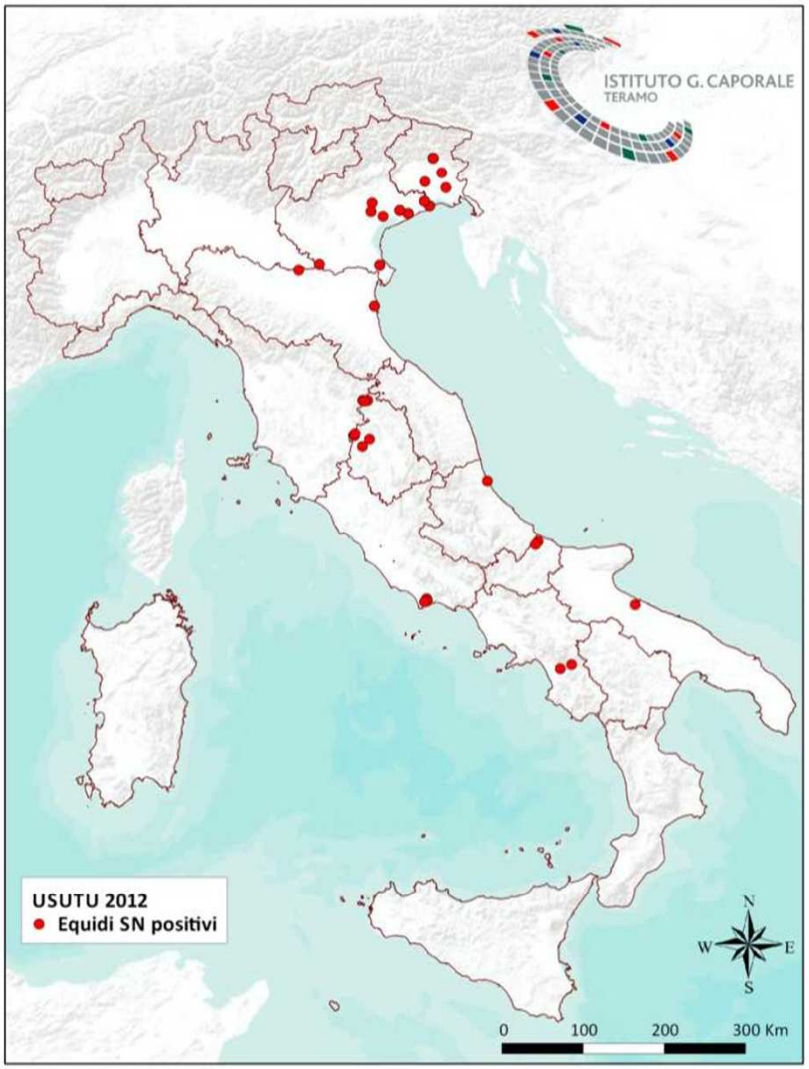
ISTITUTO G. CAPORALE
TERAMO

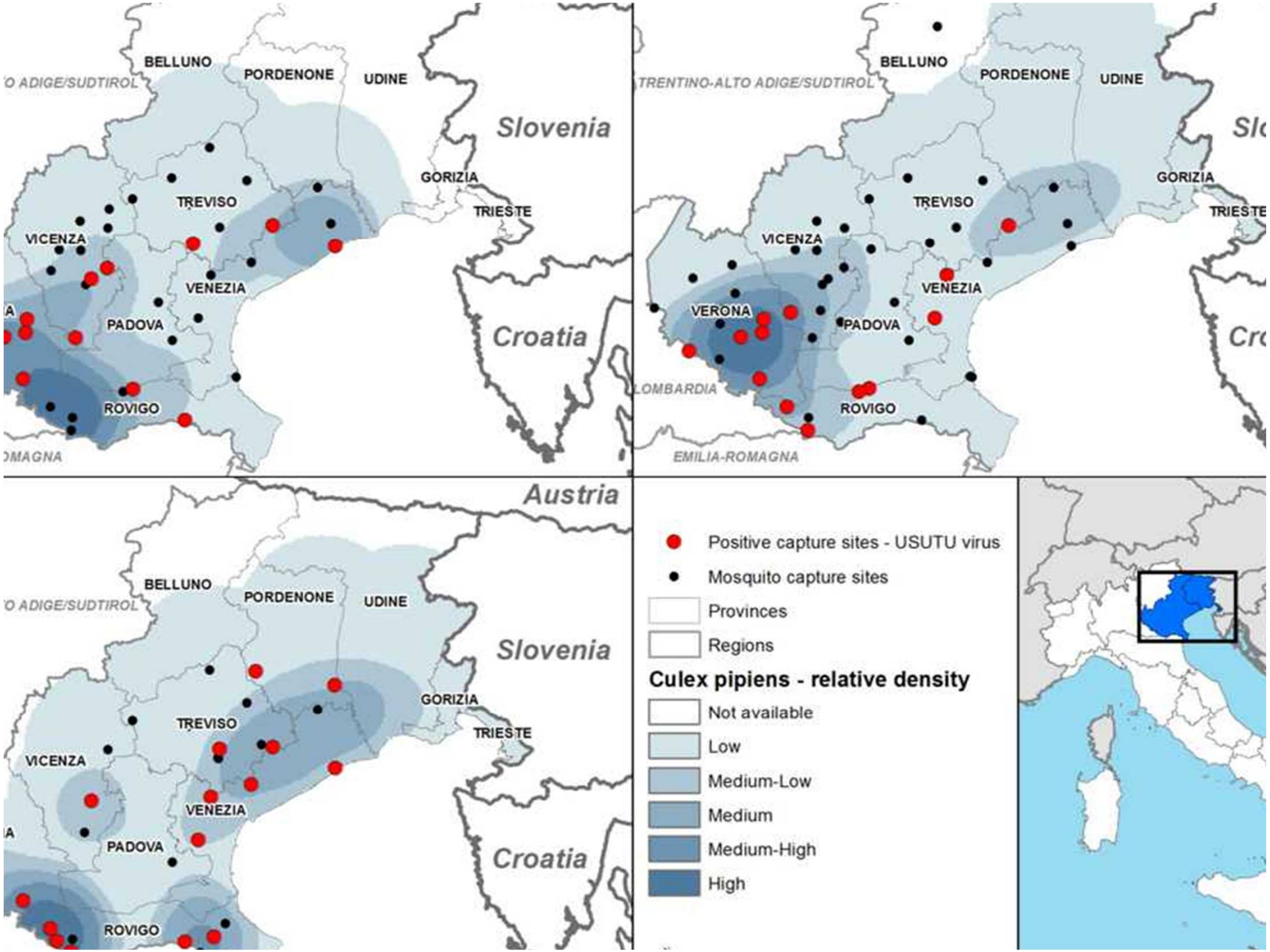



ISTITUTO G. CAPORALE
TERAMO
USUTU 2012
Pool di insetti PCR positivi
N
W
E
S
0
100
200
300 Km


ISTITUTO G. CAPORALE
TERAMO
USUTU 2012
Uccelli PCR positivi
N
W
E
S
0
100
200
300 Km

ISTITUTO G. CAPORALE
TERAMO




BELLUNO
PORDENONE
UDINE
Slovenia
GORIZIA
TRIESTE
TREVISO
VICENZA
VENEZIA
PADOVA
ROVIGO
Croatia
TRENTINO-ALTO ADIGE/SUDTIROL
VERONA
LOMBARDIA
EMILIA-ROMAGNA
Austria
Positive capture sites - USUTU virus
Mosquito capture sites
Provinces
Regions
Culex pipiens - relative density
Not available
Low
Medium-Low
Medium
Medium-High
High

USUV life cycle in Italy

Athene noctua
5,1% 95%IC 1,57-16,92

Turdus merula
37% 95%IC 27,32-47,95

Pica pica
1,1% 95%IC 0,68-1,76

Corvus corone corone
0,8% 95%IC 0,42-1,41

Garrulus glandarius
2,1% 95%IC 0,75-5,89

Ardea purpurea
100% 95%IC 22,36-98,74

Culex pipiens

Phasianus colchicus
14,3% 95%IC 3,19-52.65

Larus michahellis
7,7% 95%IC 2,35-24,29

Sturnus vulgaris
1,8% 95%IC 0,81-4,22

Columba livia
0,5% 95%IC 0,12-2,14

USUV infection in Italy

High mortality rate in the province of Treviso and in the Marche region

2009

2010

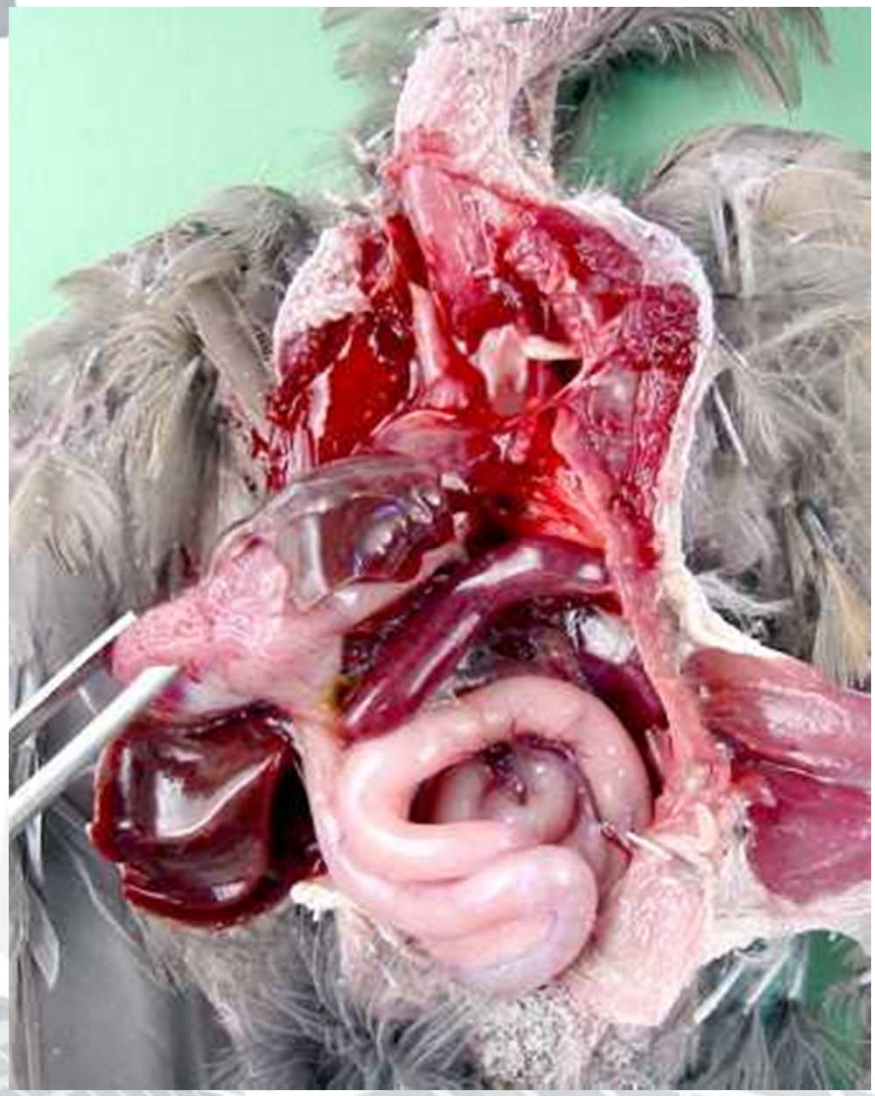
USUV infection in Italy

- Between August and October **2009** widespread anomalous deaths were reported in blackbirds of the Treviso province (**Veneto region**, **North Eastern Italy**). It has been estimated that the mortality in the different outbreaks concerned amounts to approximately one thousand birds. (Savini et al.)
- Similarly a blackbird mortality was reported in the Marche region (**PS, AN, MC**) between August and October 2010

Pathological findings

Pathological findings in an USUV-infected blackbird: enlarged and hyperaemic liver (*from Manarolla et al. 2010*)

Pathological findings



Pathological findings in an USUV-infected blackbird: enlarged and hyperaemic spleen (*from Manarolla et al. 2010*)

USUTU: human infection in Italy

At the end of the summer of 2009 in Modena (E. Romagna region), the USUV was associated with neurological disorders in two immunocompromised patients. They have been the first human cases of USUV neurological illness described worldwide.

CASE 1

www.eurosurveillance.org

Rapid communications

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

M Pecorari (pecorari.monica@policlinico.mo.it)[1], G Longo[2], W Gennari[1], A Grottola[1], A MT Sabbatini[1], S Tagliazucchi[1], G Savini[3], F Monaco[3], M L Simone[4], R Lelli[3], F Rumpianesi[1]

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, OIE 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

This article was published on 17 December 2009.
Citation style for this article: Pecorari M, Longo G, Gennari W, Grottola A, Sabbatini AM, Tagliazucchi S, Savini G, Monaco F, Simone ML, Lelli R, Rumpianesi F. First human case of Usutu virus neuroinvasive infection, Italy, August-September 2009. Euro Surveill. 2009;14(50):pii=19446. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19446

CASE 2

Eurosurveillance, Volume 14, Issue 50, 17 December 2009

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 Longo[3], A M Pierro[1], G Rossini[1], P Bonilauri[4], G E Gerunda[5], F Di Benedetto[5], A Pasetto[6], M Girardis[6], M Dottori[4], M P Landini[1], V Sambri (vittorio.sambri@unibo.it)[1]

The Ghirlandina

Modena

ISTITUTO G. CAPORALE
TERAMO

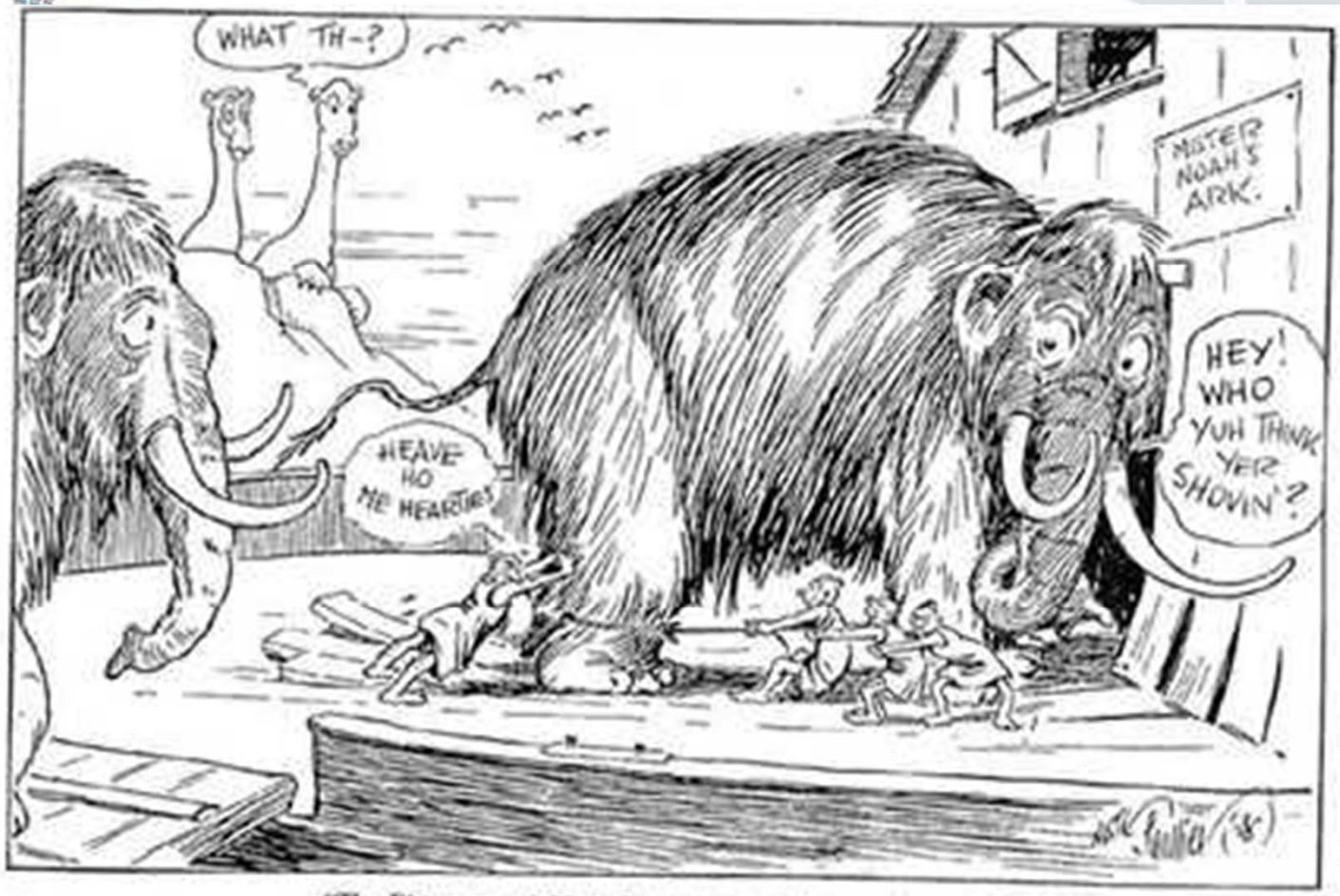
Conclusions

- Similarly to those ciruculating in Europe, the Italian North Eastern strains were pathogenic and capable of causing mortality in wild birds, particularly in blackbirds.

ISTITUTO G. CAPORALE
TERAMO

Conclusions

- Unlike the other European strains, however, the Italian North eastern strains were also able to infect humans and cause encephalitis.



"The Dinosaur and Mastodon became extinct not because of evolutionary changes, but because they were too big to be got into Noah's Ark." *Founder and President of World's Christian Fundamentals Association, in debate in Los Angeles.*

Is the Italian USUV strain particularly pathogenic?

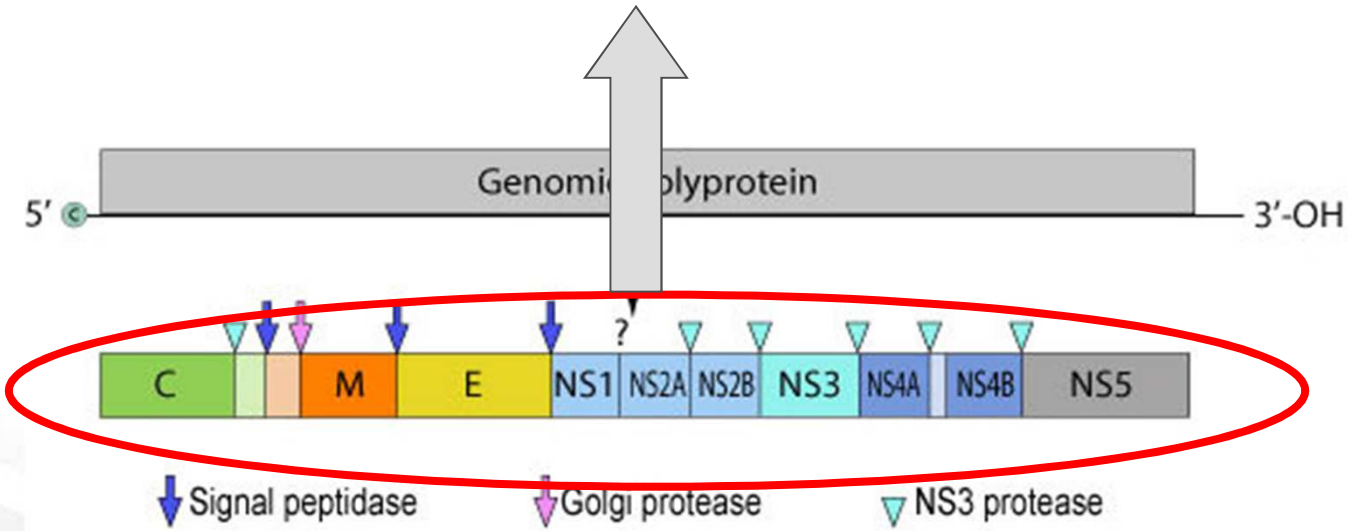
ISTITUTO G. CAPORALE
TERAMO

USUV Pathogenicity

- Neurovirulence and neuroinvasiveness of flaviviruses are mainly associated with sequence variation of the protein E and to a lesser extent to other viral proteins such as NS1 and NS2b

USUV GENE SEQUENCING

Vienna strain 2001
South African strain SAAR-1776
Budapest strain 05
Italian strain Ravenna 2009



	Vienna‘01	SAAR-1776	Budapest’05	Italy’09
Vienna‘01		97	100	99
SAAR-1776	99		97	97
Budapest’05	100	99		99
Italy’09	100	99	100	

bp

aa

Table

Percentage of nucleotidic and amino-acid sequence similarity between European and South African USUTU strains

Position bp	AY453411	AY453412	EF206350	ITA 09	Position aa	Gene	AY453411	AY453412	EF206350	ITA 09
454-56	GTT	GCC	GTT	GTT	120	C	val	ala	val	val
622-24	ACT	ACT	ATT	ACT	176	M	thr	thr	ila	thr
637-39	AAA	AAA	AAA	ACT	181		lys	lys	lys	arg
1351-53	ATG	GTG	ATG	ATG	419	E	met	val	met	met
1801-03	GGC	AGC	GGC	GGC	569		gly	ser	gly	gly
1933-35	GGC	AGC	GGC	GGC	613		gly	ser	gly	gly
2242-44	ACA	CCA	ACA	ACA	716		thr	pro	thr	thr
2464-66	AAC	AGC	AAC	AAC	790		asn	ser	asn	asn
2584-86	GAA	GAG	GAA	GGA	830	NS1	glu	glu	glu	gly
2911-13	GCA	GTA	GCA	GTA	939		asn	val	asn	val
3445-47	AGG	AAG	AGG	AGG	1117		arg	lys	arg	arg
3532-34	CAT	TAC	CAT	TAT	1146	NS2a	his	tyr	his	tyr
3685-87	ACT	ACT	ATT	ACT	1197		thr	thr	ile	thr
3895-97	AAC	GAC	AAC	AAC	1267		asn	asp	asn	asn
3898-900	TTT	CTC	TTT	TTT	1268		phe	leu	phe	phe
4066-68	ATC	ATT	ACC	ACC	1324		ile	ile	thr	thr
4900-02	ATA	ATA	ATA	GTA	1602	NS3	ile	ile	ile	val
4948-50	ATC	GTC	ATC	ATC	1618		ile	val	ile	ile
5179-81	CTG	ATG	CTG	CTG	1695		leu	met	leu	leu
5431-33	GCT	GTT	GTT	GTT	1779		ala	val	val	val
6184-86	AGA	ATA	AGA	AGA	2030		arg	ile	arg	arg
6190-02	GAG	CAG	GAG	GAG	2032		glu	gln	glu	glu
6412-14	GCT	TCC	GCT	GCT	2106		ala	ser	ala	ala
6592-94	CTT	TTT	CTT	CTT	2166	NS4a	leu	phe	leu	leu
6955-57	ATA	ATG	ATA	ATA	2287	NS4b	ile	met	ile	ile
6964-66	AGC	GGC	AGC	AGC	2290		ser	gly	ser	ser
7006-08	AAC	AAC	AAC	AGC	2304		asn	asn	asn	ser
7195-97	TTA	TTG	TTG	CTG	2367		phe	leu	leu	leu
7744-46	AAG	AGG	AAG	AAG	2550	NS5	lys	arg	lys	lys
8029-31	ATG	ATG	ATG	ATA	2645		met	met	met	ile
8503-05	ACC	GCC	ACC	ACC	2803		thr	ala	thr	thr
8641-43	AGC	GGC	AGC	AGC	2849		ser	gly	ser	ser
10183-85	CCA	CCA	CCA	CTA	3363		pro	pro	pro	leu
10375-77	AAT	GAT	AAT	AAT	3427		asn	asp	asn	asn

Comparison between the amino-acid deduced sequences of the European and South African Usutu strains

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Results

- The Italian North eastern isolate differed from Vienna and Budapest isolates in 10 and 11 amino-acids, respectively. These amino-acid substitutions were distributed throughout the entire genome.

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Results

- No differences were noticed in the amino-acid sequences of the protein E, the most commonly referred as potential site of pathogen determinants.

USUTU: human infection in Italy

Do the two cases of human encephalitis represent a coincidental event in immunocompromised individuals or the beginning of a new zoonotic expression of the Usutu virus?

USUTU: human infection in Italy

What do we know about the Usutu-infections in humans from the epidemiological point of view?

USUTU: human infection in Italy

- Dealing with a member of the genus Flavivirus the unexpected is often the most frequent event particularly with respect to disease severity and unusual clinical manifestations.
- This is probably due to the fact that most Flaviviruses have a complex life cycle which involves vertebrate and invertebrate hosts.
- Thus it's important to estimate the epidemiologic impact of USUV and the possible threat to the human population

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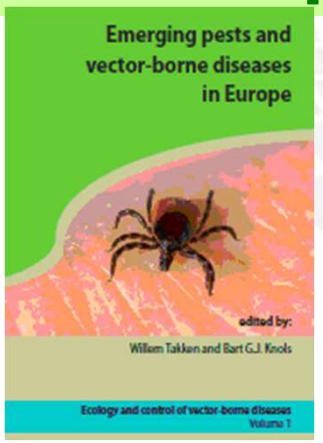
USUTU: human infection in Europe

Emergence of Usutu virus in Central Europe: diagnosis, surveillance and epizootiology

Herbert Weissenböck, Sonja Chvala-Mannsberger, Tamás Bakonyi and Norbert Nowotny

Emerging pests and vector-borne diseases in Europe

Wageningen Academic Publishers 2007, 153-168



...in human blood samples from individuals exposed to mosquitoes in USUV endemic areas (Austria), the presence of antibodies to USUV. Many of the subjects included in this study exhibited skin rash. In one case USUV RNA was demonstrated by RT-PCR.

Also in this case, USUV has been able to infect humans without inducing severe disease. Transient rash seems to be a clinical symptom associated with USUV infection. There has been no association with neurological disease in human beings.

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SEROLOGICAL and VIROLOGICAL SURVEY before 2009

Emergence of Usutu virus in Central Europe: diagnosis, surveillance and epizootiology

Herbert Weissenböck, Sonja Chvala-Mannsberger, Tamás Bakonyi and Norbert Nowotny

Emerging pests and vector-borne diseases in Europe

Wageningen Academic Publishers2007, 153-168

USUV-infections in humans

203 human blood samples from individuals exposed to mosquitoes in USUV-endemic areas were examined by RT-PCR for detection of USUV-specific nucleic acid and 208 human blood samples were investigated by HI test and by PRNT for the presence of antibodies to USUV. Many of the subjects included in this study exhibited rash of unknown origin, and they tested negative for Lyme-Borreliosis. In this pre-selected group, 83 individuals showed antibody titres to USUV between 1:20 and >1:160 in HI test, and 52 of them could be confirmed by PRNT, which results in an overall seroprevalence of 25% in this pre-selected group. In one 'fresh' case USUV nucleic acid was demonstrated by RT-PCR. These data indicate that USUV is able to infect humans without inducing severe disease; in particular, no association between neurological disease and USUV infection could be established in human beings. Transient rash, however, seems to be a clinical symptom associated with USUV infection in human beings (N. Nowotny, unpublished data, Weissenböck and Nowotny 2006).

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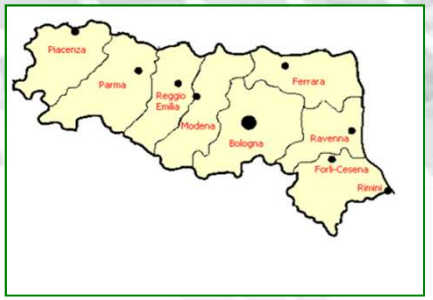
And after 2009?



Regional Reference Centre for Microbiological Emergencies (CRREM)

1. VIROLOGICAL SURVEY in E. ROMAGNA, ITALY

Journal of Clinical Virology 50 (2011) 221–223

A rapid and specific real-time RT-PCR assay to identify Usutu virus in human plasma, serum, and cerebrospinal fluid

Francesca Cavrini[a], Maria Elena Della Pepa[b], Paolo Gaibani[a], Anna Maria Pierro[a], Giada Rossini[a], Maria Paola Landini[b], Vittorio Sambri[a,b,*]

104 human specimens [cerebrospinal fluid (CSF), plasma, serum] collected in the summers of 2008 and 2009 from 44 patients with suspected meningoencephalitis WNV-negative.

7.5% (3/40) of the patients were positive in CSF for USUV RNA while plasma and sera were negative.

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2. SEROLOGICAL SURVEY in E. ROMAGNA, ITALY

Detection of Usutu-Virus-Specific IgG in Blood Donors from Northern Italy

Paolo Gaibani, Anna Pierro, Rosa Alicino, Giada Rossini, Francesca Cavrini, Maria Paola Landini, and Vittorio Sambri

Regional Reference Centre for Microbiological Emergencies (CRREM),

Between June and October 2009, 359 serum samples were collected from healthy volunteer blood donors living in the district of Ferrara.

1.1% (4/359) of the blood donors were seropositive for USUV (ELISA IgG, mNTA).

- USUV-specific IgG and neutralizing immune response
- USUV can be able to asymptomatically infect humans

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3. VIROLOGICAL SURVEY in E. ROMAGNA, ITALY

PLoS One. 2012; 7 (5)

Mosquito, Bird and Human Surveillance of West Nile and Usutu Viruses in Emilia-Romagna Region (Italy) in 2010

Mattia Calzolari[1]*, Paolo Gaibani[2], Romeo Bellini[3], Francesco Defilippo[1], Anna Pierro[2], Alessandro Albieri[3], Giulia Maioli[1], Andrea Luppi[1], Giada Rossini[2], Agnese Balzani[1], Marco Tamba[1], Giorgio Galletti[1], Antonio Gelati[4], Marco Carrieri[3], Giovanni Poglayen[5], Francesca Cavrini[2], Silvano Natalini[6], Michele Dottori[1], Vittorio Sambri[2], Paola Angelini[6], Paolo Bonilauri[1]

Emilia Romagna regional surveillance on insect borne diseases plan

The recent identification of the first 4 USUV seropositive healthy blood donors in the Emilia-Romagna region [36] is in contrast with the elevated WNV seropositivity found among blood donors in the same area [54] in 2009; this finding suggests that USUV would likely be able to asymptomatically infect humans. The discrepancy in the human epidemiologic data between USUV and WNV and the relevant level of USUV detected in the environment, suggest a lower capability of USUV to infect humans with respect to WNV.

Spread of USUV in humans in Northern Italy, Modena

Istituto Zooprofilattico Sperimentale of Teramo
Azienda Ospedaliero-Universitaria Policlinico of Modena

915 human samples analyzed for USUV and WNV infection

306 CSF from inpatients with neurological impairment
(sampling between June-November 2008-2009)

Onestep RT-PCR USUV-specific (NS5 region)

Real-time RT-PCR WNV (E region)

Nucleotide sequencig on positive samples

Origin and evolution of recent vector-borne virus incursions in the Mediterranean Basin

Italian Ministry of Health Ricerca Finalizzata 2009 IZS of Teramo

609 sera from inpatients/outpatients with different anamnesis
(sampling between July-January 2008-2011)

- ✓ One step RT-PCR USUV-specific (NS5 region)
- ✓ Real-time RT-PCR WNV (E region)
- ✓ Microneutralization assay (mNTA) for USUV/WNV

SEROLOGICAL RESULTS

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Anti-USUV Ab

Year	Neutralization test		Total tested samples	Positive samples (%)	CI(95%)
	Positive samples	Negative samples			
2008	14	230	244	5,74	3,47-9,40
2009	21	285	306	6,86	4,55-10,27
2010	4	41	45	8,89	3,62-20,79
2011	1	13	14	7,14	1,66-31,95
Total	40	569	609	6,57	4,87-8,82

USUV 6.57%

WNV 2.96%

$p<0.05\%$

Anti-WNV Ab

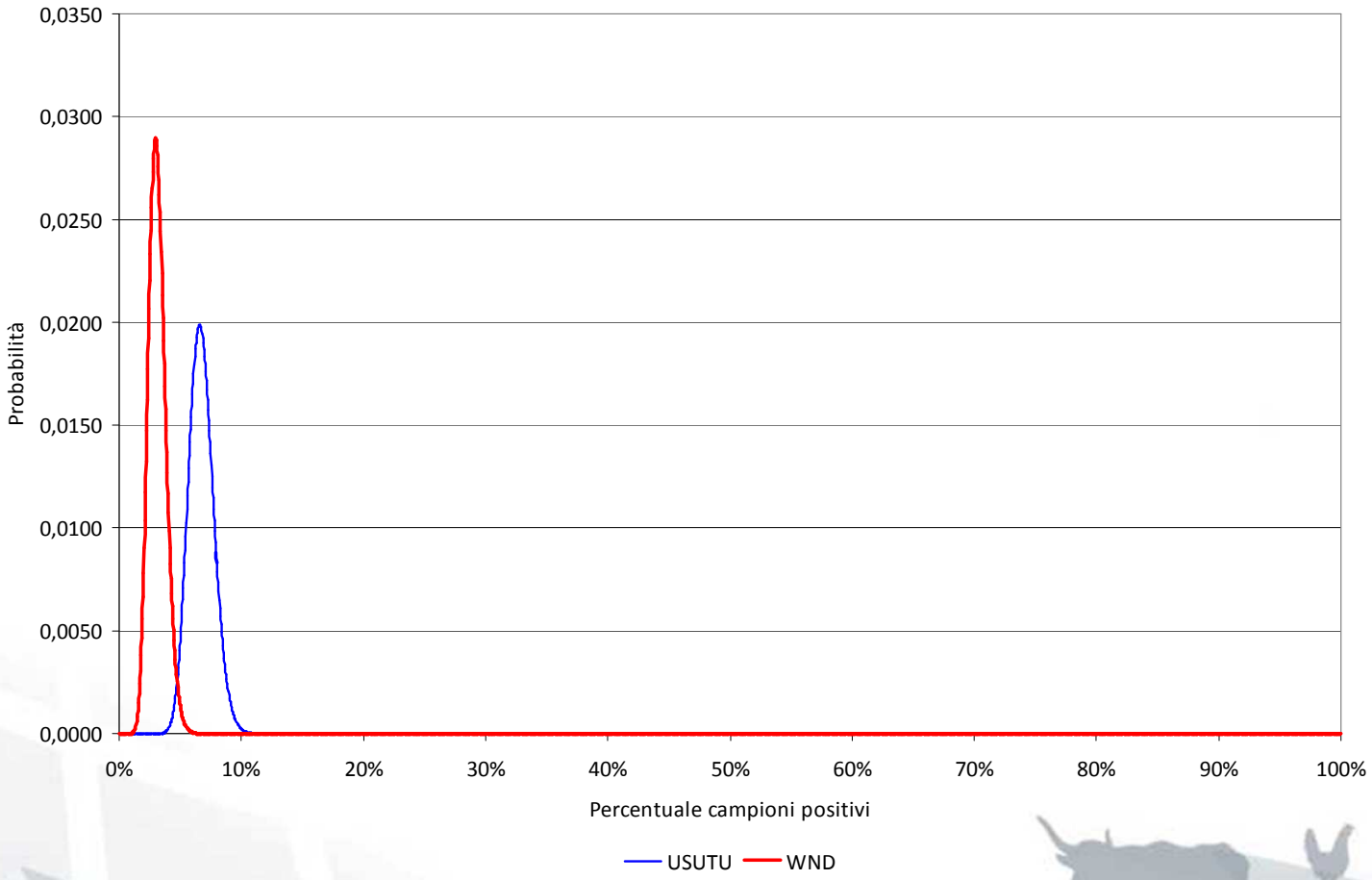
WNV seroprevalence in E. Romagna 2008/09

Blood donors **0.7-0.8%**

Farm workers **3.1%**

Year	Neutralization test		Total tested samples	Positive samples (%)	CI(95%)
	Positive samples	Negative samples			
2008	5	239	244	2,05	0,90-4,70
2009	11	295	306	3,59	2,04-6,32
2010	1	44	45	2,22	0,53-11,53
2011	1	13	14	7,14	1,66-31,95
Total	18	591	609	2,96	1,89-4,62

SEROLOGICAL RESULTS



SEROLOGICAL RESULTS

- The USUV infection in this area can't be considered sporadic because it shows a seroprevalence higher than WNV who is considered endemic in the same area

VIROLOGICAL RESULTS

USUV vs WNV					
Virus	RT-PCR		Total tested samples	Positive samples (%)	CI(95%)
	Positive samples	Negative samples			
USUV	9	906	915	0.87	0.45-1.71
WNV	0	915	915	0	0-0.33

$p < 0,005$

VIROLOGICAL RESULTS

- In all patients examined in district of Modena Usutu virus was detected more frequently than West Nile virus.
- It should be considered that in this area a human surveillance plan exists for WNV but not for USUV.

VIROLOGICAL RESULTS

WNV Real-time RT-PCR

all CSF and sera were negative

CSF = 0%; CI95%: 0%-0.97%; **Serum** = 0%; CI95%: 0%-0.49%

USUV Onestep RT-PCR

Human samples	RT-PCR		Total tested samples	Positive samples (%)	CI(95%)
	Positive samples	Negative samples			
CSF	7 *	299	306	2.29	1.13-4.64
SERUM	2 * *	607	609	0.33	0.10-1.18

* 6 CSF 2008 , 1 CSF 2009

** 2 Sera 2009

$\chi 2 = 8.03$; $p = 0.005$; gdl = 1

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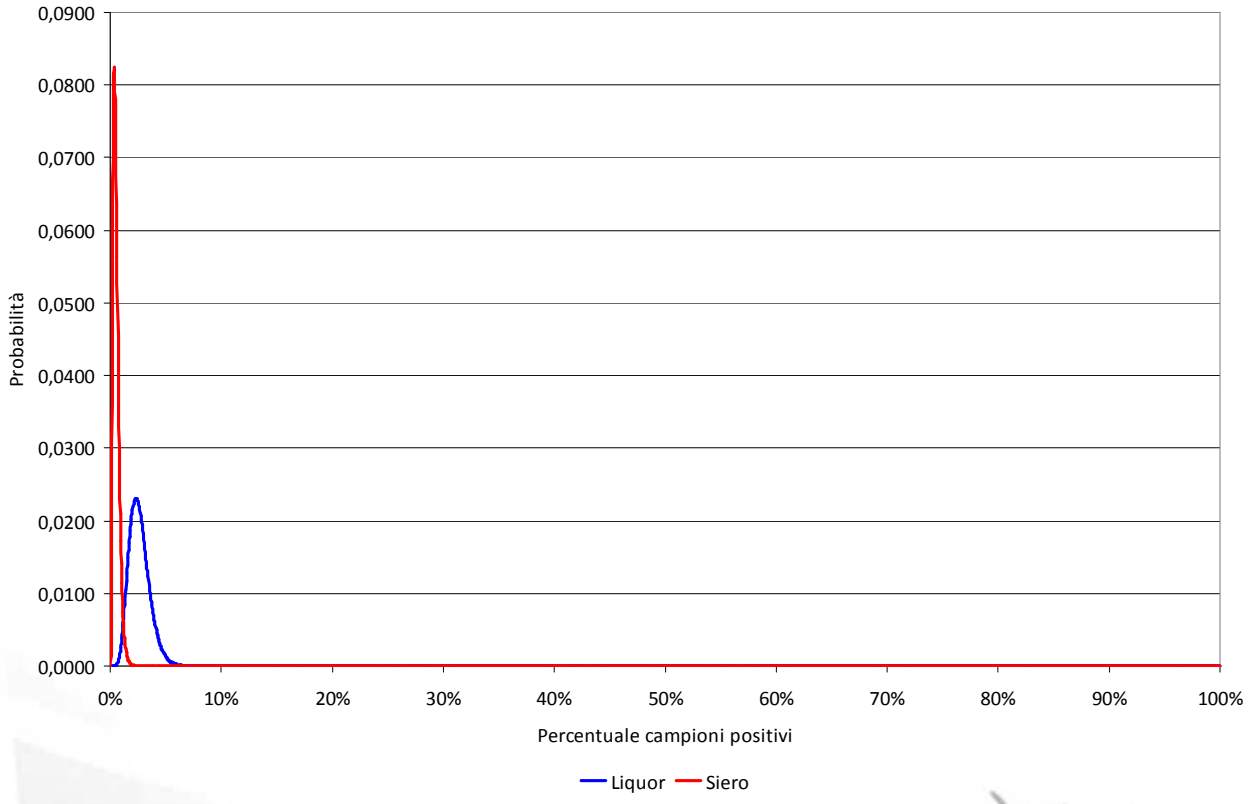
VIROLOGICAL RESULTS

- It was also possible to find a **significant association** (**c2**= 8,027; P=0,005%) between patients with neurological impairments (liquor) and presence of Usuv

VIROLOGICAL RESULTS



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Clinical diagnosis in USUV-positive patients

Year	ID Sample	Biological matrix	Diagnosis
2008	MO1_08_Hu_CSF	CSF	**acute encephalitis**
2008	MO2_08_Hu_CSF	CSF	unknown
2008	MO3_08_Hu_CSF	CSF	unknown
2008	MO4_08_Hu_CSF	CSF	unknown
2008	MO5_08_Hu_CSF	CSF	unknown
2008	MO6_08_Hu_CSF	CSF	unknown
2009	MO7_08_Hu_CSF	CSF	**acute encephalitis/polyneuritis**
2009	MO1_08_Hu_serum	Serum	dermatological infection
2009	MO2_08_Hu_serum	Serum	diabetes and neutropenia

Comparison between the USUV sequences detected in the human samples and the Genbank USUV sequences (BLAST)

The USUV sequences detected in human samples scored 97% identity to the homologous sequence of South African reference strain SAAR-1776 and high similarity percentage (99-100%) to the homologous sequences of USUV strains Vienna 2001 and Budapest 2005.

The viral sequences from humans also shared a high similarity (99-100%) with homologous USUV sequences from wild and domestic birds and mosquitoes collected in different Italian regions.

