





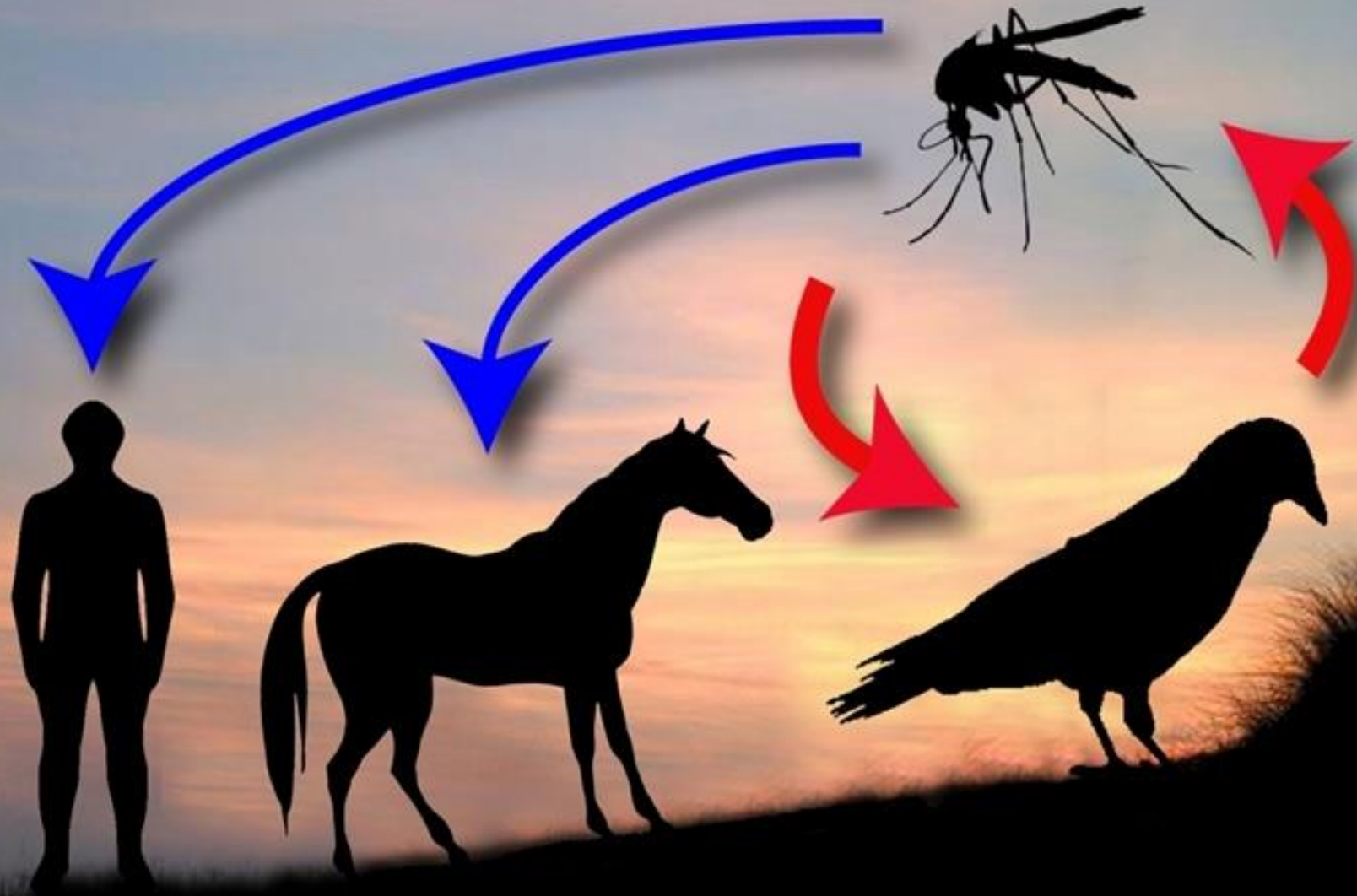
West Nile and Usutu virus – increased incidence in Europe

Team Norbert Nowotny

Institute of Virology
University of Veterinary Medicine Vienna

IPFA/PEI 26th International Workshop on Surveillance and Screening of Blood-Borne Pathogens, 22-23 May 2019, Krakow

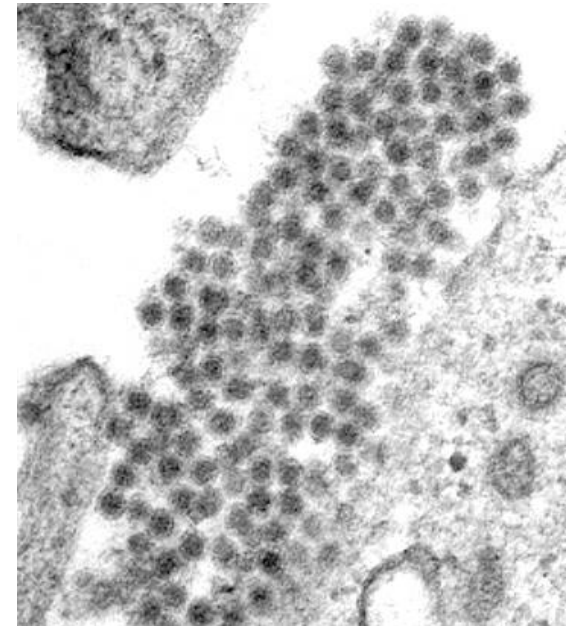
West Nile Virus Transmission Cycle



West Nile Virus

Family Flaviviridae, Genus *Flavivirus*

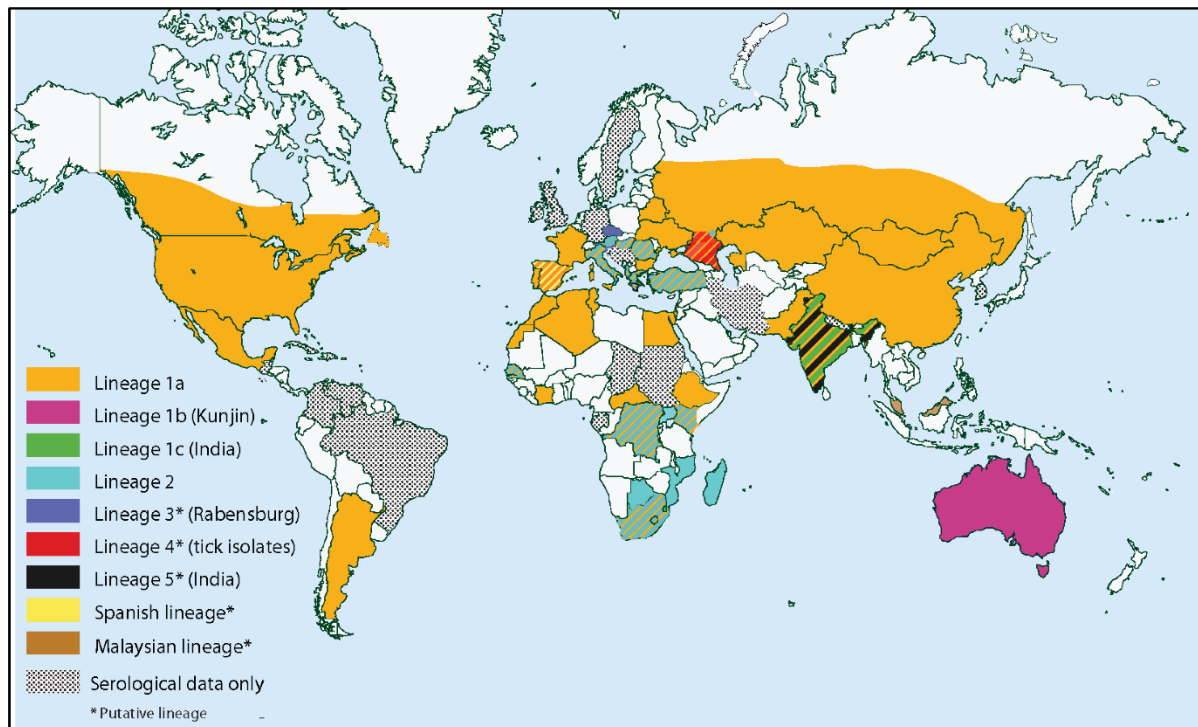
- ssRNA (positive-sense), ~11,000 nucleotides
- Human pathogens
 - Hemorrhagic fevers
 - Encephalitis
 - Febrile illness
- Three phylogenetic clusters
 - No known vector
 - Tick-borne
 - Mosquito-borne
 - Japanese encephalitis serocomplex
 - includes JEV, WNV
 - Other serocomplexes include YFV, DENV



West Nile virus epidemiology - brief history



- First isolated in Uganda in 1937
- West Nile virus (WNV), a member of the Japanese encephalitis virus (JEV) group within the genus *Flavivirus*, is the most widespread flavivirus, occurring in Africa, Europe, Asia, Australia & America
- Up to 9 different genetic lineages are known to date, however only lineage 1 and 2 are known to be pathogenic for humans.



World distribution of flaviviruses



World-wide:

Courtesy Dr. Tamás Bakonyi

WNV Neuroinvasive Disease Incidence, by County, US, 1999

n=54

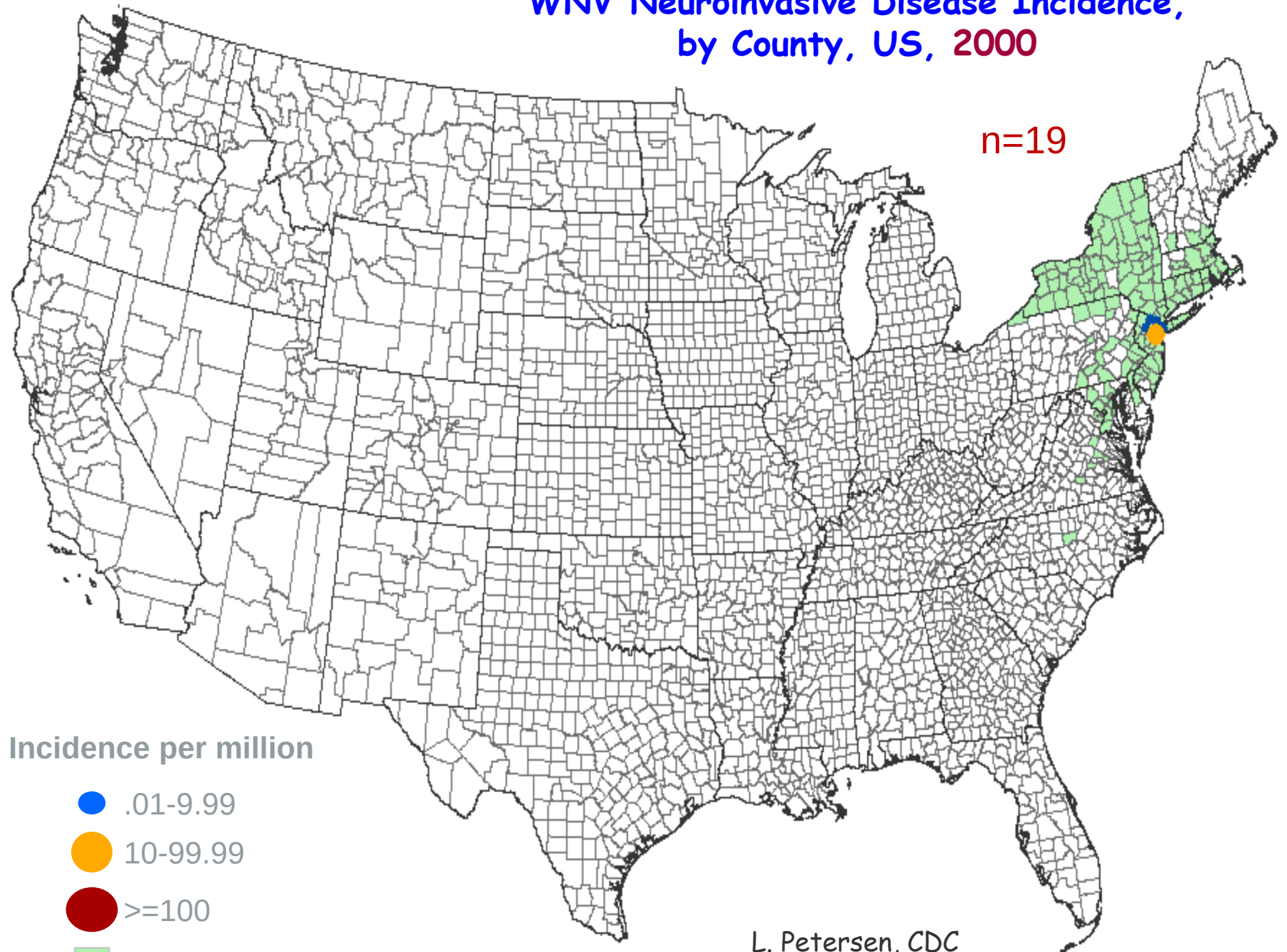
Incidence per million



L. Petersen, CDC

WNV Neuroinvasive Disease Incidence, by County, US, 2000

n=19



L. Petersen, CDC

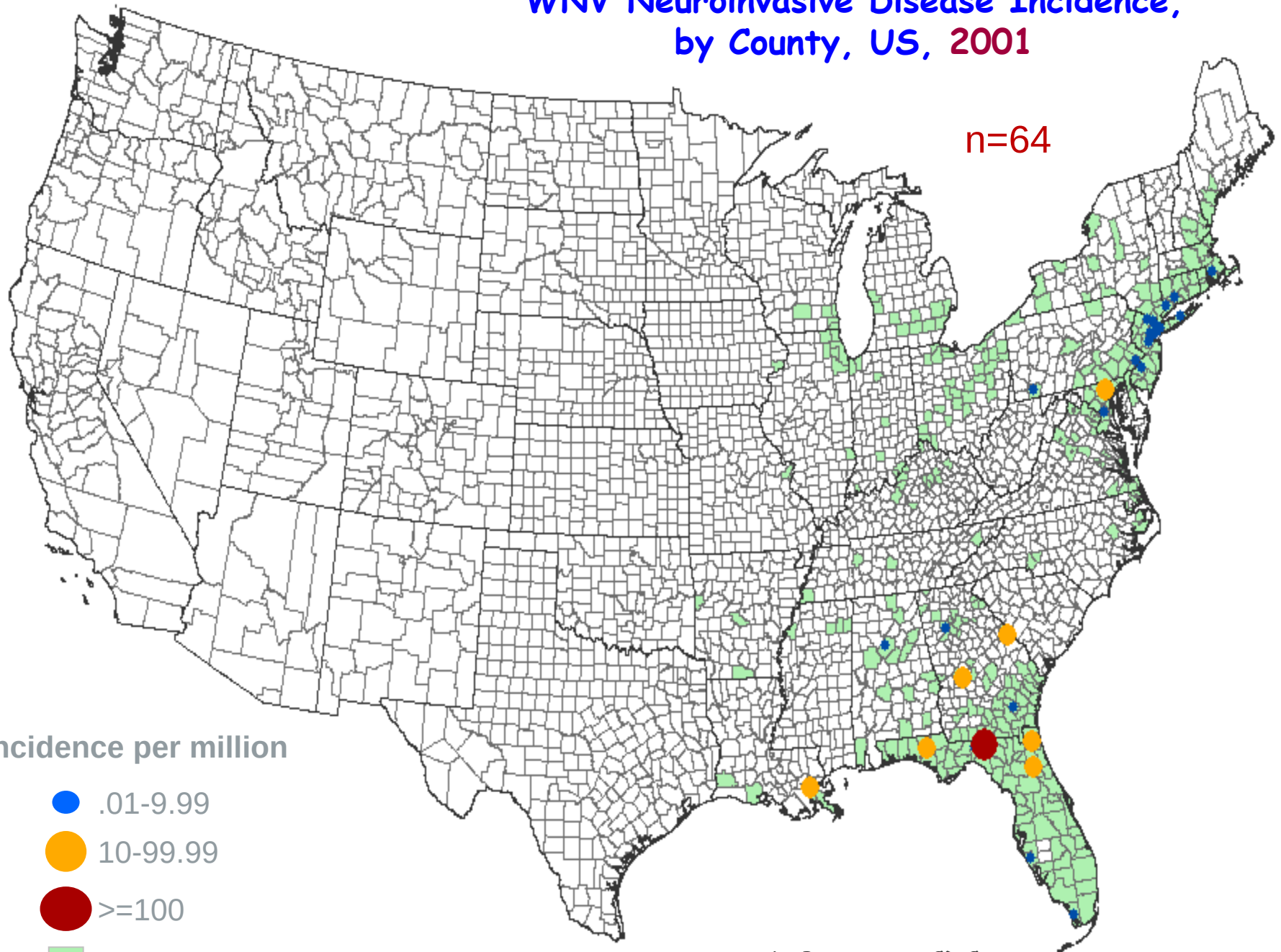
WNV Neuroinvasive Disease Incidence, by County, US, 2001

n=64

Incidence per million



Any WNV activity



L. Petersen, CDC

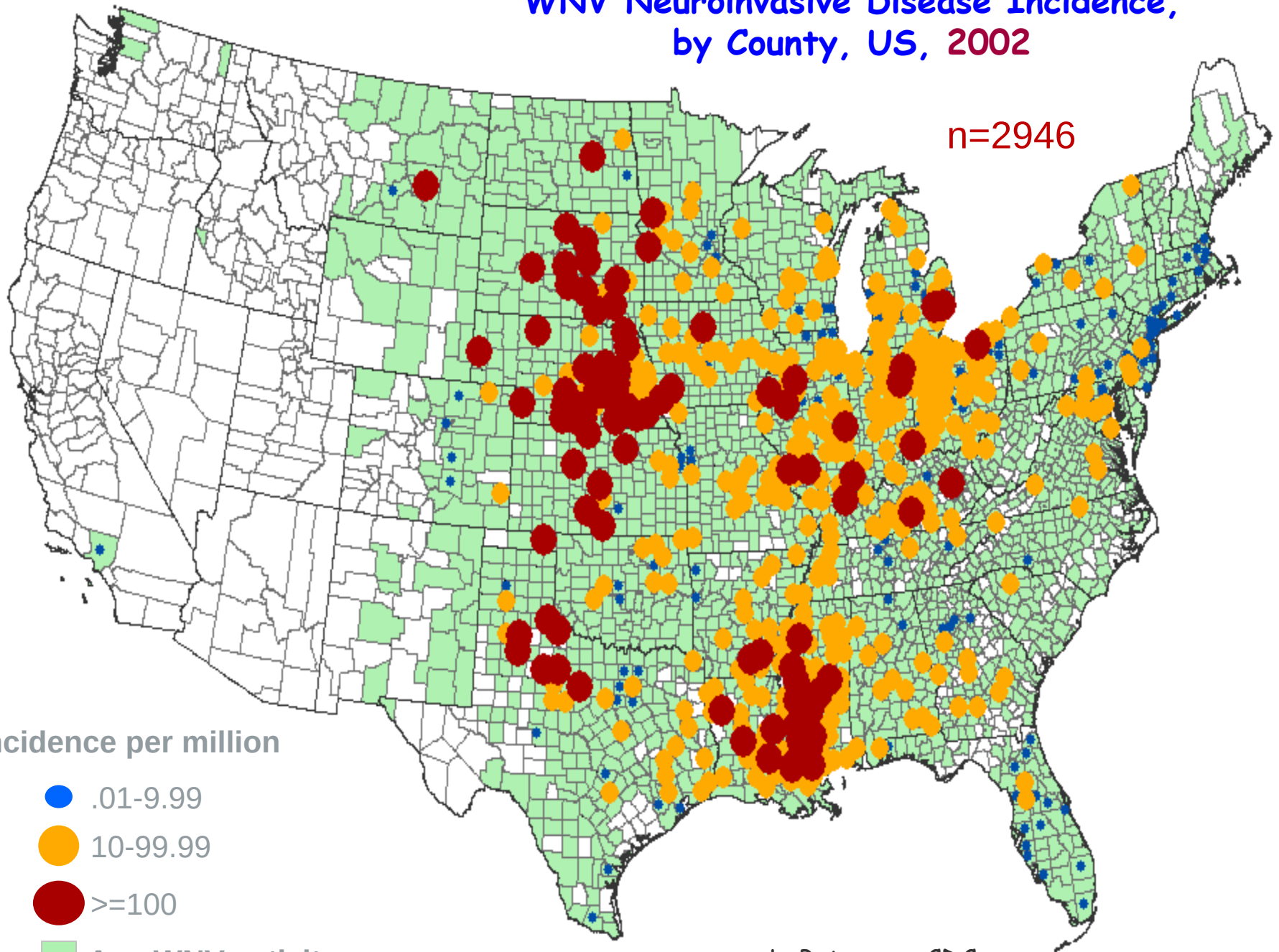
WNV Neuroinvasive Disease Incidence, by County, US, 2002