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AY453411.1	Usutu virus strain Vienna 2001 from Austria, complete genome	826	826	100%	0.0	99%
JQ219843.1	Usutu virus strain Meise H polyprotein gene, complete cds	821	821	100%	0.0	99%
JF266698.1	Usutu virus strain Italia 2009, complete genome	815	815	100%	0.0	99%
JF331432.1	Usutu virus isolate TV1-09-Tm NS5 protein gene, partial cds	815	815	100%	0.0	99%
JF331434.1	Usutu virus isolate MO-09-Hu NS5 protein gene, partial cds >gb|JF33	815	815	100%	0.0	99%
EF206350.1	Usutu virus isolate Budapest, complete genome	815	815	100%	0.0	99%
JF331433.1	Usutu virus isolate VE-09-Tm NS5 protein gene, partial cds	809	809	100%	0.0	99%
JF331431.1	Usutu virus isolate TV2-09-Tm NS5 protein gene, partial cds	809	809	100%	0.0	99%
JF331430.1	Usutu virus isolate PI-09-Cp NS5 protein gene, partial cds	809	809	100%	0.0	99%
HE599647.1	Usutu virus gene for polyprotein, genomic RNA, isolate BH65/11-02-0	804	804	100%	0.0	99%
AY453412.1	Usutu virus strain SAAR-1776 from South Africa, complete genome	758	758	99%	0.0	97%
AF013412.1	Usutu virus strain SAAR-1776 NS5 protein (NS5) gene, partial cds	752	752	99%	0.0	97%
HQ833022.1	Usutu virus isolate HU10279_09 polyprotein gene, partial cds	708	708	98%	0.0	95%
EU566860.1	St. Louis encephalitis virus strain Hubbard, complete genome	187	187	78%	6e-44	76%
EF158059.1	St. Louis encephalitis virus strain 65 V 310 polyprotein gene, partial c	187	187	78%	6e-44	76%
EF158066.1	St. Louis encephalitis virus strain GHA-3 polyprotein gene, partial cds	182	182	78%	3e-42	76%
EF158065.1	St. Louis encephalitis virus strain TNM 4-711 K polyprotein gene, part	176	176	69%	1e-40	77%
EF158062.1	St. Louis encephalitis virus strain FL 79-411 polyprotein gene, partial	176	176	78%	1e-40	76%
EF158058.1	St. Louis encephalitis virus strain Kern 217 polyprotein gene, partial c	171	171	78%	6e-39	76%
DQ525916.1	St. Louis encephalitis virus strain Kern217, complete genome	171	171	78%	6e-39	76%

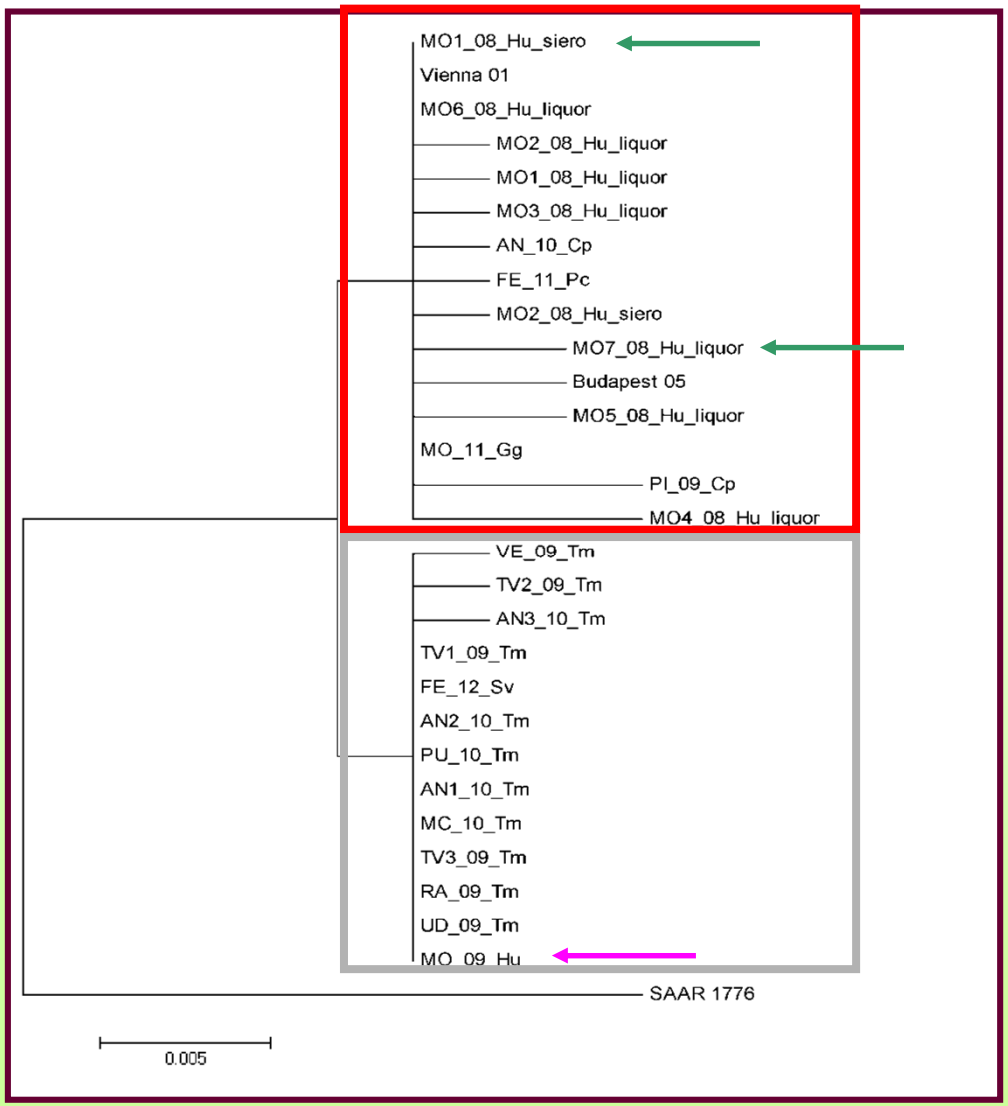
VIROLOGICAL RESULTS

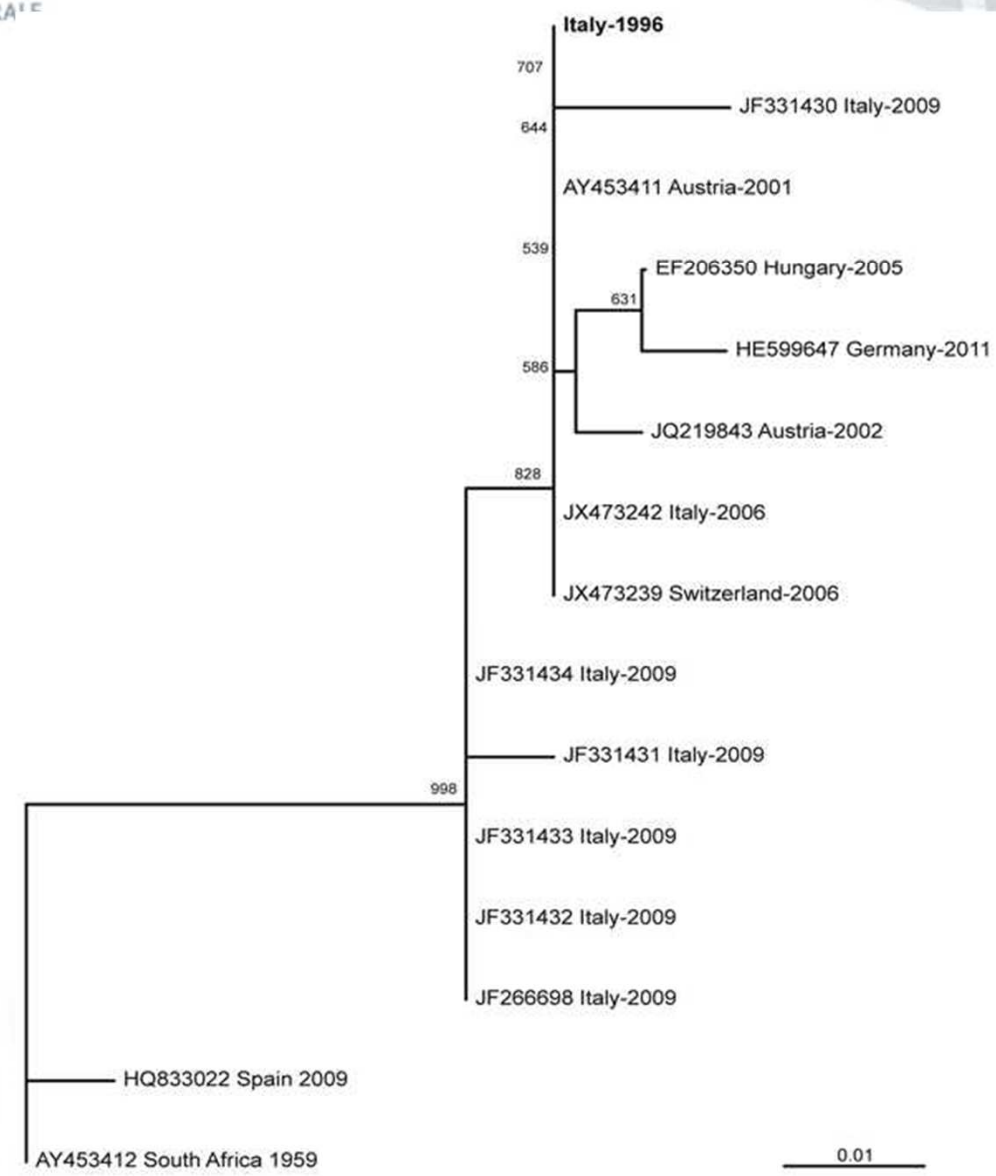
- The phylogenetic analysis of the USUV sequences obtained from human samples and of the homologous sequences of USUV strains isolated from birds and vector circulating in Italy and in Europe was done.

Year	ID Sample	Bankit/GenBank	Biological matrix	Species	Geographical origin
2008	MO1_08_Hu_CSF		CSF	Human	Modena (Emilia)
2008	MO2_08_Hu_CSF		CSF	Human	Modena (Emilia)
2008	MO3_08_Hu_CSF		CSF	Human	Modena (Emilia)
2008	MO4_08_Hu_CSF		CSF	Human	Modena (Emilia)
2008	MO5_08_Hu_CSF		CSF	Human	Modena (Emilia)
2008	MO6_08_Hu_CSF		CSF	Human	Modena (Emilia)
2009	MO7_09_Hu_CSF		CSF	Human	Modena (Emilia)
2008	MO1_08_Hu_serum		Serum	Human	Modena (Emilia)
2008	MO2_08_Hu_serum		Serum	Human	Modena (Emilia)
2010	MC_10_Tm		Organs	Blackbird (*T.merula*)	Macerata (Marche)
2010	PU_10_Tm		Organs	Blackbird (*T.merula*)	Urbino (Marche)
2011	FE_11_Pc		Organs	Pheasant (*P.colchicus*)	Ferrara (Emilia)
2011	MO_11_Gg		Organs	Eurasian jay (*G. glandarius*)	Modena (Emilia)
2012	FE_12_Sv		Organs	Common starling (*S.vulgaris*)	Ferrara (Emilia)
2010	AN_10_Cp		Mosquito	*Culex pipiens*	Ancona (Marche)
2010	AN1_10_Tm		Organs	Blackbird (*T.merula*)	Ancona (Marche)
2010	AN2_10_Tm		Organs	Blackbird (*T.merula*)	Ancona (Marche)
2010	AN3_10_Tm		Organs	Blackbird (*T.merula*)	Ancona (Marche)
2009	MO_09_Hu	JF331434	CSF	Human	Modena (Emilia)
2009	RA_09_Tm	JF331436	Organs	Blackbird (*T.merula*)	Ravenna (Emilia)
2009	TV1_09_Tm	JF331432	Organs	Blackbird (*T.merula*)	Treviso (Veneto)
2009	TV2_09_Tm	JF331431	Organs	Blackbird (*T.merula*)	Treviso (Veneto)
2009	TV3_09_Tm	JF331437	Organs	Blackbird (*T.merula*)	Treviso (Veneto)
2009	UD_09_Tm	JF331435	Organs	Blackbird (*T.merula*)	Udine (Friuli)
2009	VE_09_Tm	JF331433	Organs	Blackbird (*T.merula*)	Venezia (Veneto)
2009	PI_09_Cp	JF331430	Mosquito	*Culex pipiens*	Pistoia (Toscana)
2001	Vienna 01	AY453411	Organs	Blackbird (*T.merula*)	Austria
2005	Budapest 05	EF206350	Organs	Blackbird (*T.merula*)	Hungary
1959	SAAR 1776	AY453412	Mosquito	*Culex neavei*	South Africa

Dataset for phylogenetic analysis of NS5 gene fragment sequences (450 bp)

Phylogenetic analysis of NS5 gene fragment sequences (450 bp)




Italy-1996
707
JF331430 Italy-2009
644
AY453411 Austria-2001
539
EF206350 Hungary-2005
631
HE599647 Germany-2011
586
JQ219843 Austria-2002
828
JX473242 Italy-2006
JX473239 Switzerland-2006
JF331434 Italy-2009
JF331431 Italy-2009
998
JF331433 Italy-2009
JF331432 Italy-2009
JF266698 Italy-2009
HQ833022 Spain 2009
AY453412 South Africa 1959
0.01

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VIROLOGICAL RESULTS

- The phylogenetic analysis was carried out on a small portion of NS5 gene (450 bp).
- The hypothesis should be confirmed by phylogenetic studies conducted on other target gene (E, NS1, NS2A) or by complete sequencing of the viral genome.

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CONCLUSIONS

- The USUV infection is not sporadic in the study area given the higher virological and serological prevalence of USUV compared to WNV. (Competition between the two viruses sharing the common hosts and vectors?)

- The virus seems mainly to be associated with neurological disorders in humans.

- Different human strains of USUV have circulated in Italy between 2008 and 2011.

FUTURE PERSPECTIVES

- USUV infection should receive more attention and should be considered in the same way of infection caused by West Nile Virus.

- In areas with viral circulation, there's a need to organise an efficient human surveillance system harmonized with pre-existing veterinary surveillance systems.

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UNIVERSITÀ DEGLI STUDI
DI MODENA E REGGIO EMILIA

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Ospedaliero-Universitaria di Modena
Policlinico

"EU fiscal harmonisation means your money gets squandered by professionals."

Thanks for your attention!

WORKSHOP
Mosquitoes and sand fly—borne viruses across Europe and the Mediterranean sea

Research Opportunities and Financial Resources: Brief Introduction (done, more or less)

Brainstorming, Discussion, Ideas, Input For Future Research and Financial Support (to do….)

Gioia Capelli
IZSVE

EU Research CORDIS

http://www.cordis.europa.eu/research-eu/magazine_en.html

International Research opportunities

European projects (from FP7 to Horizon 2020)

- ERA-NET scheme (coordination of national programs)
  ANIHWA
- Transnational and interregional cooperation projects (INTERREG, ATLAS)

International Research opportunities

Other EU programs offering funding for innovation-related activities:

- LIFE (environmental and nature conservation)
- Life-Long Learning (program for Education and Training)

European research-related initiatives:

- EUREKA (pan-European projects to develop innovative products, processes and services)
- COST (European Cooperation to maximize European Synergy)

International Research opportunities

EU and international agencies
ECDC, EFSA, WHO, OIE, FAO…..

Private Foundations (more local, co-funding)

http://ec.europa.eu/ewsi/en/funding/private.cfm

Industries (more local, co-funding)

EU Research Horizon 2020

European Commission

RESEARCH & INNOVATION

Horizon 2020

European Commission > Research & Innovation > Horizon 2020 > Home

Home | What is Horizon 2020? | What's in Horizon 2020 for me? | Why Horizon 2020? | What's next? | What's your view? | Press corner

COUNTDOWN 237 DAYS

Before Horizon 2020 launch

HORIZON 2020

THE FRAMEWORK PROGRAMME FOR RESEARCH AND INNOVATION

What is Horizon 2020?

- The EU Framework Programme for Research and Innovation
- Official documents
- Questions & Answers

What's in Horizon 2020 for me?

- Excellent Science
- Competitive Industries
- Better Society

Why Horizon 2020?

- Europe 2020
- Public consultation
- Successful EU research

What's next?

- A time line for Horizon 2020
- Calendar of events
- Experts for Advisory Groups

What's your view?

- Video testimonials

Pamela Lozano

Press corner

- Press materials
- Country profiles and featured projects
- Videos by theme

EU Research Horizon 2020

The proposed support for research and innovation under Horizon 2020 will:

- Strengthen the EU's position in science with a dedicated budget of € 24 598 million. This will provide a boost to top-level research in Europe, including an increase in funding of 77% for the very successful European Research Council
- Strengthen industrial leadership in innovation € 17 938 million. This includes major investment in key technologies, greater access to capital and support for SMEs.
- Provide € 31 748 million to help address major concerns shared by all Europeans such as **climate change** developing sustainable transport and mobility, making renewable energy more affordable, ensuring food safety and security, or **coping with the challenge of an ageing population**

EU Research On going Projects on WBDs

PROJECTS

EDEN-Next : biology of vectors relevant to human and veterinary diseases

Arbo-Zoonet: Control of Emerging Viral VBZDs (WN, RVF, CCHF)

Crimean Congo Hemorrhagic Fever: diagnostics, epidemiology, prevention, therapy and preparedness.

EuroWestNile: biology, ecology and epidemiology

Healthy Futures: construction of a disease risk mapping system for malaria, Rift Valley fever and schistosomiasis in eastern Africa

ICRES: integrate the expertise of EU laboratories on CHIKV and laboratories from SE Asia

Vectorie: Risk assessment and control of WNV and CHIK virus

West Nile Shield: Epidemiology, Diagnosis and Prevention of WNV in Europe

NETWORK

VBORNET (ECDS): for Arthropod Vector Surveillance for Human Public Health

ENIVD: Diagnostics of 'Imported' Viral Diseases

CNEV: Centre National d'Expertise sur les Vecteurs

VBDs opportunities and needs

J Vector Ecol. 2008 Dec;33(2):218-24.

Interdisciplinary research in the ecology of vector-borne diseases: opportunities and needs.

Moore CG. (Fort Collins,USA)

In addition to their importance to human and animal health, vector-borne diseases are fascinating systems to study. The involvement of multiple species whose biologies and life cycles cover differing space and time scales makes it extremely difficult to predict epidemics. A single environmental factor may have opposite impacts on the system at different points in time. Patchiness at different geographical scales may have very different causes, so it is important to identify the proper scale for a particular study. New developments in **remote sensing, GIS, and spatial analysis** make it easier to tease out causes of observed patchiness. **Interdisciplinary collaboration is essential for many of the projects we carry out, but this requires awareness of the differences between disciplines and the ability to effectively communicate with each other.**

It is only by forming **multi-disciplinary groups** to focus on specific vector-host-pathogen systems that we will be able **to answer** the most interesting (and pressing) problems in our field.

VBDs critical needs

Reframing Critical Needs in Vector Biology and Management of Vector-Borne Disease
Shirley Luckhart, Steven W. Lindsay, Anthony A. James, Thomas W. Scott, 2010
PLoS Negl Trop Dis 4(2): e566.

Across these reviews, recommendations can be distilled to **five major needs**:

(1) novel **intervention tools** (e.g., new public health insecticides, biological control agents, and genetics-based instruments);
(2) improved disease **prevention strategies** (e.g., integrating different vector control strategies and combining vector control with other prevention tools, such as drugs and vaccines, to attack multiple VBDs);
(3) enhanced **surveillance methods** and data analysis;
(4) broader **integration** of scientific subdisciplines (e.g., vector biology, clinical research, natural and social environmental biology); and
(5) expanded **training opportunities**

Vector-Borne Diseases: Understanding the Environmental, Human Health, and Ecological Connections, Workshop Summary

http://www.nap.edu/catalog/11950.html

- Integration of research efforts and findings on infectious diseases in humans, livestock, and wild animals, as well as in crop and wild plants
- **Training, research, and laboratory- and field-based surveillance** in countries where diseases are likely to emerge (and especially in Asia, the source of many recently emerged zoonoses)
- More and better trained personnel, capacity, and tools for disease detection, diagnosis, and response
- Need for improved vaccines, drugs, and diagnostics
- Outbreak response plans that feature well-defined triggers for implementation
- Containment of outbreaks as local public health events
- **Measures to limit the movement of pathogens and vectors via global transportation**
- **Risk communication** that provides timely, reliable information to the public in the event of an outbreak, thereby preventing panic
- Political will sufficient to deliver economic support for these measures

Vector-Borne Diseases: Understanding the Environmental, Human Health, and Ecological Connections, Workshop Summary
http://www.nap.edu/catalog/11950.html

Field studies of vectors are crucial to answering many of these questions; however, as several participants who engage in such research attested, this work is **not well funded**.

"Some research is being done on methods for reducing the risk of Lyme disease through tick population suppression and other field intervention strategies, but this effort has been meager compared to that already invested in vaccines [that were withdrawn from the market].
One can only imagine what impact [the money invested in developing the discontinued Lyme vaccine, conservatively estimated at $200 million] would have [had] upon research to answer some basic questions about tick ecology, such as what limits the geographic distribution of Lyme disease vectors" (Fish, 2001a).

About the costs of surveillance and control

The cost of screening donated blood for WNV in USA is approximately **three times** the current CDC budget for surveillance, prevention, and control of the virus (Custer et al., 2005; Korves et al., 2006; Petersen, 2008).

Thus, in order to convince the pharmaceutical industry, as well as governments, that vector-borne diseases are worth solving, **researchers will need to provide evidence of economic benefit** and opportunities for strategic investment

Vector-Borne Diseases: Understanding the Environmental, Human Health, and Ecological Connections, Workshop Summary , 2008

VBDs what to do

"Without the ability to predict specific outbreak events, continuing surveillance to detect virus transmission is necessary, and must be linked to effective response.

……..

In a sense, arbovirus epidemics are like floods, hurricanes, or tornadoes: they occur sporadically, but their precise time and location cannot be predicted.

However, advanced preparation, continued research to identify and robustly define environmental and other risk factors for large outbreaks, and a well-developed response plan can mitigate the resulting damage and loss of life.

For now, this must be our response to the continuing threat of WNV."

Beasley et al, 2013; Antiviral Research, Available online 26 April 2013

Challenges in VBDs

DRIVERS

- Insecticide and drug resistance
- demographic and social changes
- climate change
- ……

CONTROL

- vaccines
- alternative approaches to vector control (IVM)
- training programs for health care workers
- cost/benefit analyses
- …….

BIOLOGY/ECOLOGY

- Interaction host/pathogen/vector/ environment
- genetic change of pathogens and vectors
- …….

NEW TECHNOLOGIES

- next generation sequencing
- epidemiological tools (remote sensing, GIS, spatial analysis….)
- …….
- ……

A role for EPIZONE?

EPIZONE European Research Group (ERG) plays a central role in prevention and control of animal diseases and will contribute to limiting both the risks and damage caused by those diseases in the EU and beyond

In EPIZONE over 300 scientists worldwide strive for the common EPIZONE goal:

to improve, standardise, and develop (new):

- diagnostic methods
- vaccines, intervention strategies
- surveillance, epidemiology studies
- risk analyses

The future

Your turn

Institut für Mikrobiologie der Bundeswehr
München

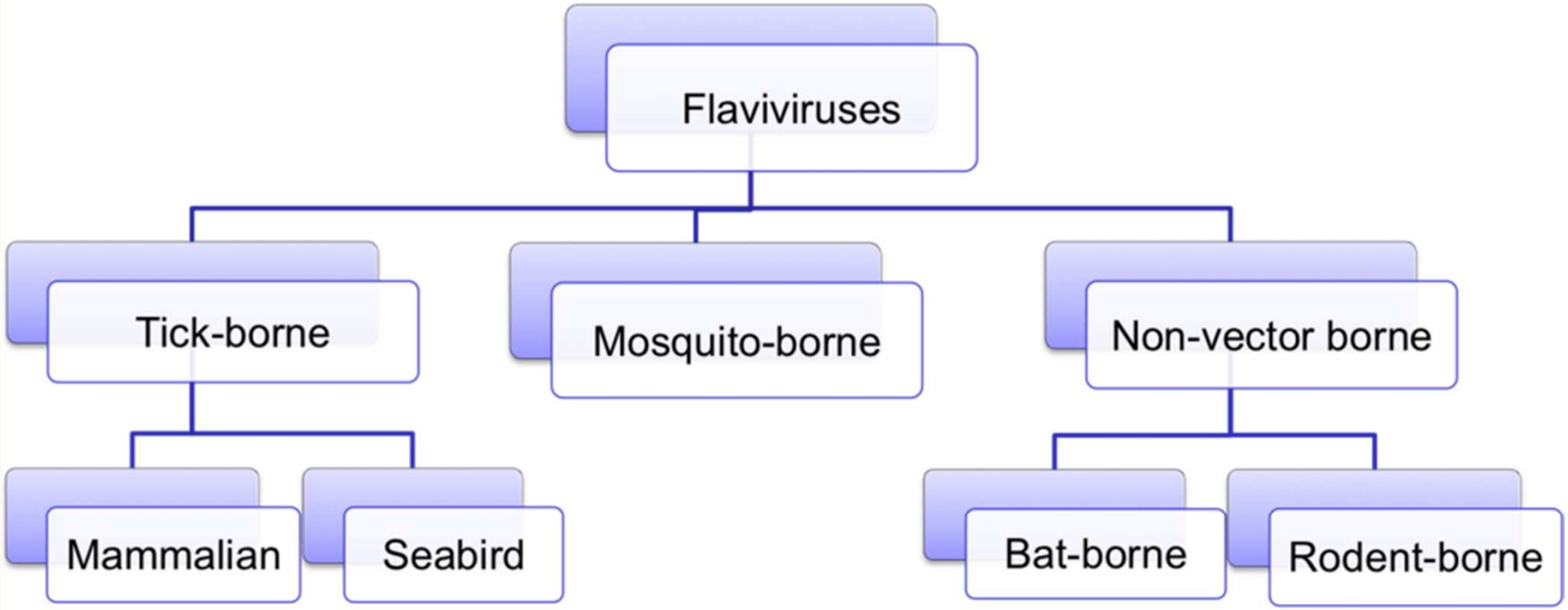
Risk of introduction and spread of flaviviruses other than Dengue, West Nile and Usutu virus

Lt.Col. MC Dr. Gerhard Dobler

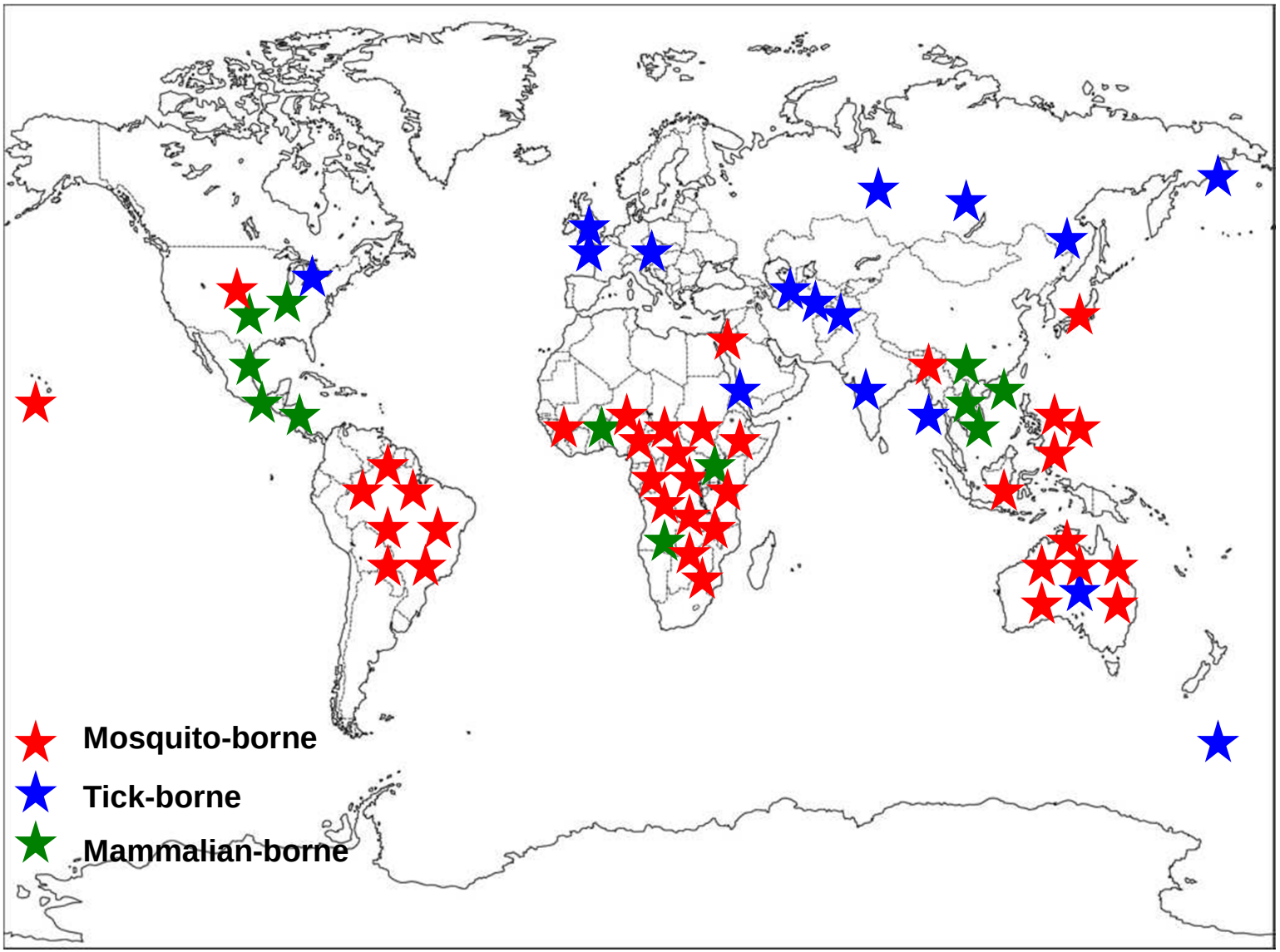
Overview

- Flaviviruses
- Historical spread of flaviviruses
- Spread of tick-borne flaviviruses
- General mechanisms for the spread of flaviviruses

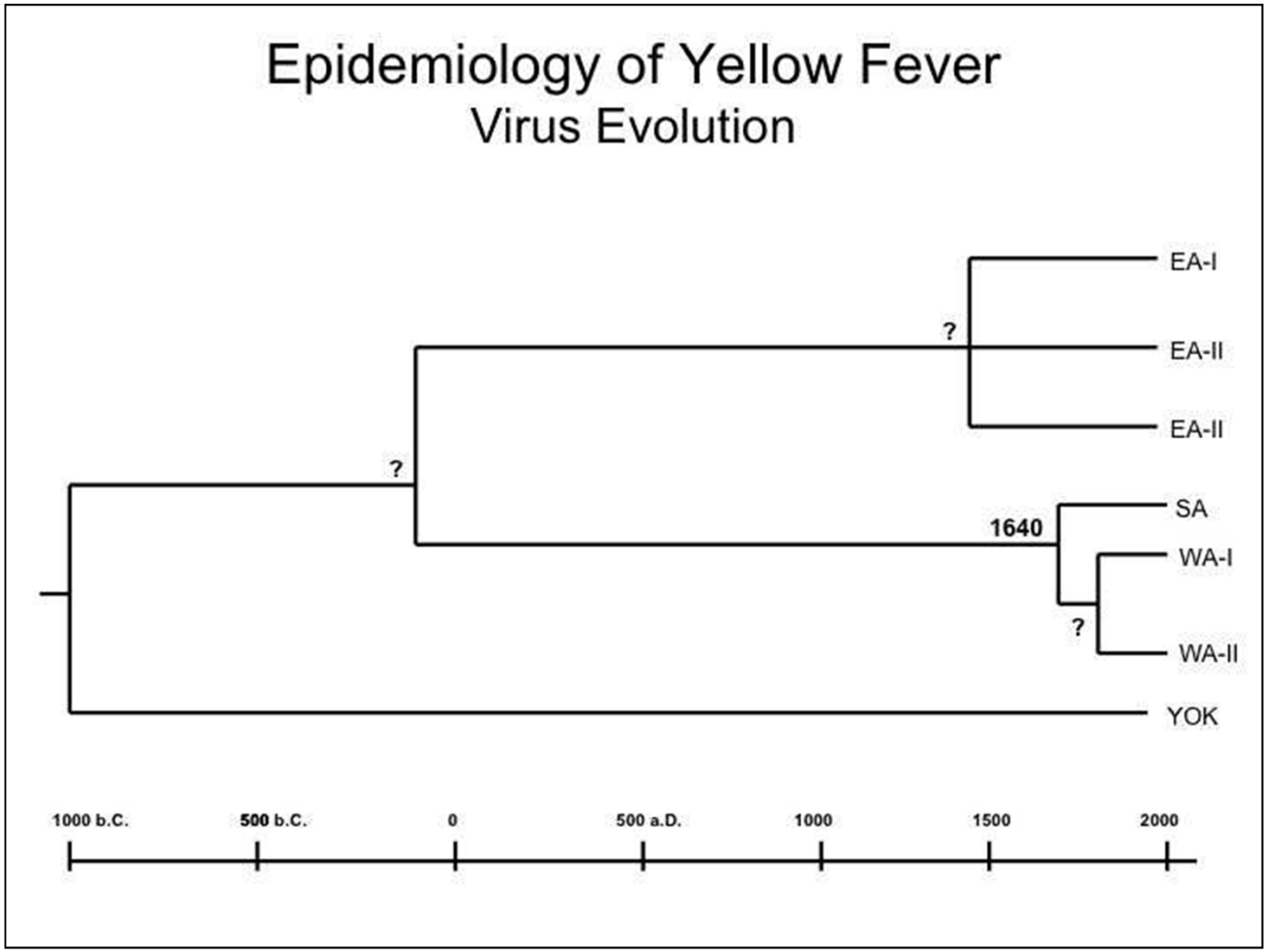
Genus: Flavivirus



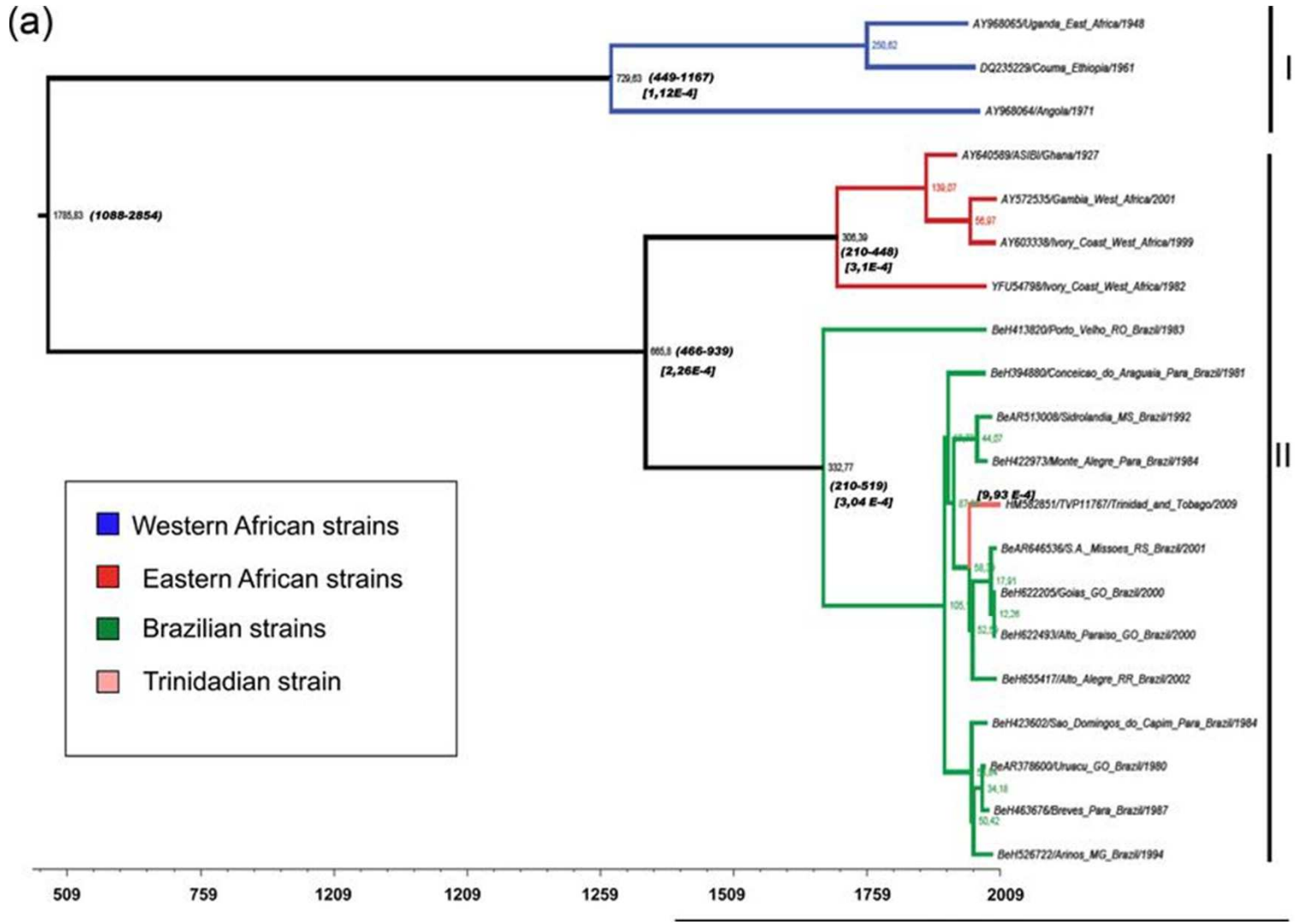
Worldwide distribution of flaviviruses



Yellow fever

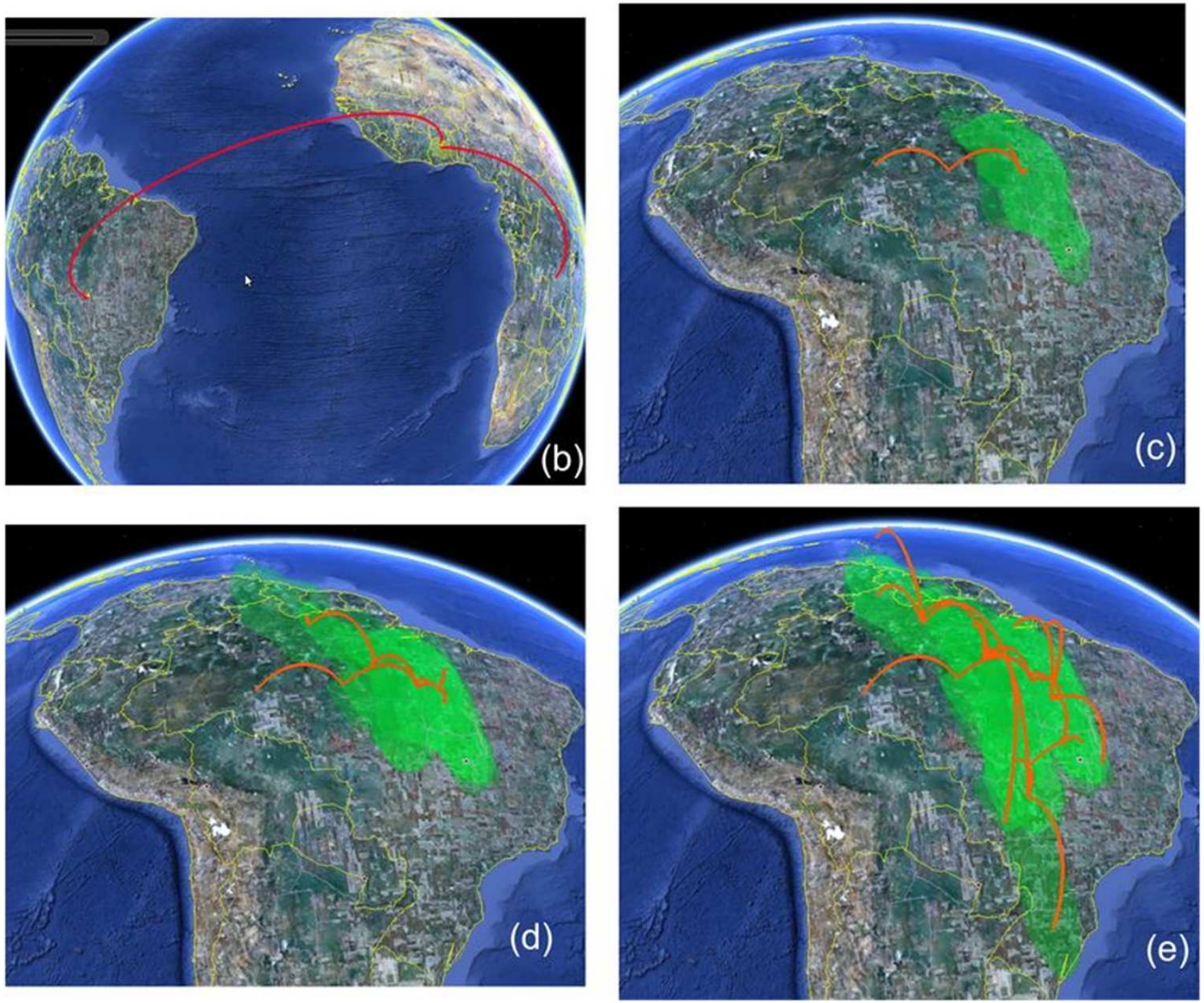


Phylogeny of YFV in South America



Nunes et al., J Virol 2012

Spread of yellow fever virus



Nunes et al., J Virol 2012

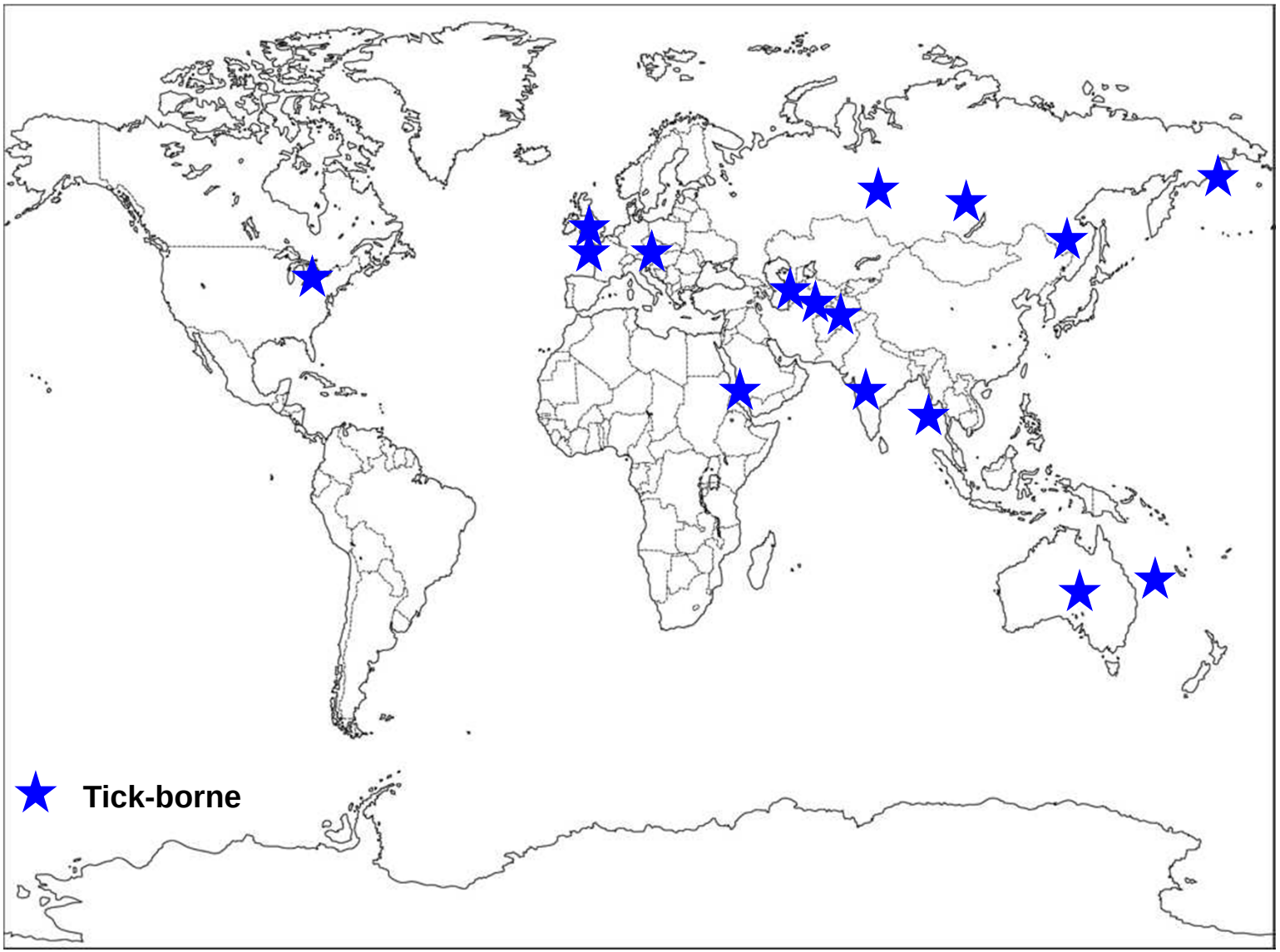
YFV spread

How did YFV come from West Africa to Central and South America ?

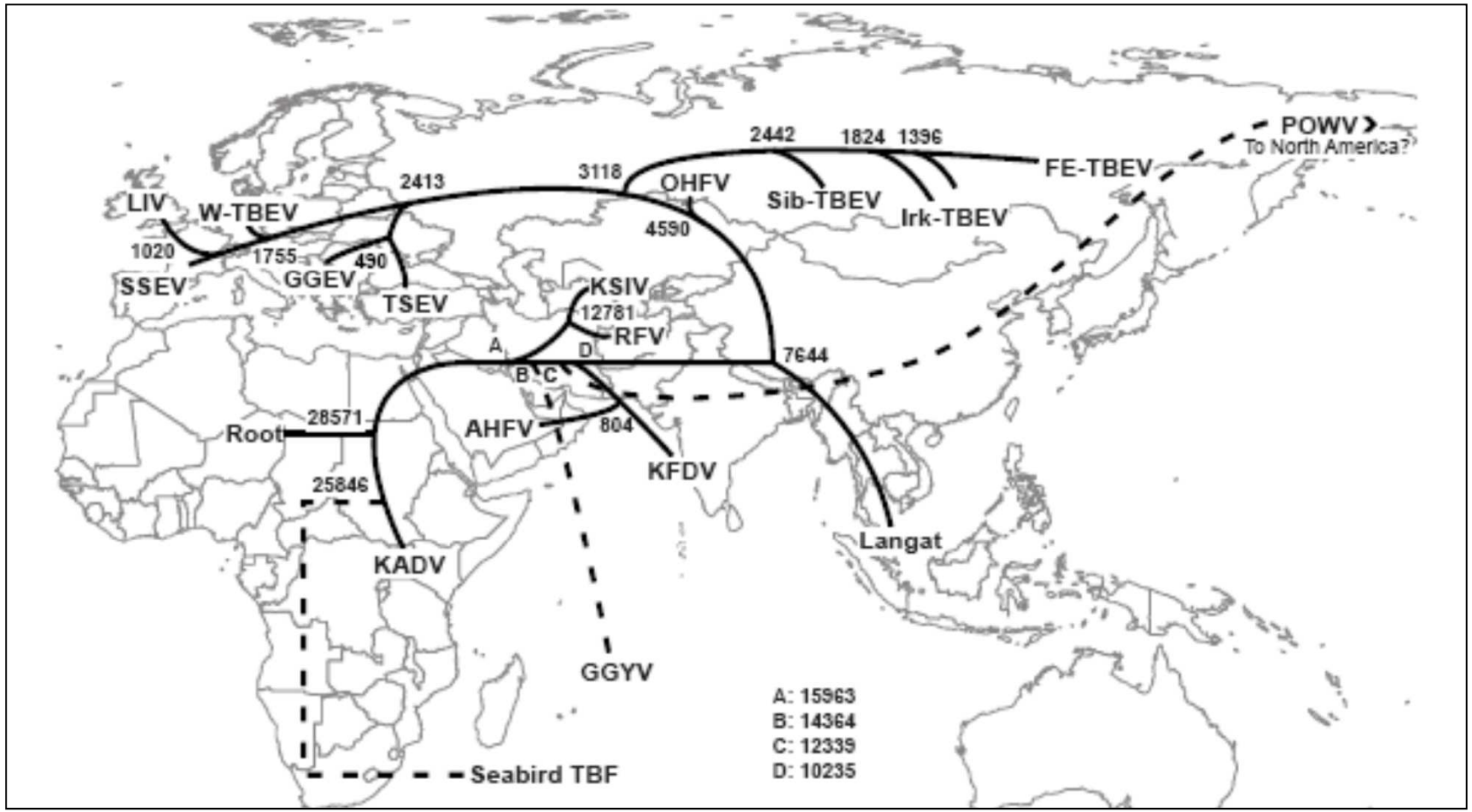
By human activities !

- Not by a viremic passenger !
- Infected vectors on board ?
- Continuous transmission cycle by infected vectors and viremic humans (slaves) during ship travel

Tick-borne flaviviruses



Historical spread of tick-borne flaviviruses



Heinze et al. J Virol 2012

How do tick-borne flaviviruses spread ?

Spread of TBE virus in Russia

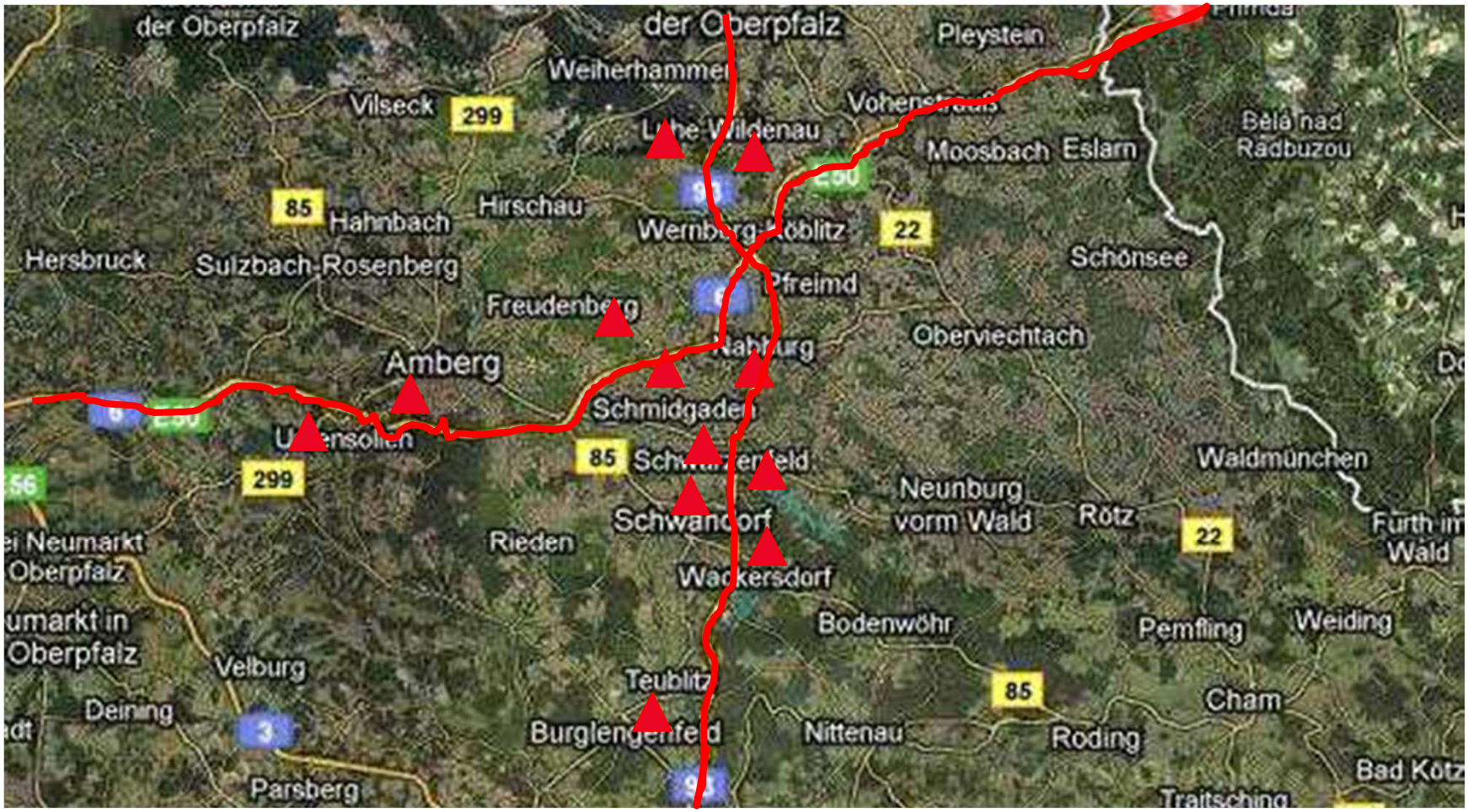
Spread of TBE virus along the Transsiberian Highway and along the Transsiberian Railway

Time of spread during construction of the highway resp. railway

Spread by tick carrying animals like horses, dogs, domestic farm animals, livestock, synanthropic species of birds and mammals

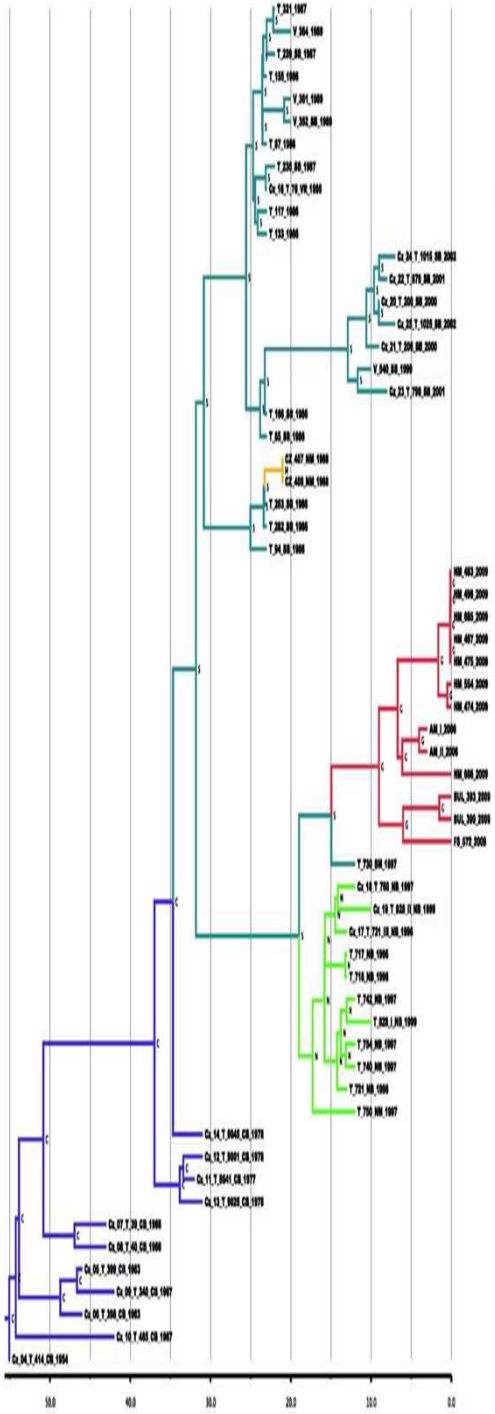
Kovalev et al. J Gen Virol, 2009

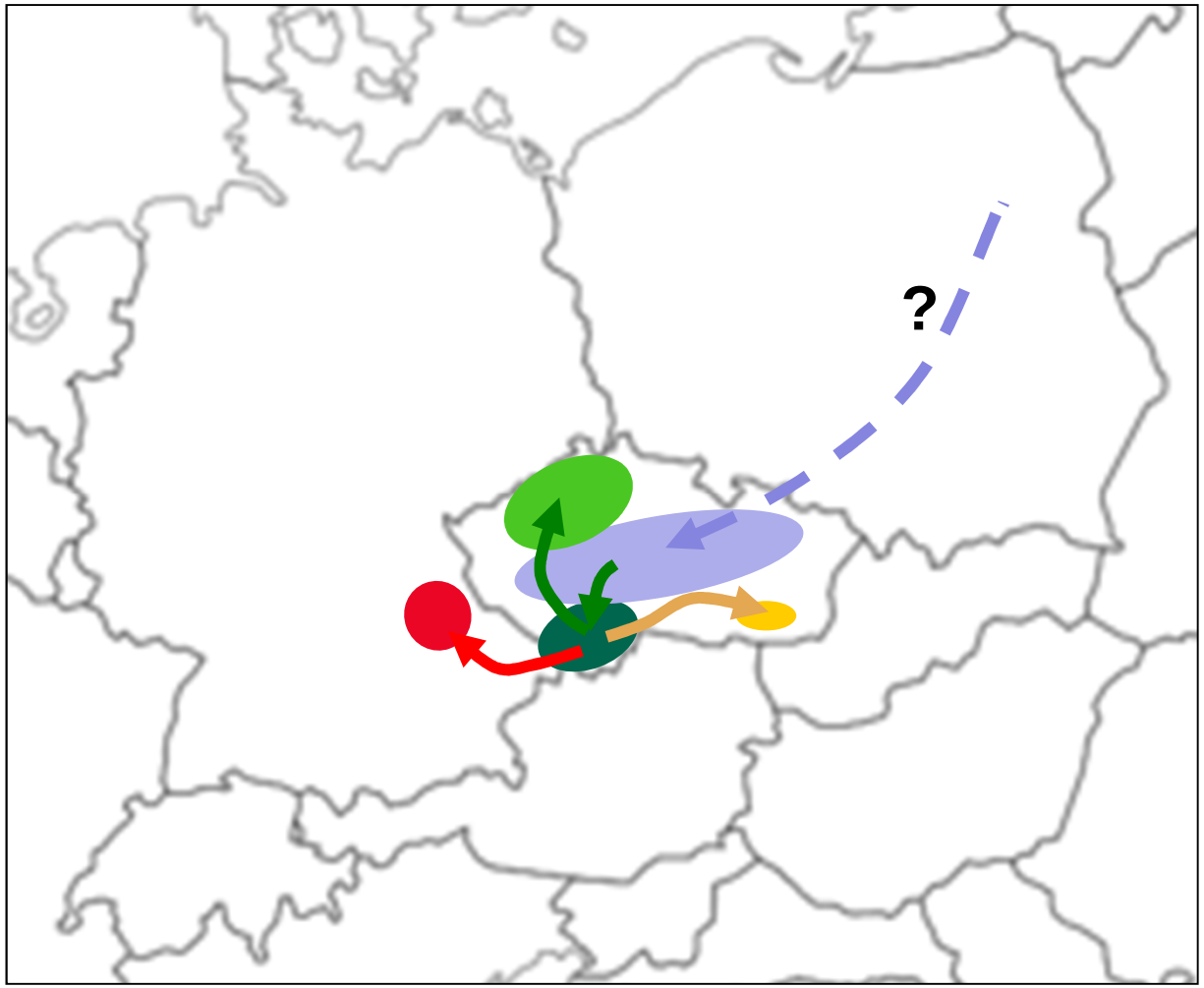
TBE cases in SE-Germany



Working hypothesis:

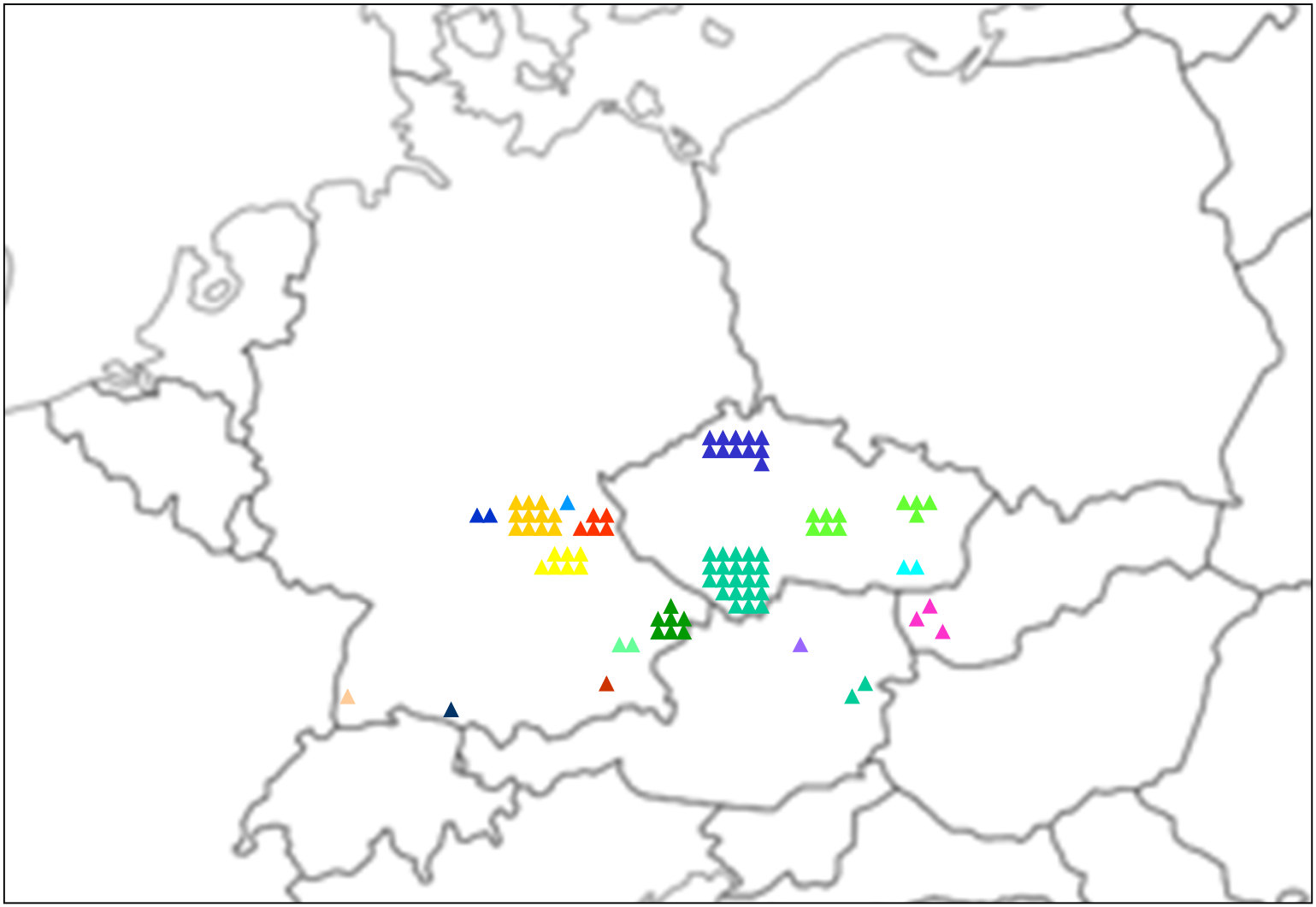
TBE virus spread along the construction of highway from Prague to Nuremberg.

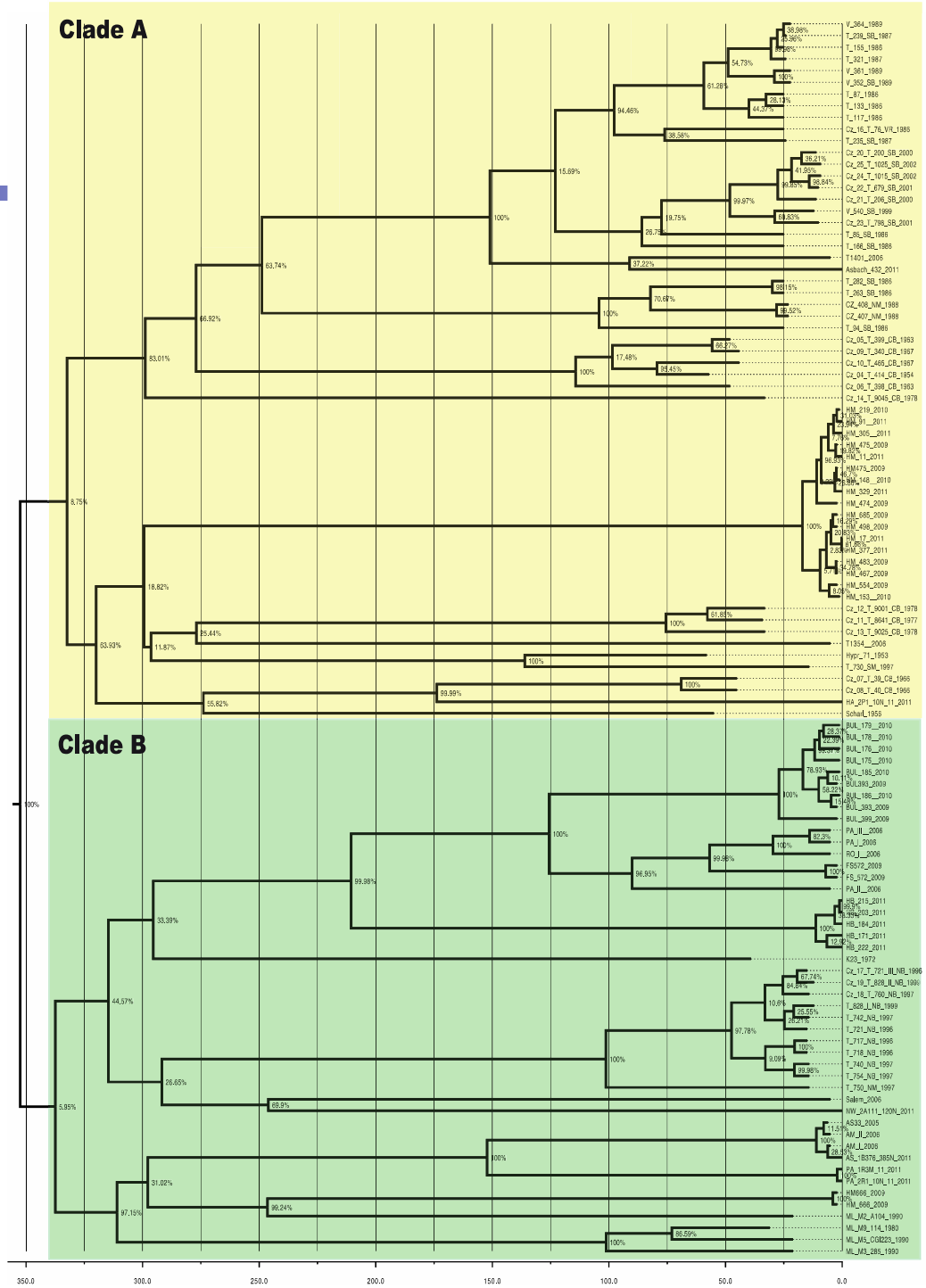




Weidmann et al. 2011)

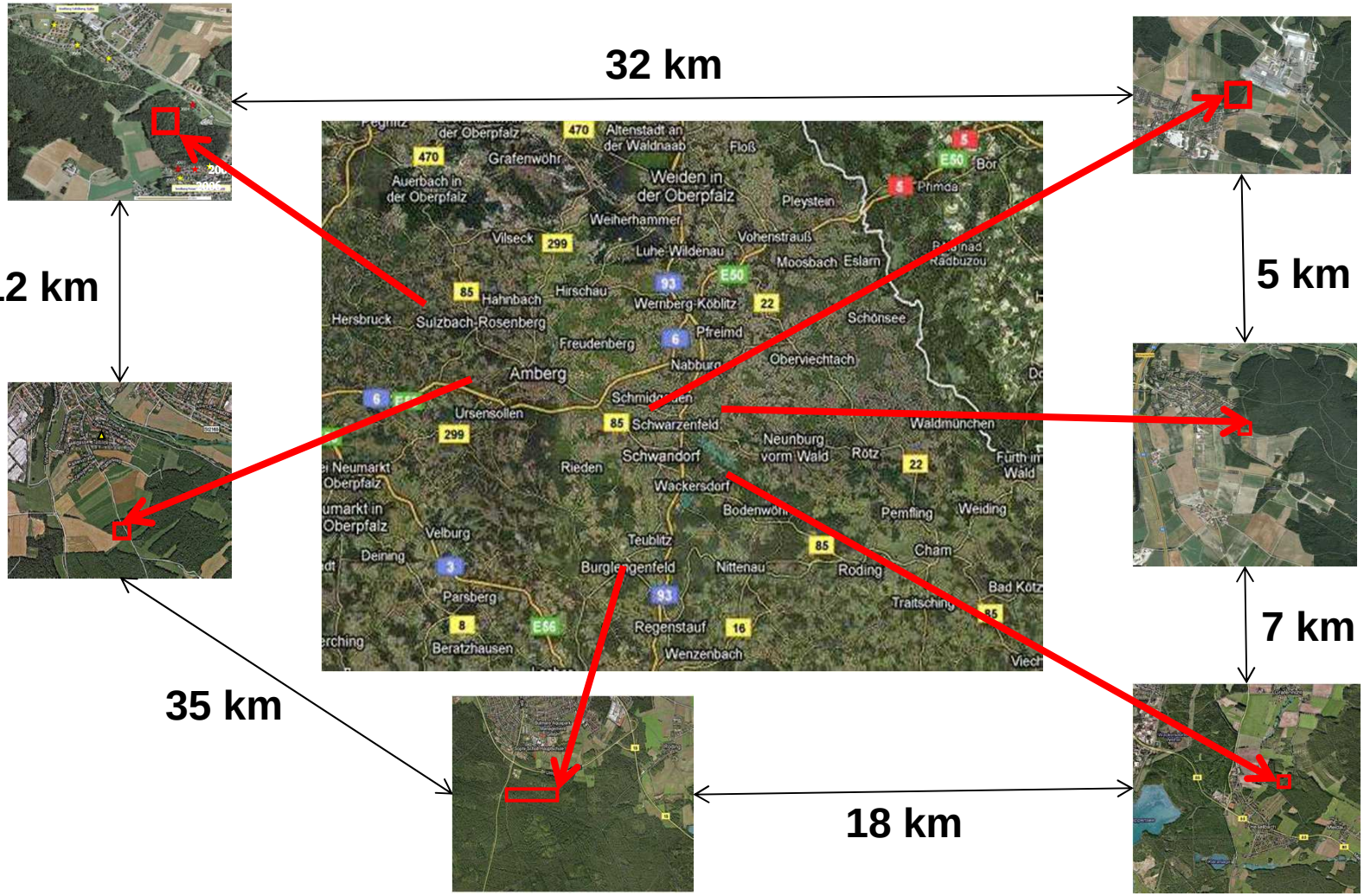
Localisation of 102 analysed TBEV strains





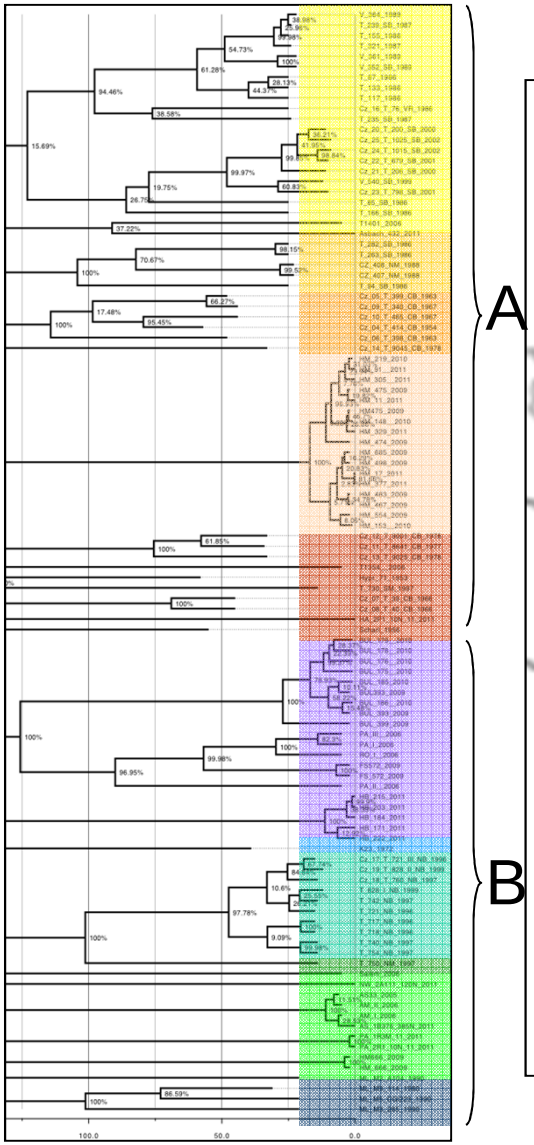
Lt.Col. MC Dr. G. Dobler

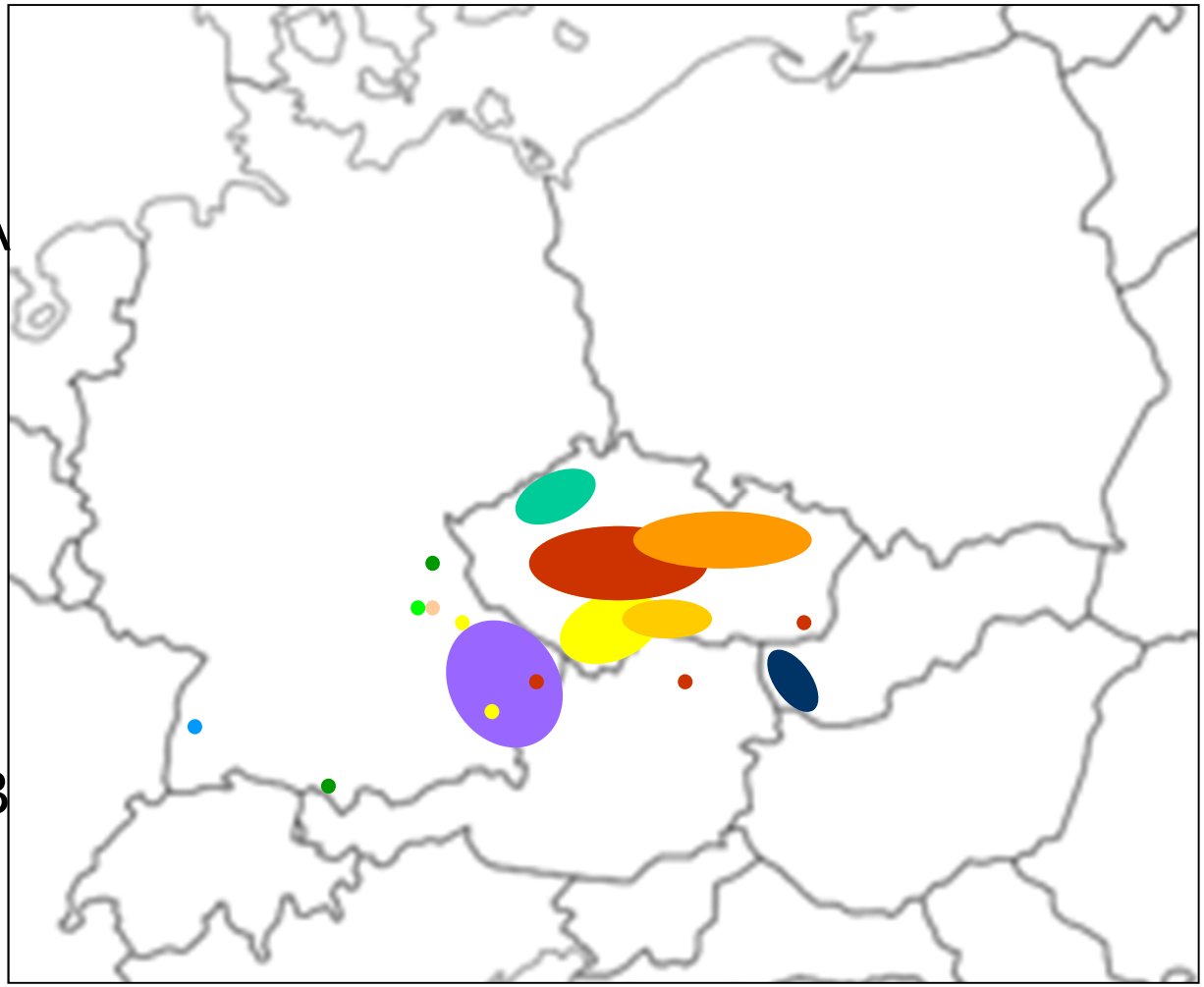
Geography of TBE foci in Eastern Bavaria



Is there a continuous spread from natural focus to natural focus ?

Phylogeography of TBE virus in Central Europe

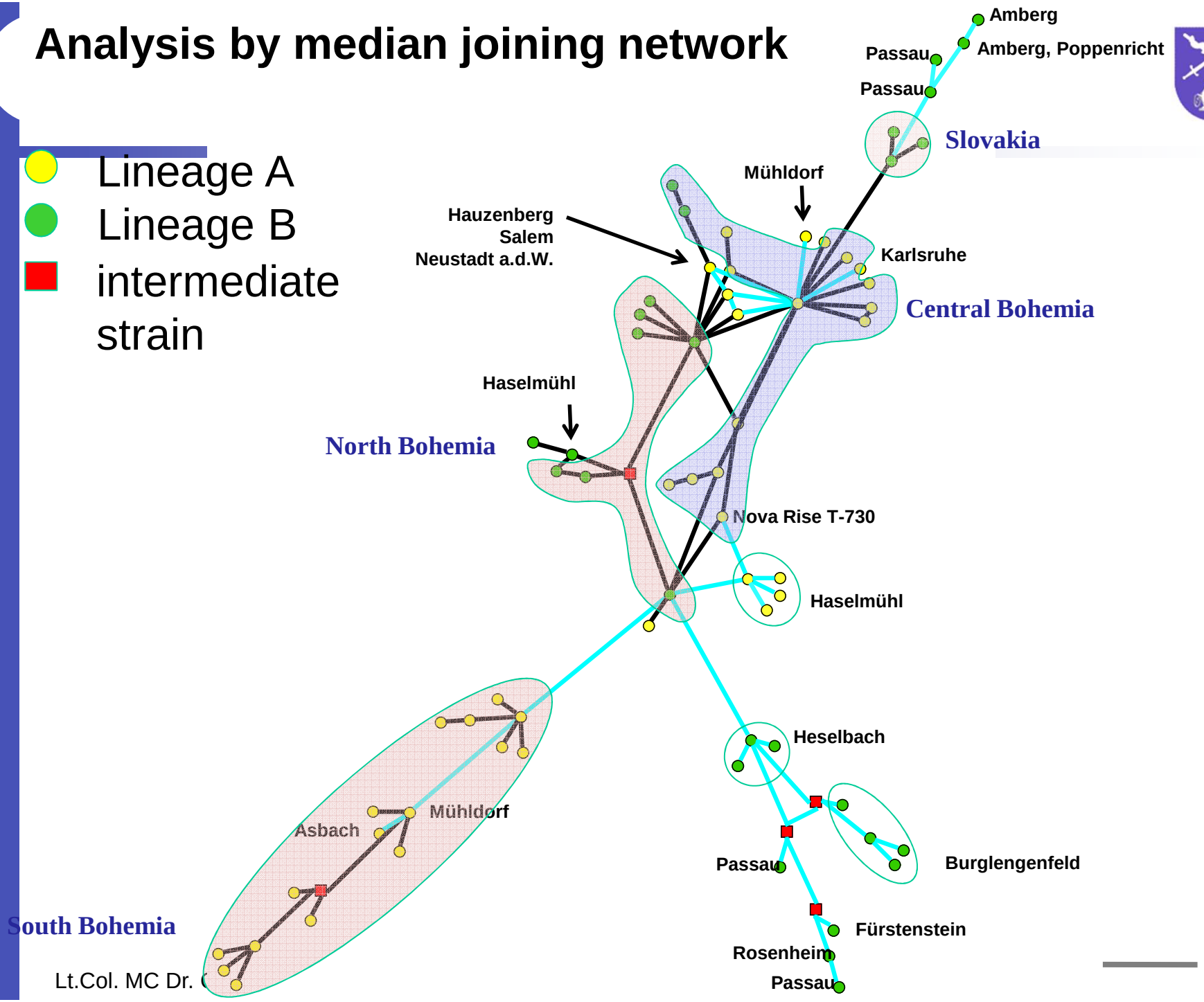




Lt.Col. MC Dr. G. Dobler

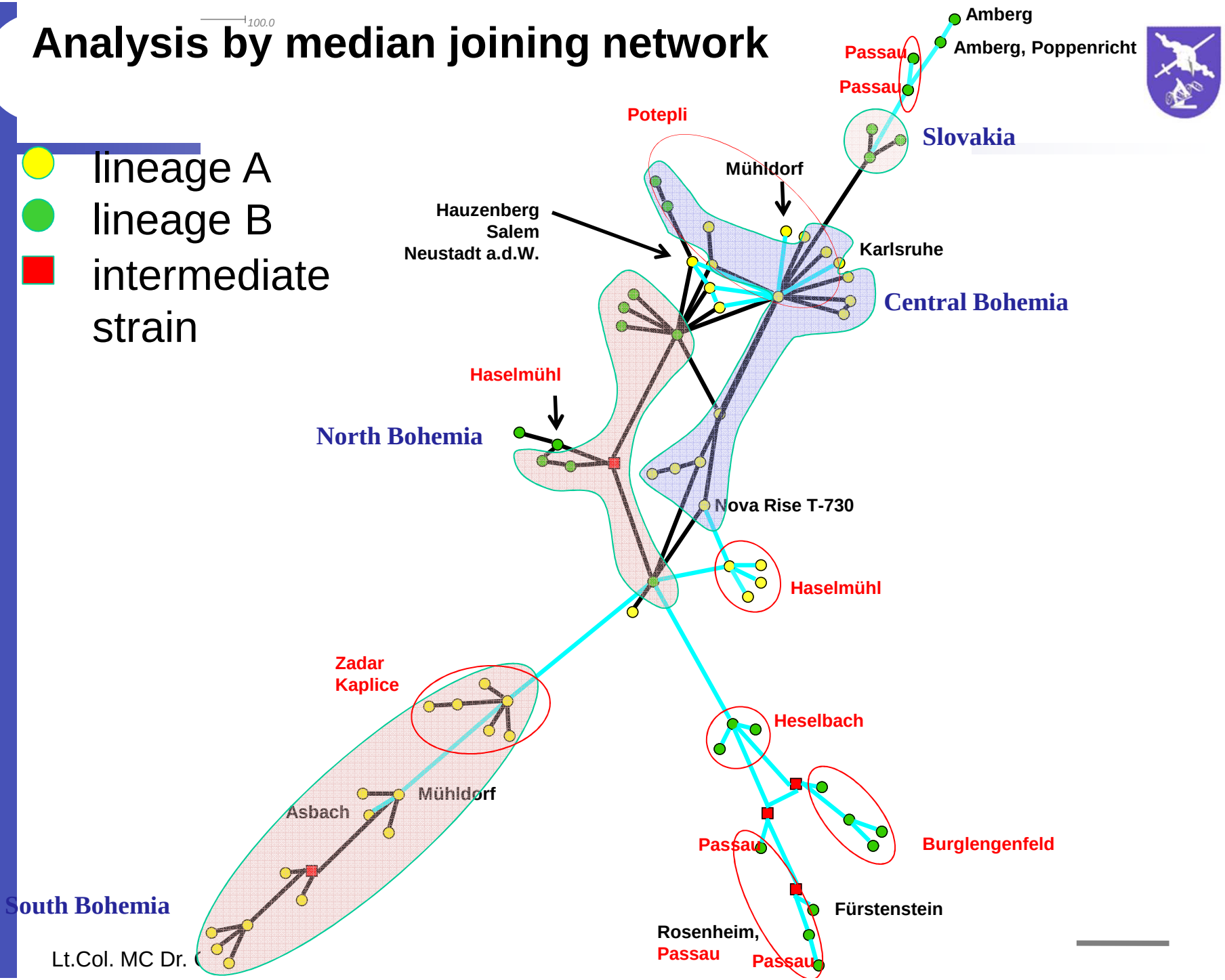
Analysis by median joining network

- Lineage A
- Lineage B
- intermediate strain

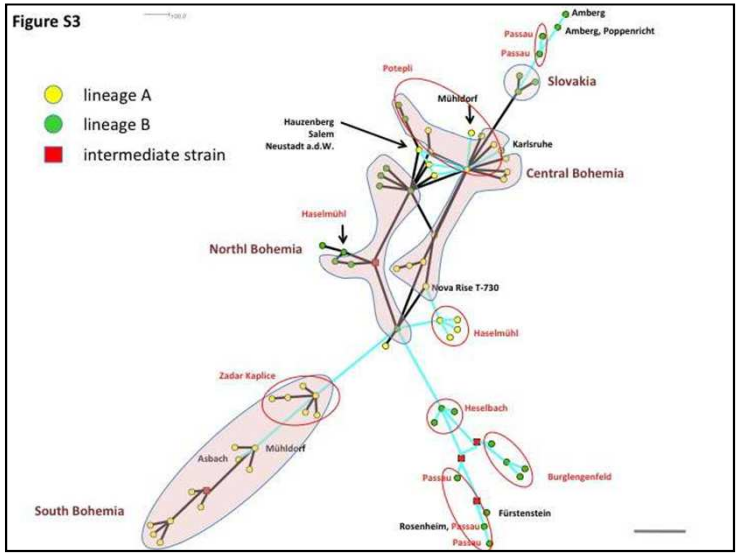


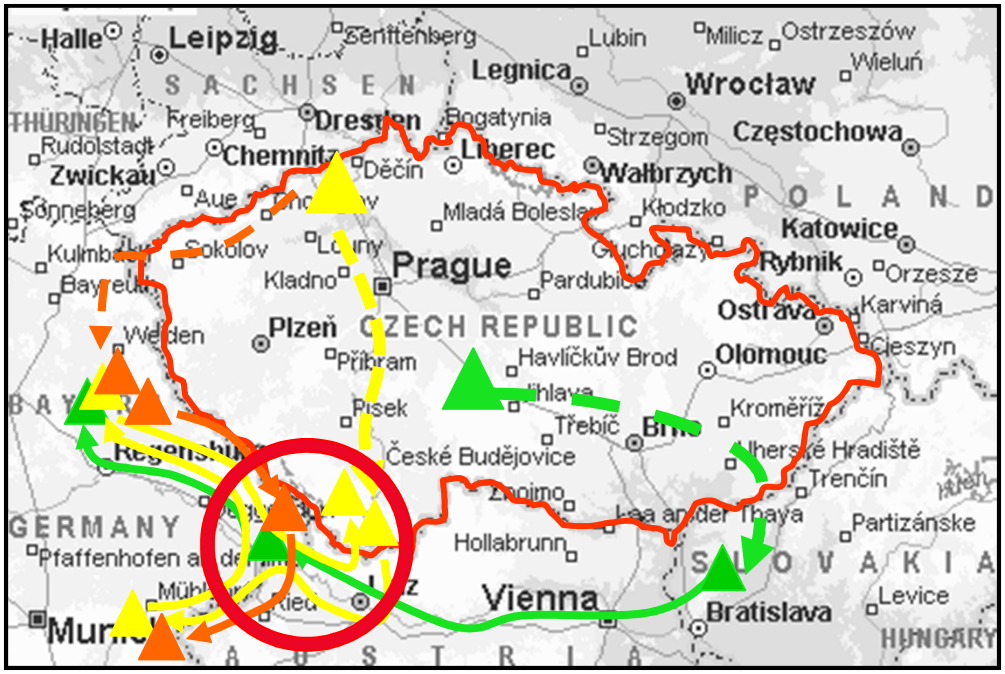
Analysis by median joining network

- lineage A
- lineage B
- intermediate strain

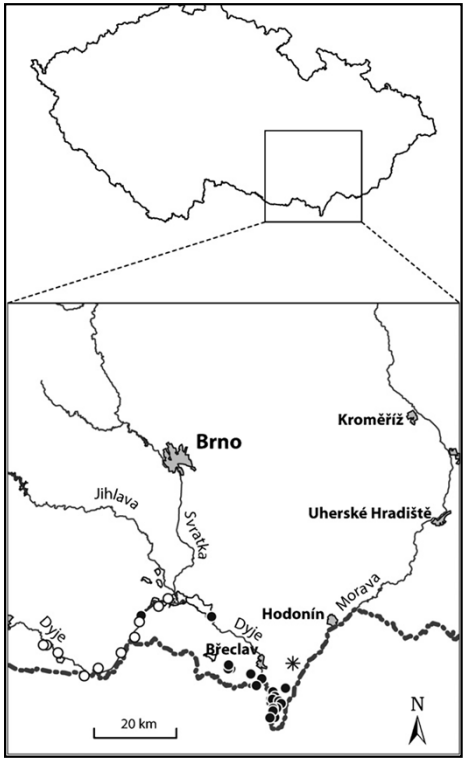


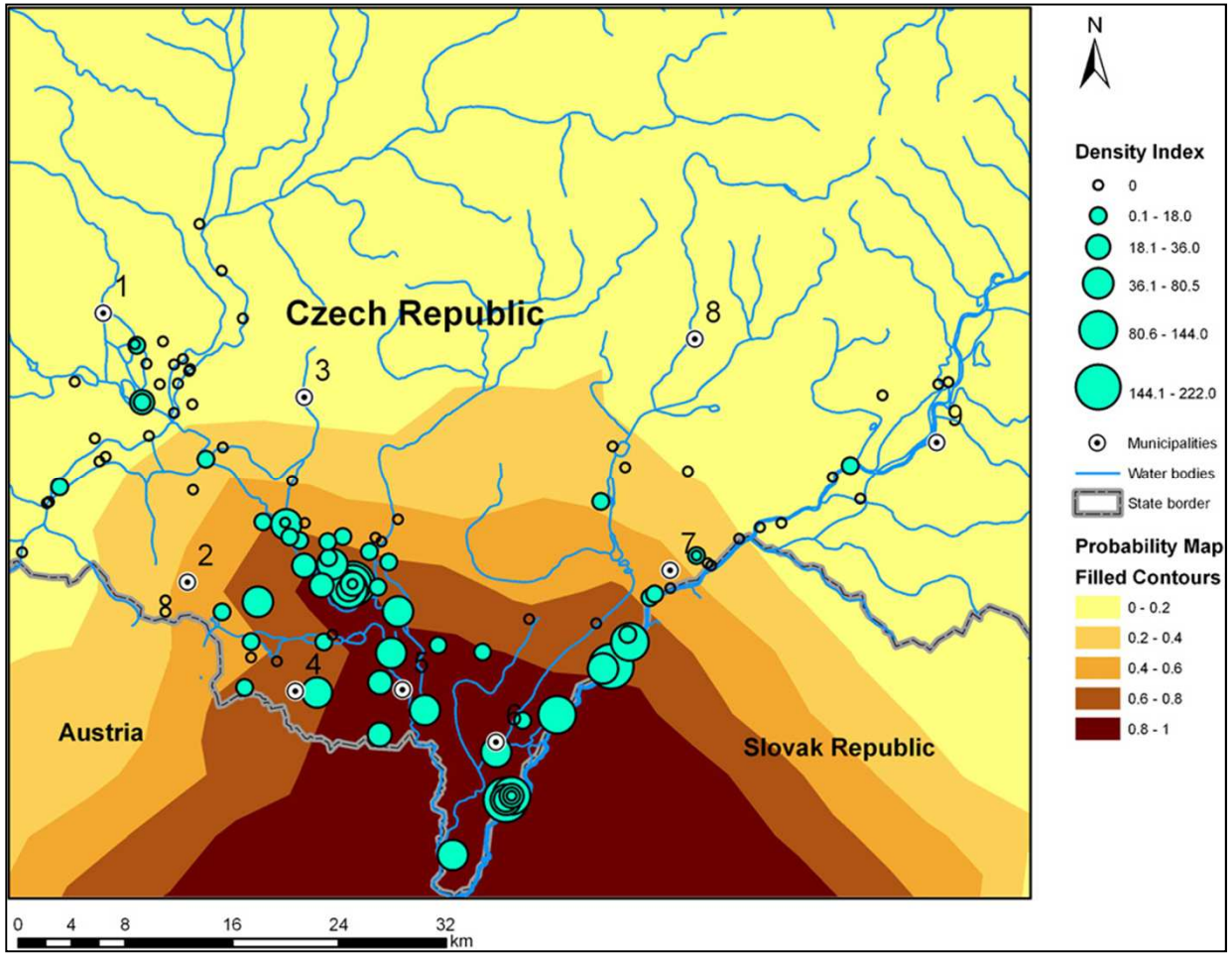
TBEV continuous spread in Central Europe





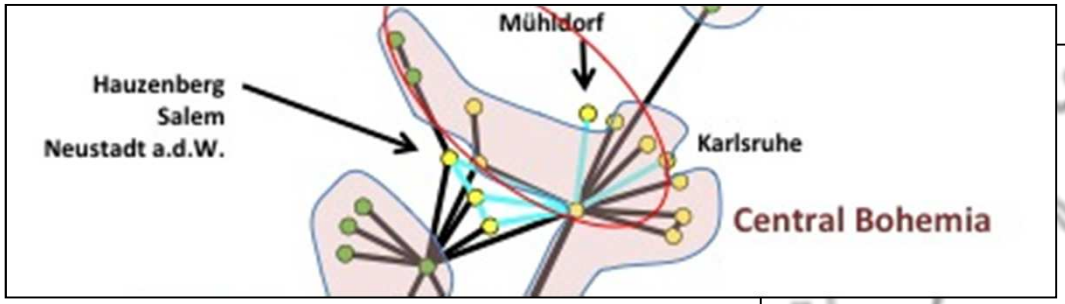
Spread of Dermacentor reticulatus in SE Czech Republik

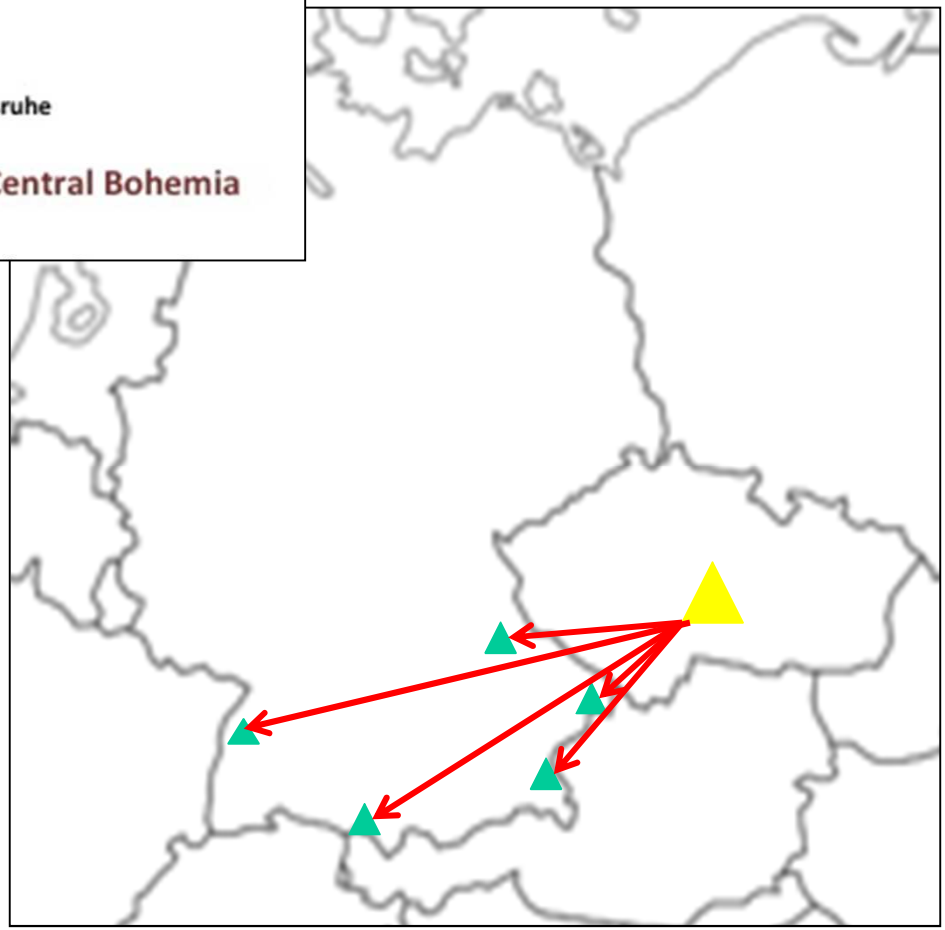




Stoky et al. Vet Parasitol 2011

Focal spread of TBEV in Central Europe





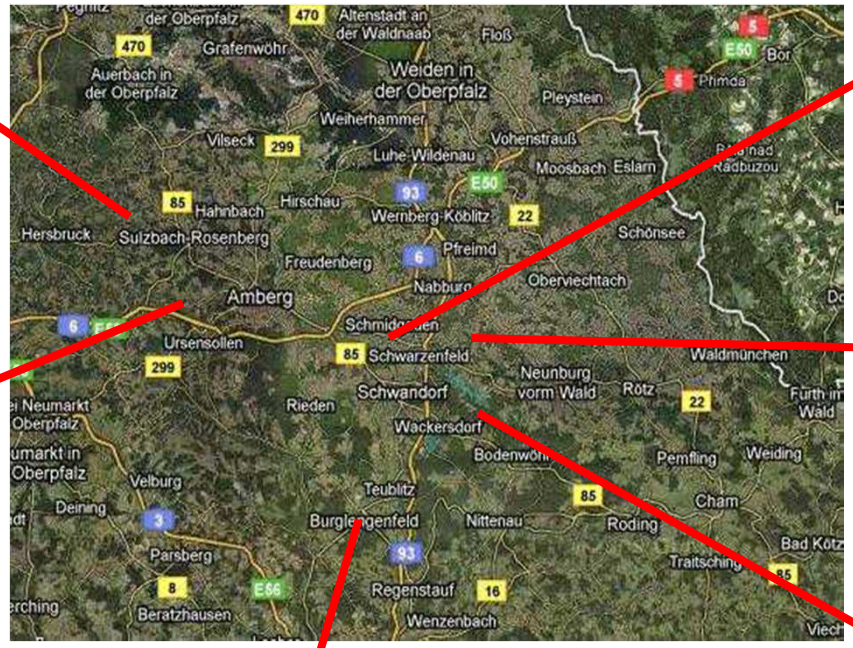
Missing virus links?