n=2946

Incidence per million



Any WNV activity



L. Petersen, CDC

WNV Neuroinvasive Disease Incidence, by County, US, 2003

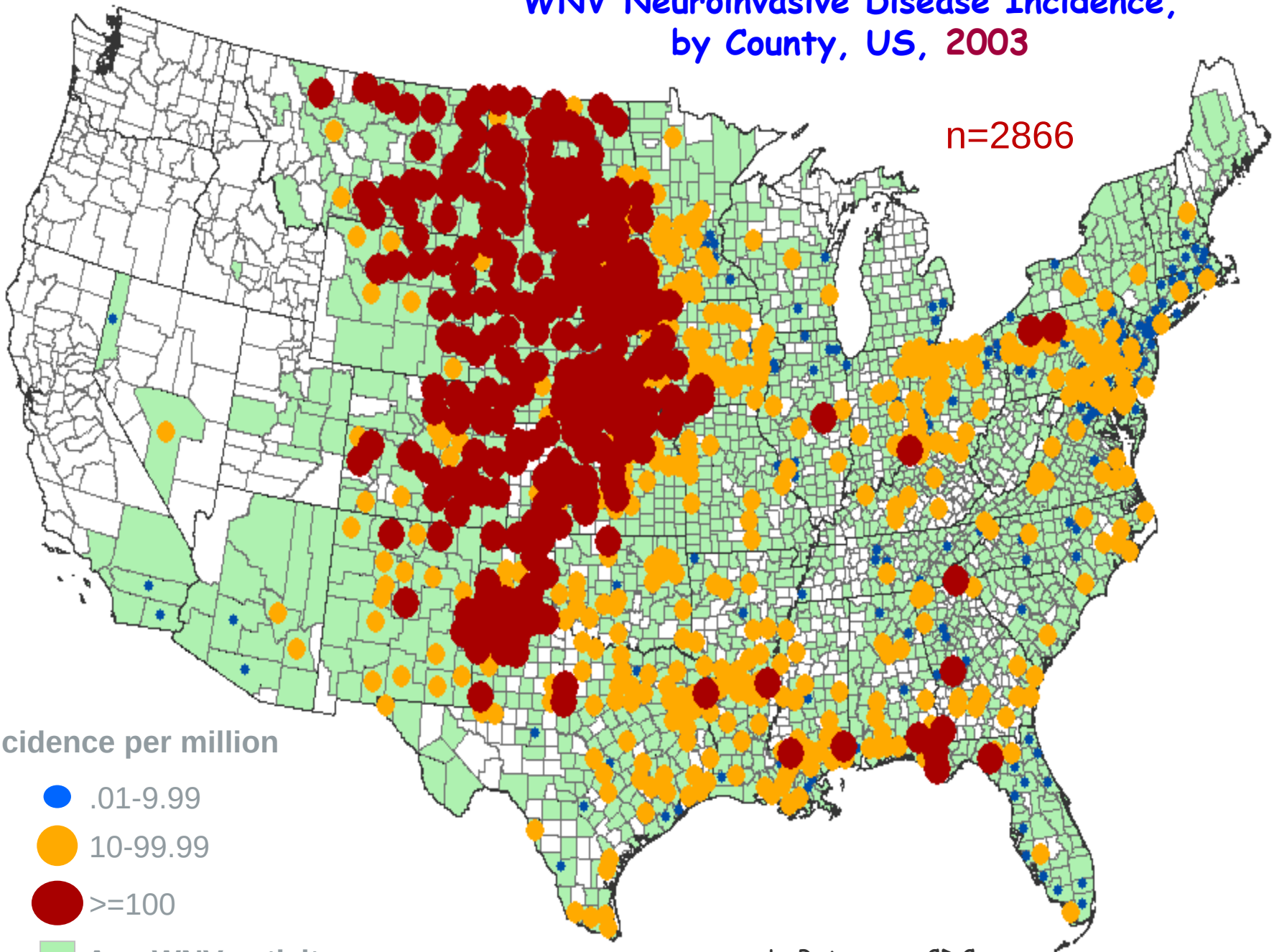
n=2866

Incidence per million



Any WNV activity

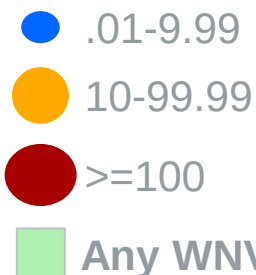
L. Petersen, CDC



WNV Neuroinvasive Disease Incidence, by County, US, 2004

n=1142

Incidence per million



L. Petersen, CDC

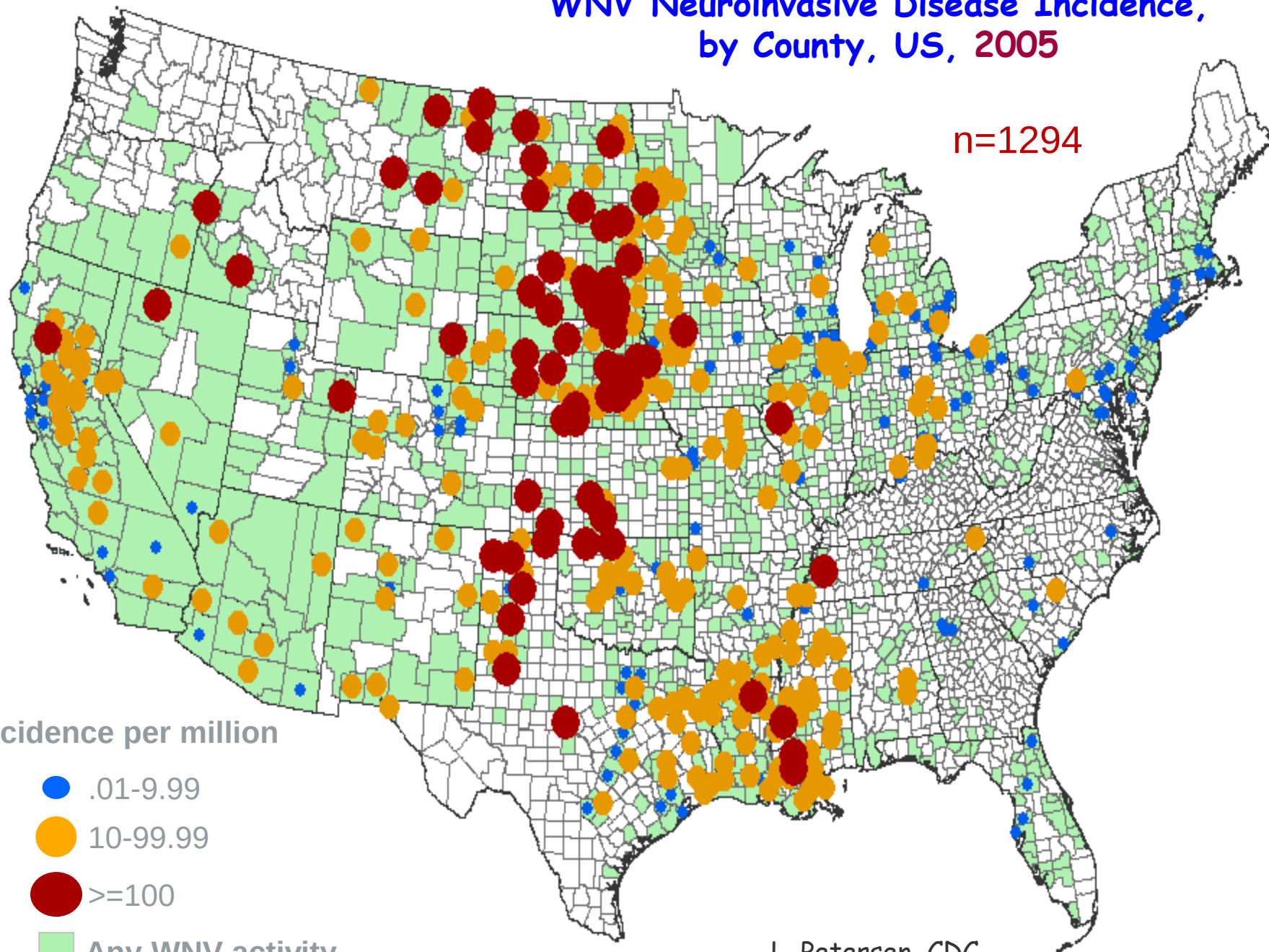
WNV Neuroinvasive Disease Incidence, by County, US, 2005

n=1294

Incidence per million



Any WNV activity



L. Petersen, CDC

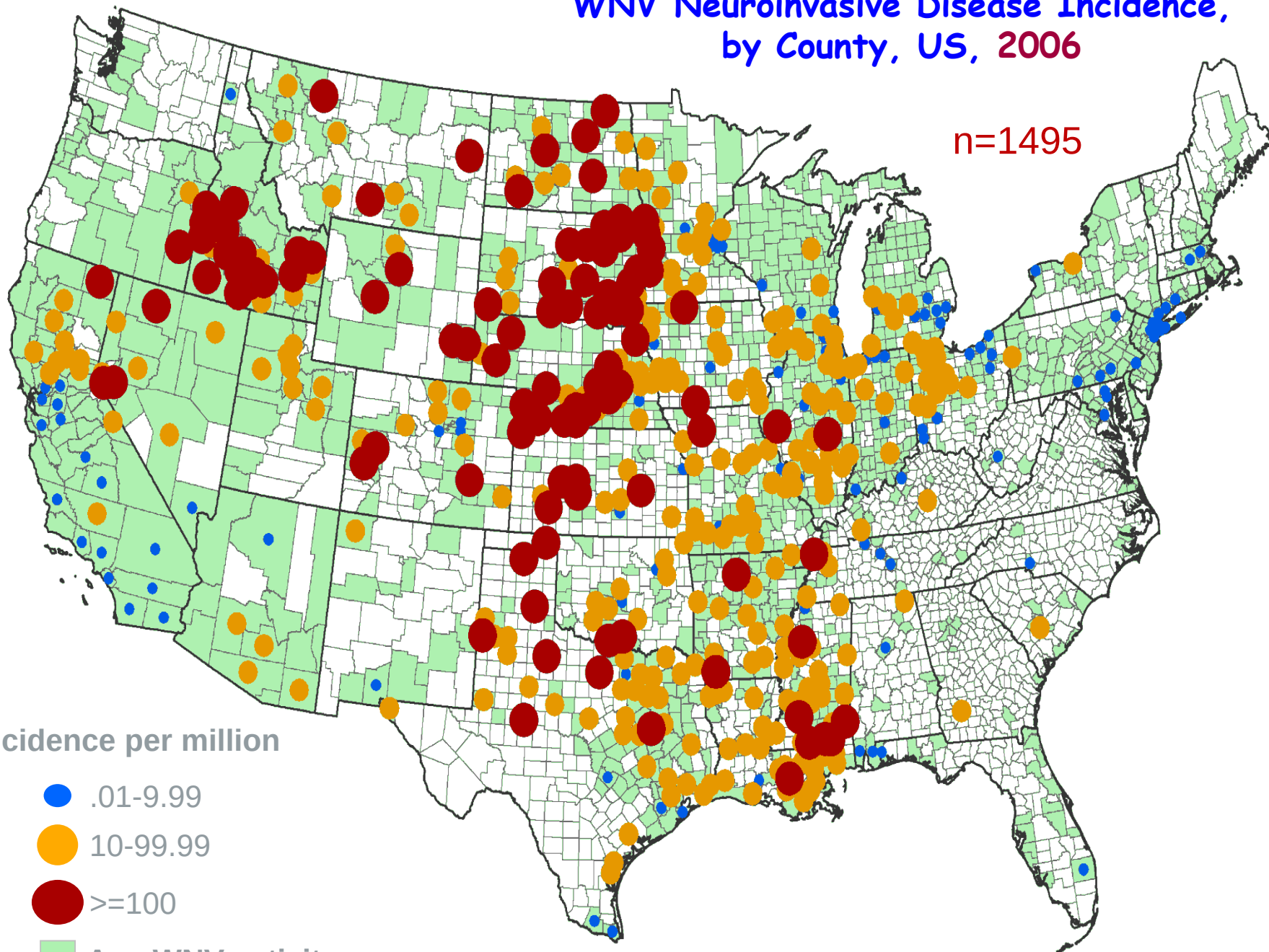
WNV Neuroinvasive Disease Incidence, by County, US, 2006