Birds, bats ?

Geography of TBE foci in Eastern Bavaria

32 km: 50 years

12 km: > 350 years



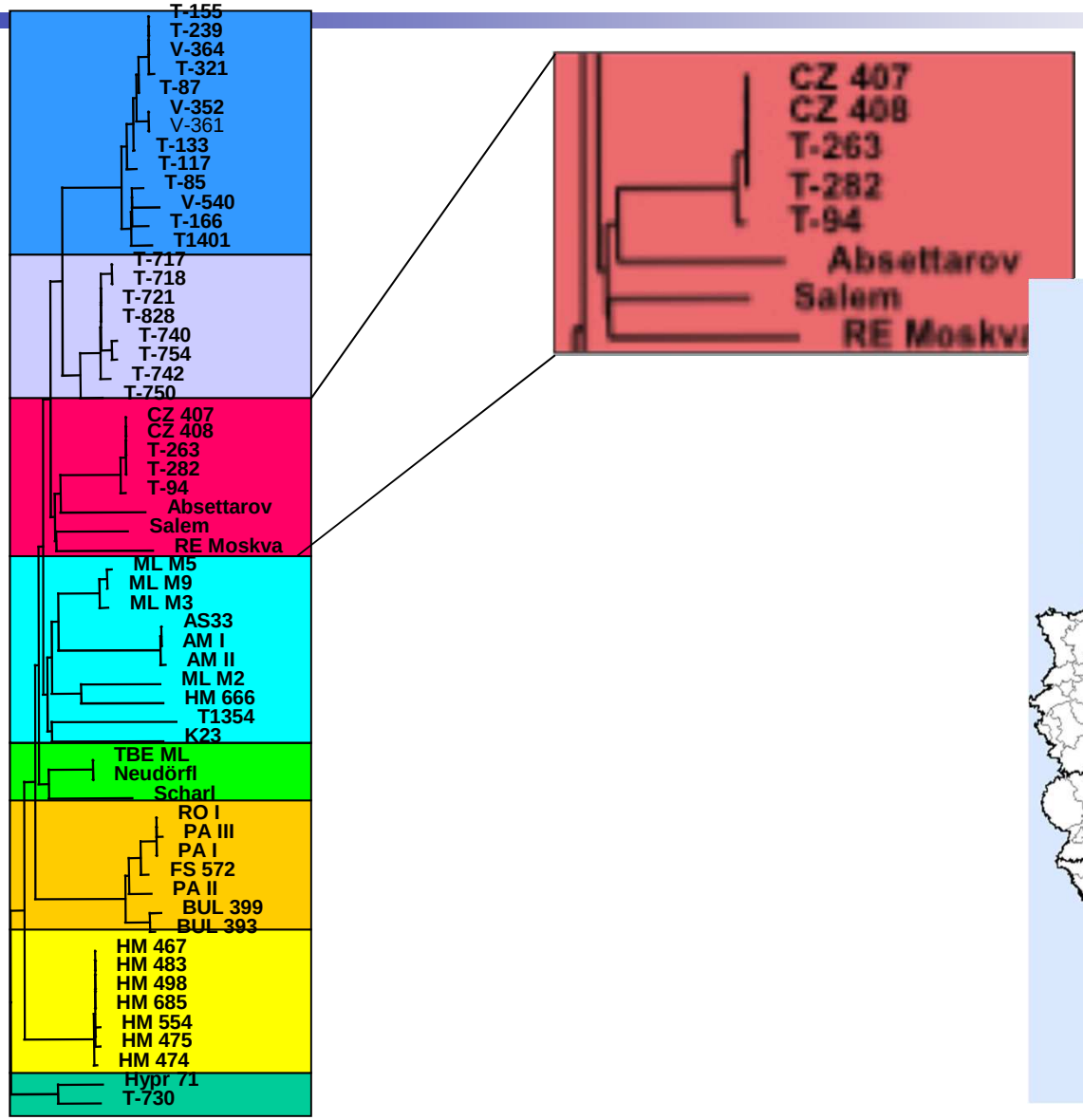
5 km: > 350 years

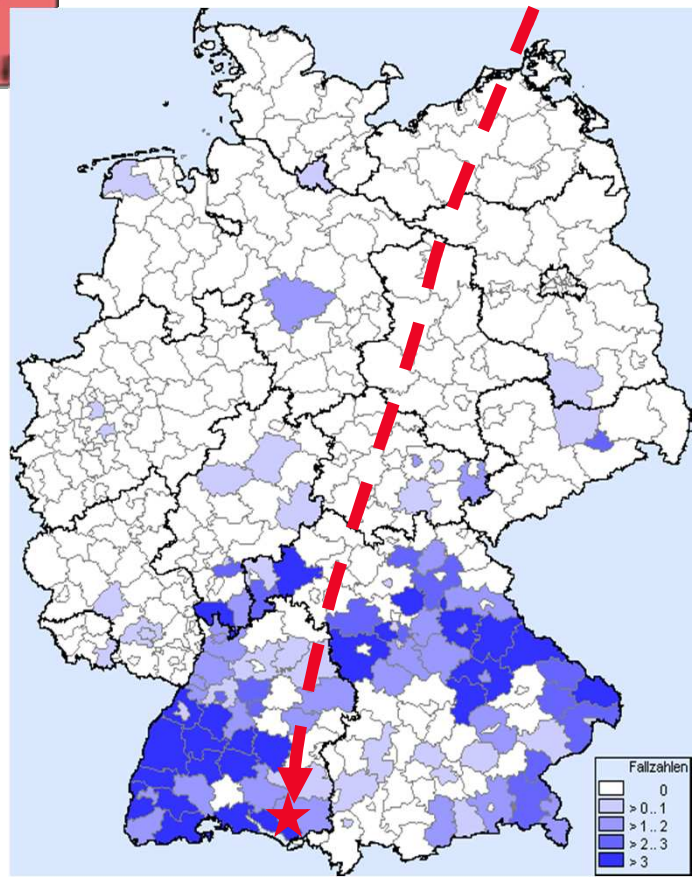
7 km: > 350 years

35 km: 210 years

18 km: 210 years

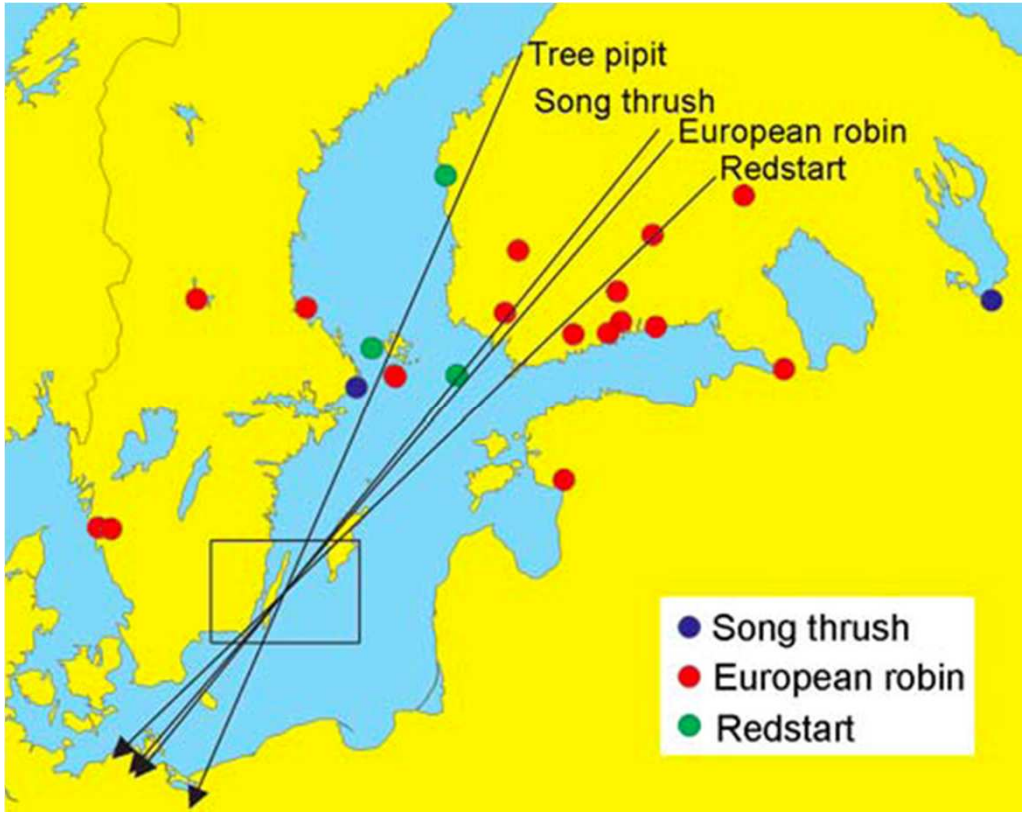
TBEV Phylogeny in Central Europa





LILV

Birds as carrier of TBE virus ?



Waldenström et al. 2007

1/100 bird carrier of ticks

1/100 ticks carrier of virus

1/10.000 birds carrier of virus

Conclusions

- TBE virus may spread along artificial aisles (railroads, highways)
- TBE virus spread along natural aisles (river courses)
- TBE virus can spread over long distances with migrating birds and possibly also by migrating bats
- In Eastern Bavaria so far no continuous spread within closely located TBE foci
- Several independant introductions of TBE virus

➔ continuous spread of TBE virus

➔ sporadic spread of TBE virus over long distances

Conclusions

Mechanisms of spread

- Spread by viremic birds (?)
- Spread by virus infected tick-carrying birds
- Spread by viremic mammals (rodents, bats ?)
- Spread by virus infected tick-carrying mammals (rodents, pets, livestock)

Live Animal Imports into Europe 2005-2009
(according to TRACES)

Species	Europe	Asia	Total
Horses	75,043	47,733	0
Swine	2,052	0	2,052
Poultry	607,348	214,054	821,402
Primates	934	24,295	25,229
Birds	384,025	20,329	404,354
Reptiles	74,935	904,852	979,787
Rodents	132,795	246,421	379,116
Total	1,277,132	1,467,684	2,744,814

Acknowledgements

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Institute of Animal Hygiene, University Leipzig
Martin Pfeffer

Czech Academy of Sciences, Ceske Budejovice
Daniel Ruzek

Czech Central Military Institute of Health, Ceske Budejovice
Karel Krivanec

Workshop EPIZONE: IZS Brescia, 9-10 May 2013

Arboviruses (pathogenic for animals) circulating in Europe, or at risk of introduction

Zdenek Hubalek

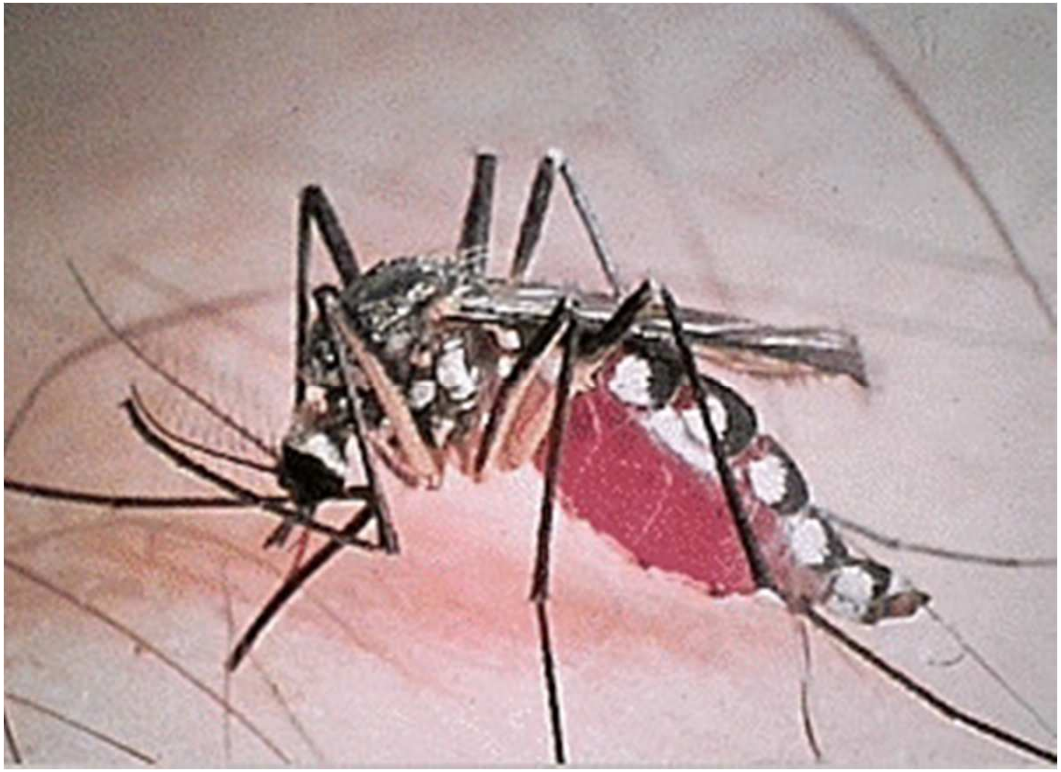
(1) Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic;

(2) Masaryk University Brno

Animal pathogenic arboviruses *(44)*

1. Tick-borne: LI, TBE, OHF, KFD, NSD, BHA, THO, ASF *(8)*
2. Mosquito-borne: EEE, WEE, VEE, GET, SFV, MVE, JE, WN, USU, ITM (BAG), TEM, WSL, LAC, SSH, CV, RVF, PHS *(17)*
3. Sandfly-borne: VS-I, VS-NJ, VS-A, CHP, COC *(5)*
4. Midge-borne: MD, AKA, AIN, SHU, SHA, SB, AHS, KAS (CHU), BT, EHD, IBA, EE, BEF, KOT *(14)*

Mosquito-borne viruses



Eastern equine encephalomyelitis (EEE) virus

Family: *Togaviridae*
Genus: *Alphavirus*
Vector: *Culiseta melanura*
Hosts: birds
Animal disease: encephalomyelitis of horses with high mortality; occasionally extended epizootics (USA 1938: 184,000 horses infected); epizootics in pheasants
Geographic distribution: North America

Western equine encephalomyelitis (WEE) virus

Family: *Togaviridae*
Genus: *Alphavirus*
Vector: *Culex tarsalis*
Hosts: birds
Animal disease: encephalomyelitis of horses, mortality lower than in EEE
Geographic distribution: North and South America

Venezuelan equine encephalomyelitis (VEE) virus

Family: ***Togaviridae***
Genus: ***Alphavirus***
Vector: ***Aedes* spp.**
Hosts: rodents, horse; birds
Animal disease: horses and dogs (also cats, sheep, goat)
– fever, diarrhea, occasionally fatal encephalitis
Geographic distribution: South and Central America, Florida

Japanese encephalitis (JE) virus

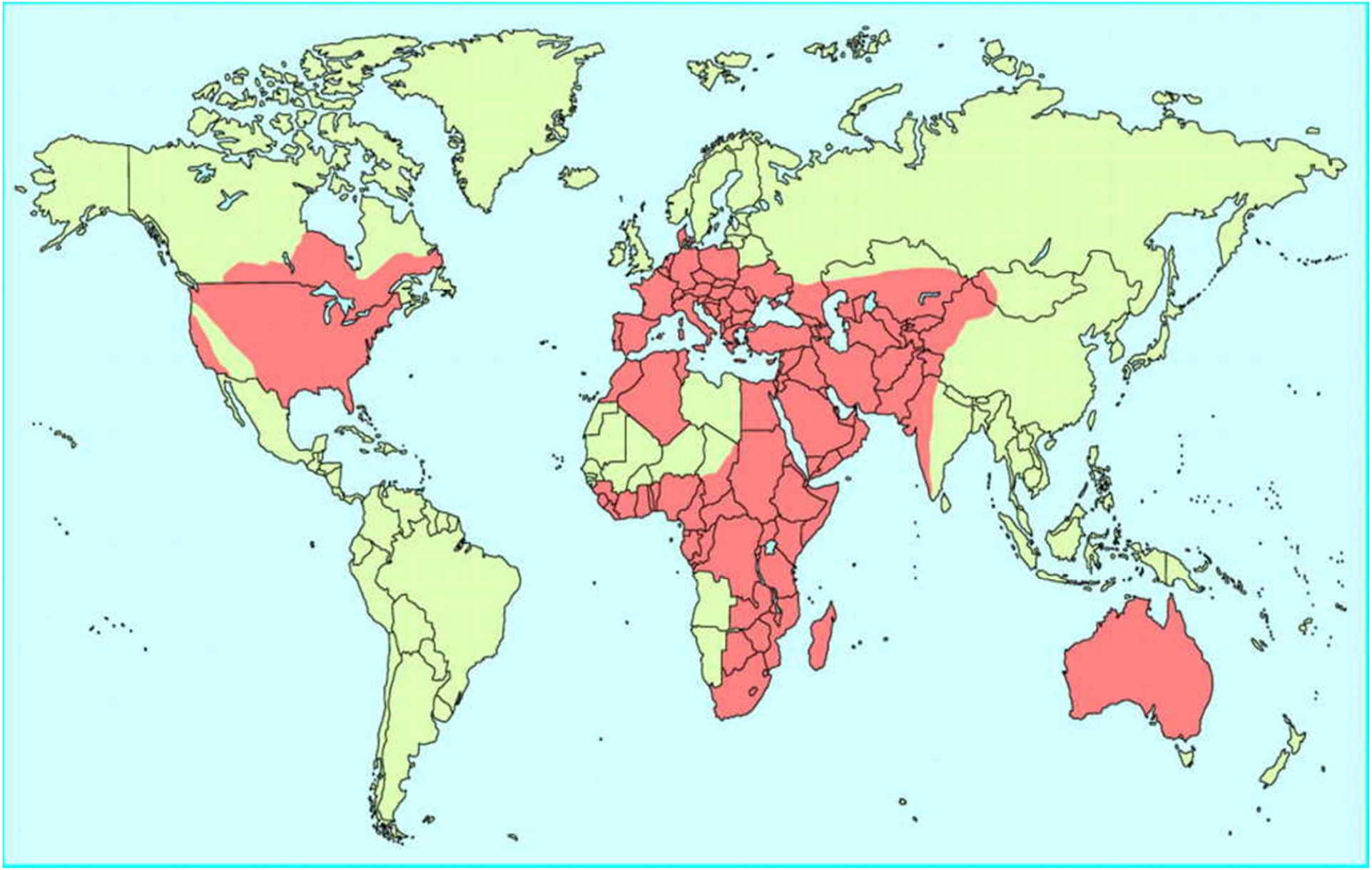
Family: *Flaviviridae*
Genus: *Flavivirus*
Antigenic group: Japanese encephalitis
Vector: *Culex* **spp.**
Hosts: birds, pig
Animal disease: occasionally encephalitis in horses, aborts in pigs
Geographic distribution: Southeast Asia

West Nile (WN) virus

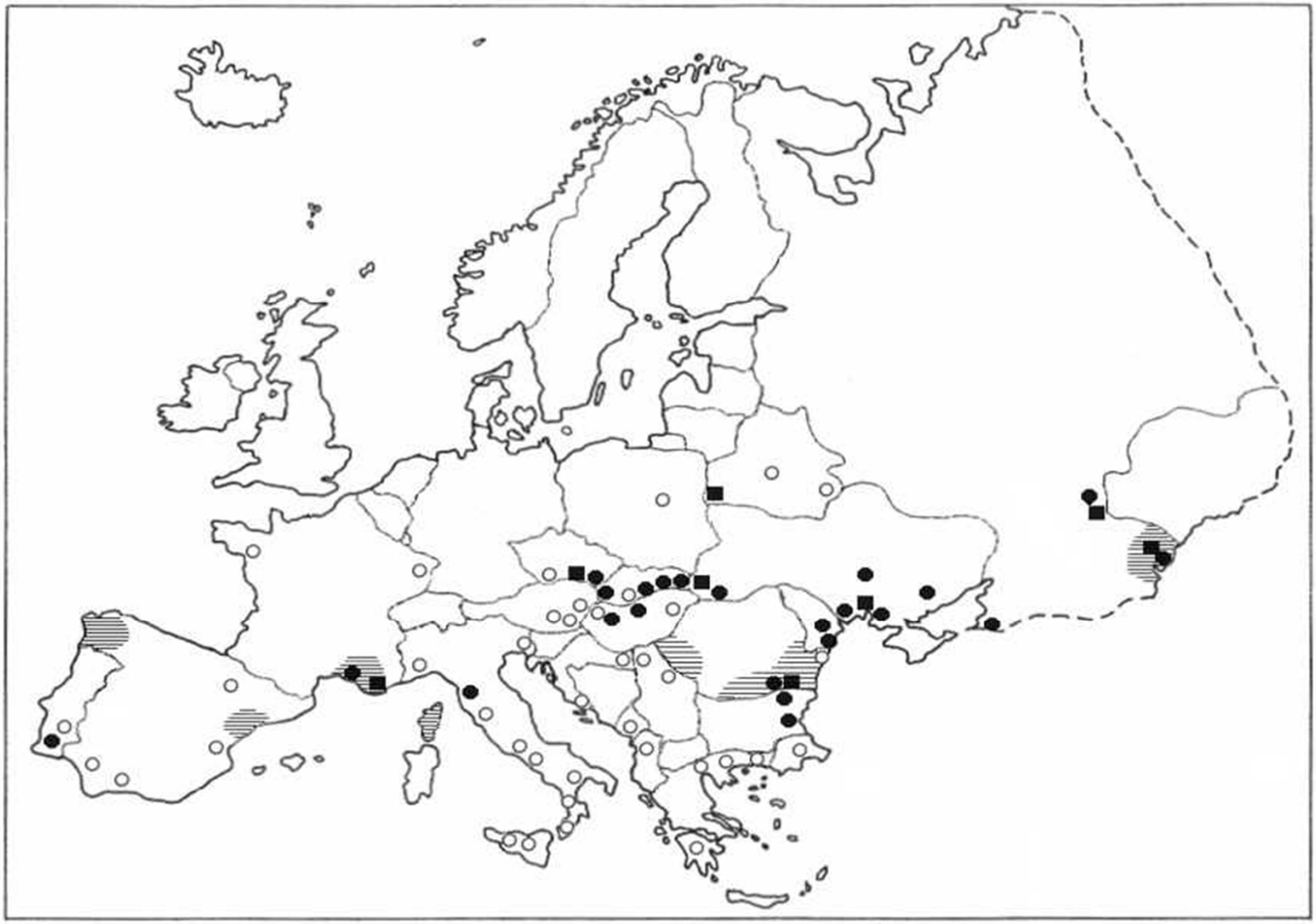
Family: *Flaviviridae*
Genus: *Flavivirus*
Antigenic group: Japanese encephalitis
At least 2 lineages
Vector: *Culex* spp., other mosquitoes
Hosts: birds

Geographic distribution of WNV

(Solomon et al. 2003)



West Nile virus, Europe



WNV hosts

Birds: WNV detected in 317 avian spp.

- **Corvids** and **raptors** exhibit a high mortality rate (>90%) in N. America
- Some birds reveal a high viraemia; many infected are asymptomatic

Mammals rarely

- **Man** and **horse:** "dead end"

Amphibians, reptiles sometimes

WND symptoms in birds

- depression, ataxia
- encephalitis, paralysis
- myocarditis
- sometimes necrotic hepatitis, splenitis and pancreatitis

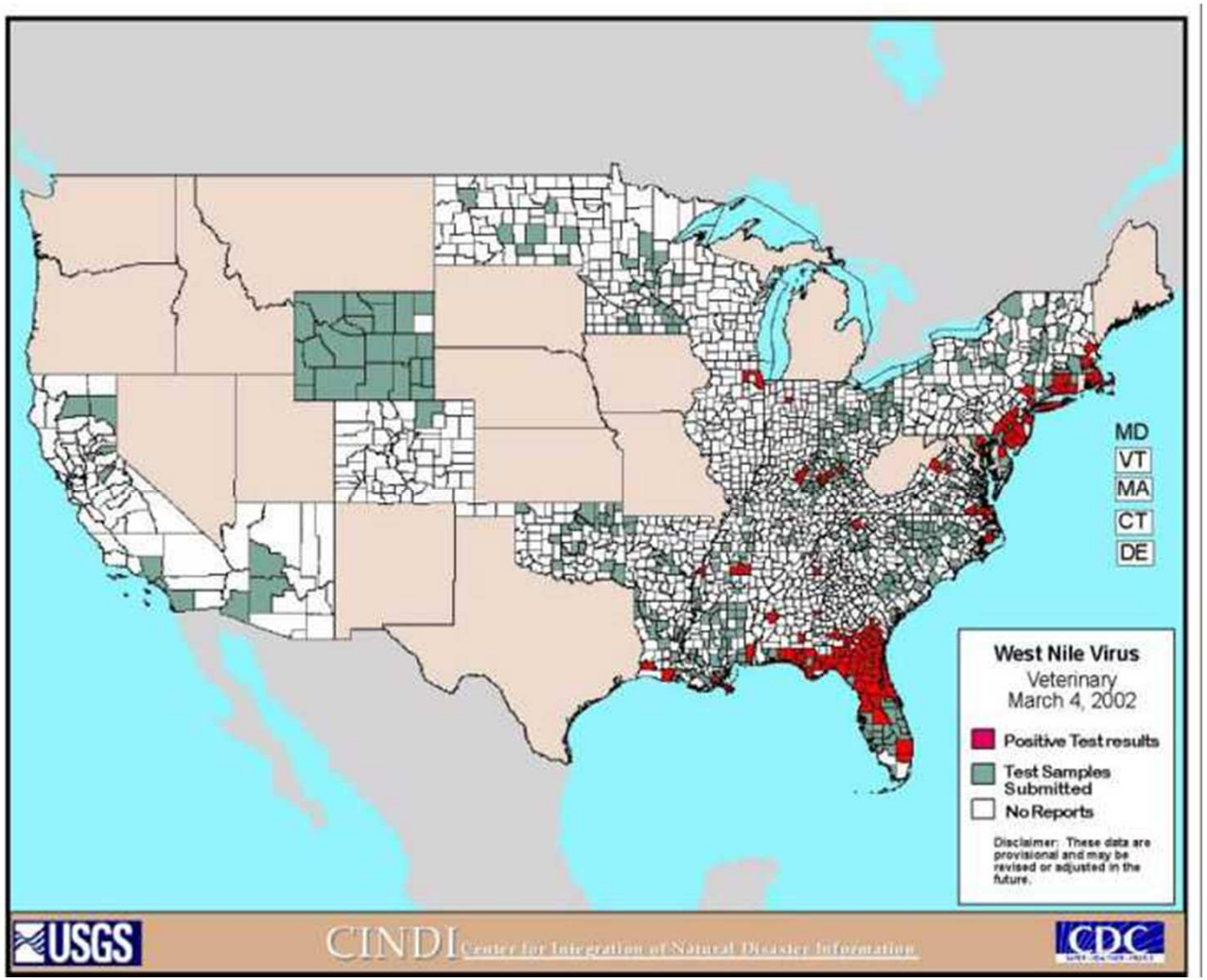
WND symptoms in horses

Febrile neuroinvasive ilness:
polioencephalomyelitis with ataxia, pareses, paralysis (up to tetraplegia)

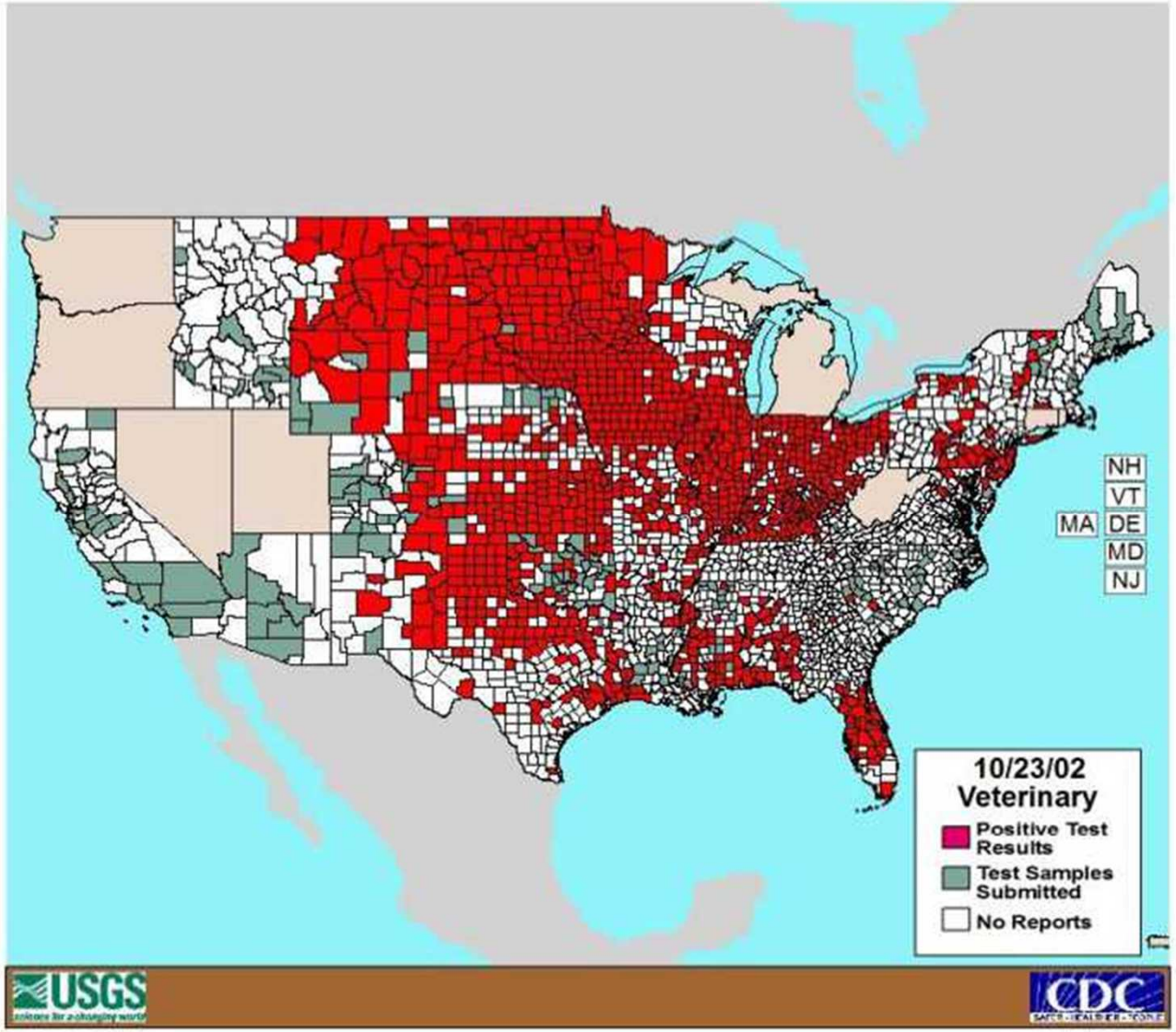
WNV activity: USA, 1999-2006

	1999	2000	2001	2002	2003	2004	2005	2006
U.S. states	**4**	**12**	**27**	**44**	**46**	**47**	**48**	**43**
Humans	**62**	**21**	**66**	**4156**	**9862**	**2539**	**3000**	**4261**
dead	*7*	*2*	*9*	*284*	*264*	*100*	*119*	*161*
Horses	**25**	**63**	**738**	**9157**	**4146**	**1341**	**1072**	**1086**
dead/eut	*9*	*24*	*245*	Hundr.	Hundr.			
Birds	**295**	**4323**	**7333**	**14122**	**11350**	**7074**	**5204**	**4106**
Mosquit (**+**pools)	**15**	**481**	**919**	**4943**	**7725**	**4755**	**11263**	**11898**

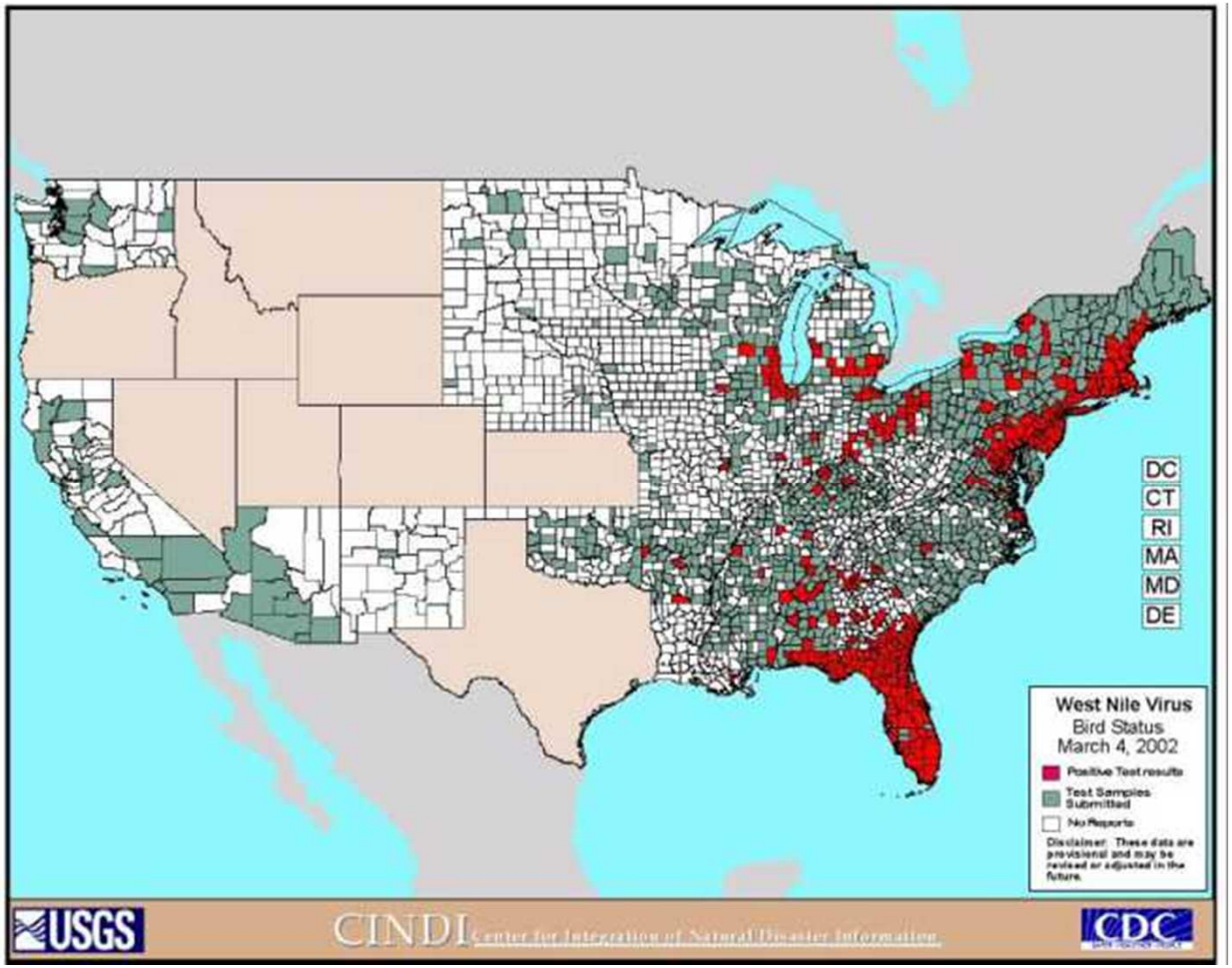
WND in horses, March 2002



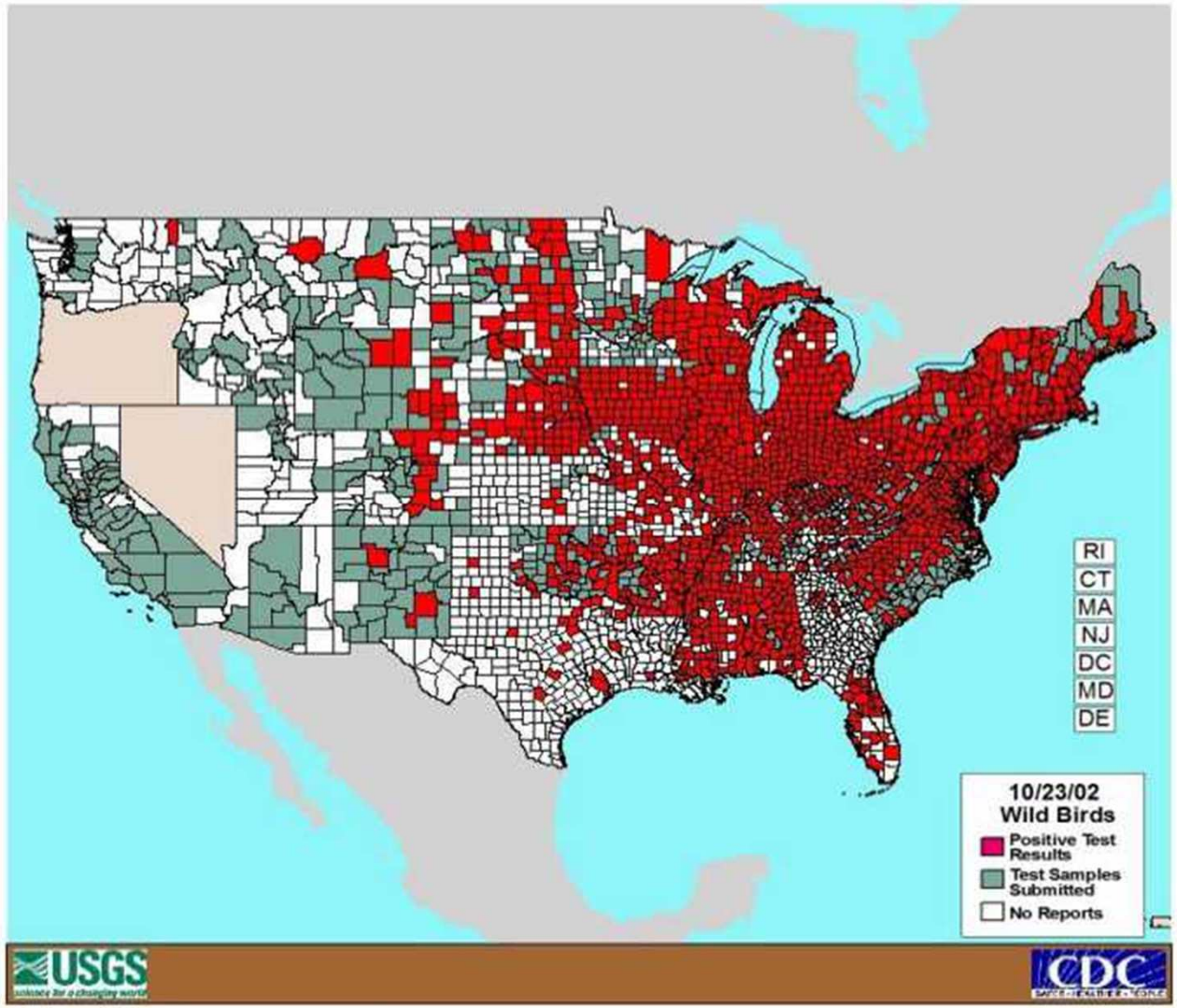
WND in horses, Oct. 2002



Wild bird mortality (WNV), March 2002



Wild bird mortality (WNV), Oct. 2002



WNV disease in Europe, **2010**

	Humans	Horses
No. cases:	**811**	166

WNV disease in Europe, **2011**

	Humans	Horses
Total:	**270**	**86**
Russia	135	
Greece	100	20
Italy	14	65
Romania	10	
Albania	2	
Ukraine, Maced.	9	
Spain		1

WNV disease in Europe, **2012**

In humans, WNF was reported in 9 European countries (ECDC data):

Russia (399 cases), Greece (161 cases), Serbia (67 cases), Italy (50 cases), Romania (14 cases), Hungary (10 cases), Macedonia (6 cases), Croatia (5 cases), Kosovo (4 cases).

Total: **716 human cases**.