n=1495

Incidence per million

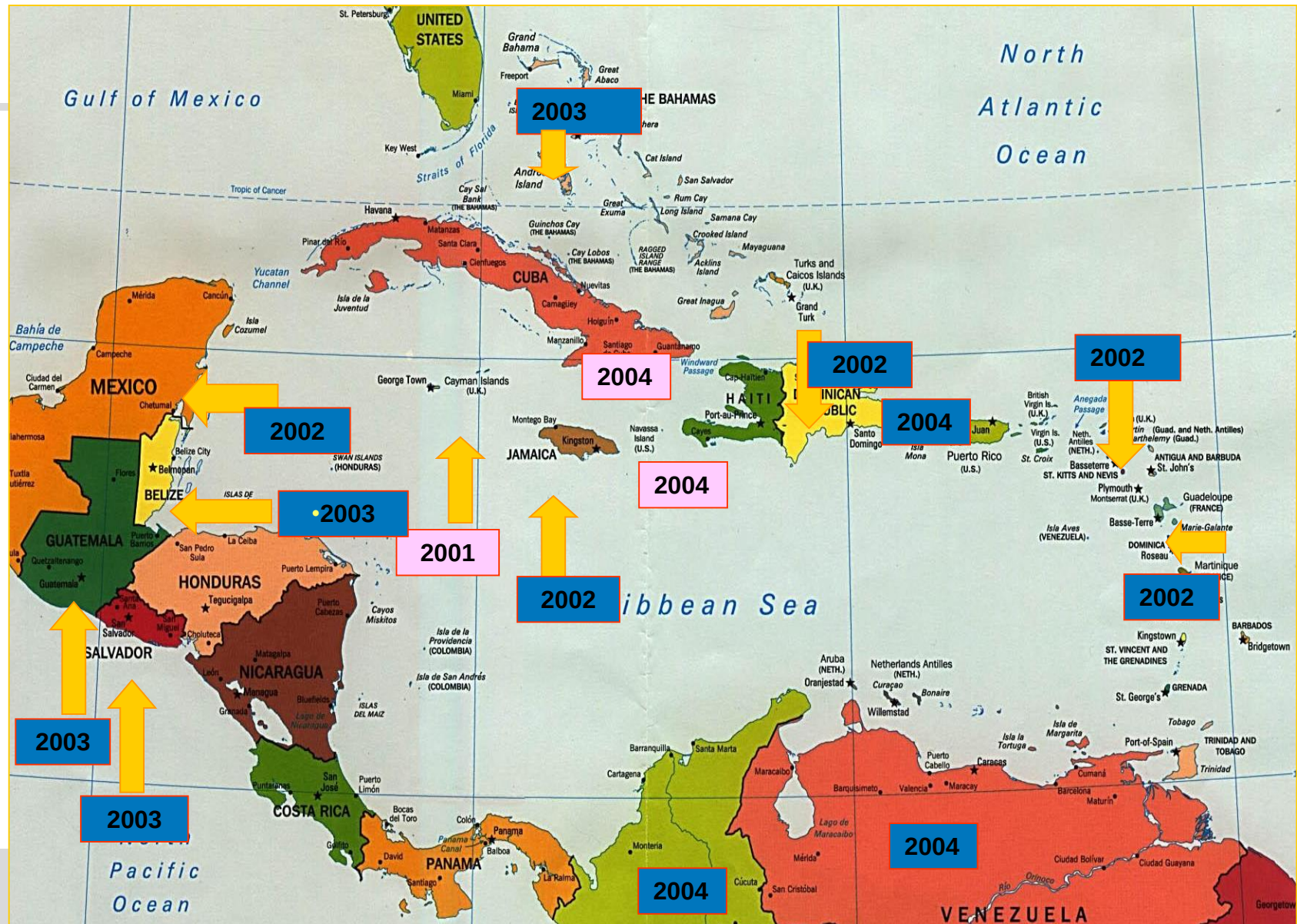


Any WNV activity



L. Petersen, CDC

West Nile Virus in Latin America



Hungary

2003 encephalitis in
geese + human cases



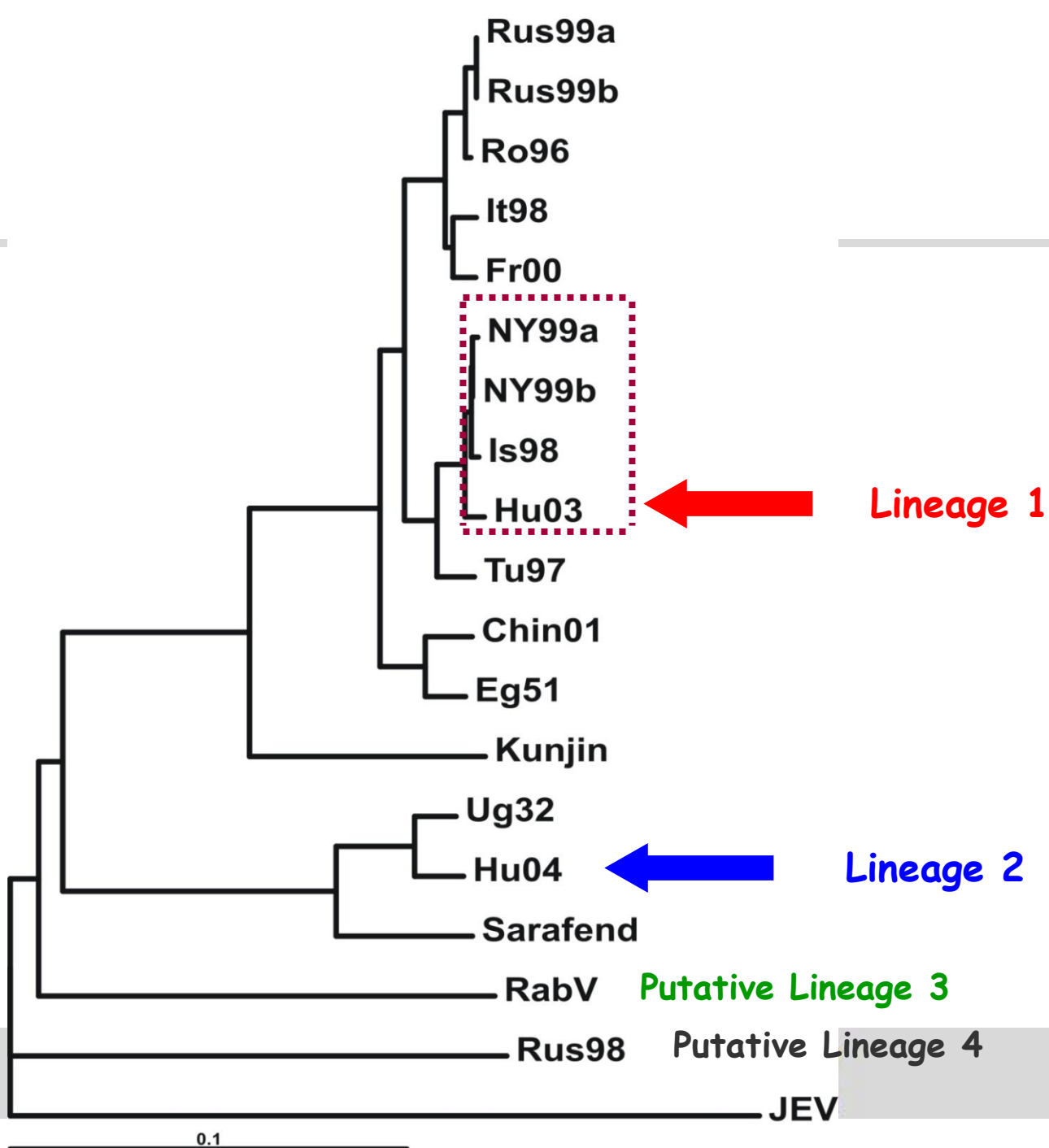
2004 and 2005
encephalitis in
birds of prey



Birds of prey, 2004 & 2005

- In August 2004, a goshawk (*Accipiter gentilis*) fledgling showed CNS symptoms and died in a national park in south-eastern Hungary (in very close proximity to the 2003 cases in geese)
- Surprisingly, sequencing of the whole genome revealed a lineage 2 WNV, which was until that restricted to sub-Saharan Africa, and was never before detected in Europe.





RESEARCH