Usutu (USU) virus

Family: *Flaviviridae*
Genus: *Flavivirus*
Antigenic group: Japanese encephalitis
Vector: *Culex* **spp.**
Hosts: wild birds
Animal disease: encephalitis of certain passerine birds (blackbirds) and raptors
Geographic distribution: Africa, Europe

Israeli turkey meningoencephalitis (ITM) virus

Family: *Flaviviridae*
Genus: *Flavivirus*
Antigenic group: Japanese encephalitis
Synonym: Bagaza virus
Animal disease: meningoencephalitis of turkeys, partridges and certain raptors
Geographic distribution: Israel, Africa, Spain (Bagaza virus)

Wesselsbron (WSL) virus

Family: *Flaviviridae*
Genus: *Flavivirus*
Antigenic group: yellow fever (YF)
Vector: *Aedes* **and** *Culex* **spp.**
Animal disease: epizootics in sheep – aborts, death of newborn lambs and gravid sheep. Fever in cattle and pigs.
Geographic distribution: Africa (south)

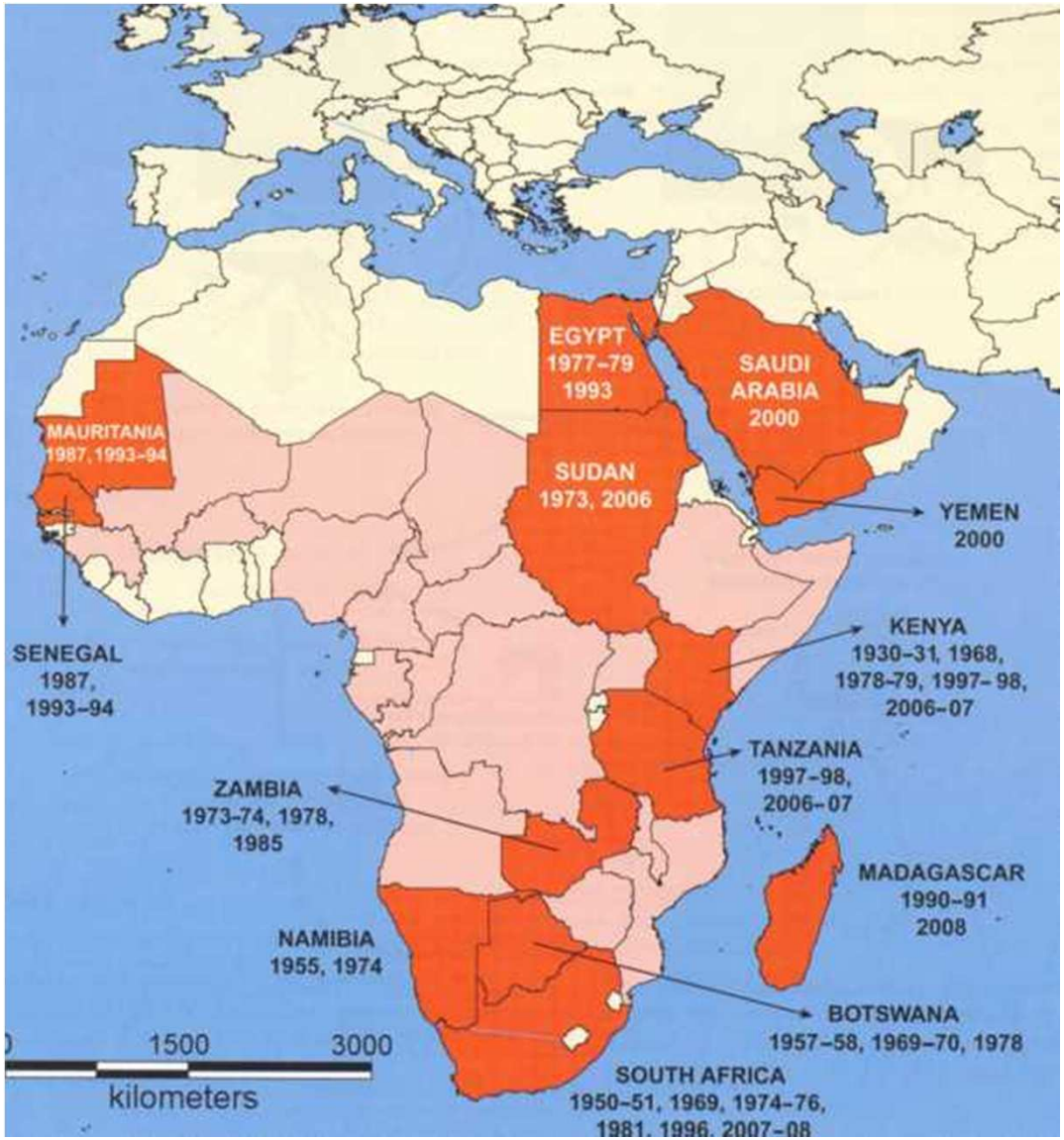
Cache Valley (CV) virus

Family: *Bunyaviridae*
Genus: *Orthobunyavirus*
Antigenic group: Bunyamwera
Vector: *Culiseta inornata*
Animal disease: epizootics of congenital abnormalities in sheep (arthrogryposis, hydranencephaly) observed since 1986 (Texas)
Geographic distribution: North America

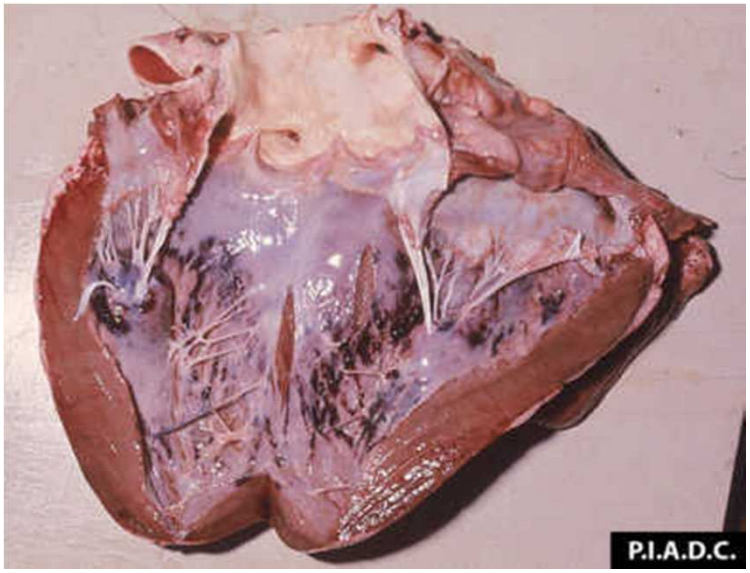
Rift Valley fever (RVF) virus

Family: *Bunyaviridae*
Genus: *Phlebovirus*
Vector: mosquitoes (midges, sandflies?)
Animal disease: fever and aborts in ruminants (especially sheep), death newborn animals. Bloody diarrhoea. Often in epizootics. Most susceptible are lambs, kids and calves. 1951: about 100,000 lams in South Africa
Geographic distribution: Africa, Arabian Peninsula

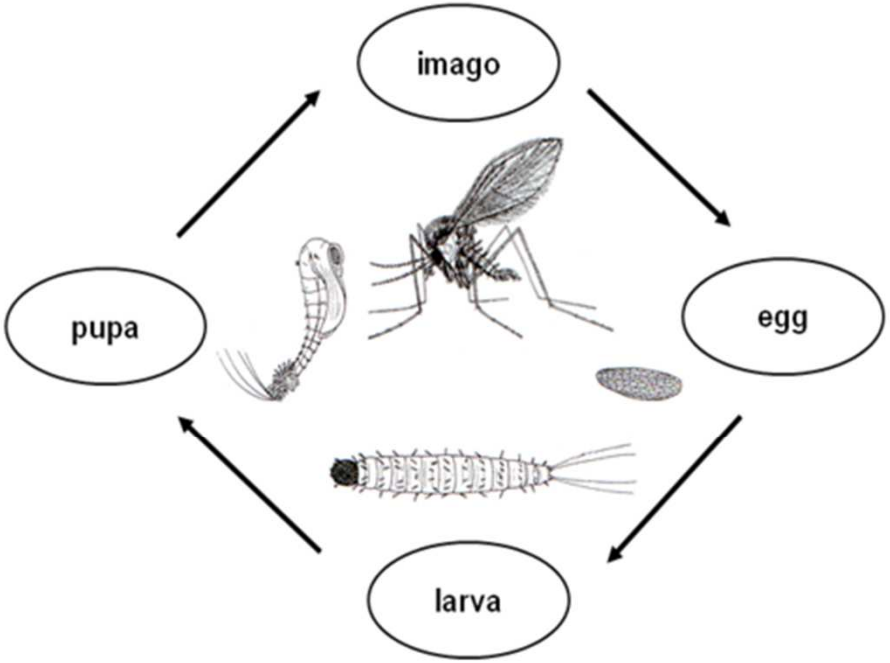
RVF


EGYPT
1977–79
1993
SAUDI
ARABIA
2000
MAURITANIA
1987, 1993–94
SUDAN
1973, 2006
YEMEN
2000
SENEGAL
1987,
1993–94
KENYA
1930–31, 1968,
1978–79, 1997–98,
2006–07
TANZANIA
1997–98,
2006–07
ZAMBIA
1973–74, 1978,
1985
MADAGASCAR
1990–91
2008
NAMIBIA
1955, 1974
BOTSWANA
1957–58, 1969–70, 1978
0
1500
3000
kilometers
SOUTH AFRICA
1950–51, 1969, 1974–76,
1981, 1996, 2007–08

RVF



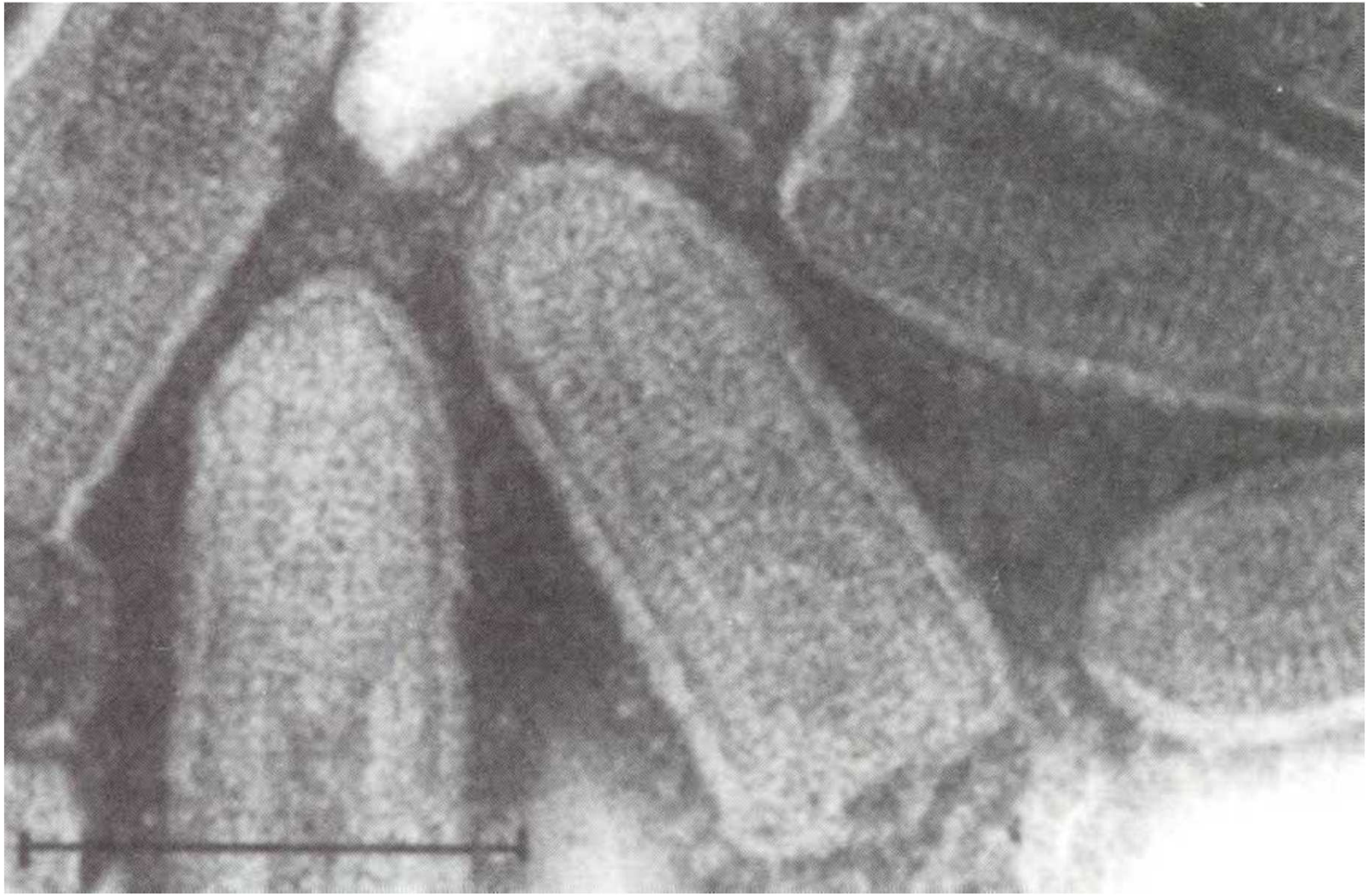
Sandfly-borne viruses



Vesicular stomatitis – New Jersey (VS-NJ) virus

Family: *Rhabdoviridae*
Genus: *Vesiculovirus*
Antigenic group: Vesicular stomatitis
Vector: sandflies, probably also other insects
Geographic distribution: North and South America

VSV - Indiana



VSV

FIGURE 18.8 Extensive (craterous) ulcer in the tongue of a horse with vesicular stomatitis. *(Courtesy of R. Bowen, Colorado State University.)*

Vesicular stomatitis – Alagoas (VS-A) virus

Family: *Rhabdoviridae*
Genus: *Vesiculovirus*
Antigenic group: Vesicular stomatitis
Geographic distribution: South America

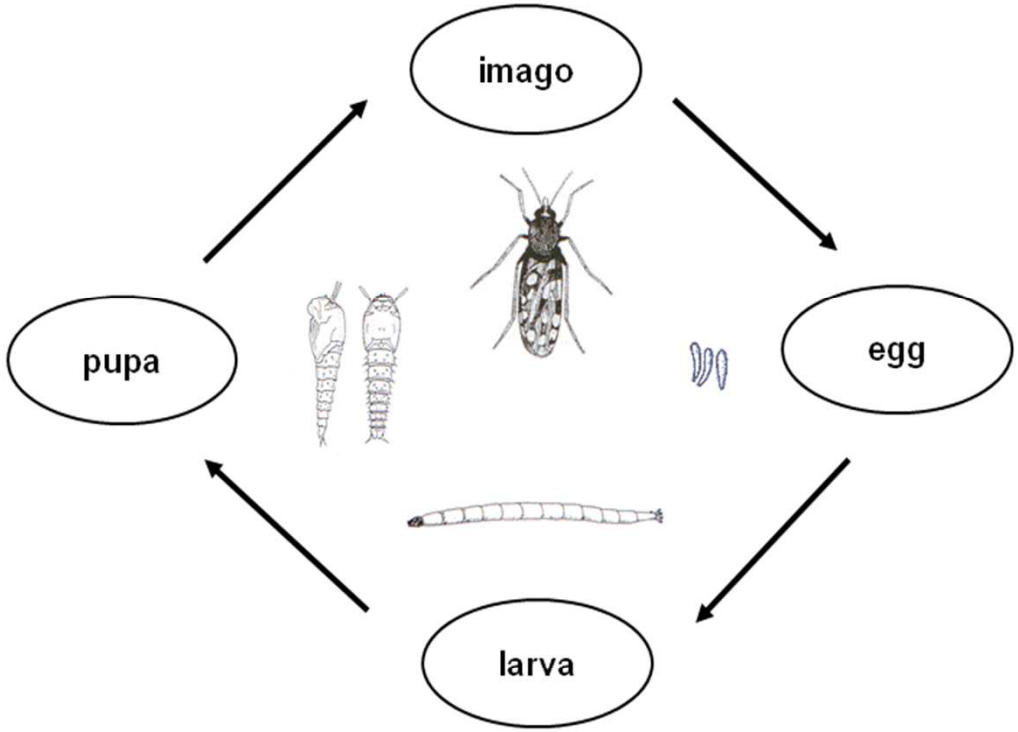
Chandipura (CHP) virus

Family: *Rhabdoviridae*
Genus: *Vesiculovirus*
Antigenic group: Vesicular stomatitis
Geographic distribution: Africa, Asia

Cocal (COC) virus

Family: *Rhabdoviridae*
Genus: *Vesiculovirus*
Antigenic group: Vesicular stomatitis
Geographic distribution: South America

Midge (*Culicoides*)-borne diseases



Bovine ephemeral fever (BEF) virus

Family: *Rhabdoviridae*
Genus: *Ephemerovirus*
Antigenic group: BEF
Vector: *Culicoides*
Animal disease: febrile illness in cows
Geographic distribution: Africa, Asia, Australia

African horse sickness (AHS) virus

Family: *Reoviridae*
Genus: *Orbivirus*
Antigenic group: AHS
Vector: *Culicoides*
Animal disease: fever, pumonary oedema, supraorbital oedema, carditis (hydrothorax, hydropericarditis)
Geographic distribution: Africa, Middle East

Bluetongue (BT) virus

Family: ***Reoviridae***
Genus: ***Orbivirus***
Antigenic group: **BT**
26 serotypes
Animal disease: catarrhal fever in sheep with exanthema, crusts and cyanosis around mouth, oedema of the head and neck, haemorrhagies, pulmonary oedema
Geographic distribution: ***Africa, Asia, Europe, America***

BT – sheep tongue (cyanosis)

BT

Epizootic haemorrhagic disease of deer (EHD) virus

Family: *Reoviridae*
Genus: *Orbivirus*
Antigenic group: EHD
Synonym: Ibaraki = EHD-2
Animal disease: oedema, necrosis of GIT membranes, haemorrhagies, muscular degeneration
Geographic distribution: North America, Africa, India (Ibaraki)

Equine encephalosis (EE) virus

Family: *Reoviridae*
Genus: *Orbivirus*
Antigenic group: EHD
7 serotypes
Vector: *Culicoides* **spp.**
Animal disease: encephalitis in horses
Geographic distribution: Africa, Israel

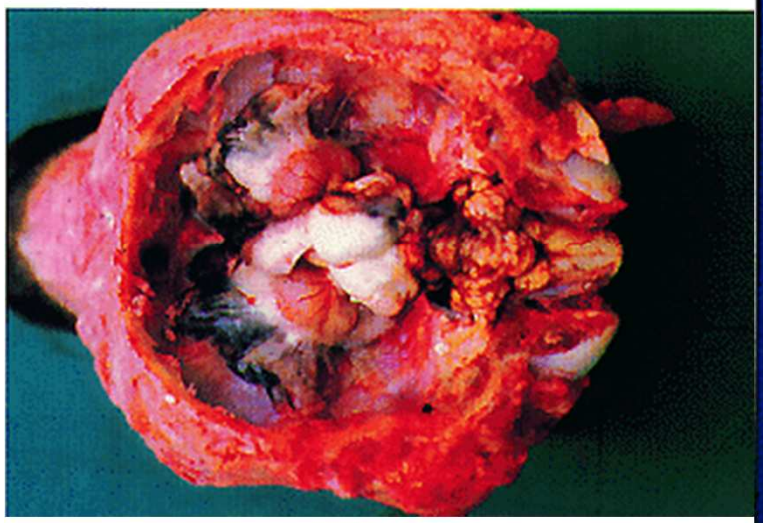
Main Drain (MD) virus

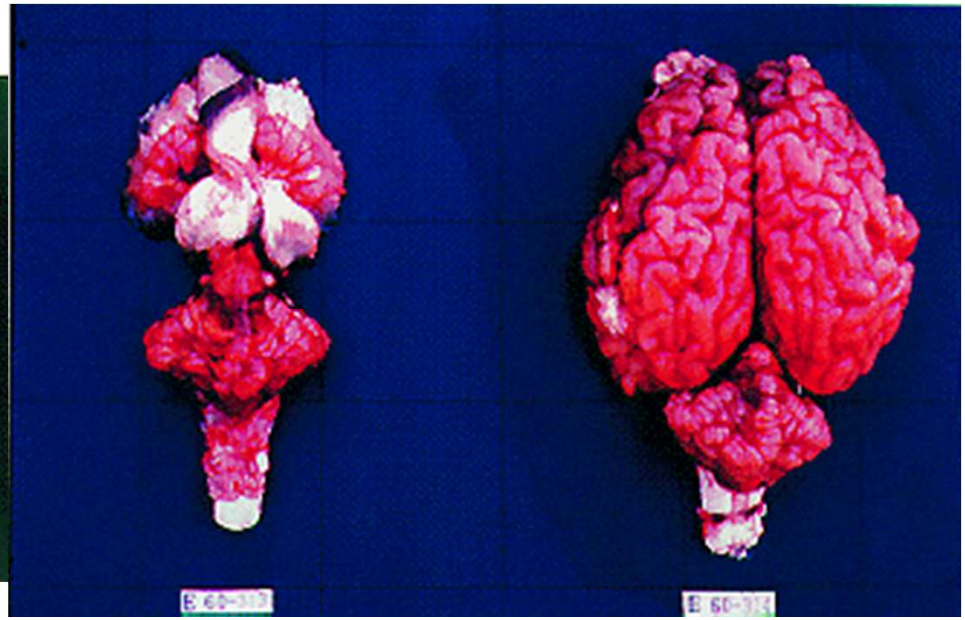
Family: *Bunyaviridae*
Genus: *Orthobunyavirus*
Antigenic group: Bunyamwera
Vector: *Culicoides variipennis*
Animal disease: encephalomyelitis in horses (5 cases)
Geographic distribution: North America (California)

Akabane (AKA) virus

Family: *Bunyaviridae*
Genus: *Orthobunyvirus*
Antigenic group: Simbu
Animal disease ("Akabane disease"): ruminants (cattle, sheep, goat) - abortion, miscarriage, premature birth, foetal arthrogryposis, hydranencephaly, muscle dystrophy
Geographic distribution: Japan, South-East Asia, Africa, Australia, Israel, Turkey
Prevention: attenuated and inactivated vaccines are available in enzootic regions

AKA





Aino (AIN) virus

Family: *Bunyaviridae*
Genus: *Orthobunyvirus*
Antigenic group: Simbu
Animal disease: no signs in adults, but malformations in calves and sheep – infections between the 120th and 180th days of gestation; stillbirths, premature births, birth defects (arthrogryposis, scoliosis, sunken eyes, cataracts, maxillary retraction, dental irregularities, hydranencephaly, cerebellar hypoplasia), weakness, blindness or poor eyesight, neurologic signs (ataxia, torticollis, paresis, swimming movements, opisthotonus, circular walking)
Geographic distribution: Asia (Japan) and Australia

Shuni (SHU) virus

Family: *Bunyaviridae*
Genus: *Orthobunyavirus*
Antigenic group: Simbu
Animal disease: encephalitis in horses (7 cases in Zimbabwe and South Africa)
Geographic distribution: Africa

Shamonda (SHA) virus

Family: *Bunyaviridae*
Genus: *Orthobunyavirus*
Antigenic group: Simbu
Animal disease: arthrogryposis and hydranencephaly in newborn calves
Geographic distribution: Africa (Nigeria), Japan (2002)

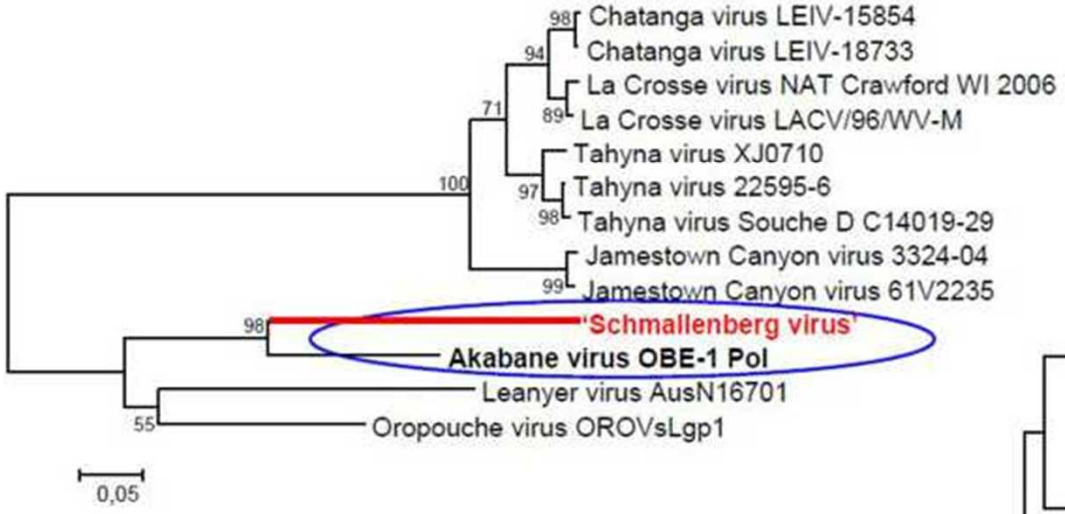
Schmallenberg (SB) virus

Family: *Bunyaviridae*
Genus: *Orthobunyavirus*
Antigenic group: Simbu

Phylogeny

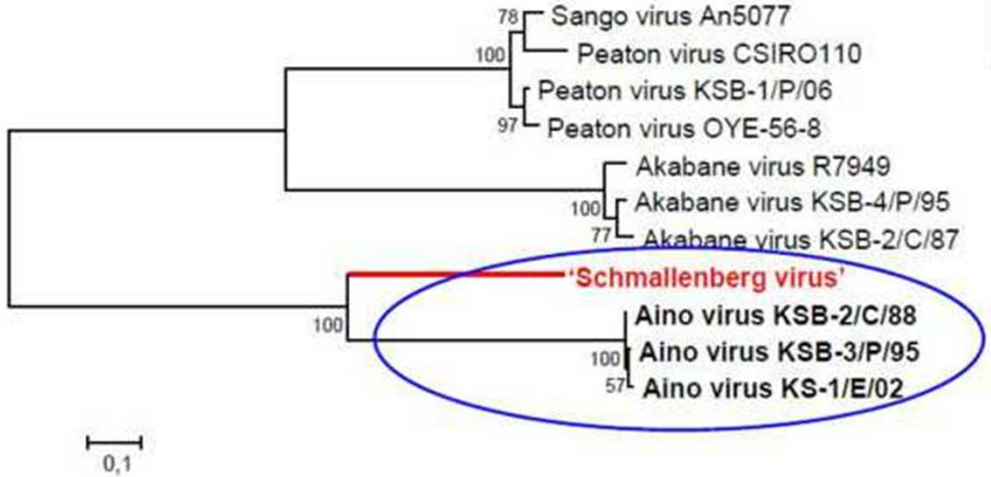
L segment

145 amino acids / 70% similarity to Akabane virus



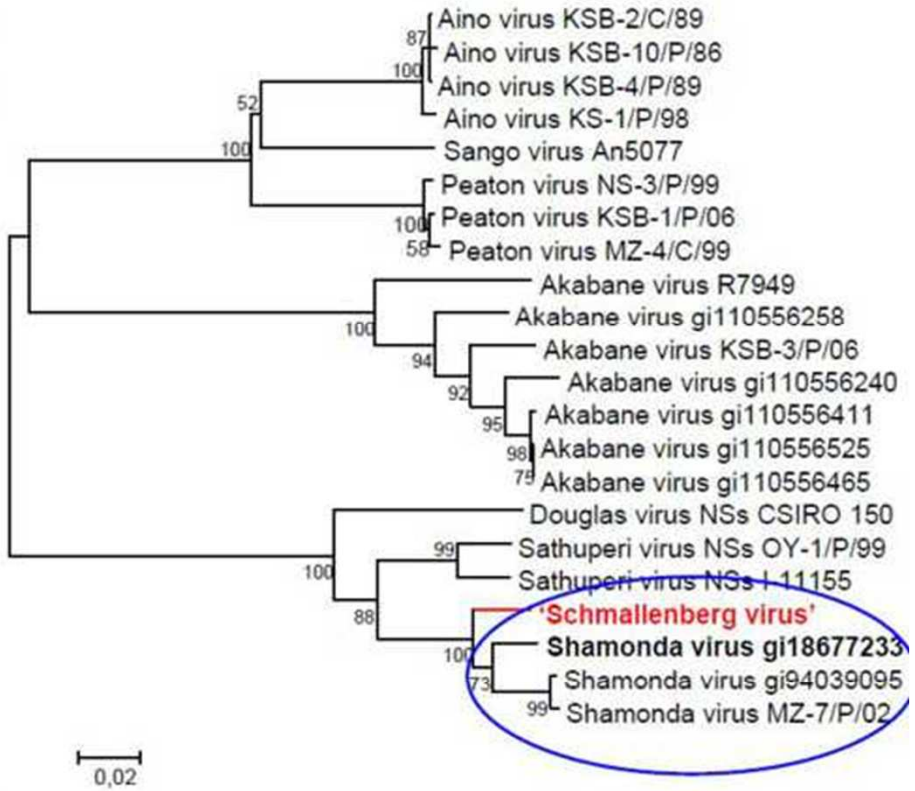
M segment

251 amino acids / 48% similarity to Aino virus



S segment

451 nucleotides / 96% similarity to Shamonda virus



L- and M: Neighbour Joining trees, Jones-Taylor-Thornton, 1000 bootstrap replicates

S: Neighbour Joining tree; Tamura Nei parameter; 1000 bootstrap replicate

An emerging disease in ruminants in Europe

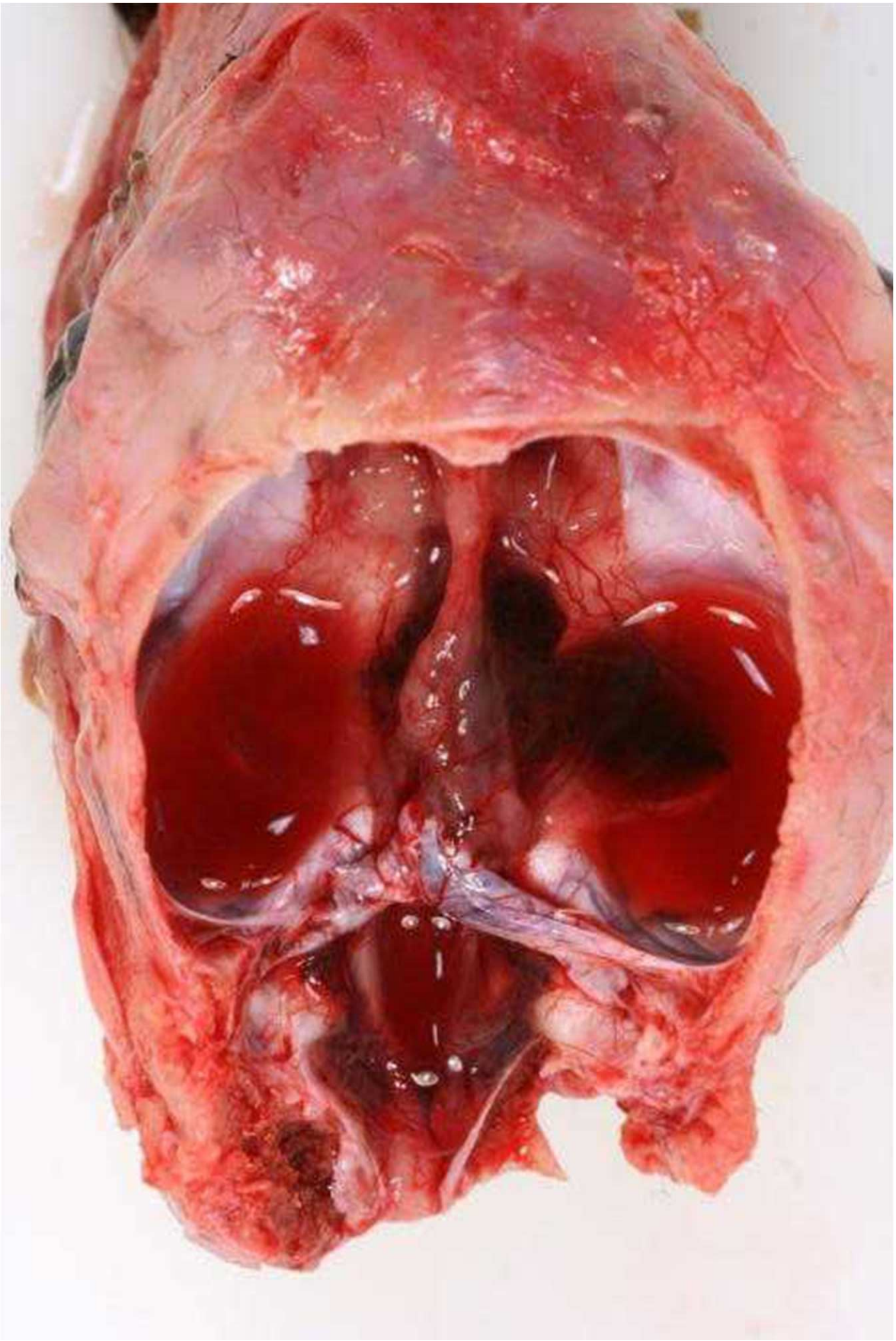
- Summer 2011:
  - Reports on a new illness in dairy cows in Germany (Schmallenberg, North Rhine-Westphalia) and in the Netherlands:
    - increased body temperature (>40°C)
    - impaired general condition, anorexia
    - reduced milk yield (by up to 50%)

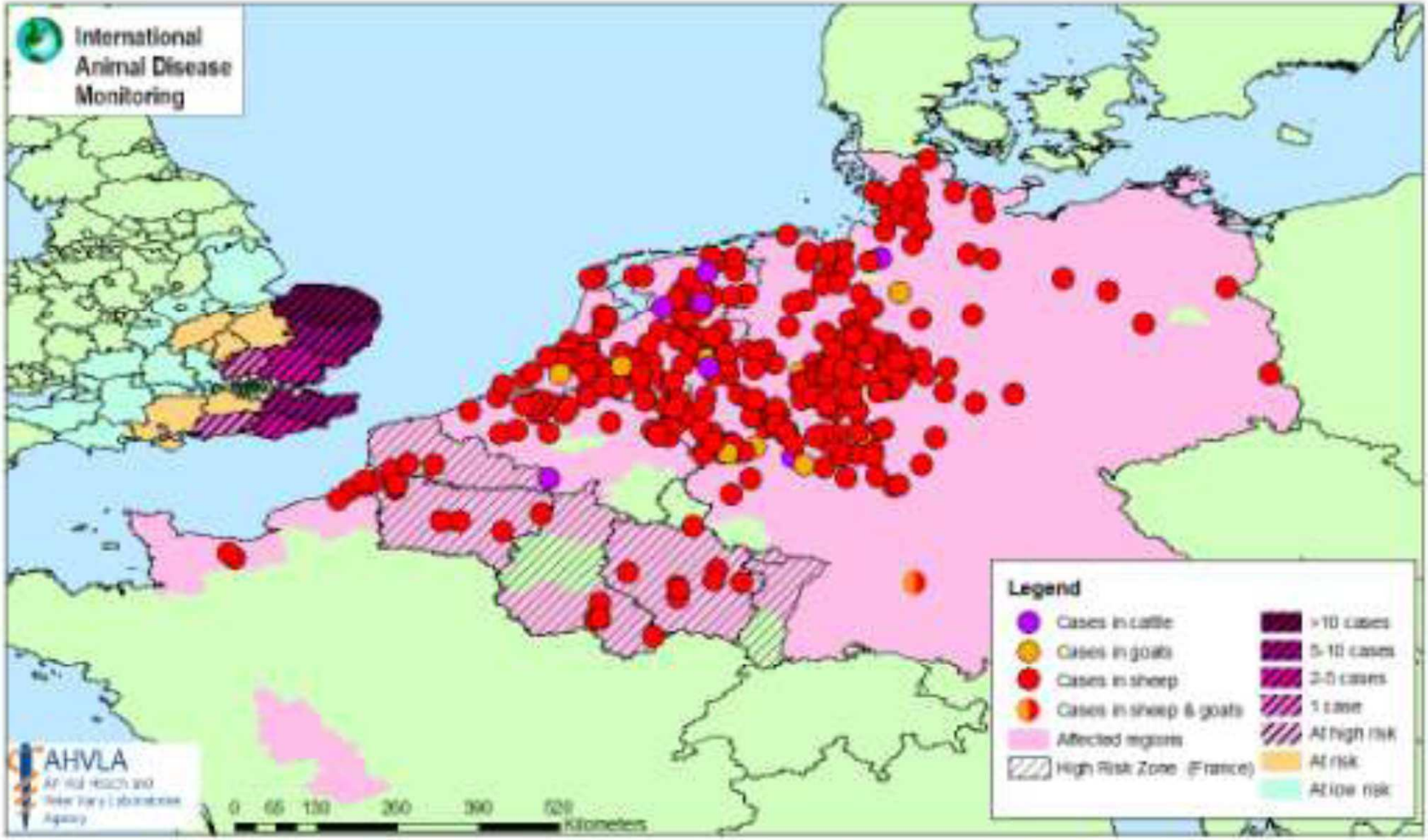
    → the signs disappeared after some days
  - Sequencing and metagenome data analysis revealed *Orthobunyavirus*-like sequences most closely related to Akabane, Aino and Shamonda viruses

Schmallenberg virus

Clinical signs

- **Cattle**
  - fever (40.5 °C)
  - anorexia, diarrhoea
  - poor condition, reduced milk yield (50%)
  - congenital disorders rarely seen
- **Sheep**
  - fever (40.5 °C)
  - congenital disorders
    - Lambs are sometimes born alive, mostly not viable
    - Arthrogryposis, hydranencephaly, ankylosis, torticollis, scoliosis, brachygnatia, cerebellar hypo- and aplasia, enlarged thymus





Outbreaks of Schmallenberg disease in cattle, sheep and goats, and UK counties by density of cases and at risk of vector incursion in summer 2011

Actual Scale 1:8,000,000

Date Prepared 10/02/2012

Map prepared by IDM

Conclusion

Important arboviral diseases of animals

(those underlined have occurred in Europe)

1. **Tick-borne**: LI, NSD, ASF
2. **Mosquito-borne**: EEE, WEE, VEE, WN, USU, ITM, CV, RVF
3. **Sandfly-borne**: VS
4. **Midge-borne**: AKA, SB, AHS, BT, EHD, EE, BEF

Mosquito only flaviviruses

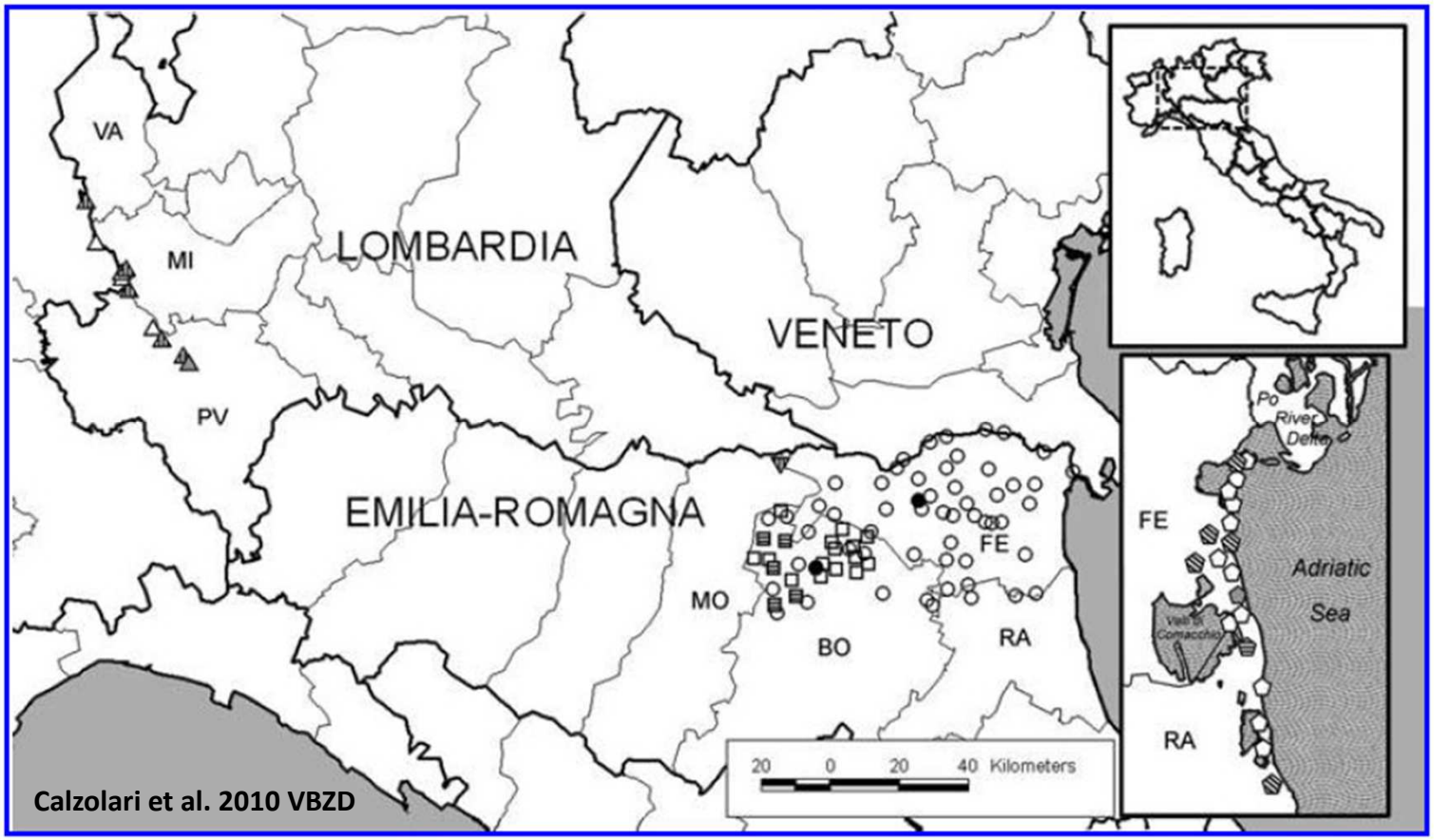
Mattia Calzolari

Istituto Zooprofilattico Sperimentale
della Lombardia e
dell'Emilia Romagna "B. Ubertini"

Mosquito surveillance

- Mosquito surveillance to detect arbovirus was performed, since 2007, in “Parco Lombardo delle Valli dell Ticino” (Lombardia) and “Lidi Ferraresi” (Emilia-Romagna).

- After the first reported WNVD case in horse in Emilia Romagna in 2008 Regional Health Authority set a plane, to implement the National Plan Activity.
- This plan was based also on mosquito monitoring.



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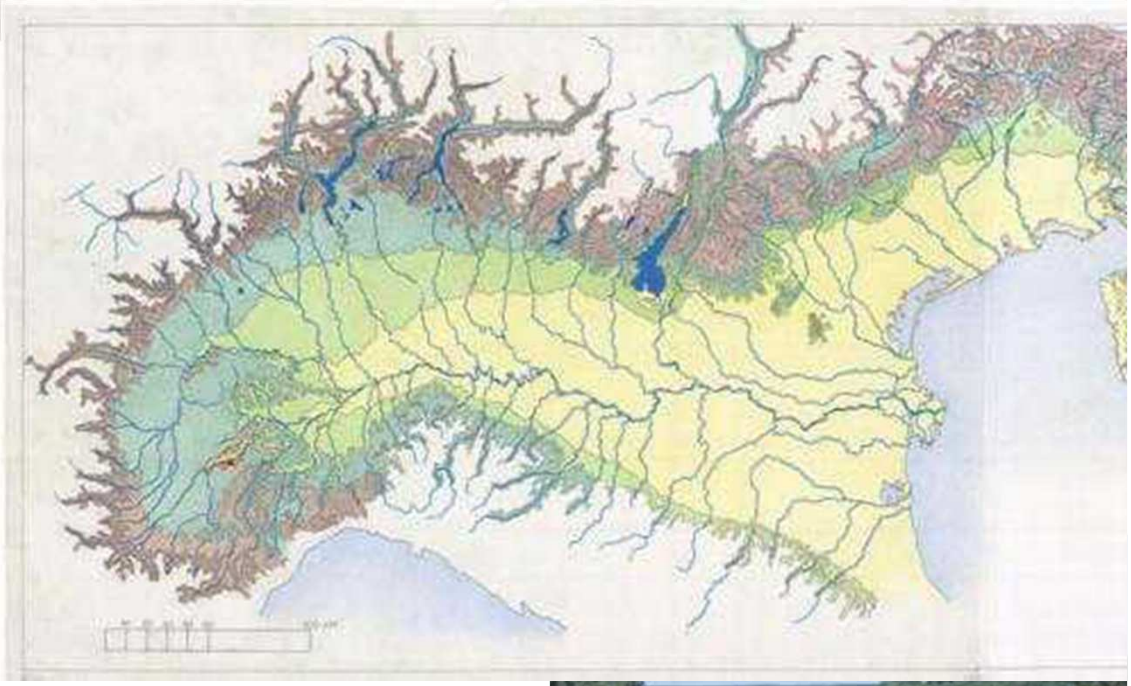
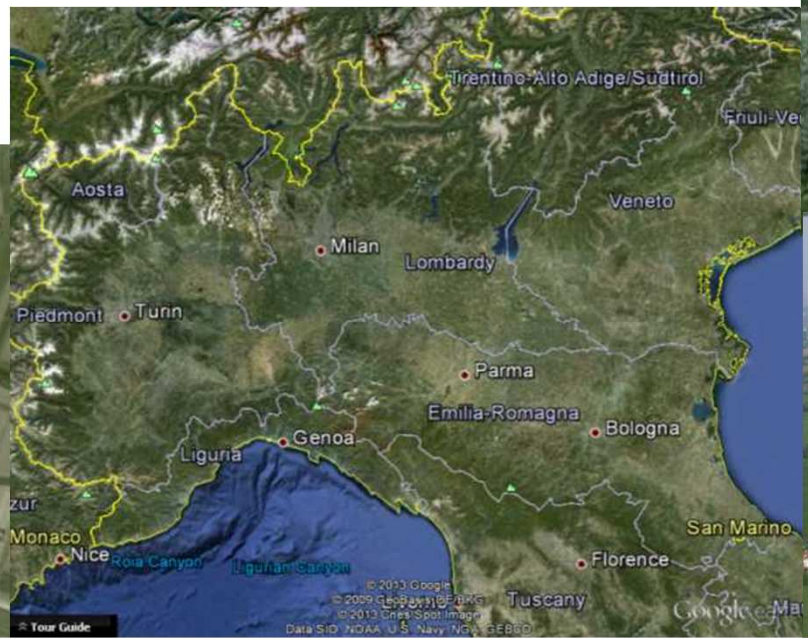
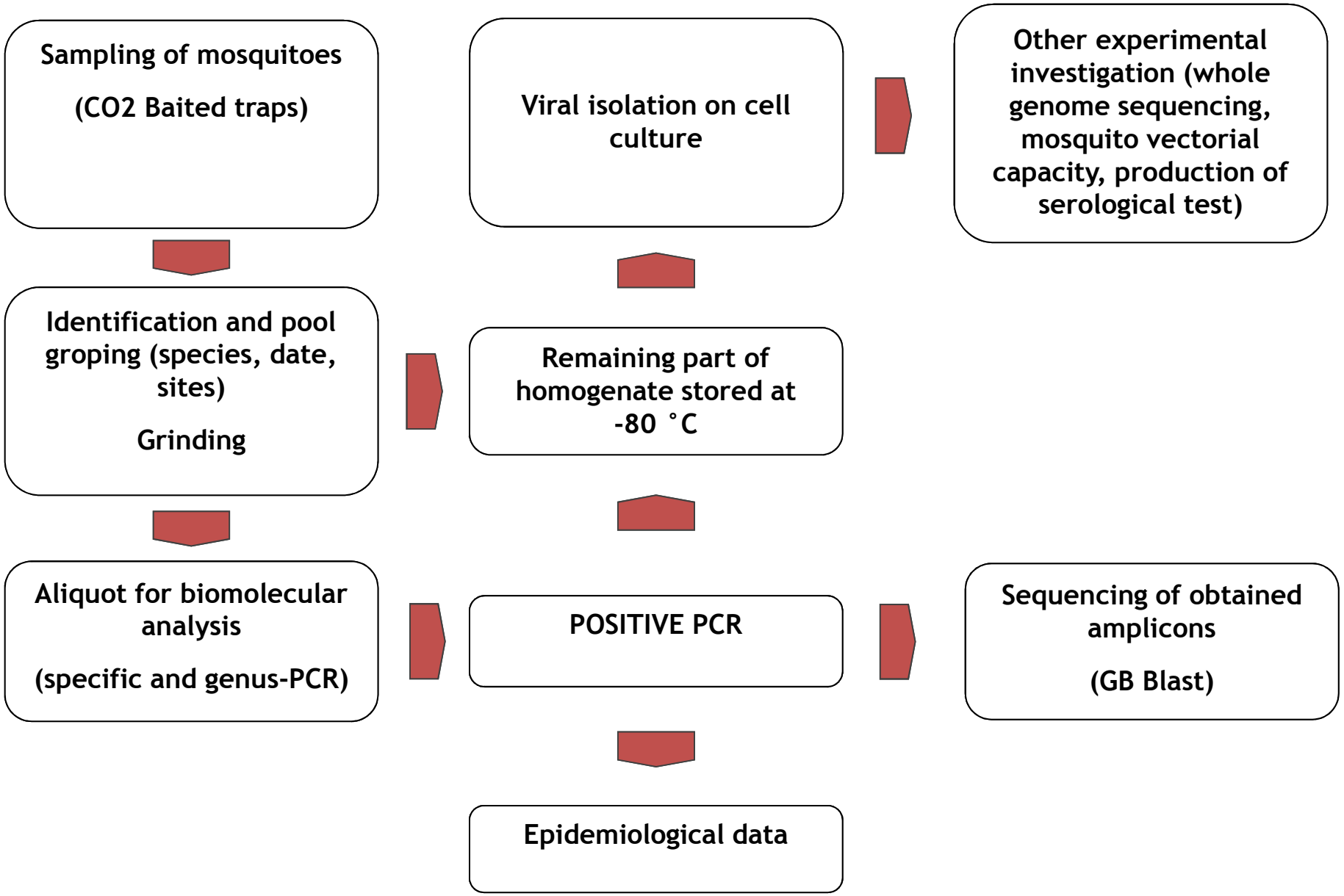
Surveyed areas

- Surveyed areas are sited in Pianura Padana.
- Sub-continental climate.
- High population density (abundant urbanized and industrial areas).
- Intensive agriculture and animal husbandry.
- Scarcity of natural areas.





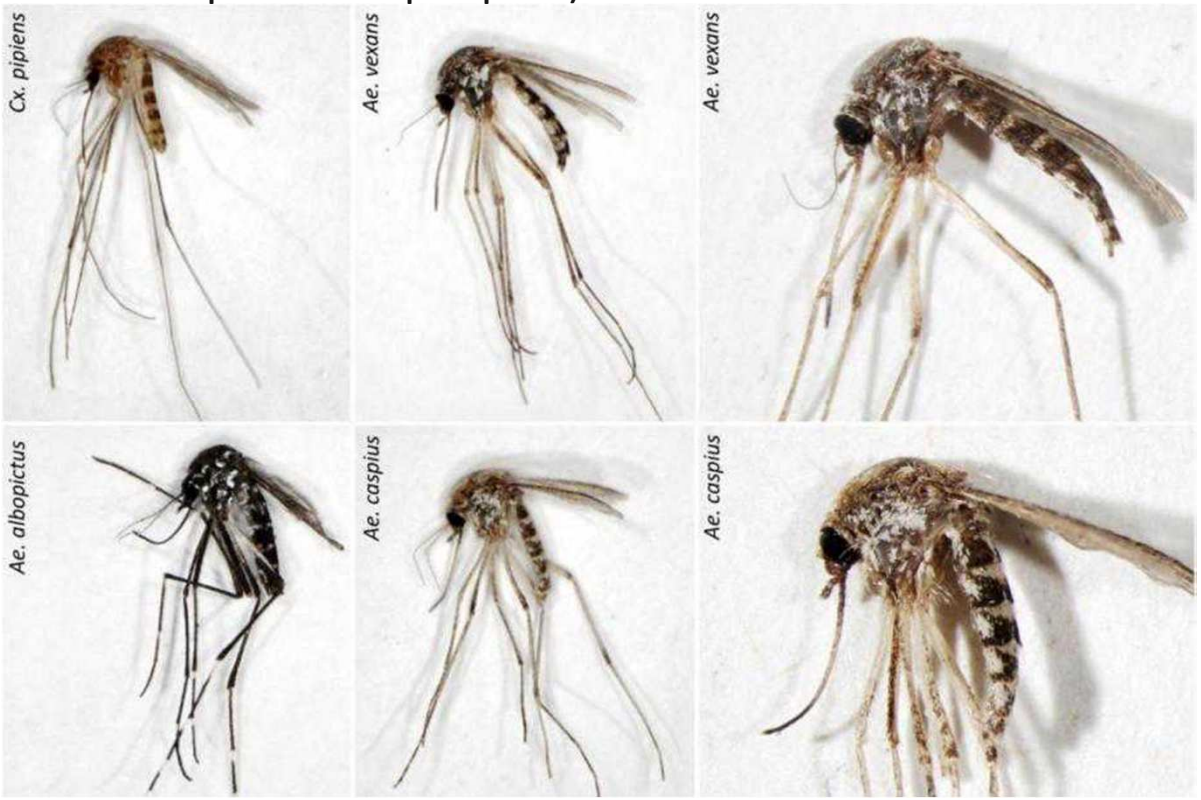
Mosquito surveillance in E-R



Mosquito surveillance in E-R

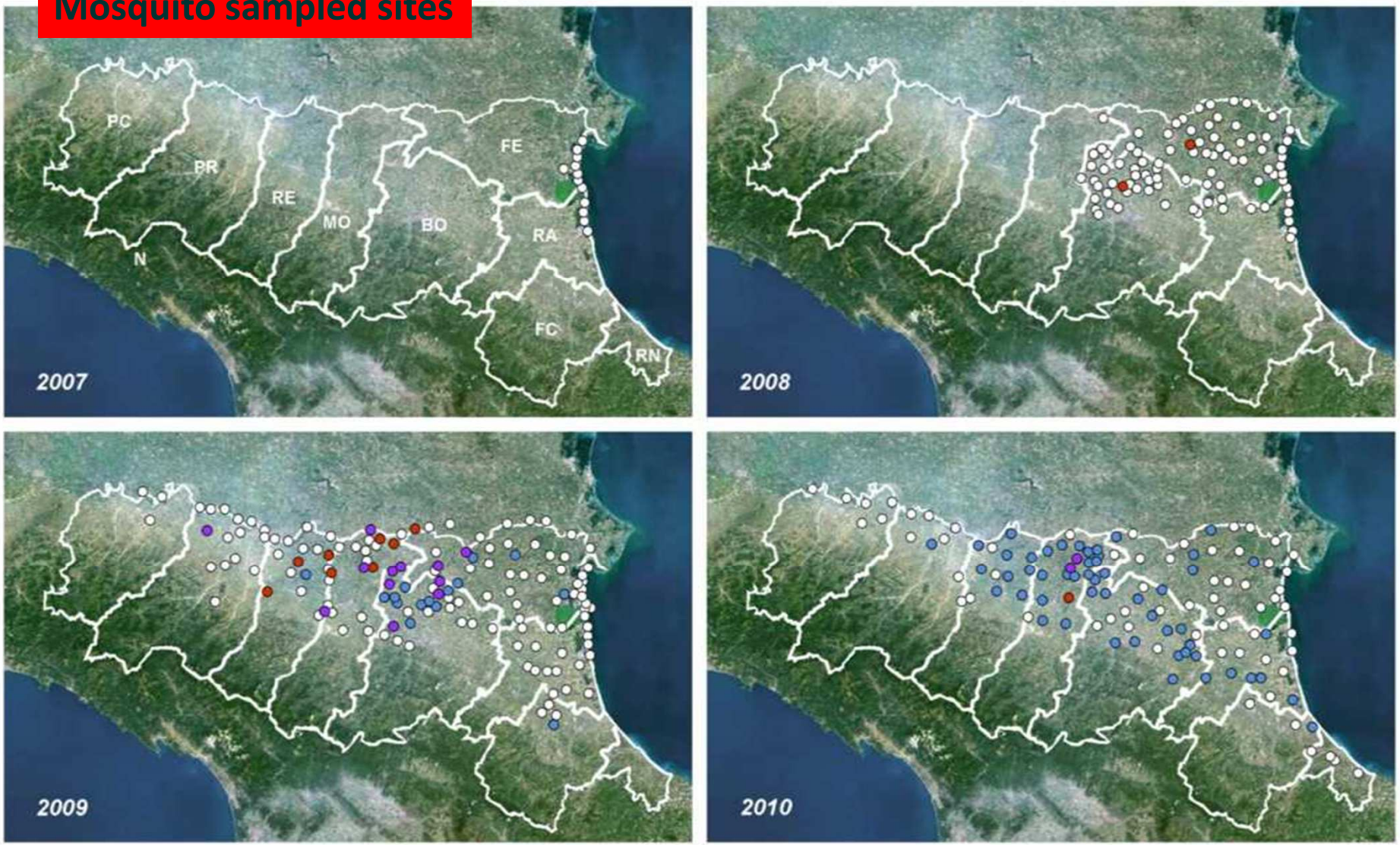
- Collection of mosquitoes (attractive traps carbon dioxide baited) overnight, every two week.

- Identified and pooled according to species, place and date of sampling (with a maximum of 200 specimens per pool).



- Pools were ground and aliquot of the samples were submitted to species specific (USUV and WNV) or genus (flavivirus and orthobunyavirus) specific PCRs.
- The remaining part was stored for the viral isolation.

Mosquito sampled sites



Surveyed area and sampled sites were improved over the years.

Results I

SPECIE	2008		2009		2010		2011		2012		Total	
Ae.albopictus	720	86	1,227	108	1,855	131	2,451	193	1,613	123	7,866	641
Ae.caspius	14,068	283	29,667	340	18,135	367	27,390	467	23,036	311	112,296	1,768
Ae.detritus	17	3	162	7	46	2					225	12
Ae.dorsalis			13	1							13	1
Ae.geniculatus			8	3	532	5					540	8
Ae.vexans	1,029	30	4,597	60	17,697	185	6,140	114	4,813	101	34,276	490
Aedes spp.	8	2									8	2
An.maculipennis s.l.	186	10	559	30	296	38	873	64	409	22	2,323	164
An.plumbeus	1	1	2	2	15	4	5	3			23	10
Cq.richiardii					167	4	143	4	100	3	410	11
Cs.annulata	3	3	6	4	2	2					11	9
Culex spp.	1	1									1	1
Culiseta spp.	1	1			1	1					2	2
Cx.modestus	10	4	246	26	1,107	27	201	15	921	20	2,485	92
Cx.pipiens	31,409	387	157,604	1,337	399,599	2,419	236,850	1,758	190,830	1,281	1,016,292	7,182
Total	47,453	811	194,091	1,918	439,452	3,185	274,053	2,618	221,722	1,861	**1,176,771**	**10,393**

Ochlerotatus an *Stegomyia* taxa are considered an *Aedes* sub-genus

Mosquitoes sampled in E-R.

The more abundant tested specie was *Culex pipiens* (86.4%), then *Aedes caspius* (9.5%) *Aedes vexans* (2.9%) and *Aedes albopictus* (0.7%).

Results II

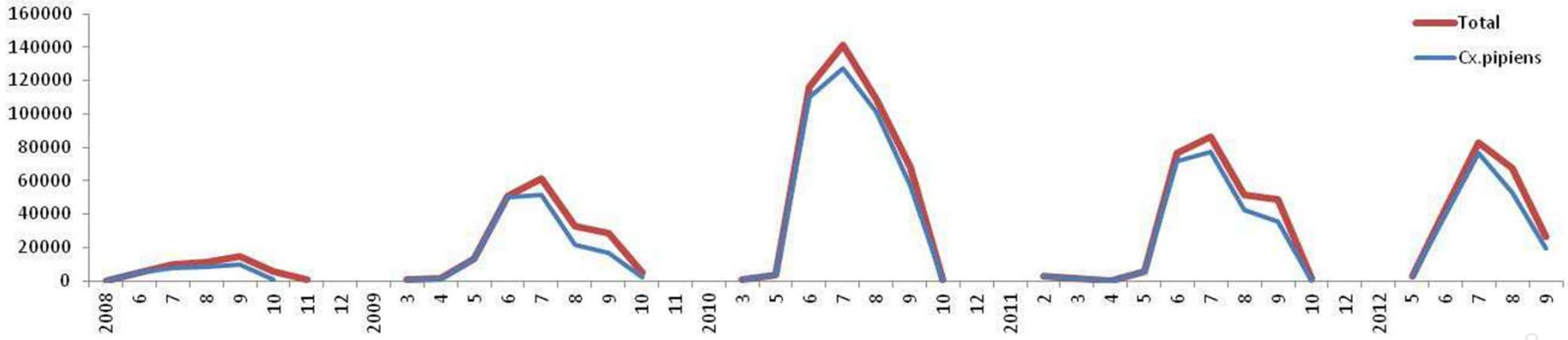
	2008	2009	2010	2011	2012
Mosquitoes					
Cx pipiens pools	387	1259	2367	1632	1281
WNV positive (%)	2 (0.5)	27 (2.1)	3 (0.1)	0	0
USUV positive (%)	-	54 (4.3)	89 (3.8)	74 (4.5)	78 (6.1)
Ae. albopictus pools	86	108	131	192	123
USUV positive (%)	-	2 (1.9)	2 (1.5)	6 (3.1)	2 (1.6)

WNV was detected until 2010 only in *Cx. pipiens*

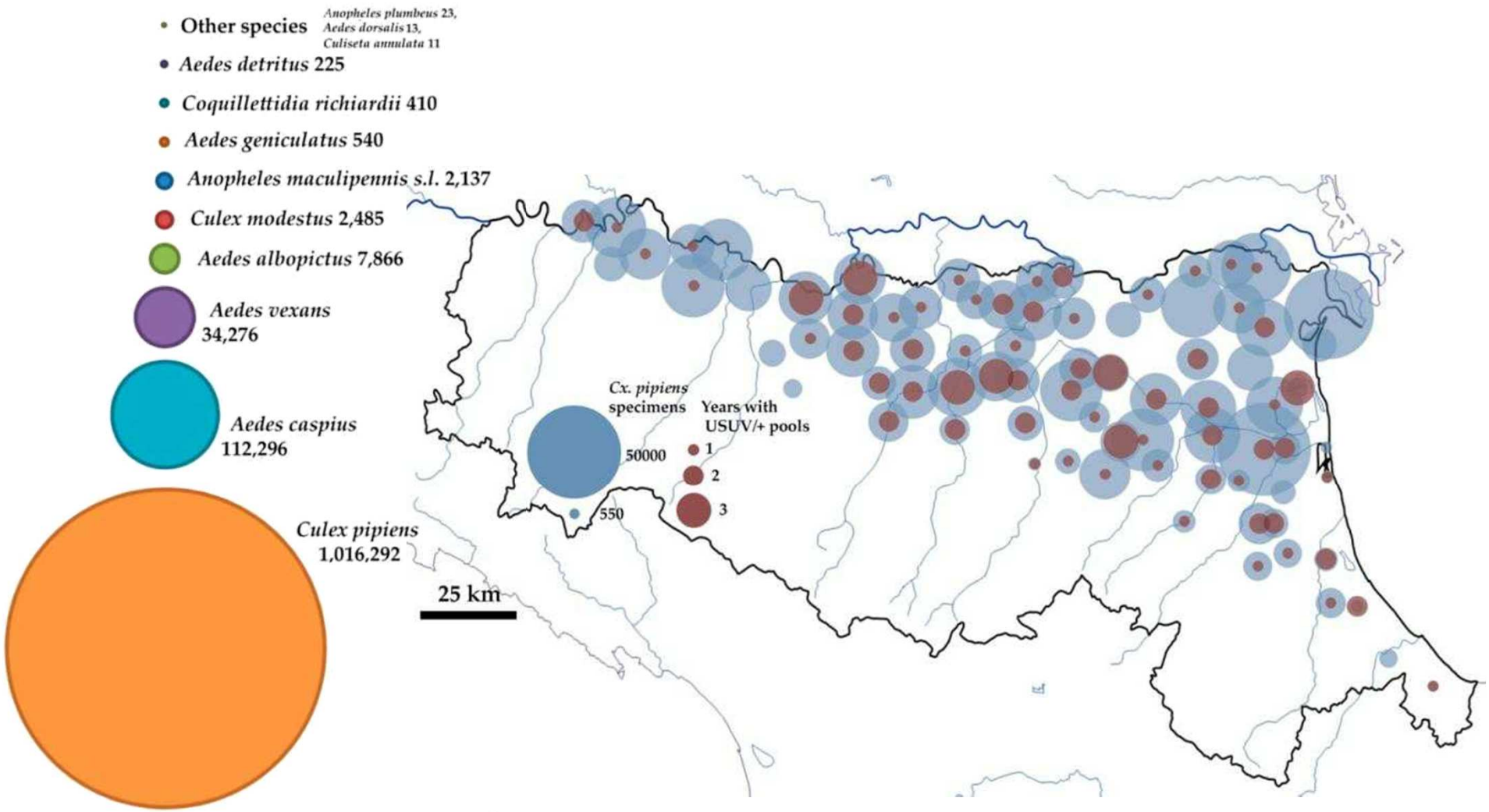
USUV was detected from 2009 in *Cx. pipiens* and other species

Also the two orthobunyaviruses Tahyna virus and Batai virus were detected

Tested mosquitoes over the years .



Results III

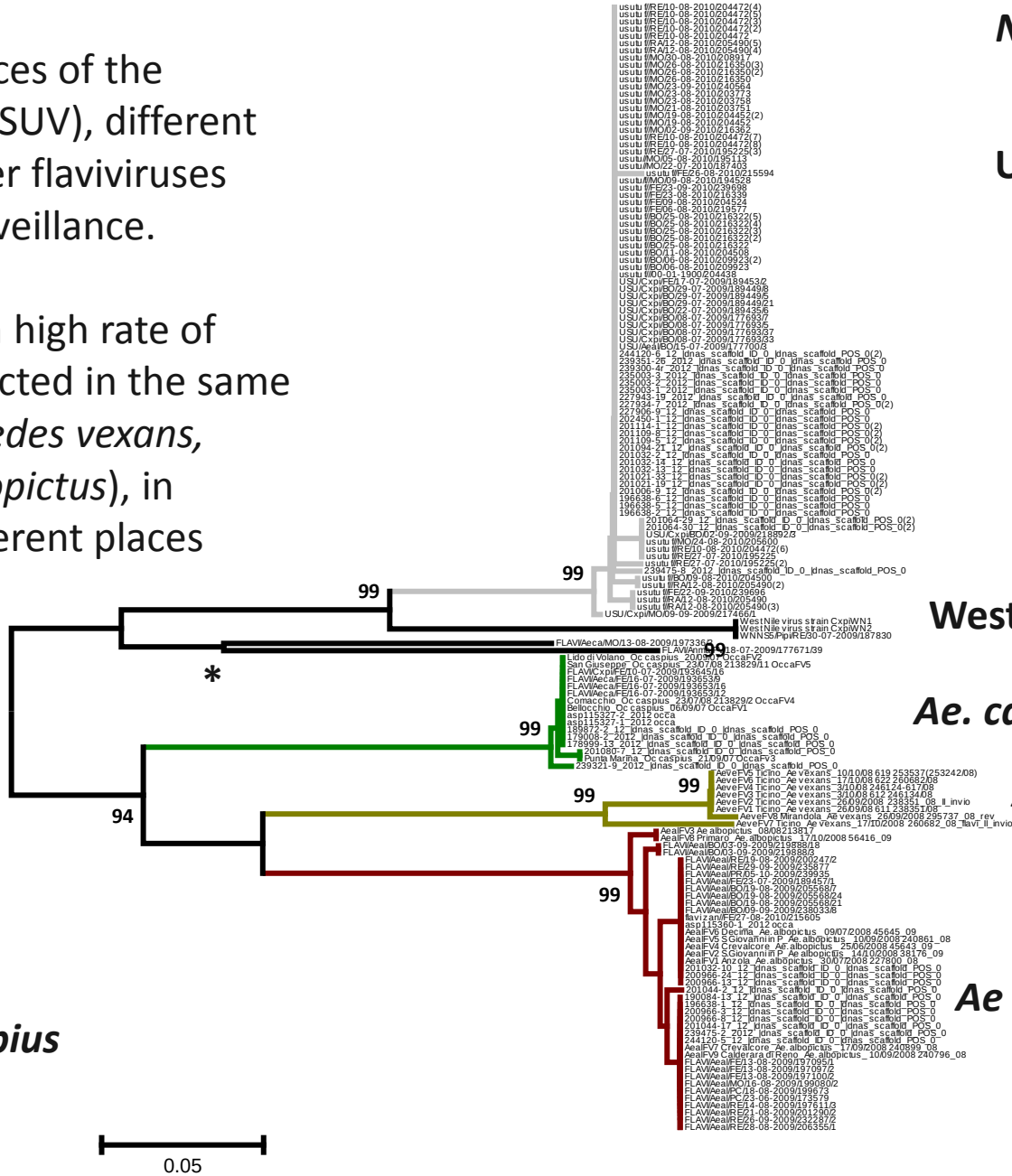


Sites sampled in all the three years from 2010 to 2012 with the total number of *Culex pipiens* mosquito tested (azure) and detection of USUV-positive pools (red).

Unespected results

In addition to the sequences of the surveyed viruses (WNV, USUV), different sequences related to other flaviviruses were detected during surveillance.

Groups of sequences with high rate of identity were mainly detected in the same species of mosquitoes (*Aedes vexans, Aedes caspius, Aedes albopictus*), in different years and in different places strongly suggest the presence of viruses not yet discovered.



***NS5* 156 bp**

Usutu virus

West Nile virus

***Ae. caspius* seq.**

***Ae vexans* seq.**

***Ae albopictus* seq.**

*** Two sequences *Ae. caspius* *An. maculipennis***

Mosquito-only flavivirus

- Interestingly similar sequence were detected from other groups in Europe, in similar surveillance plans in different mosquito species.
- Six groups of sequences were detected.
- The sequences from *Aedes albopictus* are related to a virus isolated in Japan in 2009
- Isolation of Ochlerotatus flavivirus was successful in Portugal and Spain

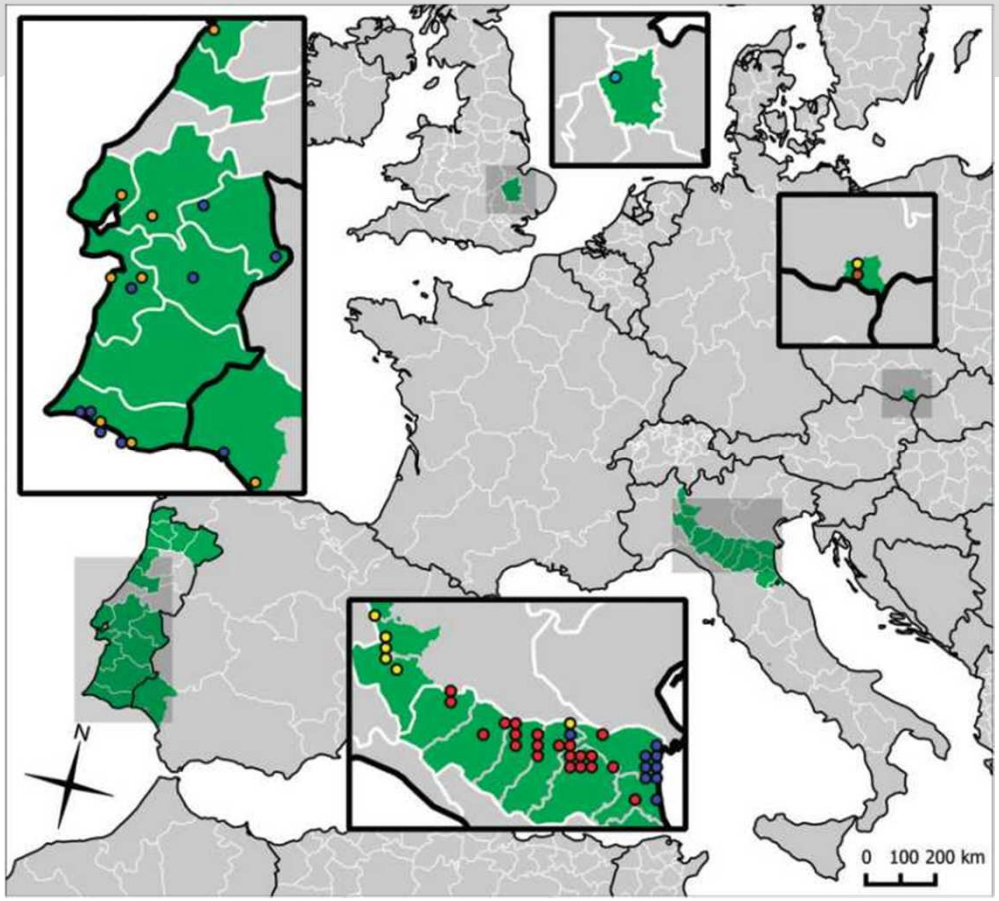


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Table 2. Characteristics of the reported MOFs with the reference to the mosquito species, number, period, area and environment of detection

Virus	Mosquito species	No. detections (in GenBank)	Collection period	Collection years	Country	Collection area	Environment
Aedes flavivirus	*Aedes albopictus*	32 (29)*	June–October	2008, 2009, 2010	Italy	Pianura Padana	Floodplain
Ochlerotatus flavivirus	*Aedes caspius/detritus Culex pipiens/perexiguus/theileri*	33 (22)†	March–October	2007, 2008, 2009, 2010	Italy, Portugal, Spain	Lidi Ferraresi, Algarve, Alentejo, Andalusian	Inland and tidal wetland
Aedes vexans flavivirus	*Aedes vexans*	9 (9)‡	July–November	2008, 2009	Czech Republic, Italy	Pianura Padana, South Moravia	Inland wetland
Czech *Aedes vexans* flavivirus	*Aedes vexans*	4 (4)§	August	2009	Czech Republic	South Moravia	Pond
Culex theileri flavivirus	*Culex theileri*	15 (9)‖	March–October	2007, 2008, 2009, 2010	Portugal, Spain	Alentejo, Algarve, Centro, Ribatejo Andalusian	Inland and tidal wetland
Aedes cinereus flavivirus	*Aedes cinereus*	17¶	June–July	2010	UK	Cambridgeshire	Inland wetland

Mosquito-only flavivirus

•The reported sequences are related to similar sequences detected worldwide from field collected mosquitoes (or sand flies) or obtained by viruses isolated from mosquitoes.

•These sequences grouped together in a distinct branch respect other flaviviruses.

• These viruses were previously defined **Mosquito only flaviviruses** or **Insect specific flaviviruses,** to distinguisch them to the other flaviviruses.

•This group of viruses could represents a primordial form of flaviviruses with replication restricted to mosquitoes and unable to infect vertebrate cells.

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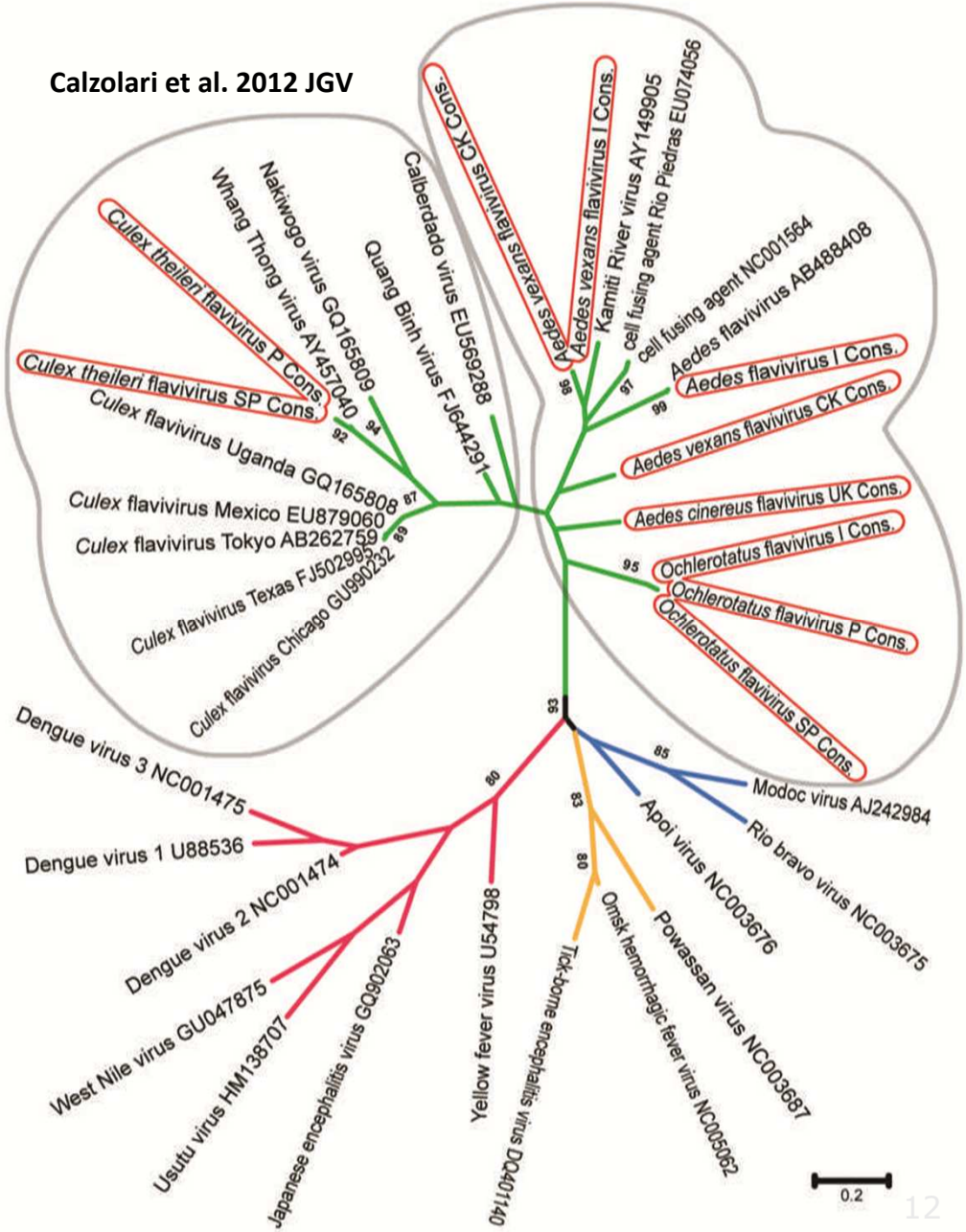
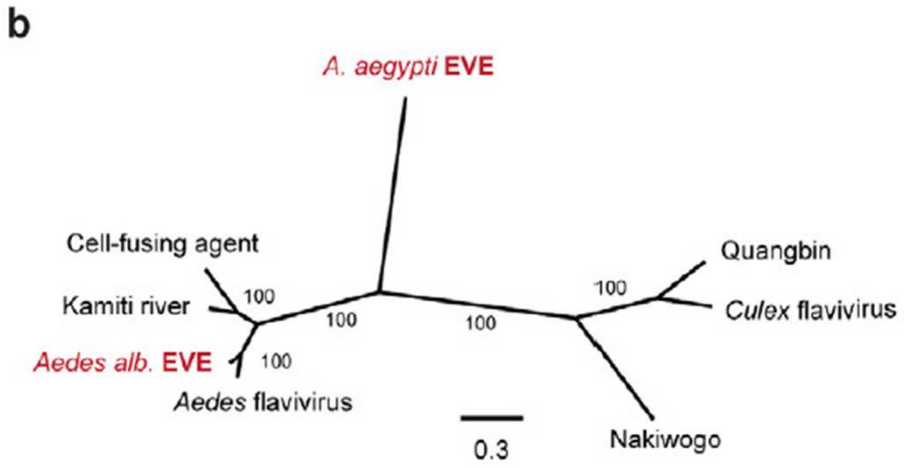


Integration/DNA Form

To complicate the picture…..

- Presence of sequences closely related to MOF integrated in genomic DNA of mosquitoes were reported (Crochu et al. 2004, Katzourakis & Gifford 2010)
- Detection of sequences in DNA form in first passage of cell cultures of Culex flavivirus (Cook et al. 2006, Cook et al. 2009)



Katzourakis & Gifford 2010

Total nucleic acid extracted from C6/36 cultures infected with Culex flavivirus , (passage 5, day 6 post-infection)

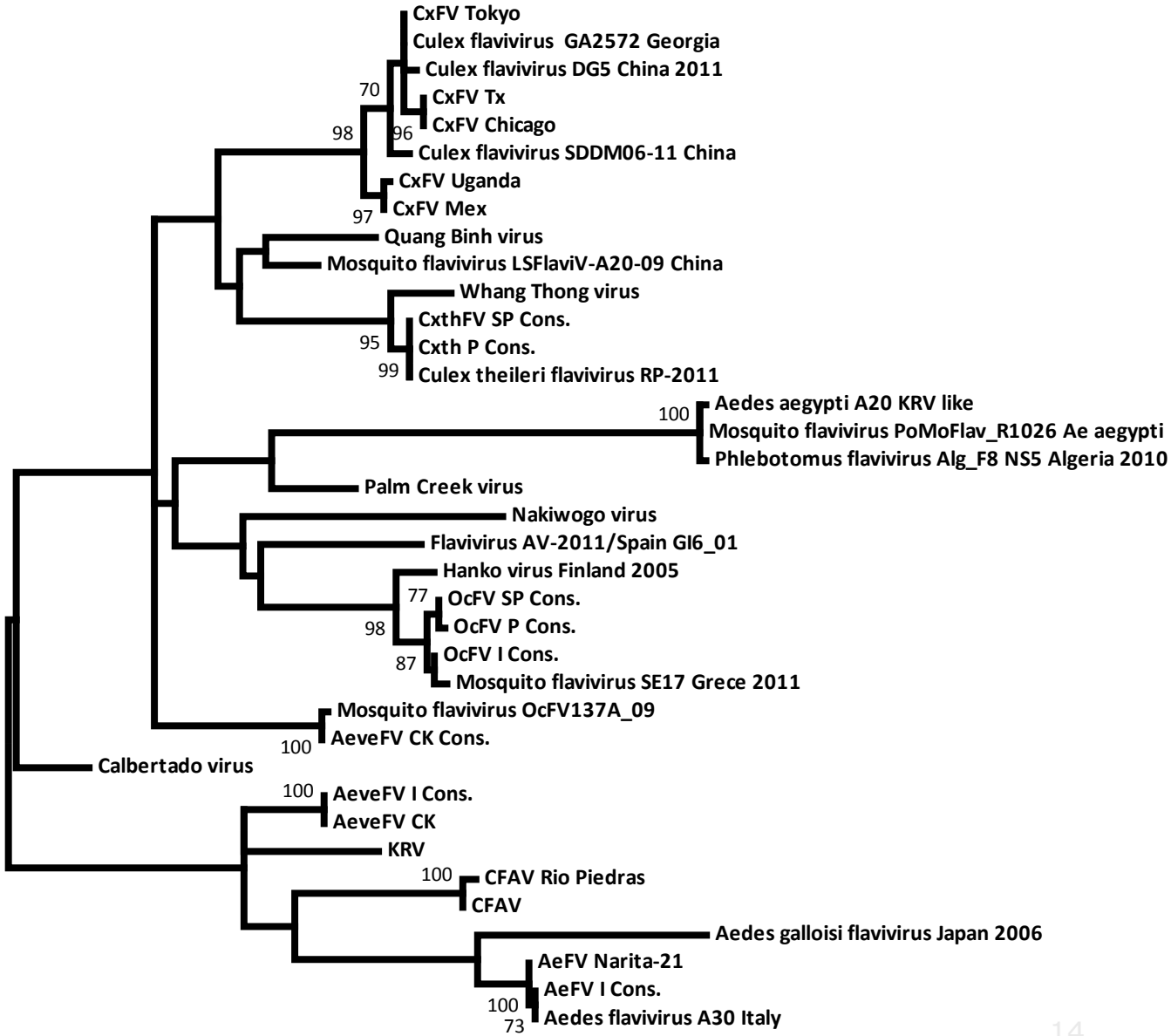
Sample	Primers	No RT classic PCR		RT-PCR one-step		No RT classic PCR	
		Supernatant	Cells	Supernatant	Cells	Cells +DNase	Cells no DNase
H4A1	NS3 verif S/R	−	+	+	+	−	+
	NS5 verifB S/R	−	+	+	+	−	+
H3E6	ENV verif S1/R1	−	+	+	+	−	+
	NS3 verif S/R	−	+	+	+	−	+
	NS5 verifB S/R	−	+	+	+	−	+

Cook et al. 2009 JGV

MOF sequences in GB

•Number of sequences ascribable to these viruses were increasingly reported over the years in GenBank database.

•Only a short part of the NS5 gene can be aligned between all detected sequences.



Maximum Likelihood method based on the Kimura 2-parameter Model.
There were a total of **112** positions in the final dataset. Evolutionary analyses were conducted in MEGA5

MOFs worldwide

Name	Isolation	Mosquito species	Countries	First det.
Cell fusing agent virus	x	*Aedes aegypti* cell line, *Aedes albopictus, aedes aegypti*	Puerto Rico, Mexico	1975
Kamiti River virus	x	*Aedes macintoshi*	Kenya	1999
Flavivirus AV 2011 Spain genomic		*Aedes vexans, Culiseta annulata, Aedes spp.*	Spain	2001
Quang Binh virus	x	*Culex tritaeniorhynchus*	Vietnam	2002
Culex flavivirus	x	*Culex spp.*	Japan, US, China, Guatemala, Mexico Uganda	2003
Calbertado virus	x	*Culex tarsalis, Culex pipiens*	Canada and Northern America	2003
Aedes aegypti flavi		*Aedes aegypti*	Detected integratet in DNA form, detected in RNA form in Portuga (Madeira Is.)	2003
Aedes gallosi flavivirus		*Aedes gallosi*	Japan	2003
Ochlerotatus flavivirus/Hanko virus	x	*Aedes caspius/detritus, Culex pipiens/perexiguus/theileri*	Italy, Spain, Portugal, Finland, Greece	2005
Culex theileri flavivirus/Wang Thong virus		*Culex theileri, Culex fuscocephala*	Portugal, Spain, Thailand	2007
Aedes vexans flavivirus		*Aedes vexans*	Italy, Czechland	2008
Nakiwogo virus	x	*Mansonia africana*	Uganda	2008
Aedes flavivirus	x	*Aedes* mosquitoes, *Aedes albopictus*	Japan, Italy, Missouri	2009
Czech Aedes vexans flavivirus		*Aedes vexans, Aedes caspius*	Czechland, Italy	2009
Mosquito LSF china		*Culex tritaeniorhynchus*	China	2009
Aedes cinereus flavivirus		*Aedes cinereus*	UK, Italy	2010
Palm Creek virus	x	*Coquillettidia xanthogaster*	Australia	2010

Carachteristic

•Same of these viruses are present worldwide (Culex flavivirus reported in America, Asia, Africa).

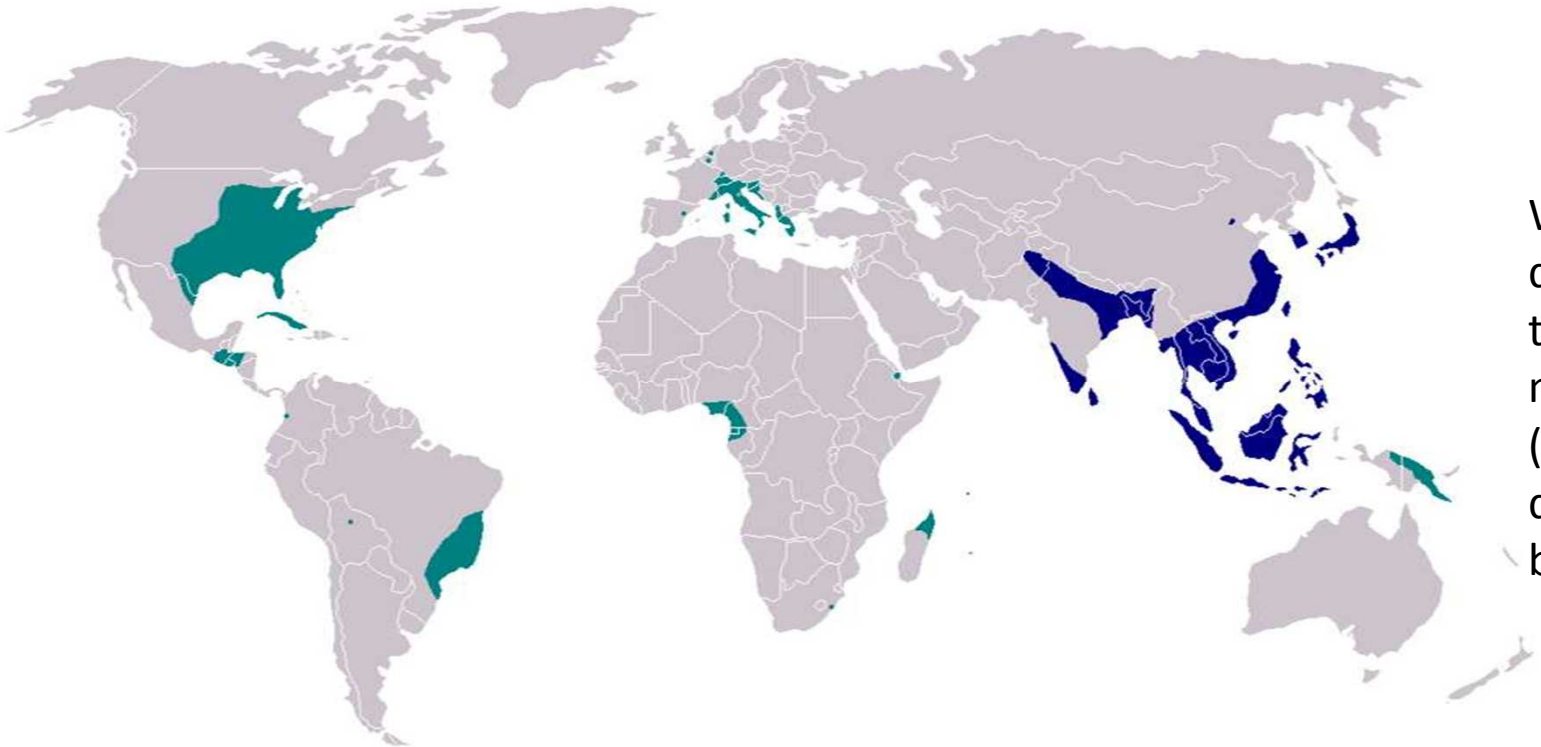
•Many of these viruses are detected in several mosquito species, sometime belonging to different genera (Ochlerotatus flavivirus detected in *Aedes caspius/detritus, Culex pipiens/perexiguus/theileri*).

• Some of these viruses seem to be diffused in specific environment (eg. Ochlerotatus flavivirus in wetlands)

- •Presence of environmental factors favoring MOF persistence.
- •Influence of MOF on the bionomic of infected mosquitoes that favor their survival in particular environments.