Lineage 1 and 2 Strains of Encephalitic West Nile Virus, Central Europe

Tamás Bakonyi,^{*†} Éva Ivanics,[‡] Károly Erdélyi,[‡] Krisztina Ursu,[‡] Emöke Ferenczi,[§]
Herbert Weissenböck,^{*} and Norbert Nowotny^{*¶}

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 12, No. 4, April 2006



Bird Migration Via the Middle East & Mediterranean



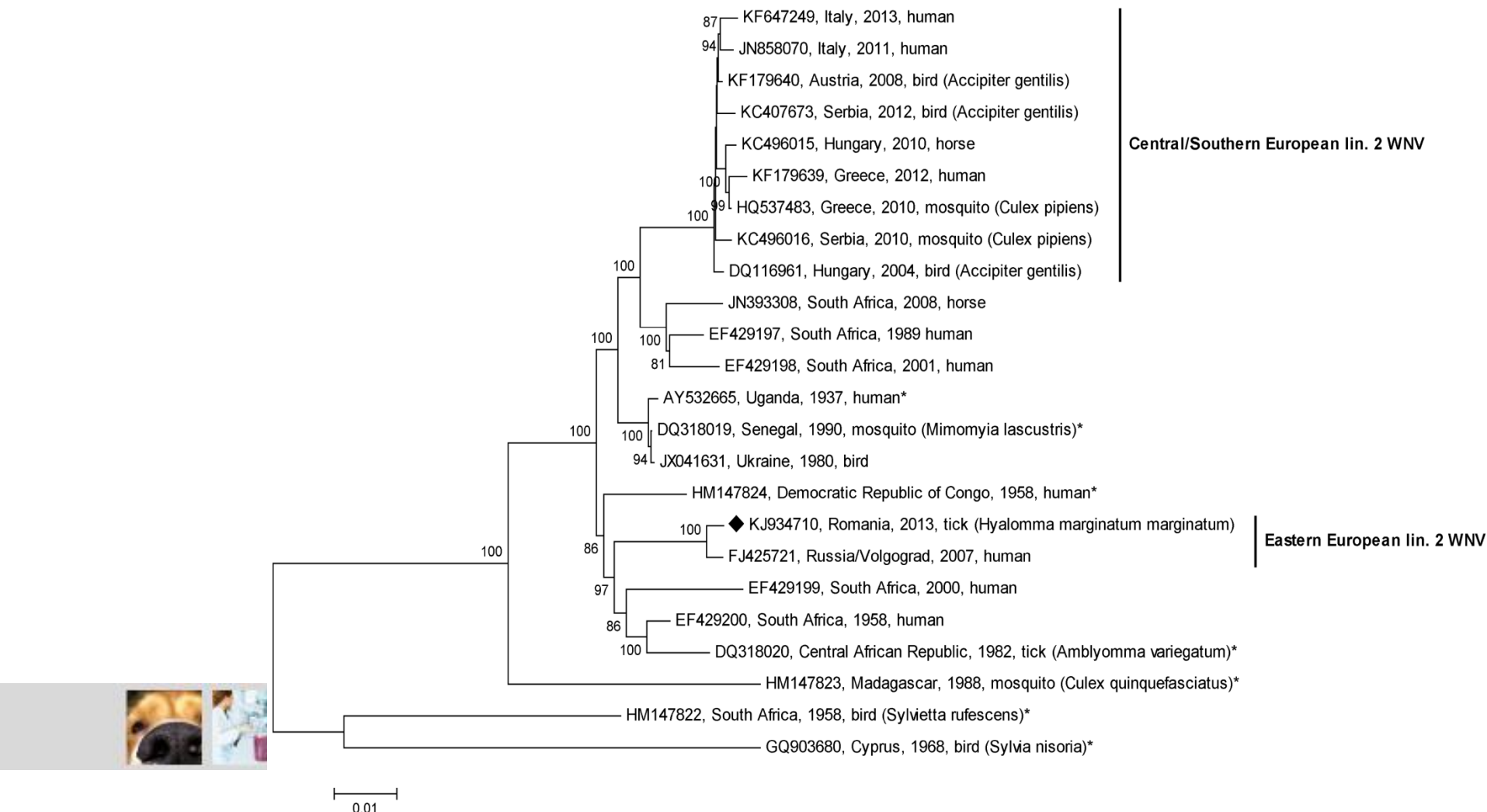
Source: Dr. Yossi Leshem

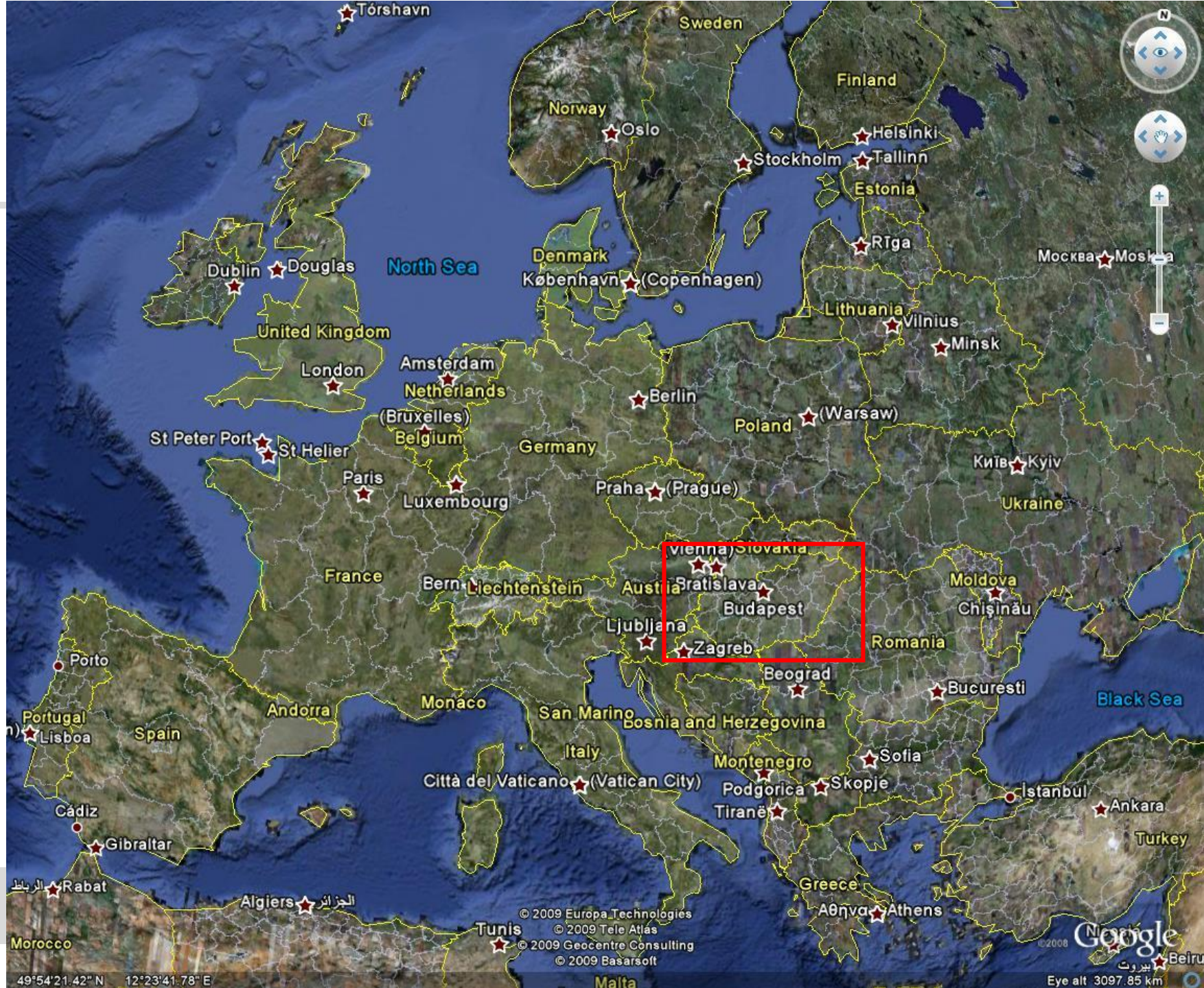


The Complete Sequence of a West Nile Virus Lineage 2 Strain Detected in a *Hyalomma marginatum marginatum* Tick Collected from a Song Thrush (*Turdus philomelos*) in Eastern Romania in 2013 Revealed Closest Genetic Relationship to Strain Volgograd 2007