Aedes flavivirus

- Aedes flavivirus, isolated for the first time in Japan from *Aedes albopictus*, was also detected (and isolated) in Europe and US, probably the virus traveled with mosquito eggs.
- Why the virus is still present in Tiger mosquitoes "emigrated" worldwide?
- Could this virus give some competition advantage to infected mosquitoes?
- We also detect this virus in pools of *Culex pipiens* (unpublished data).



Worldwide distribution of the Tiger mosquito (original distribution in blue)

Characteristics

Viruses probably exclusive of insects :

isolation of these viruses only from insect cell culture

unsuccessful attempts to grow or to isolate these viruses in vertebrate cell cultures (Hobson-Peters et al. 2013; Bolling et al. 2011; Crabtree et al. 2003, 2009; Hoshino et al. 2007, 2009; Morales-Betoulle et al. 2008; Sang et al. 2003; Stollar & Thomas, 1975).

No risk for human

Viruses unable to replicate in vertebrates probably do not represent a health risk.

The lack of a vertebrate host distinguish MOFs from other flaviviruses and raises the question of how these viruses **persist in the environment**.

Vertical transmission:

transmission from adult mosquitoes to their offspring has been reported

MOF detection in mosquito males and immature stages has been observed

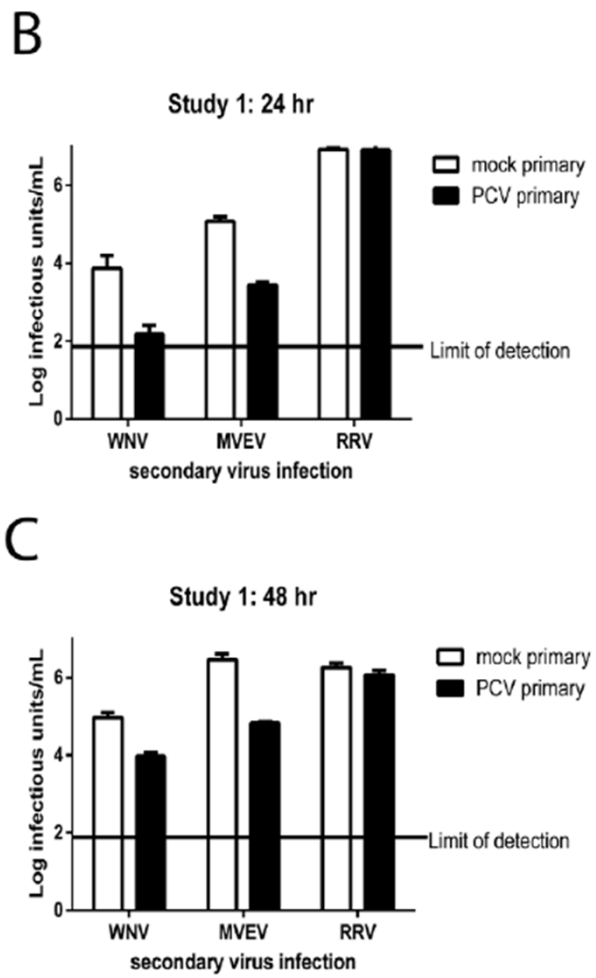
Oral rute: Laboratory studies have demonstrated the ability of KRV to infect *Aedes aegypti* mosquitoes via the oral route (Lutomiah et al., 2007).

Interactions with other flaviviruses

Superinfection phenomena: prior infection with one flavivirus can inhibit replication of a related flavivirus.

Reported in cell cultures (C6/C36)
•WNV and CxFV (Boiling et al 2010)
•Murray Valley encephalitis virus/WNV (Kunjin virus) and Palm Creek virus (Hobson-Peters et al. 2013)

Conversely:
•the enhancement of WNV transmission in mosquitoes inoculated simultaneously with CxFV Izabal has been reported (Kent et al.2010)
•a positive association between CxFV and WNV was reported in field collected mosquitoes (Newman et al. 2011)



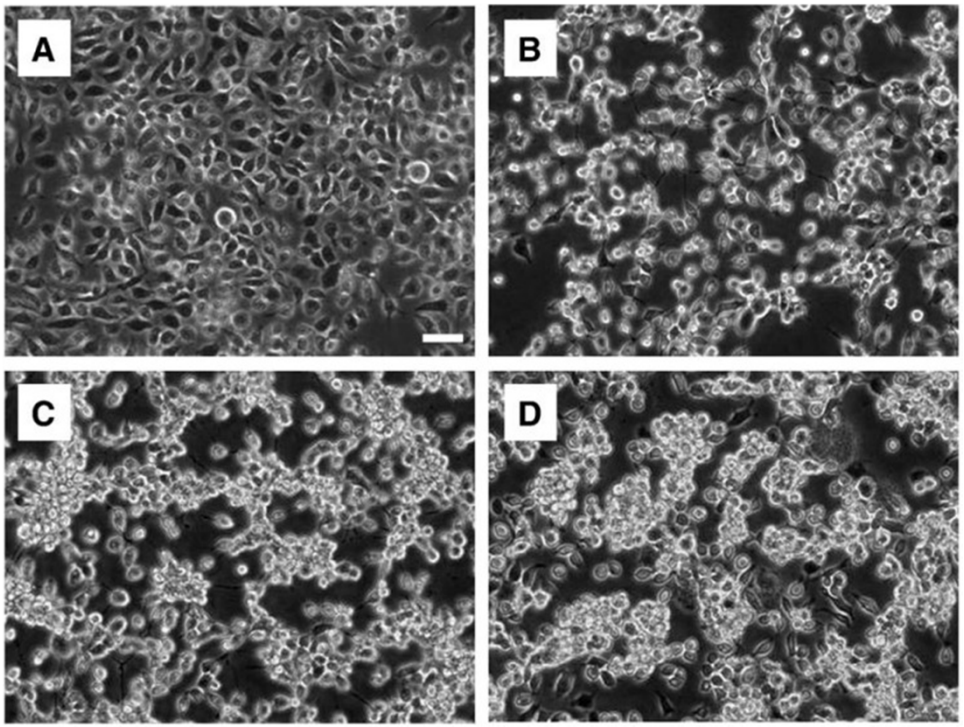
Hobson-Peters et al. 2013

Isolation

Difficulties in characterization of MOFs:

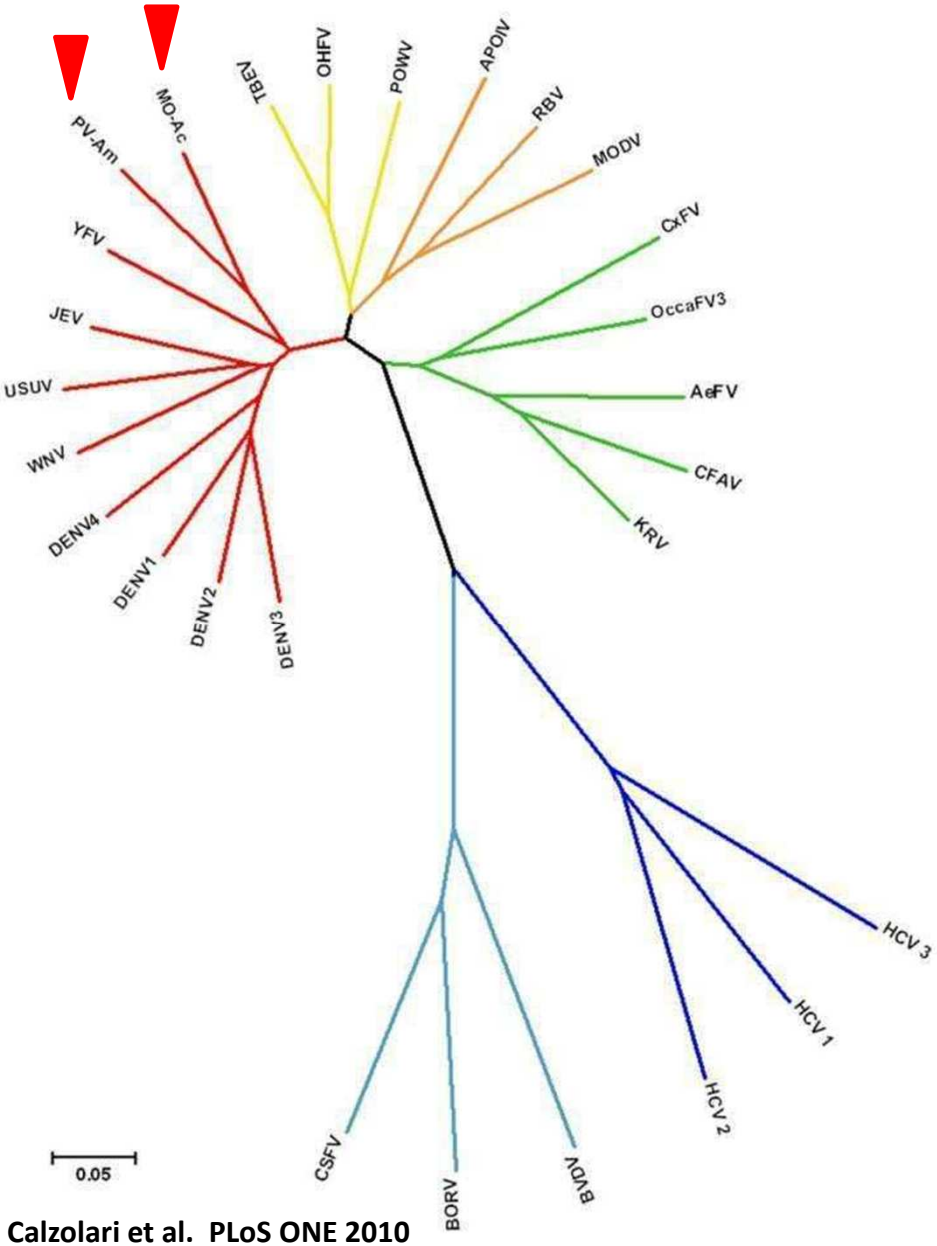
•Difficult isolation in cell cultures: the MOF CPE can be weak (Hoshino et al. 2009) or strain dependent (Kim et al. 2009), or only visible after a number of blind passages.

•The virus can go undetected by RT-PCR in cell-culture medium during early passage after inoculation (Bolling et al. 2011).



Phase contrast micrographs of control (uninfected) C6/36 cells (A), AEFV-infected cells (B), CXFV (Surabaya strain)-infected cells (C), and CFA (Surabaya strain)-infected cells
(D) at 4 d post-infection. Cell monolayers were inoculated with stock viruses that had been passaged three times. Scale bar, 50 µm. **From Hoshino et al. 2009**

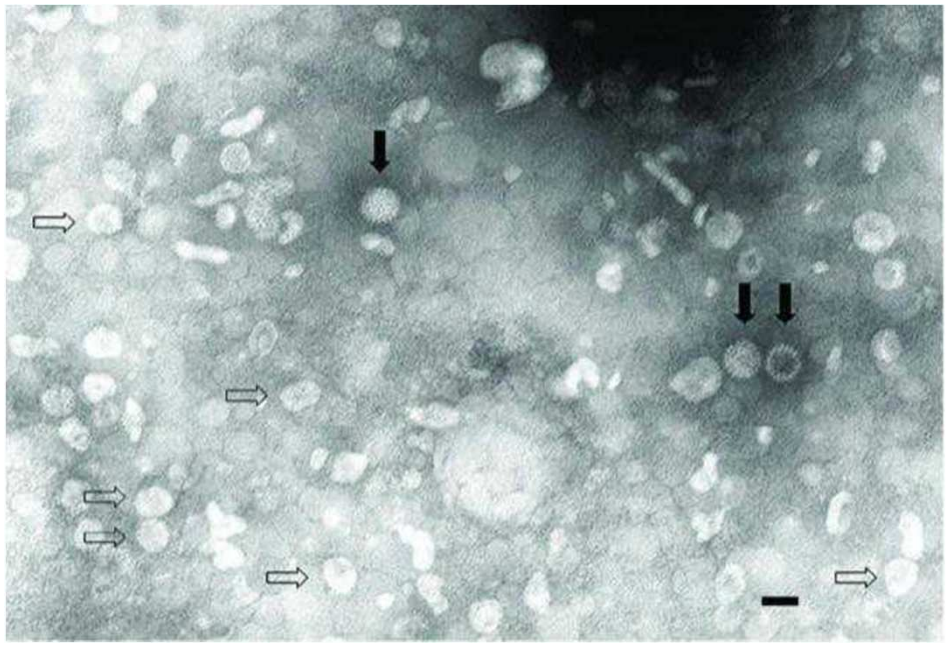
Other unexpected results



Calzolari et al. PLoS ONE 2010

- Two sequences (obtained by the flavivirus genus PCR) are more similar to the arthropod-transmitted flavivirus.

- Isolation of unknown viruses (from PCR negative pools) producing cytopathic effect on mosquito cell line (characterization in progress).



Cited in Calzolari et al. 2010 VBZD

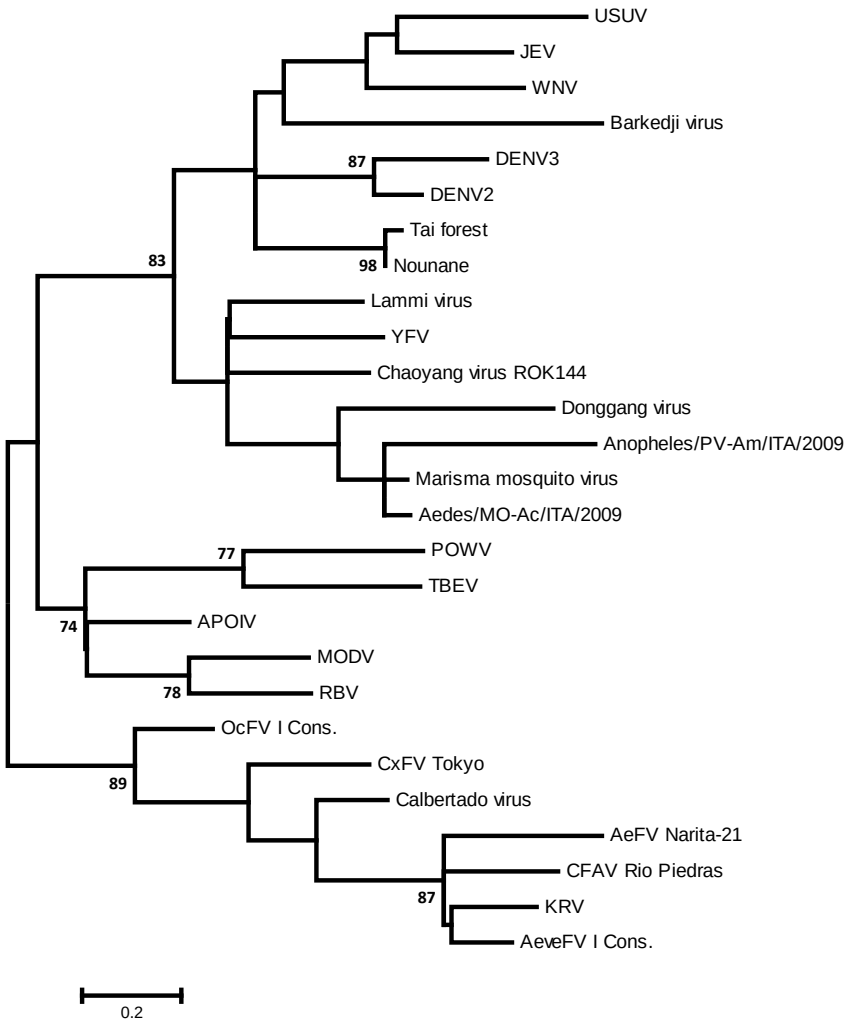
Other insect-specific flavivirus

NS5

One of these sequences has a good identity with Marisma mosquito virus, recently isolated in Spain (Vazquez et al 2011).

- These two sequences grouped with other recently isolated mosquito viruses and Mosquito-borne viruses rather than MOF
- No vertebrate hosts were reported for these viruses
- No cytopathic effect on vertebrate cell line

Name	Mosquito species	Counties	Year
Marisma mosquito virus	*Aedes caspius*	Spain, Italy	2003
Lammi virus	*Aedes cinereus*	Finland	2004
Nounanè virus	*Uranotaenia mashonaensis*	Ivory Coast	2004
Chaoyang virus	*Aedes vexans*	China, Korea	2008
Donggang	*Aedes spp*	China	2009
?	*Anopheles maculipennis*	Italy	2009



Conclusions

The abundance of worldwide reports of described viruses highlights the ubiquity of these viruses in different mosquito species, and suggests that many of these viruses have yet to be discovered.

Much of the ecology of MOFs is still largely unknown, and the scarcity of knowledge on the cycle and characteristics of these viruses highlights the need for further study.

- The cycle of these viruses (DNA form, integration, possible reservoirs)
- Eventually influence of these viruses on bionomics of mosquitoes
- Interaction of these viruses with pathogenic flaviviruses

Thank you!

For information and collaborations

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Emerging sandfly-borne phleboviruses around the Mediterranean

Pr. Rémi CHARREL

UMR_D 190 – Emergence des Pathologies Virales

Aix-Marseille Univ - IRD - EHESP – IHU Méditerranée Infection

Marseille, FRANCE

Questions to be addressed

- Co-circulation of different phleboviruses in the same geographic area raises the question of respective impact on human/animal health

  The antigenic cross-reactivity between many phleboviruses renders the answer to this problem more complex than initially beleived

- The lack of available full-length genome sequences of phleboviruses hampers the development of real-time RT-PCR tests for diagnostic purpose of known phleboviruses

- The absence of field studies (such as supported by the Rockefeller foundation after WWII) has left a large number of phleboviruses undiscovered: these virus are likely to play an important role in ecology and human and veterinary medicine

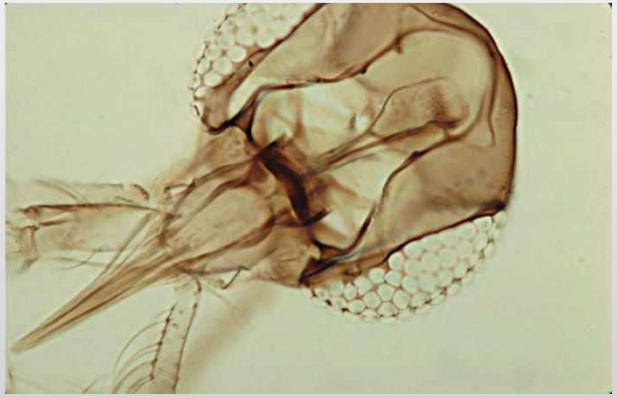
Phlebotomine sandflies

- ✓ Order : *Diptera*
- ✓ Family : *Psychodidae*
- ✓ Genus : *Phlebotomus, Sergentomyia, Lutzomyia*
- ✓ > **800 species**

- ✓ Size : 2 to 5 mm
- ✓ Shelter in dark corners during the day
- ✓ Active during the night
- ✓ Close to humans and domestic animals
- ✓ Temperate countries = summertime
- ✓ Tropical countries = all the year

Vector role

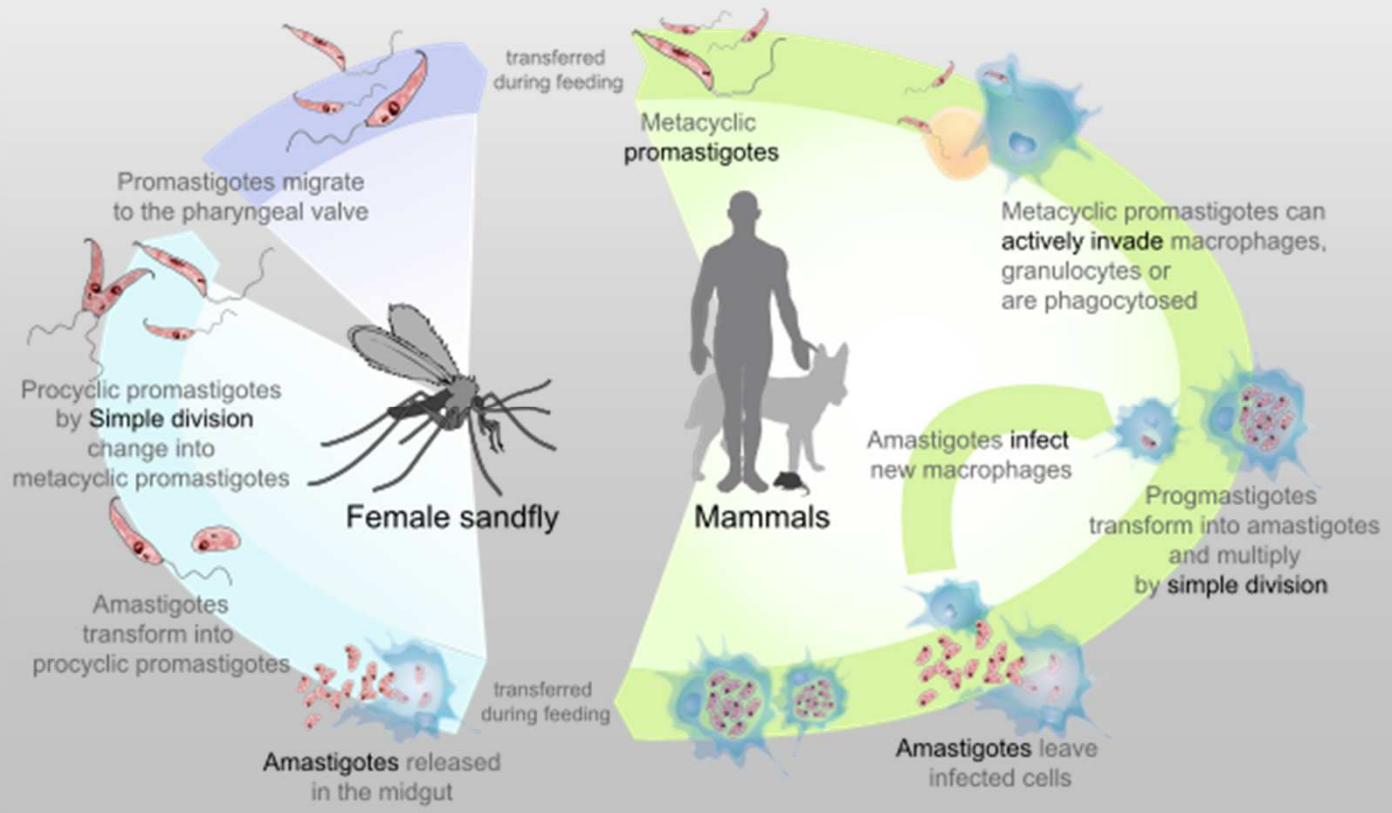
- ✓ Female : hematophagous (blood meal → develop eggs)
- ✓ **Anthropophilic** species



- ✓ 98 species recognized or suspected as vector of pathogens (WHO, 2010)
  - → Viral diseases : papatasi fever, toscana virus, sicilian…
  - → Parasitic diseases : leishmaniasis
  - → Bacterial diseases : bartonellosis (*Bartonella baciliformis*) (*Lutzomyia)*

Leishmaniasis

- ✓ Caused by flagellate protozoan parasites
- ✓ In the genus *Leishmania*
- ✓ **20** species and subspecies **pathogenic for human**



Leishmaniasis

✓ **Tegumentary leishmaniasis**

→ Cutaneous and mucocutaneous forms

→ Incidence : **1.5 million cases/year**

→ Disfigurement, social and phsychological stigma

✓ **Visceral leishmaniasis** (VL): *L. donovani* and *L. infantum*

→ Most severe form : parasites migrate to vital organs

→ Incidence = **500 000 cases/year**

→ Fatal if untreated

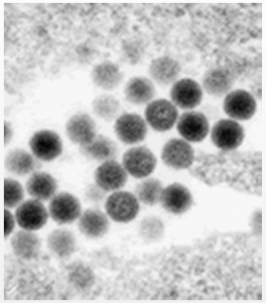
✓ **Asymptomatic forms**

Designed by WHO as one of the 10 priority tropical diseases

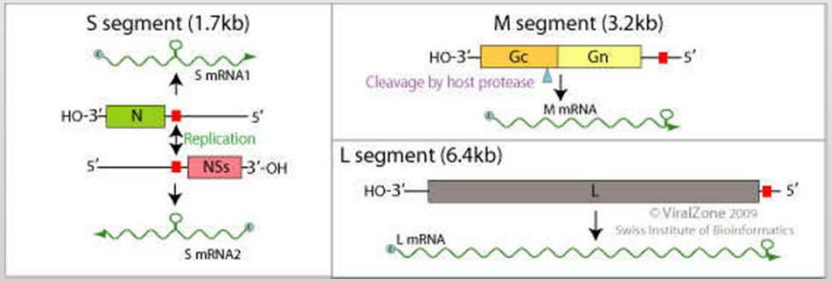
Viruses

- ✓ **Arthropod-borne viruses** transmitted by sandflies :
  - → *Reoviridae* (Changuinola virus)
  - → *Rhabdoviridae* (Irririvirus, vesicular stomatitis virus …)
  - → ***Bunyaviridae*** *:* genus ***Phlebovirus***

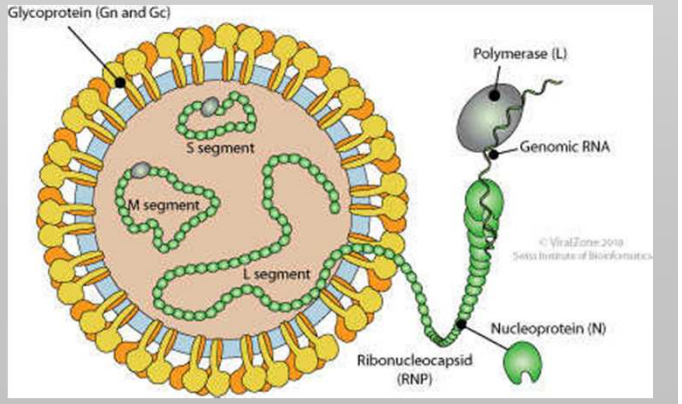
Phleboviruses



- ✓ **The genus *Phlebovirus***
  - → Enveloped virus
  - → Size is 85-120 nm
  - → Trisegmented single stranded RNA genome, negative polarity



- ✓ Segmented **RNA genome**
  - → 3 different sized segments
  - → S = 1.7 kb, Nucleocapsid and NS
  - → M = 3.2 kb, Glycoproteins G_N & G_C + NS
  - → L = 6.4 kb, RNA polymerase



Phleboviruses

9 species grouping 37 viruses

- **Bujaru virus**
  - ✓ Bujaru
  - ✓ Munguba
- **Chandiru virus**
  - ✓ Alenquer
  - ✓ Chandiru
  - ✓ Itaituba
  - ✓ Nique
  - ✓ Oriximina
  - ✓ Turuna
- **Chilibre virus**
  - ✓ Cacao
  - ✓ Chilibre
- **Frijoles virus**
  - ✓ Frijoles
  - ✓ Joa
- **Punta Toro virus**
  - ✓ Buenaventura
  - ✓ Punta Toro
- **Rift Valley fever virus**
  - ✓ Belterra
  - ✓ Icoaraci
  - ✓ Rift valley fever
- **Salehebad virus**
  - ✓ Arbia
  - ✓ Salehebad
- **Sandfly fever Naples virus**
  - ✓ Karimabad
  - ✓ Sandfly fever Naples
  - ✓ Sabin
  - ✓ Tehran
  - ✓ Toscana
- **Uukuniemi virus**
  - ✓ EgAN
  - ✓ Fin V
  - ✓ Grand Arbaud
  - ✓ Manawa
  - ✓ Murre
  - ✓ Oceanside
  - ✓ Ponteves
  - ✓ Precarious point
  - ✓ RML
  - ✓ St Abbs head
  - ✓ Tunis
  - ✓ Uukuniemi
  - ✓ Zaliv Terpeniya

+ 16 unclassified viruses

- **Tentative species**
  - ✓ Sandfly fever Sicilian
  - ✓ Aguacate
  - ✓ Anhanga
  - ✓ Arboledas
  - ✓ Arumowot
  - ✓ Caimito
  - ✓ Chagres
  - ✓ Corfou
  - ✓ Gabek Forest
  - ✓ Gordil
  - ✓ Itaporanga
  - ✓ Odrenisrou
  - ✓ Pacui
  - ✓ Rio Grande
  - ✓ Saint-Floris
  - ✓ Urucuri

+ 10 new unclassified viruses

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Old World sandfly-transmitted viruses

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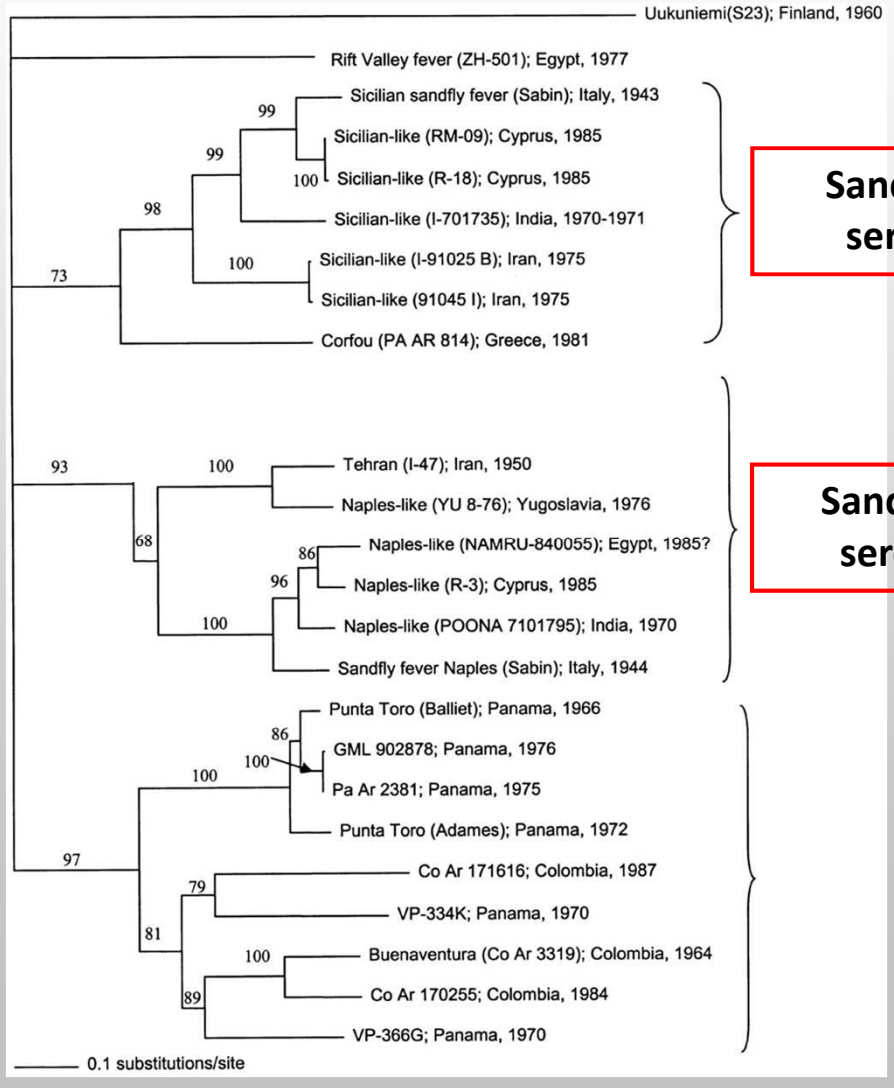
+ > 10 new unclassified viruses

Phlebovirus infections

✓ **In the Old-World before 1970**



Sandfly Sicilian serocomplex

Sandfly Naples serocomplex

✓ Vector = ***P. papatasi***

✓ **Clinical picture**

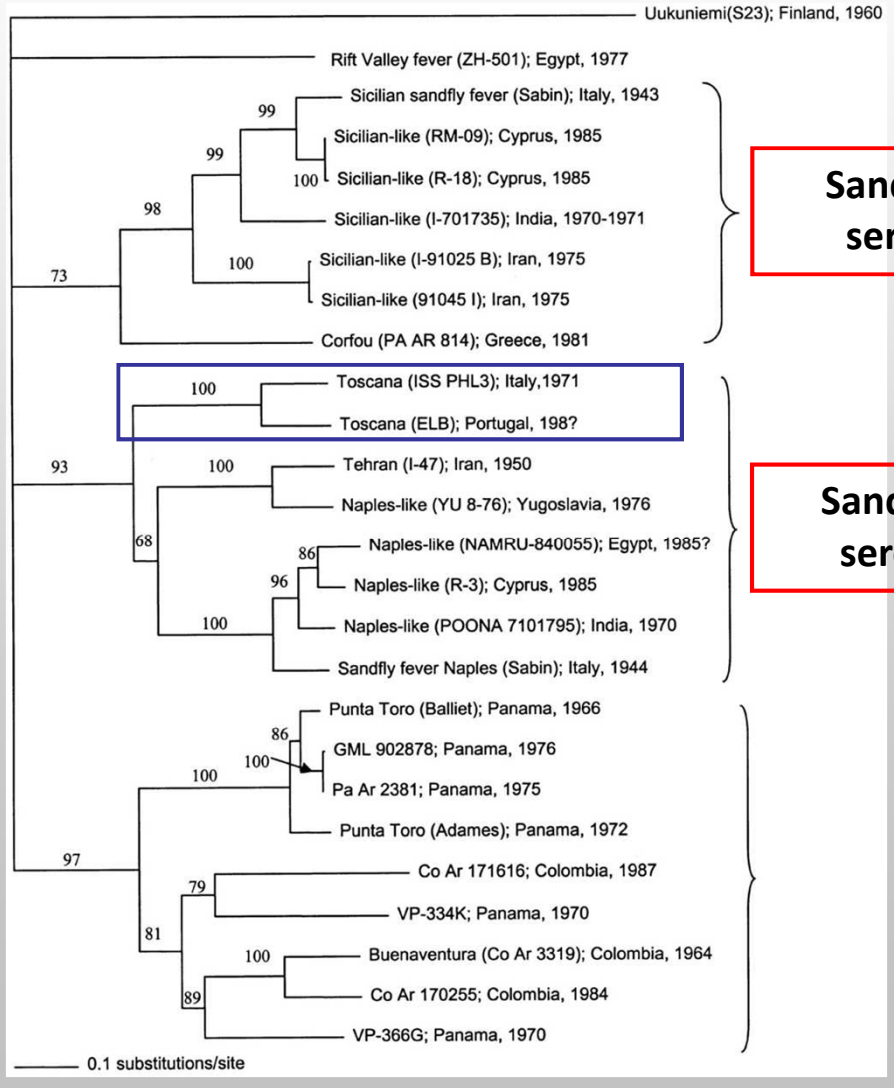
→ Summer fever

→ Mild febrile syndrome

→ No fatality recorded

Phleboviruses infection

✓ **In 1971 : Toscana virus**



Sandfly Sicilian serocomplex

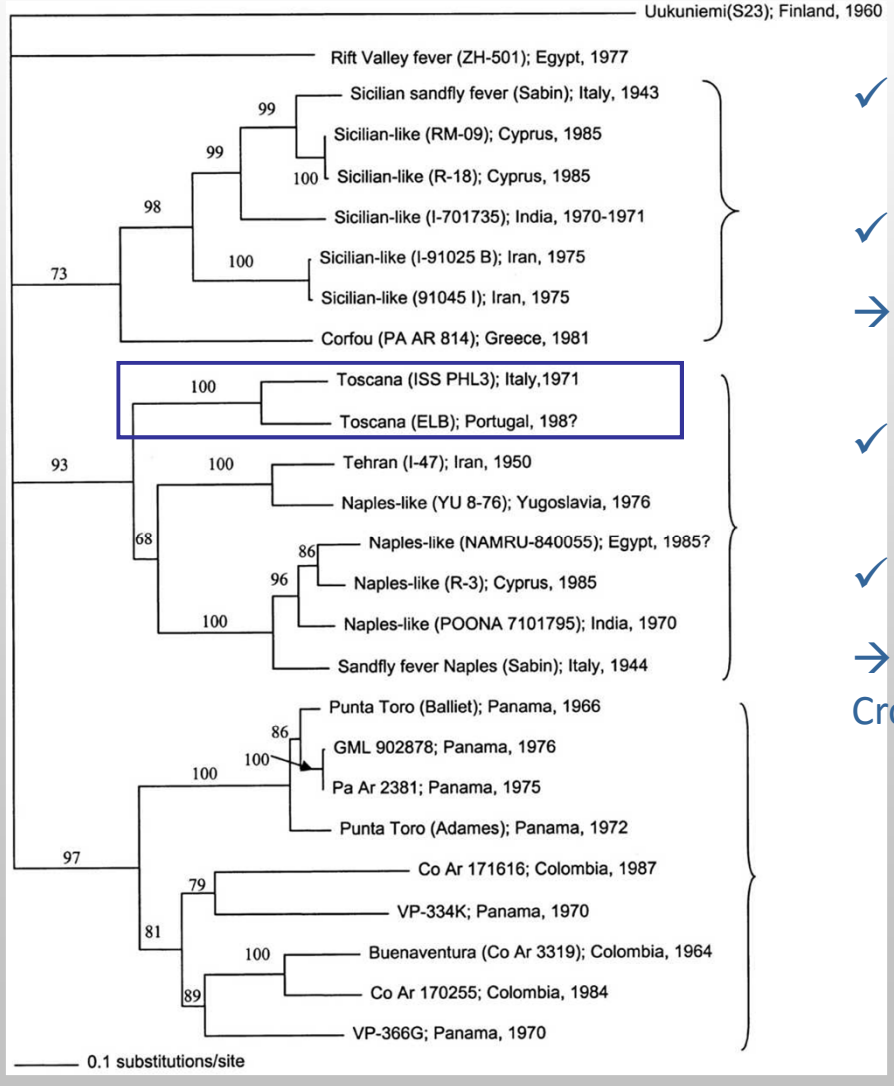
Sandfly Naples serocomplex

✓ First isolated in Italy, 1971

✓ From *P. perniciosus* & *P. perfiliewi*

Phleboviruses infection

✓ **During 80's & 90's : Toscana virus**



✓ Human disease demonstrated in 1985

✓ Tropism for the CNS

→ Meningitis and encephalitis

✓ 1st cause of summer meningitis in Italy in 80's

✓ Serological data and virus isolation

→ Portugal, France, Spain, Greece, Cyprus, Turkey, Croatia

The only sandfly-transmitted virus that demonstrates neurotropic activity

Toscana virus outside of Italy

- **Spain 1988 & 2003: serologic and molecular evidence:**
  - **15 strains isolated from patients,**
  - **26% seroprevalence rate in Granada (*Mendoza-Montero et al 1998 Clin Infect Dis*)**
- **France 2004: serologic and molecular evidence (*Hemmersbach-Miller et al 2004 Eur J Intern Med, Peyrefitte et al 2005 Emerg Infect Dis*).**
- **France 2005-2009**
  - **seroprevalence 12% in healthy blood donors southeastern France**
  - **detection of TOSV RNA**
    - **in patients with meningitis**
    - **in *P perniciosus* in Marseille and Nice**
    - **in *Sergentomyia minuta***

➔ Toscana virus is in the top-3 of causes of meningitis in France and Spain

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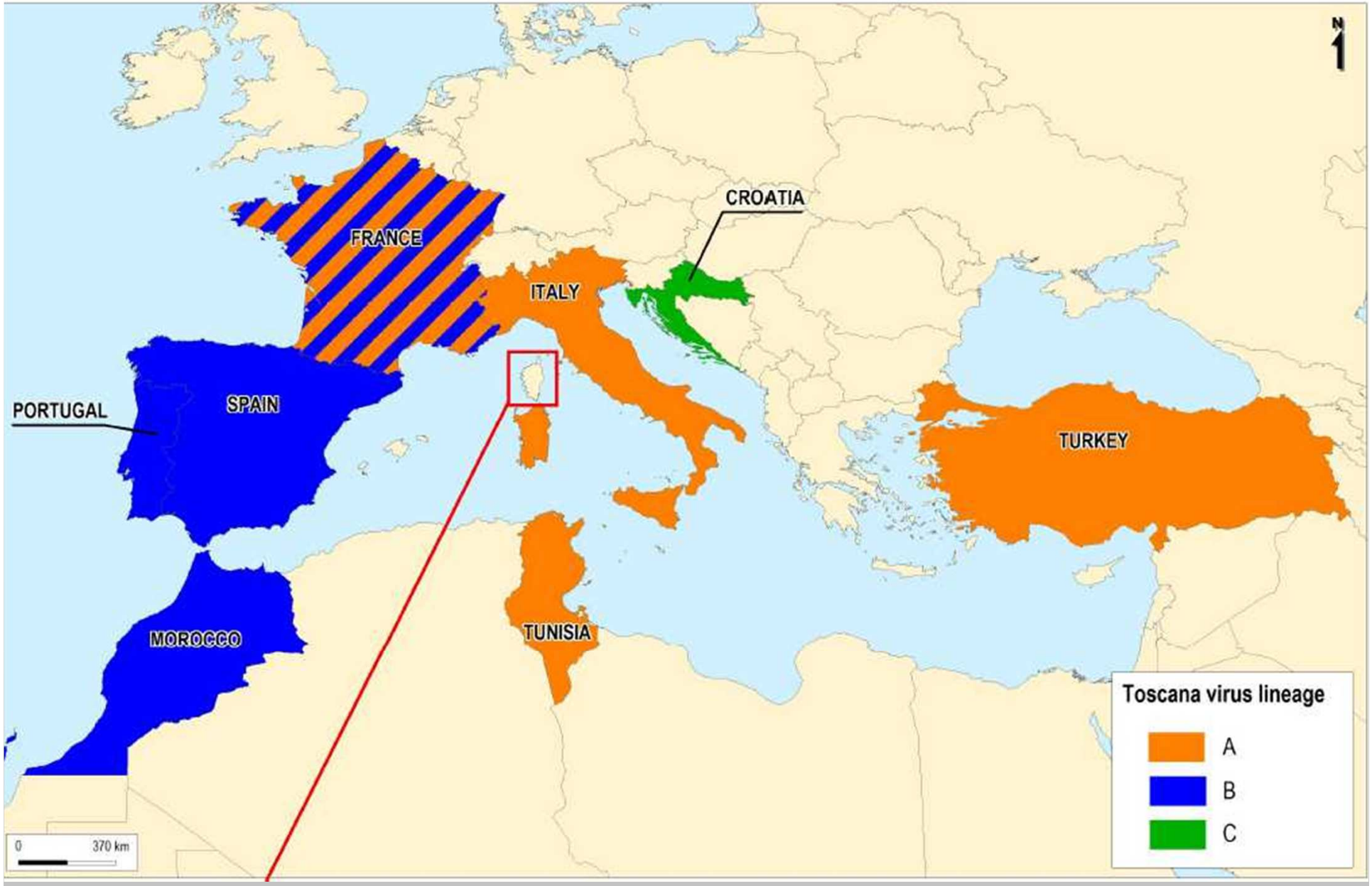
➔ Toscana virus is in the top-3 of causes of meningitis in France and Spain

- **Turkey 2009: serologic and molecular evidence (*Ergunay et al Clin Microbiol Infect 2010*).**
- **Greece 2010 (*Papa et al 2010*)**
- **Elba island 2010 (*Gabriel et al 2010, Sonderegger et al 2009*)**
- **Morocco**

A - TOSCANA VIRUS GENETIC LINEAGES AROUND MEDITERRANEAN



Disease in humans and Toscana virus

clinical forms of the infection

• meningitis: brutal onset (70%), headache (100%), fever (76-97%), nausea and vomiting (67-88%), myalgias (18%).
neck rigidity (53-95%),
Kernig sign (87%),
poor levels of consciousness (12%),
tremors (2.6%), paresis (1.7%),
nystagmus (5.2%)

CSF > 5-10 cells, normoglyco- proteinorachia leucocytosis (29%) or leucopenia (6%)

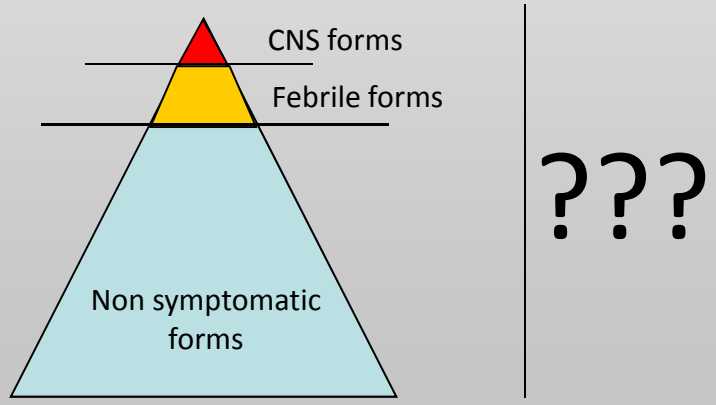
mean duration of the disease is 7 days
outcome is usually favorable.

- meningoencephalitis (Baldelli et al 2004)
- encephalitis (Dionisio et al 2001)
- deafness

• other disease manifestations not involving the CNS
- no published data exist to suggest that TOSV could cause other clinical syndromes

• febrile illness
self-limiting febrile illness without CNS manifestation
usually neither hospitalized nor investigated further

• asymptomatic or pauci-symptomatic
seroprevalence studies suggest that a large proportion of infections by TOSV are a- or pauci-symtomatic



UMR190 Aix Marseille université IRD Institut de recherche pour le développement EHESP

Phleboviruses infection

✓ **Since 2000**

→ Several teams studied phleboviruses

	Vector(s)	Clinical Syndromes
Sandfy Fever Sicilian Virus (SFSV)	*P. papatasi*	Sandfy fever (Papatacci fever, 3-day fever)
Sandfy Fever Naples Virus (SFNV)	*P. papatasi*	Sandfy fever (Papatacci fever, 3-day fever)
Toscana Virus (TOSV)	***P. perniciosus*** ***P. perfiliewi***	**Sandfly fever** **Aseptic meningitis**

✓ Progressively become extinct in western Europe

✓ Currently **Toscana virus**

→ In the **top 3 viruses** causing meningitis during summertime

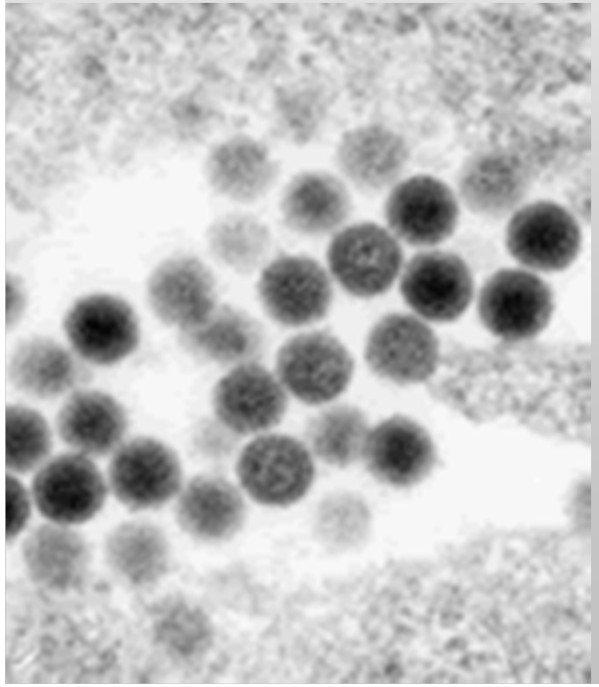
- ✓ Phlebovirus infections :
  - → Known for a long time
  - → But remained neglected
  - → Few data available on their epidemiology

- ✓ Bibliographic research in PubMed for the last 10 years :
  - → "Toscana virus" = 234 references
  - → "Leishmaniasis" >10 000
  - → Other emerging viruses : "Chikungunya" = 955
    "West Nile" = 3 500
    "Dengue" = 4 000

Data of distribution of known viruses are up-to-date ?
Missing viruses in the phylogeny?

→ Virus discovery using field studies

New phleboviruses



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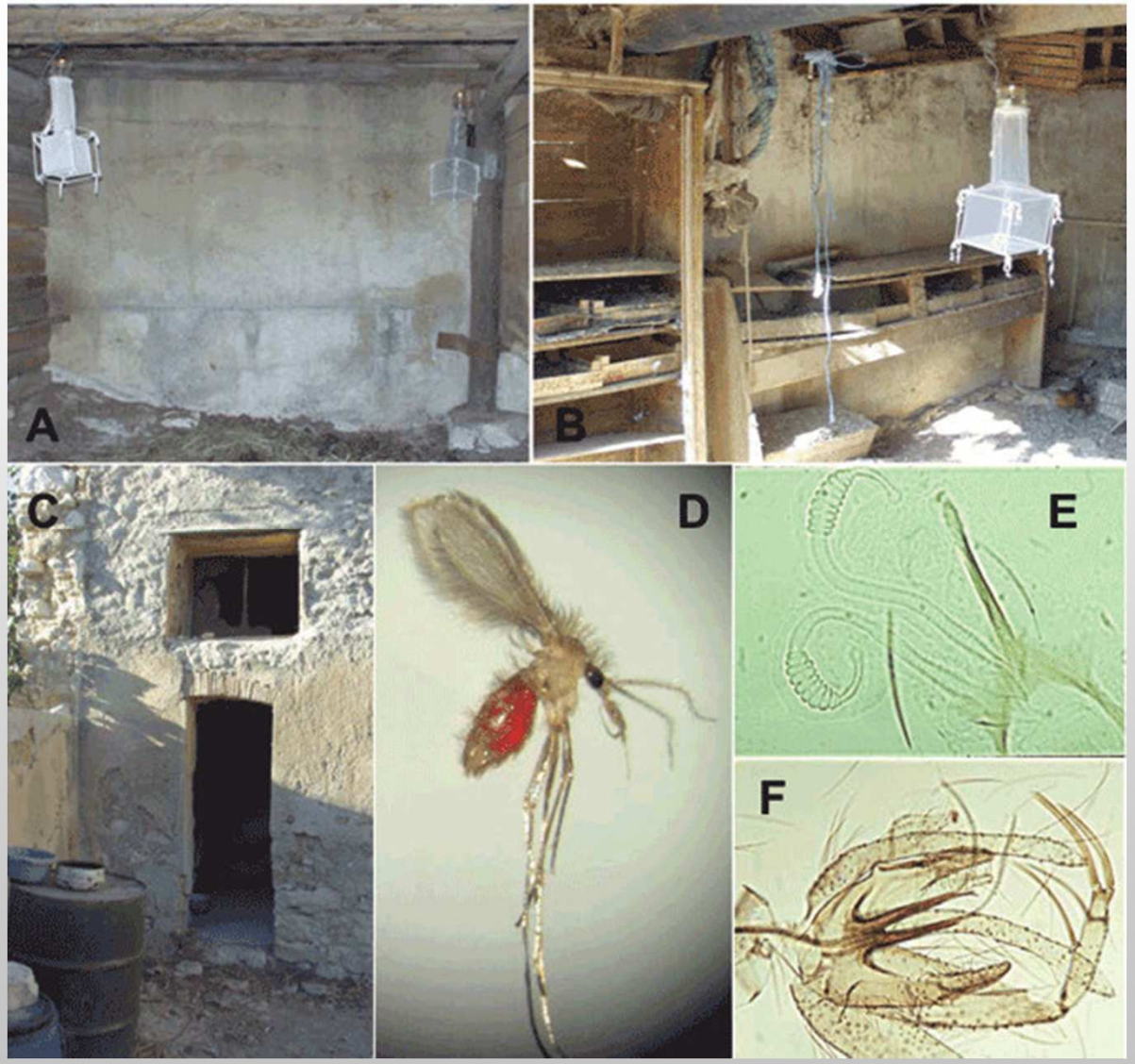
Sandfly trapping



Field Work

- ✓ **CDC** miniature light traps
  - → Inside house, animal housing facilities (sheep, goat, rabbits…)
  - → From dusk to down

- ✓ Each morning, sandflies were :
  - → Collected and identified
  - → Pooled by species, gender and trapping place, max 30
  - → Preserved in dry ice, liquid nitrogen, - 80°C

Viral infectivity

Lab work

✓ Need for an **entomologic plateform** to perform field samples

→ Defrost samples only one time = preserve viral infectivity

✓ Virus **isolation**

= using cells cultures

✓ Virus **detection**

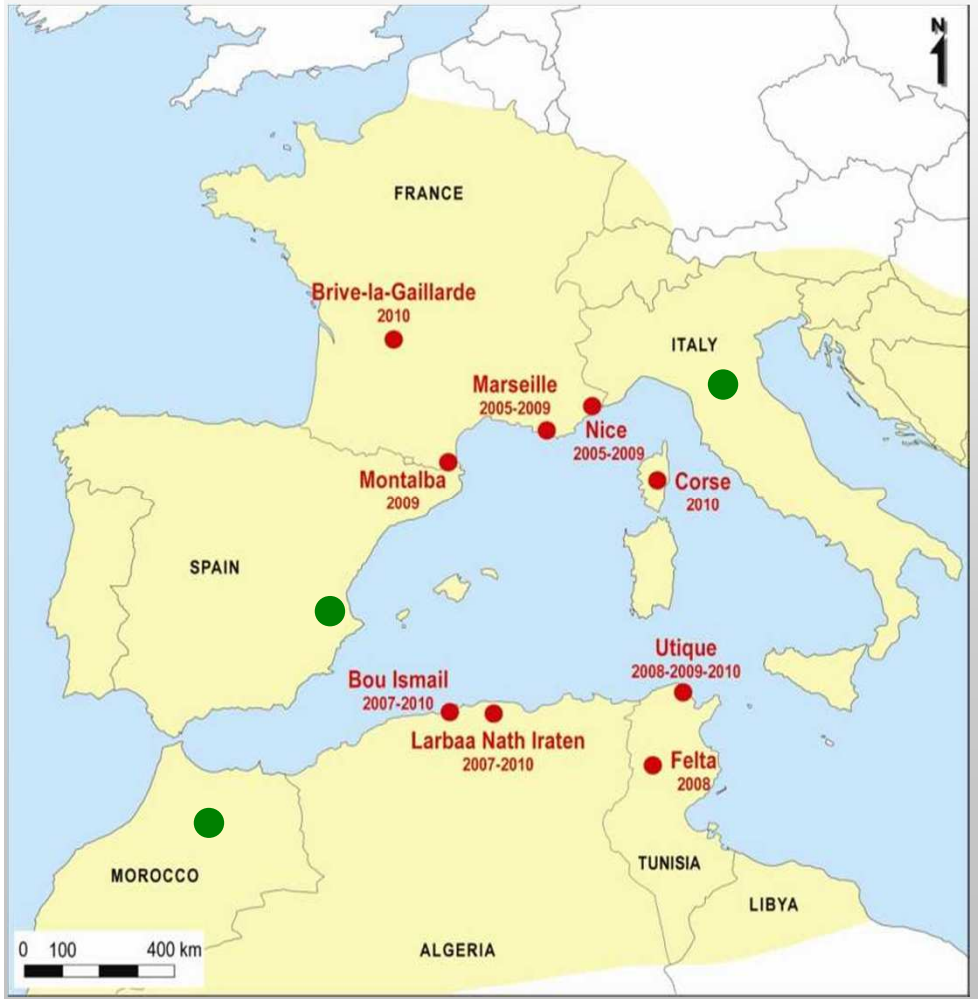
= molecular biology tools, to detect the presence of phlebovirus RNA

✓ **Genetic characterization** of newly discovered phleboviruses

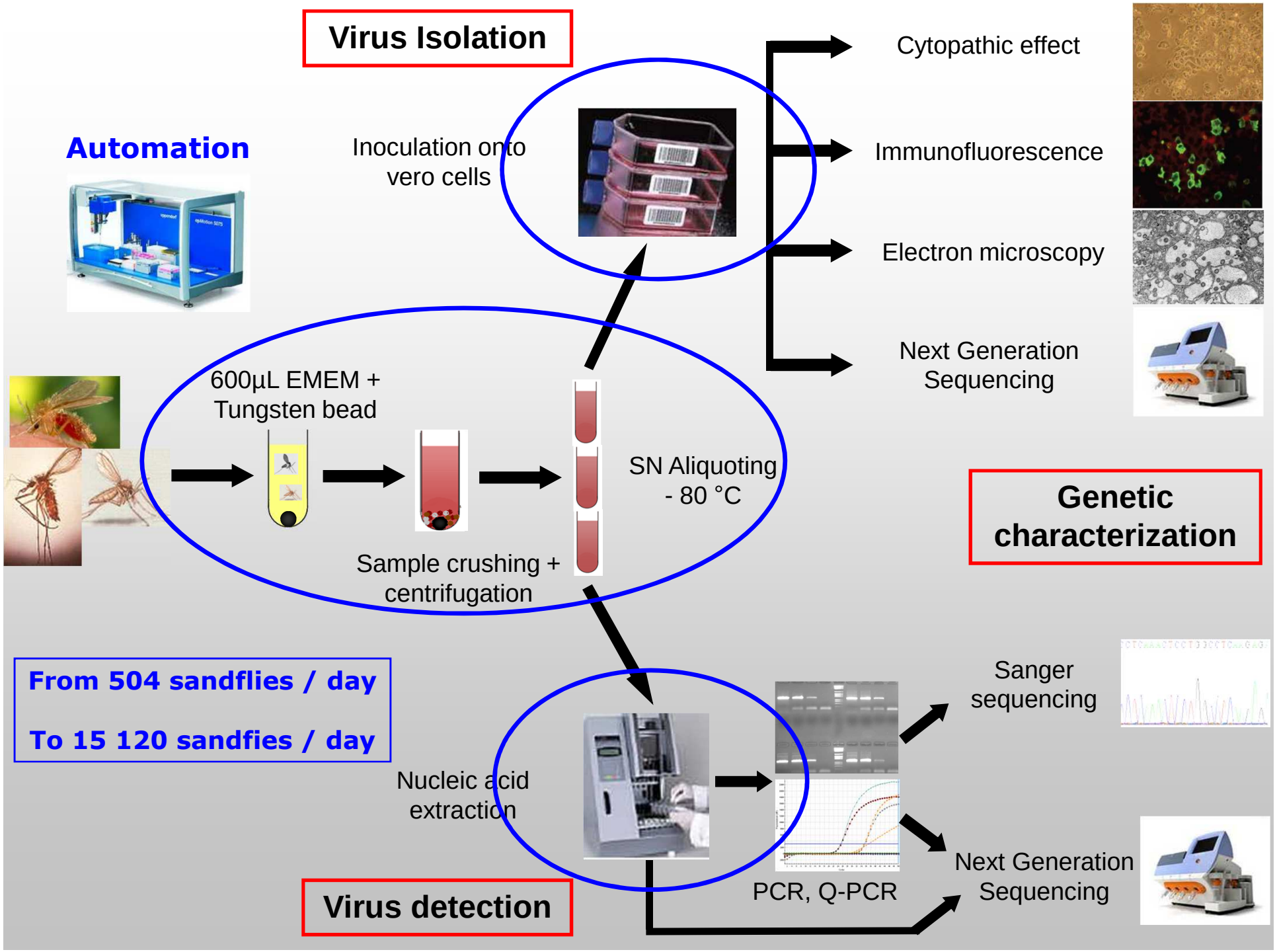
= Sequencing and phylogeny analysis

Field Work

Aim : to detect, to isolate and to characterize existing and/or new phleboviruses in sandfly populations

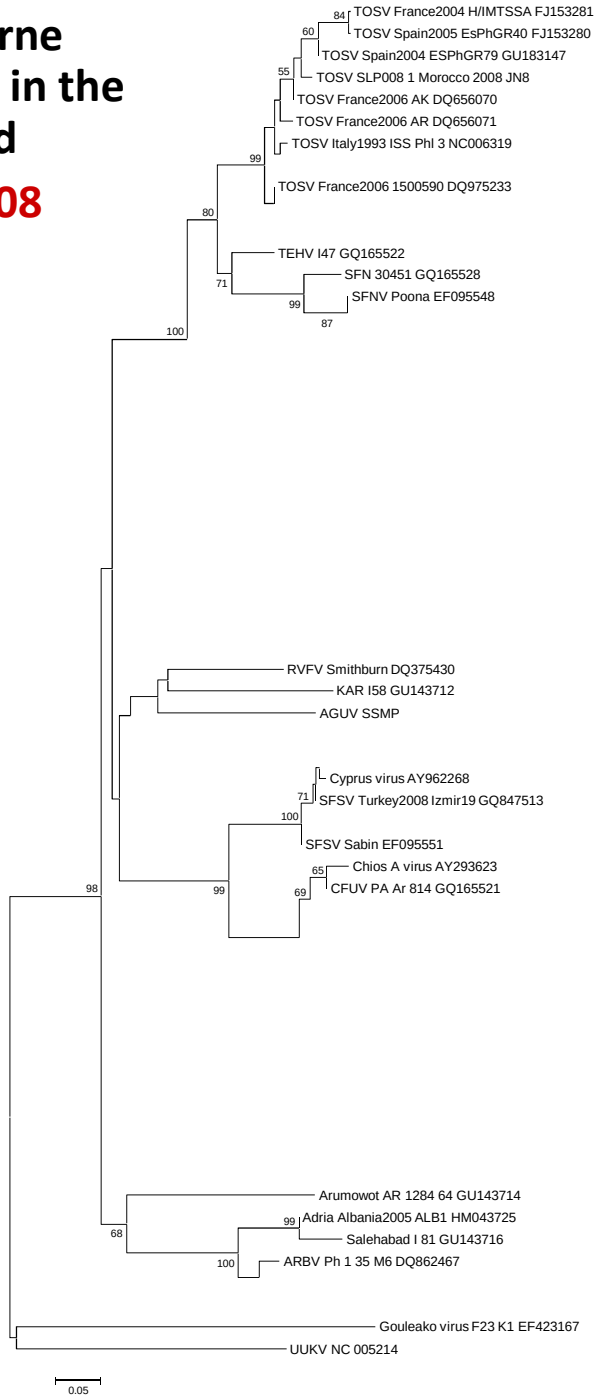


- Done by our group
  - ✓ **12** sandfly trapping campaigns :
    - → Summers 2007, 2008, 2009, 2010
    - → France, Tunisia Algeria
    - → Turkey in 2012 and 2013
- Done by other groups

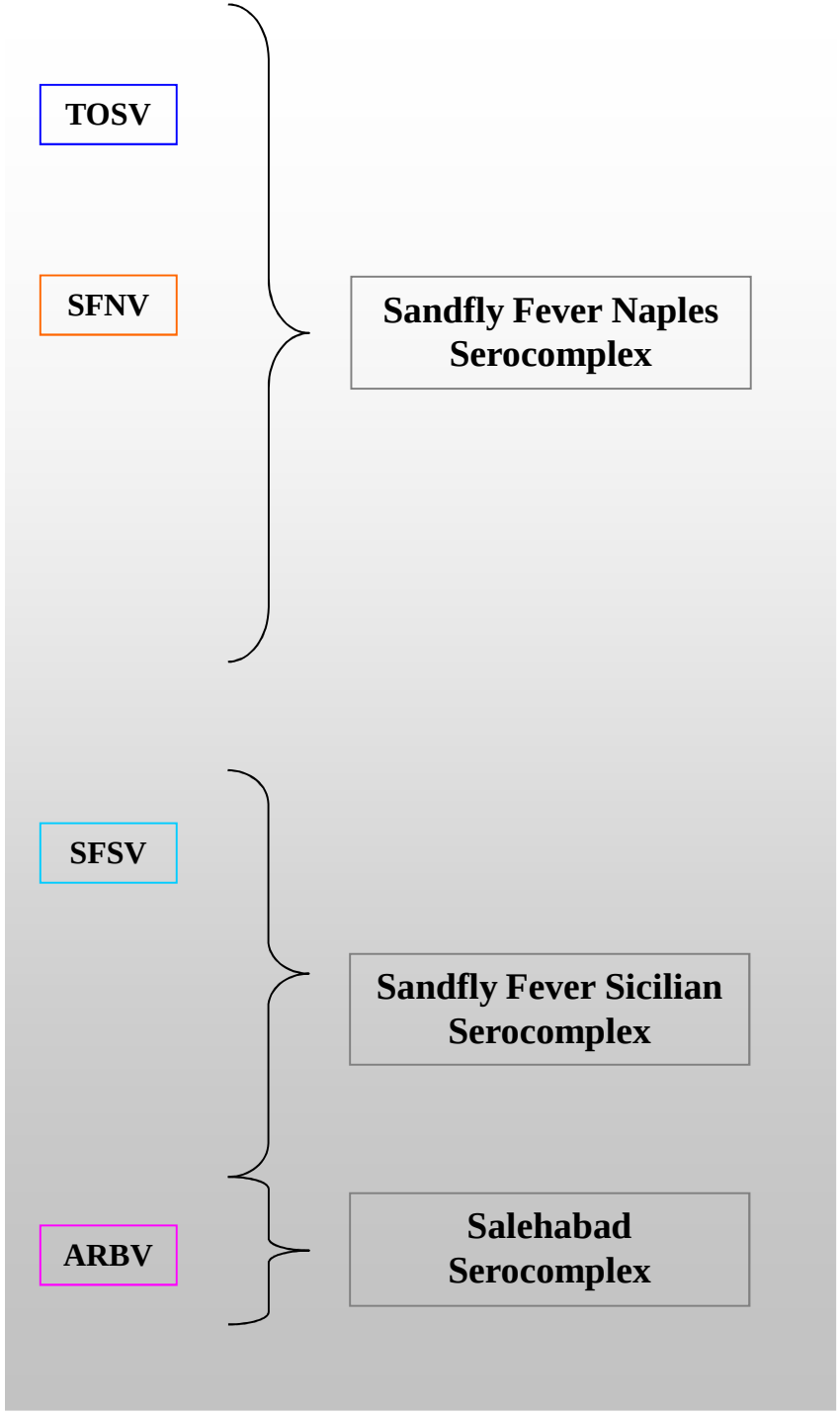

Virus Isolation
Automation
Inoculation onto vero cells
Cytopathic effect
Immunofluorescence
Electron microscopy
Next Generation Sequencing
600µL EMEM + Tungsten bead
Sample crushing + centrifugation
SN Aliquoting - 80 °C
Genetic characterization
From 504 sandflies / day
To 15 120 sandfies / day
Nucleic acid extraction
PCR, Q-PCR
Sanger sequencing
Next Generation Sequencing
Virus detection

Sandfly-borne phleboviruses in the old world

Before 2008

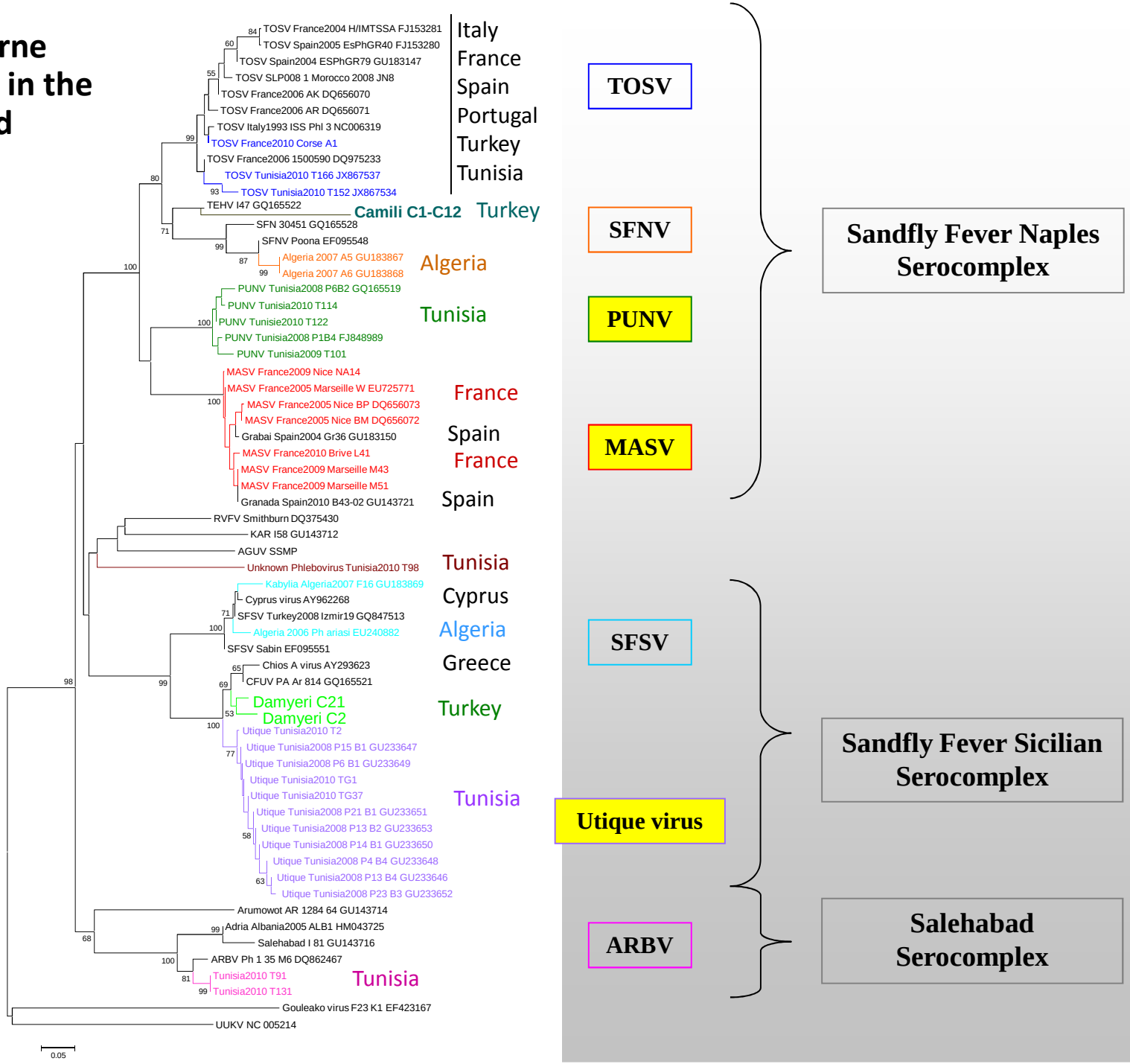


L-segment, AA



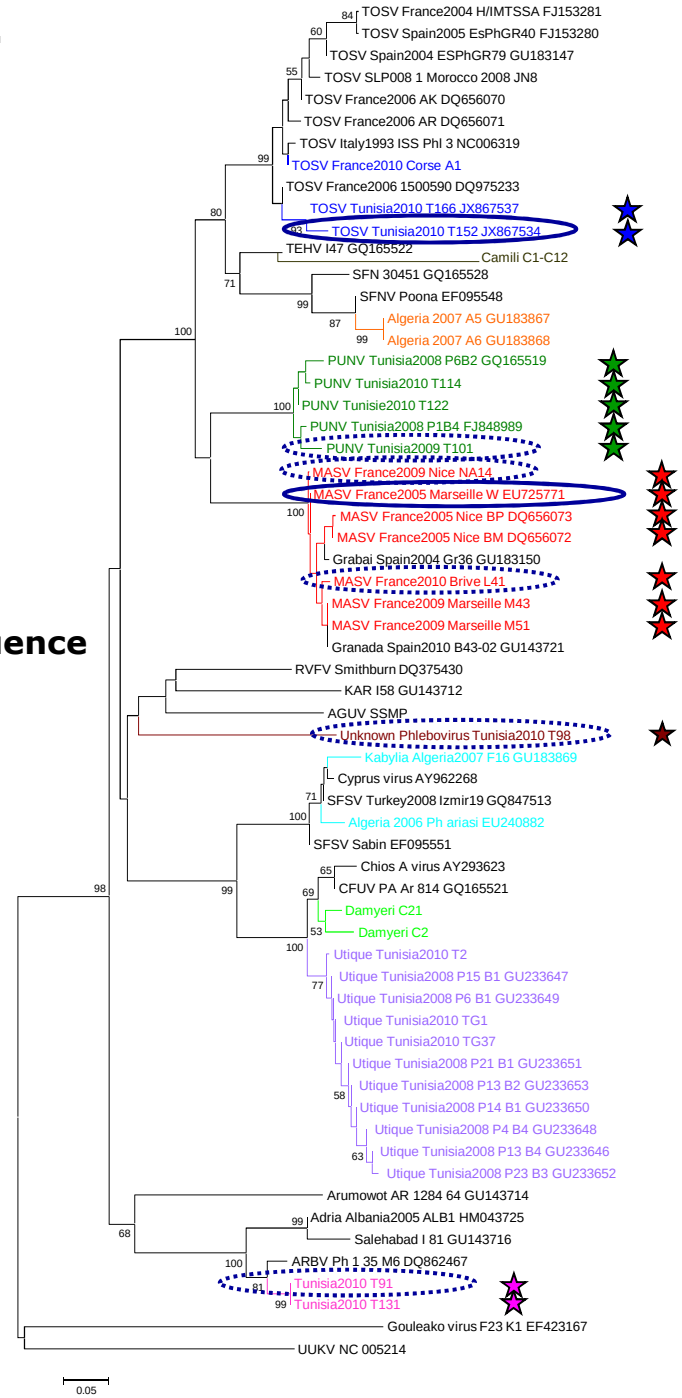
Sandfly-borne phleboviruses in the old world

Today



L-segment, AA

Now …



Isolated

Full genetic sequence

Done

Ongoing

L-segment, AA

TOSV

SFNV

PUNV

MASV

Sandfly Fever Naples Serocomplex

SFSV

Utique virus

Sandfly Fever Sicilian Serocomplex

ARBV

Salehabad Serocomplex

Complete genome sequence using Next Generation Sequencing

- ✓ To obtain **full genetic sequence** from isolated viruses
  - → Classic PCR = 1 year of work for 1 virus
  - → Full sequence of TOSV Tunisia = 1 NGS run
  - → but big amount of data to manage

- ✓ Better understanding of phleboviruses phylogeny with full-lengh genome sequences

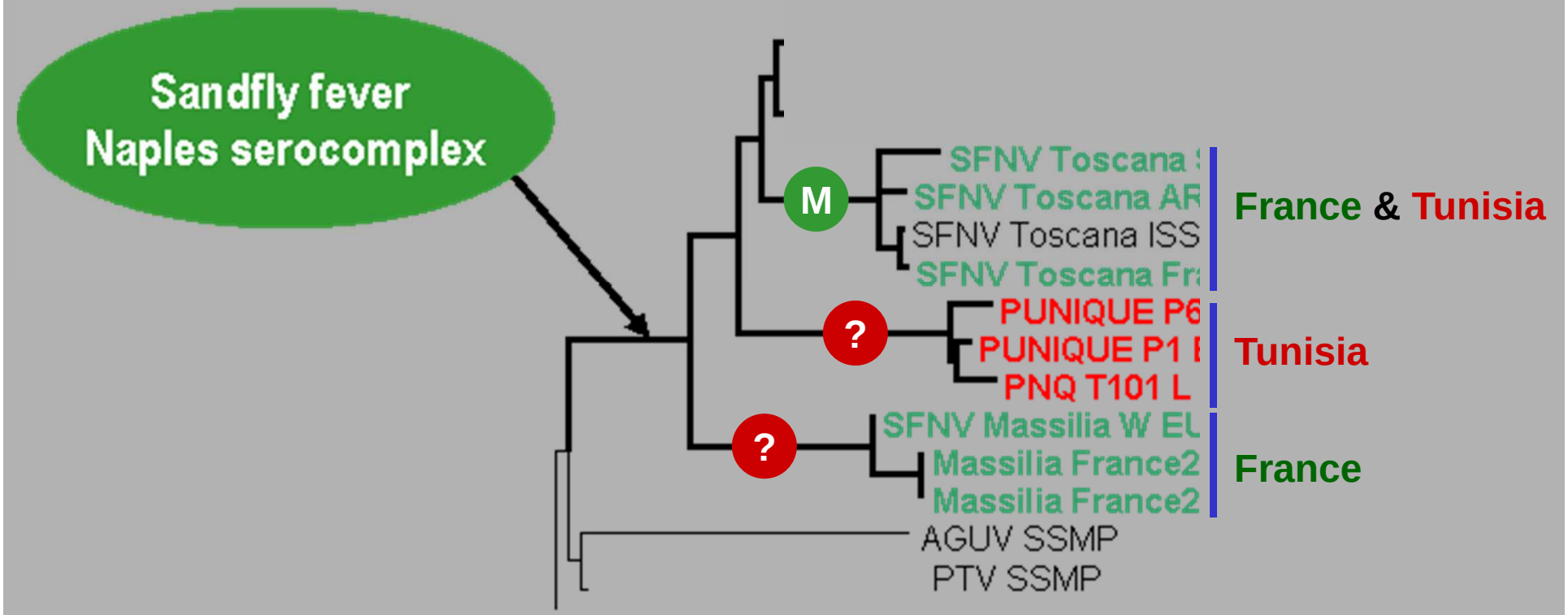
- ✓ … also a new tool for **direct microorganism discovery**!
  - → Directly using mix of insects
    - → e.g. : Paraiso Escondido, flavivirus in sandfly from Ecuador
  - → Possibility to sequence non isolated viruses

Question

- Co-circulation of different phleboviruses in the same geographic area raises the question of respective impact on human/animal health

  - seroprevalence studies with the capacity to discriminate between the different viruses (neutralization tests) because other tests are cross-reactive

    - Naples / Toscana / Tehran / Massilia / Punique / Granada
    - Sicilian / Utique / SFSV Turkey / SFSV Algeria



- How to distinguish different phleboviruses from each others using antibody studies ???

- The affinity / avidity of the antibodies is proportional to the antigenic closeliness of the virus: the more similar the viruses, the more cross-reactive the antibodies

- However techniques such as IF, ELISA are not discriminative enough
  - therefore the only possibility is to use Neutralisation tests

Toscana virus versus Punique virus in Tunisia

- Questions :
  - is Punique virus capable to infect humans ???
  - What are the respective proportions of seroprevalence against Toscana & Punique in humans

Toscana virus versus Punique virus in Tunisia

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- First step : Elisa tests
  - 1 273 human sera
  - From different regions of Tunisia
  - Tested by Elisa for the presence of antibodies against phleboviruses

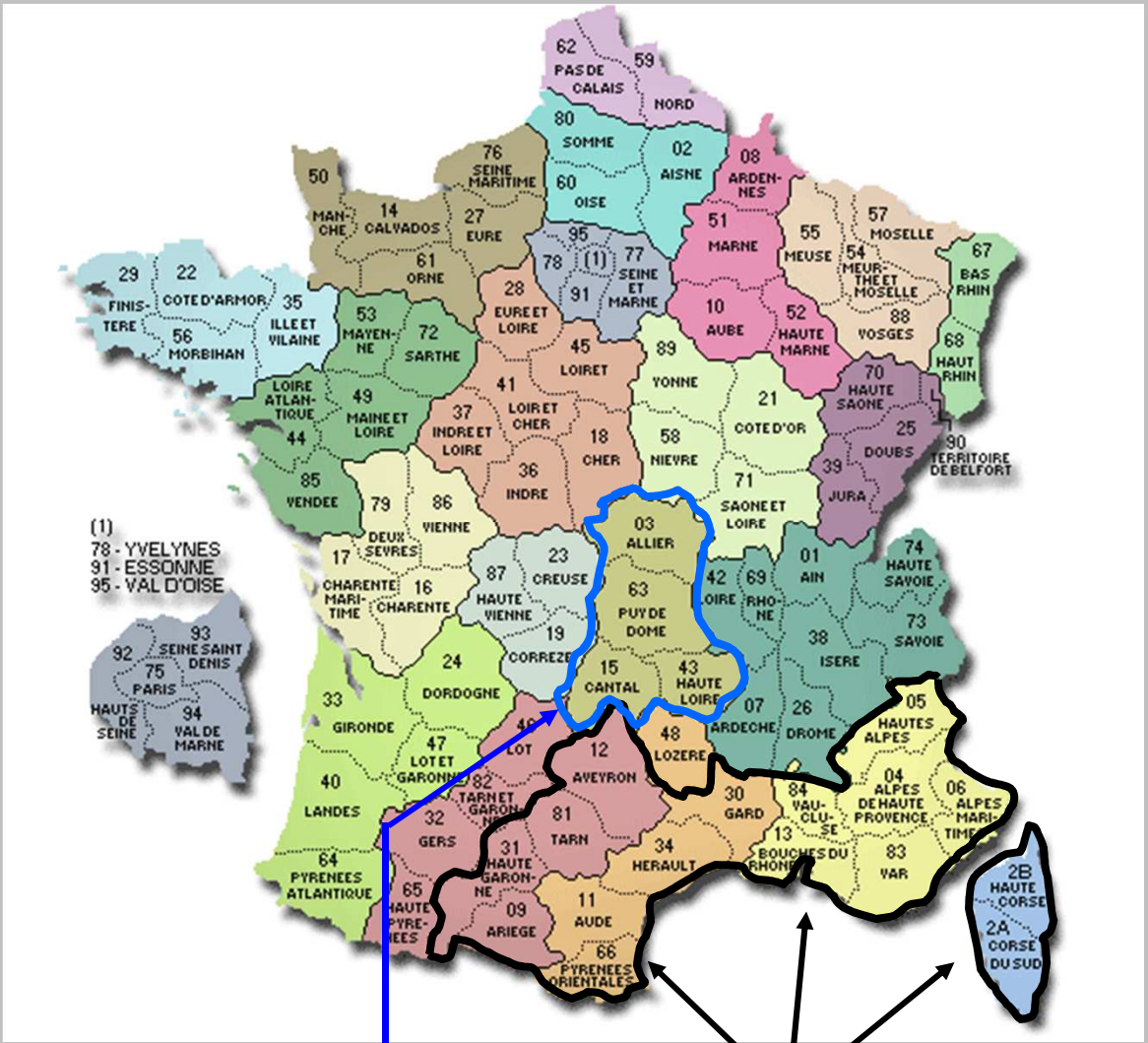
➡ 516 sera Elisa positive

In France

- ✓ Isolation of to viruses in south of France
  - → TOSV and MASV
  - → Distinct but antigenetically and genetically closely related
  - → Within the Sanfdly fever Naples serocomplex
- ✓ TOSV = recognised human pathogen
- ✓ MASV : no evidence suggesting that it is capable
  - → To infect humans
  - → To cause a disease

→ Seroprevalence study using comparative VNT
(i) To determine wether MASV is **able to infect humans**
(ii) to estimate and to compare the respective **involvement of TOSV and MASV in human infections** in South of France

ArboMED / PRIAM project: arboviruses in the Mediterranean
Risk perception of arboviral diseases in the Mediterranean



control region

studied regions

- Blood donor samples
- 3 study regions / 1 control region
- 14,000 sera collected in 2-weeks
- IgG seroprevalence
- tested for different arboviruses including phleboviruses
- tested for *Leishmania* Ab
- tested for saliva Ab

- collection period: Sept-Oct 2012

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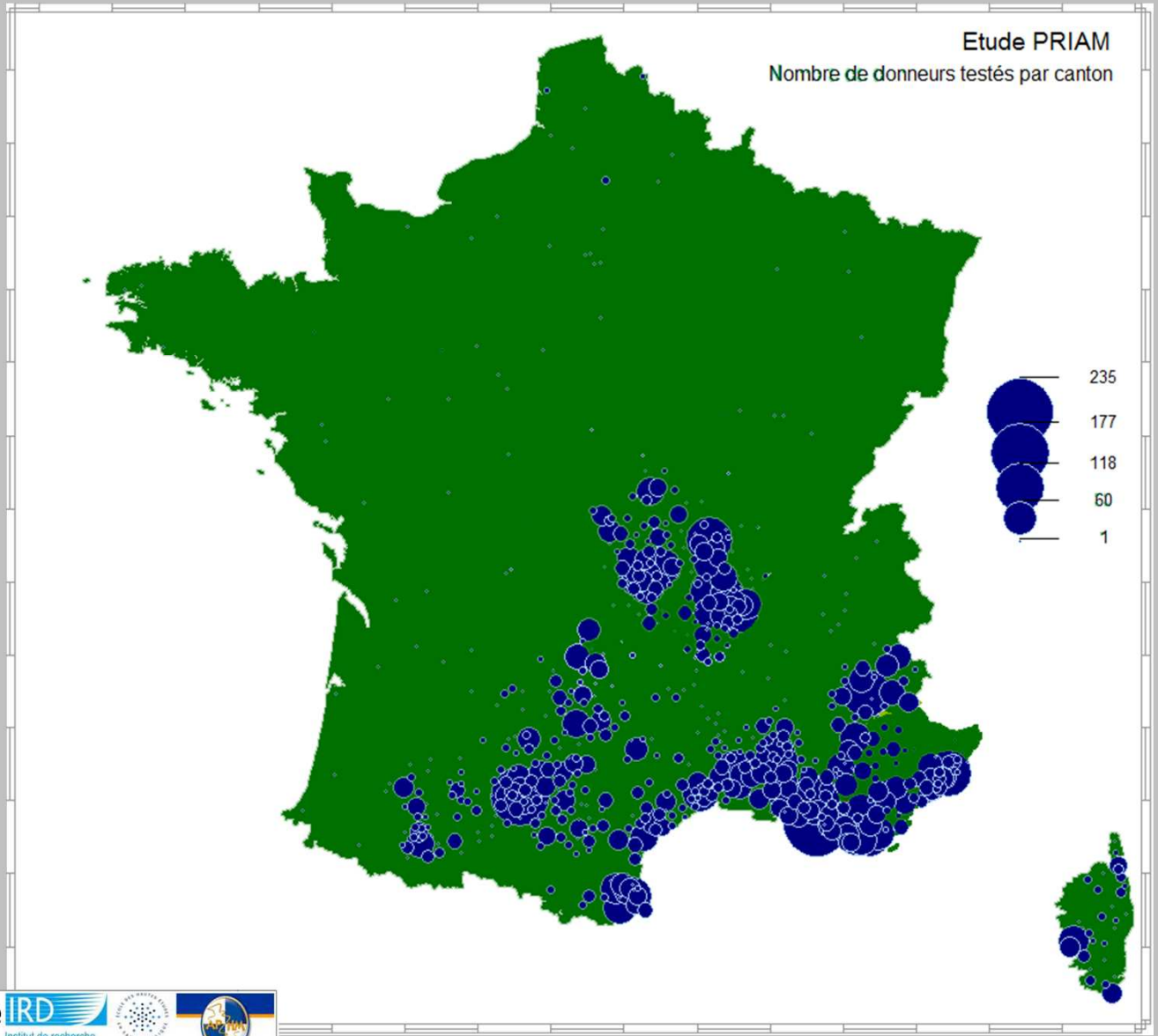
PRIAM / ArboMED project



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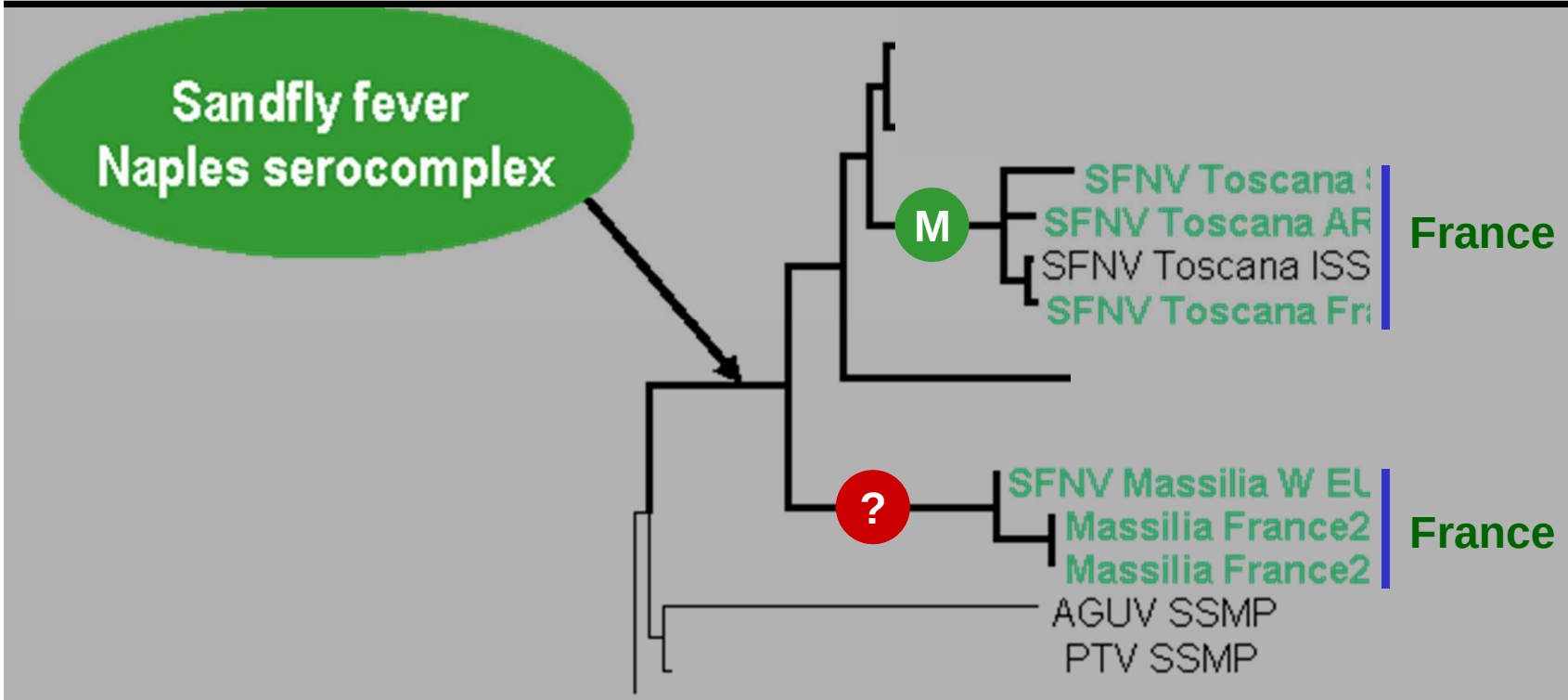
Same methodology applied for sera from France



- **Toscana virus and Massilia virus are present in France (Charrel et al *Emerg Infect Dis* 2007, Charrel et al *Vector borne zoonot dis* 2009)**
- **what is the respective role of Toscana virus and Massilia virus ???**
- **ELISA results**

-

Questions to be solved and Future directions

- Co-circulation of different phleboviruses in the same geographic area raises the question of respective impact on human/animal health

  - seroprevalence studies with the capacity to discriminate between the different viruses (neutralization tests) because other tests are cross-reactive

    - Naples / Toscana / Tehran / Massilia / Punique / Granada
    - Sicilian / Utique / SFSV Turkey / SFSV Algeria

- impact on human health ➔ specific PCR tests to be used in diagnostics in virology labs
  Massive sequencing using NGS is necessary to increase the number of sequences available in the databaess

- Continue and Extend virus discovery programs based on multidisciplinary teams (entomologists, ecologists, virologists, veterinarian, …)

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Thank you for your attention