Jolanta Kolodziejek¹, Mihai Marinov², Botond J. Kiss², Vasile Alexe², Norbert Nowotny^{1,3*}

¹ Viral Zoonoses, Emerging and Vector-Borne Infections Group, Institute of Virology, University of Veterinary Medicine Vienna, Vienna, Austria, ² Danube Delta National Institute for Research and Development, Tulcea, Romania, ³ Department of Microbiology and Immunology, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Oman

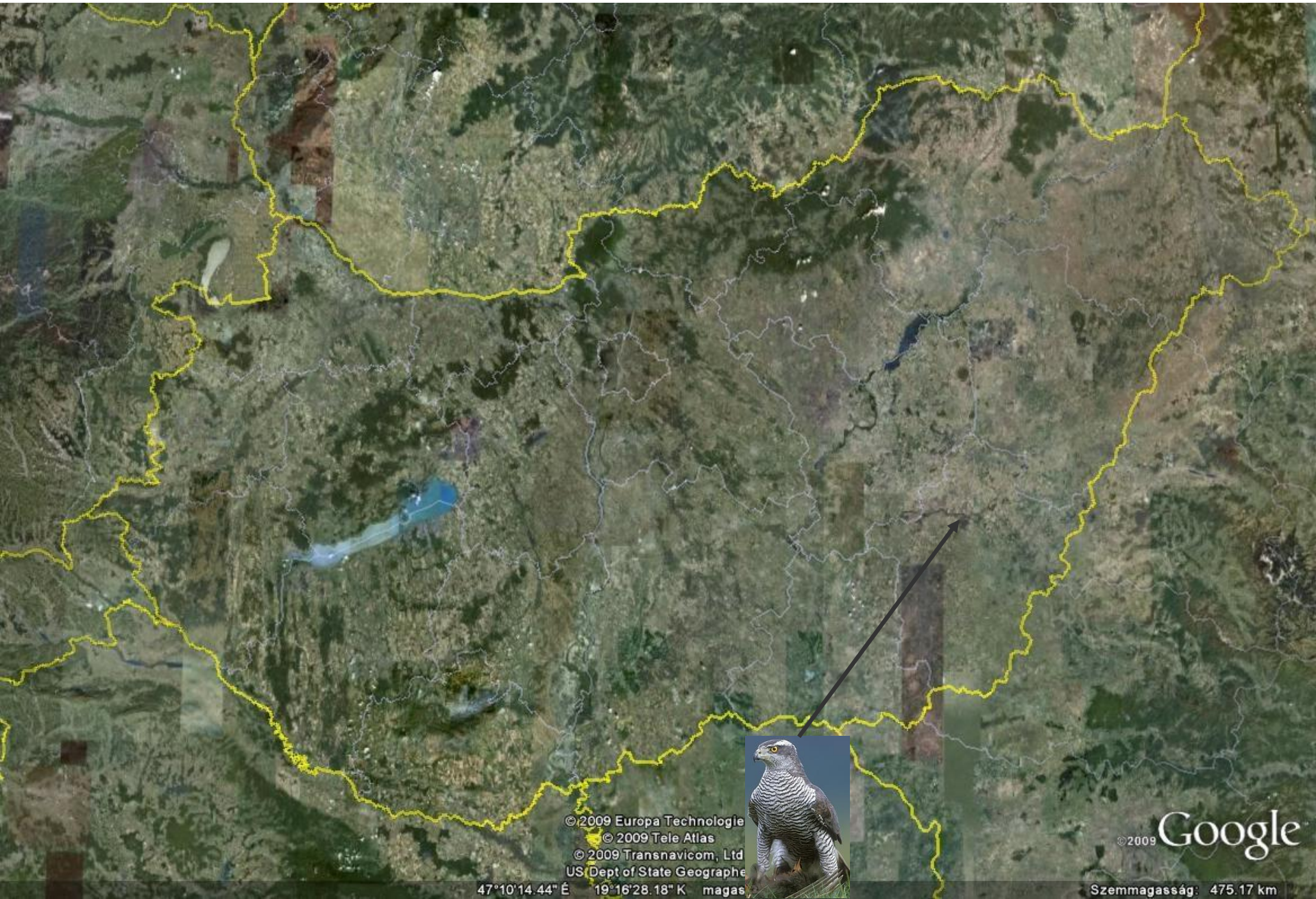




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Google

Eye alt 3097.85 km

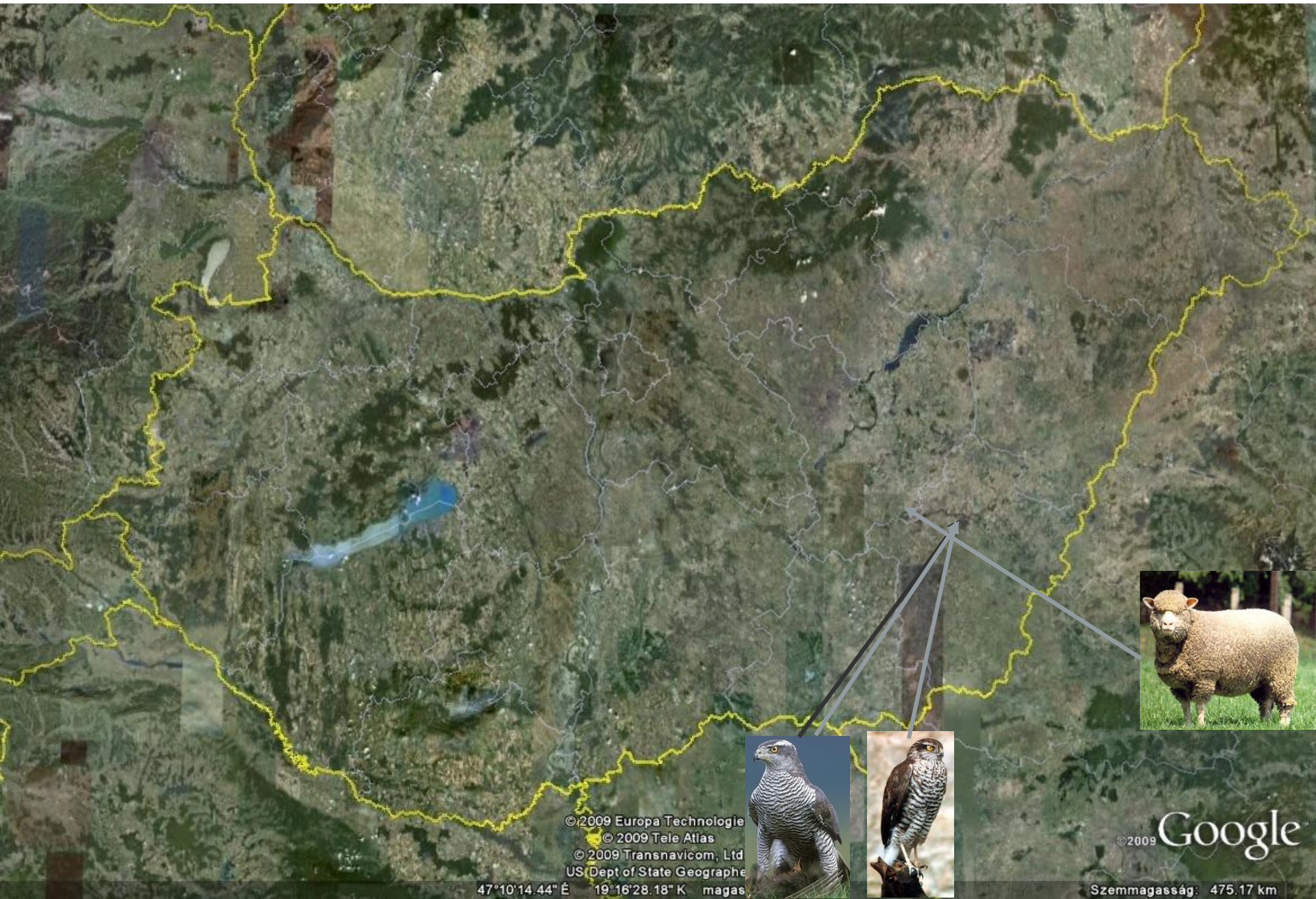


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US Dept of State Geographer

47°10'14.44" É 19°16'28.18" K magas

© 2009 Google

Szemmagasság: 475.17 km



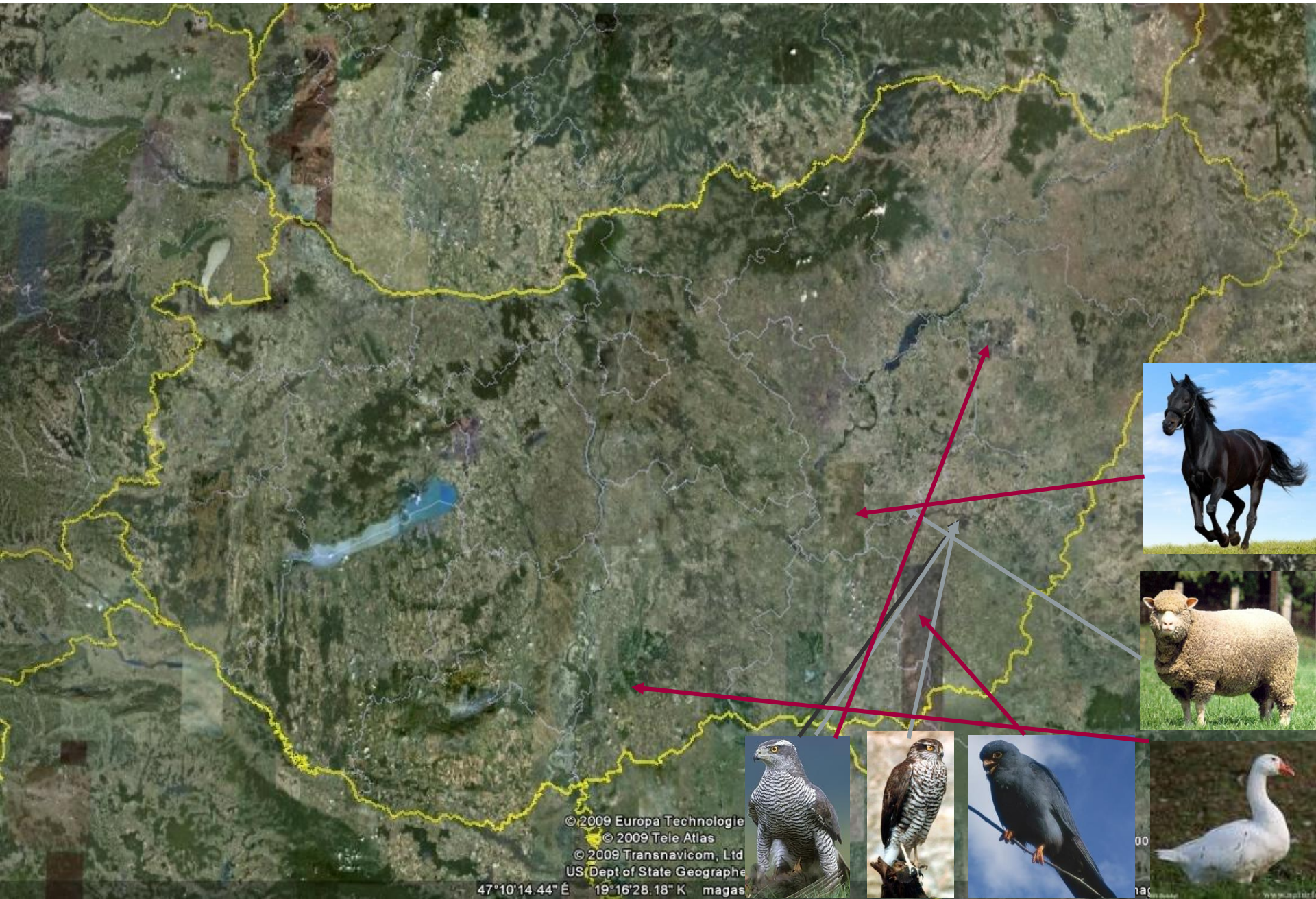
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47°10'14.44" É 19°16'28.18" K magas

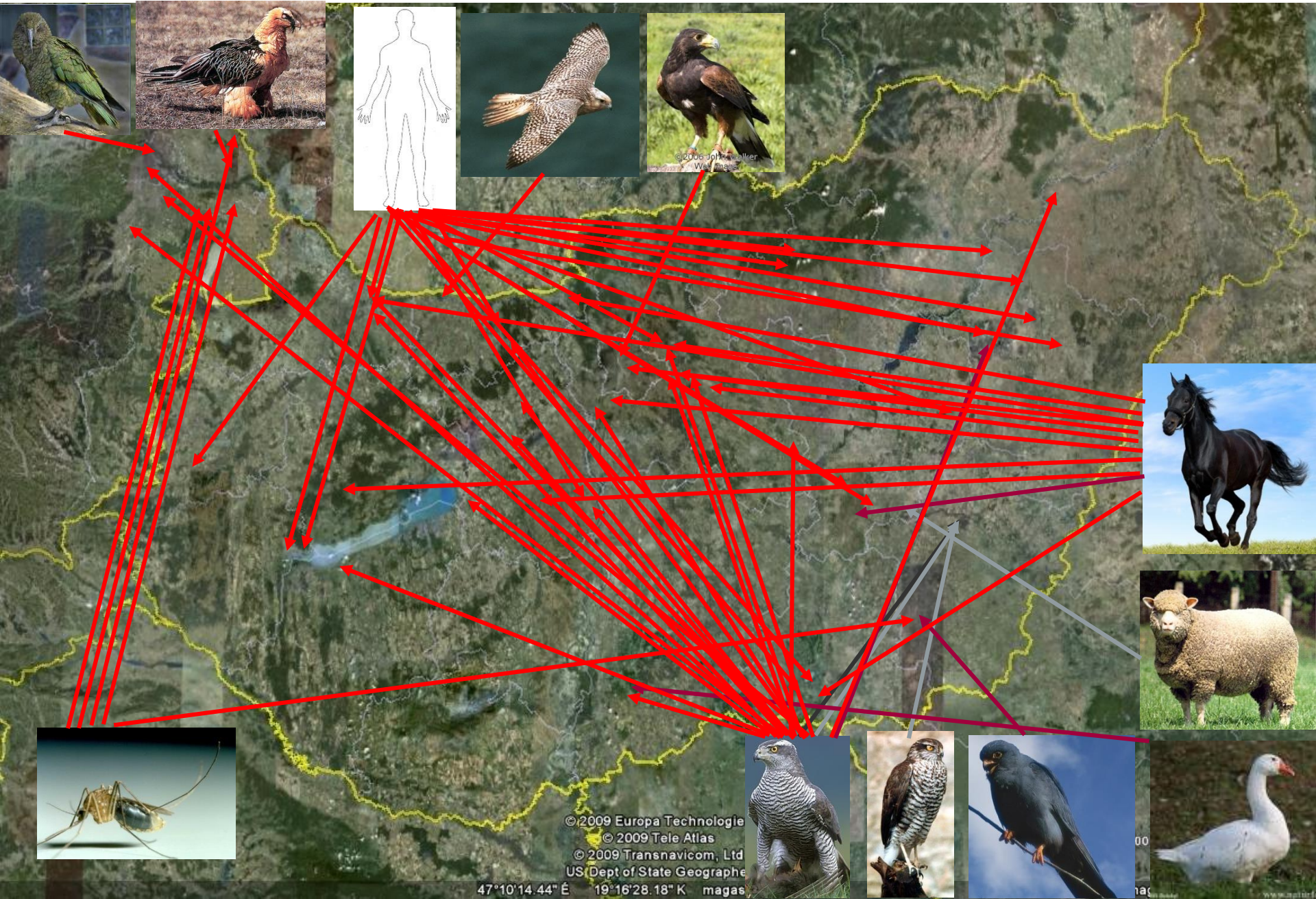
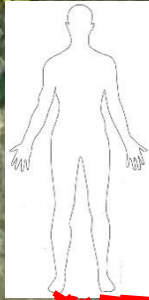
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Szemmagasság: 475.17 km

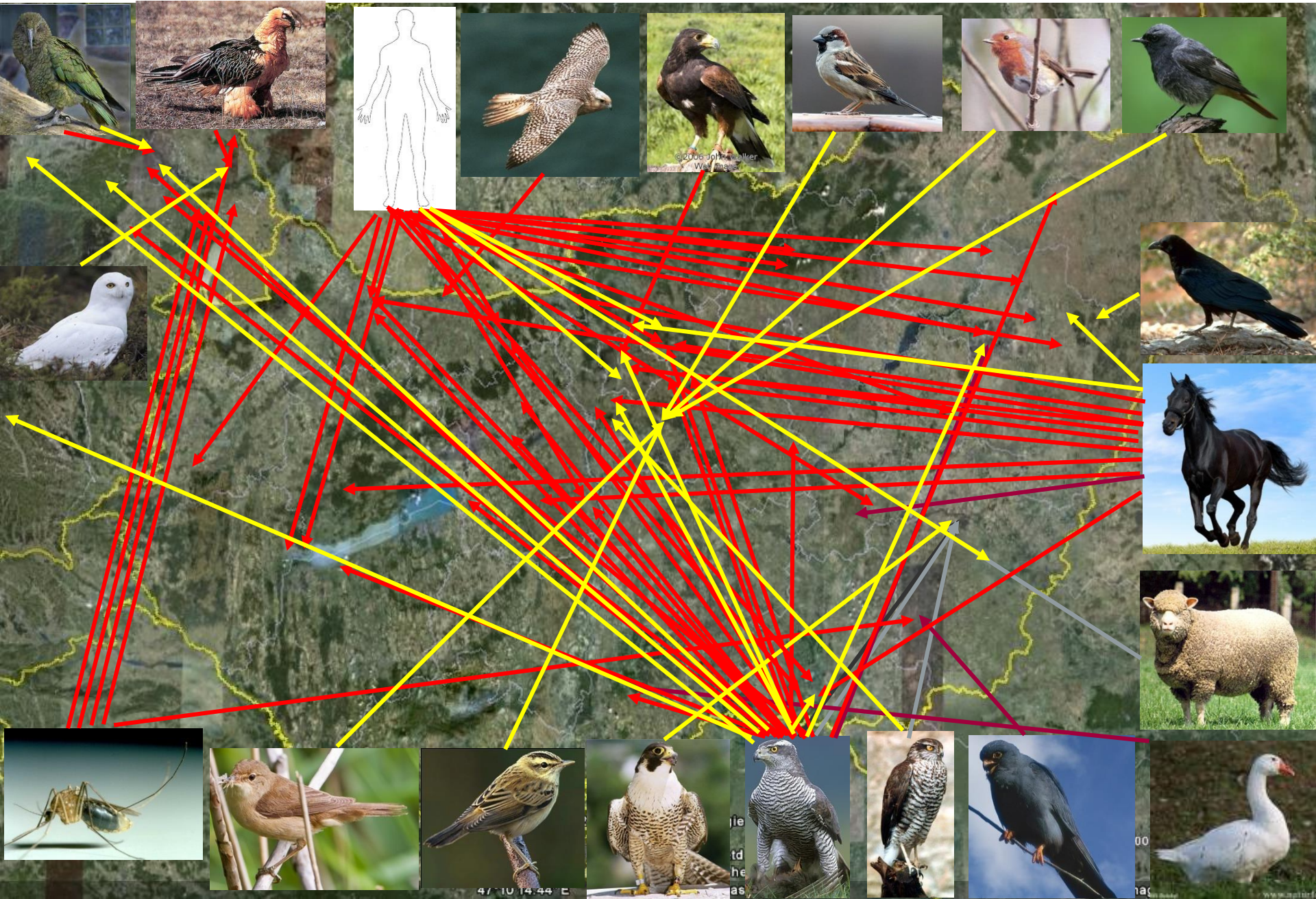
WNV cases 2004-2005-2007



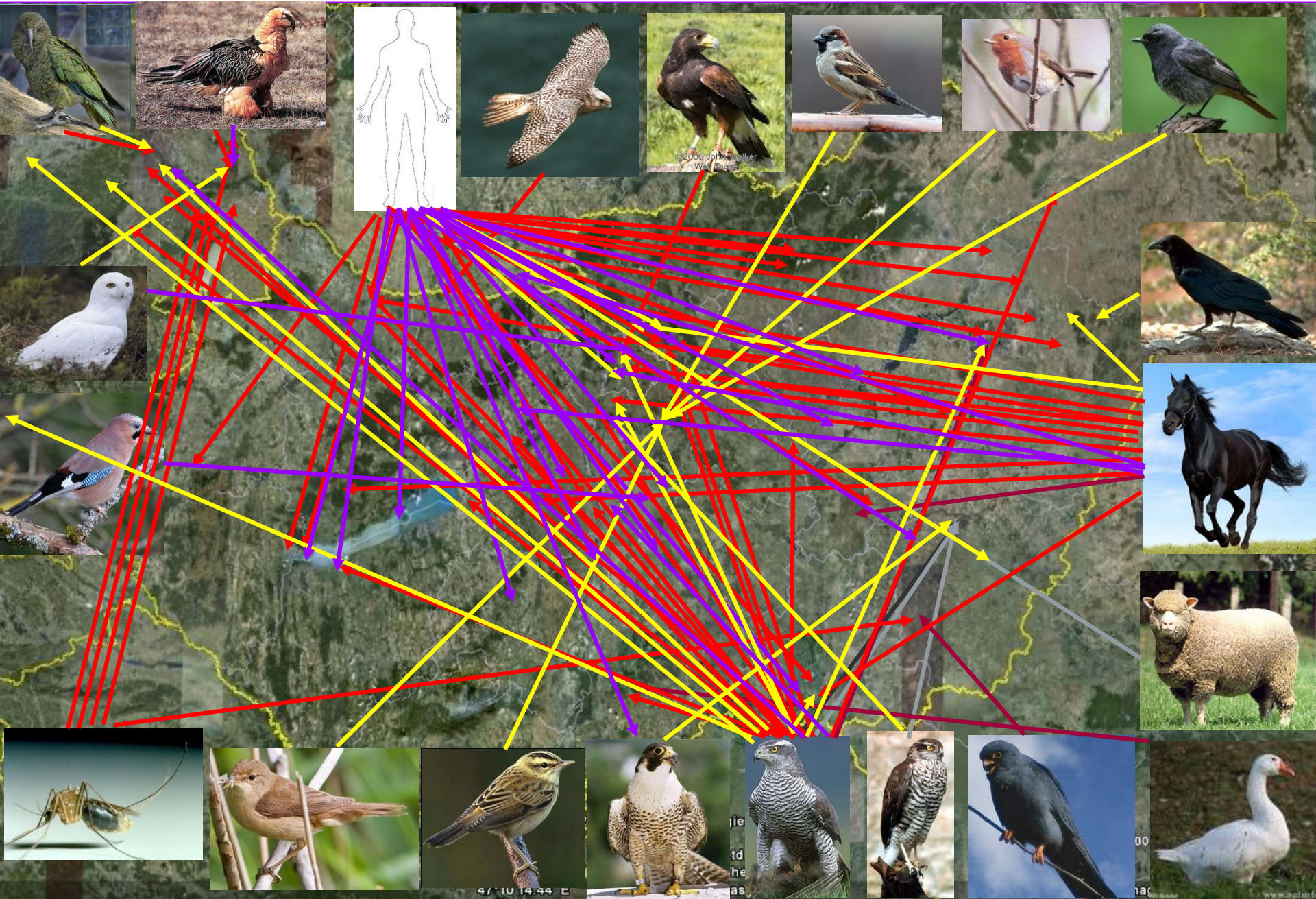
WNV cases 2004-2005-2007-2008

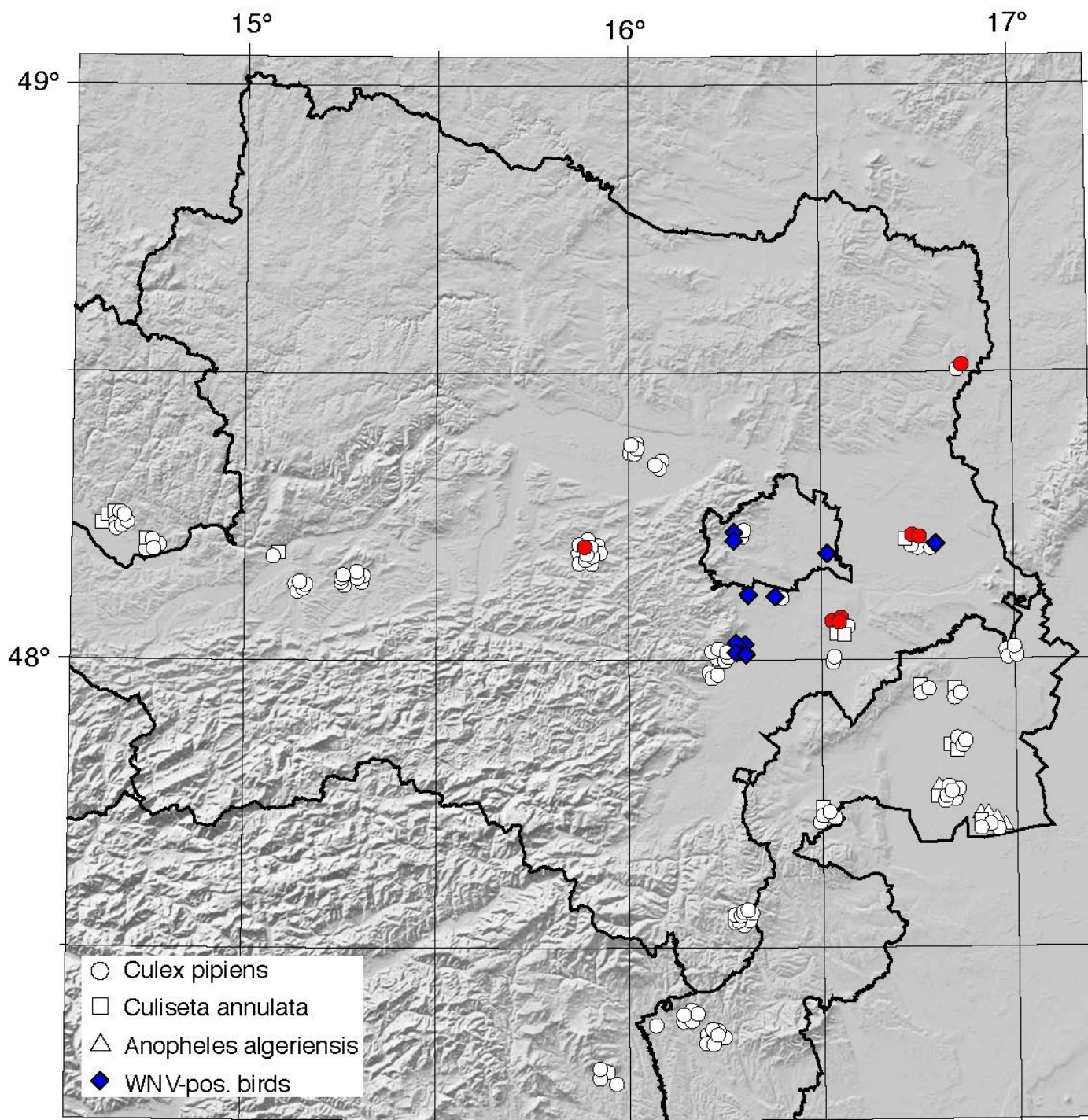


WNV cases 2004-2005-2007-2008-2009



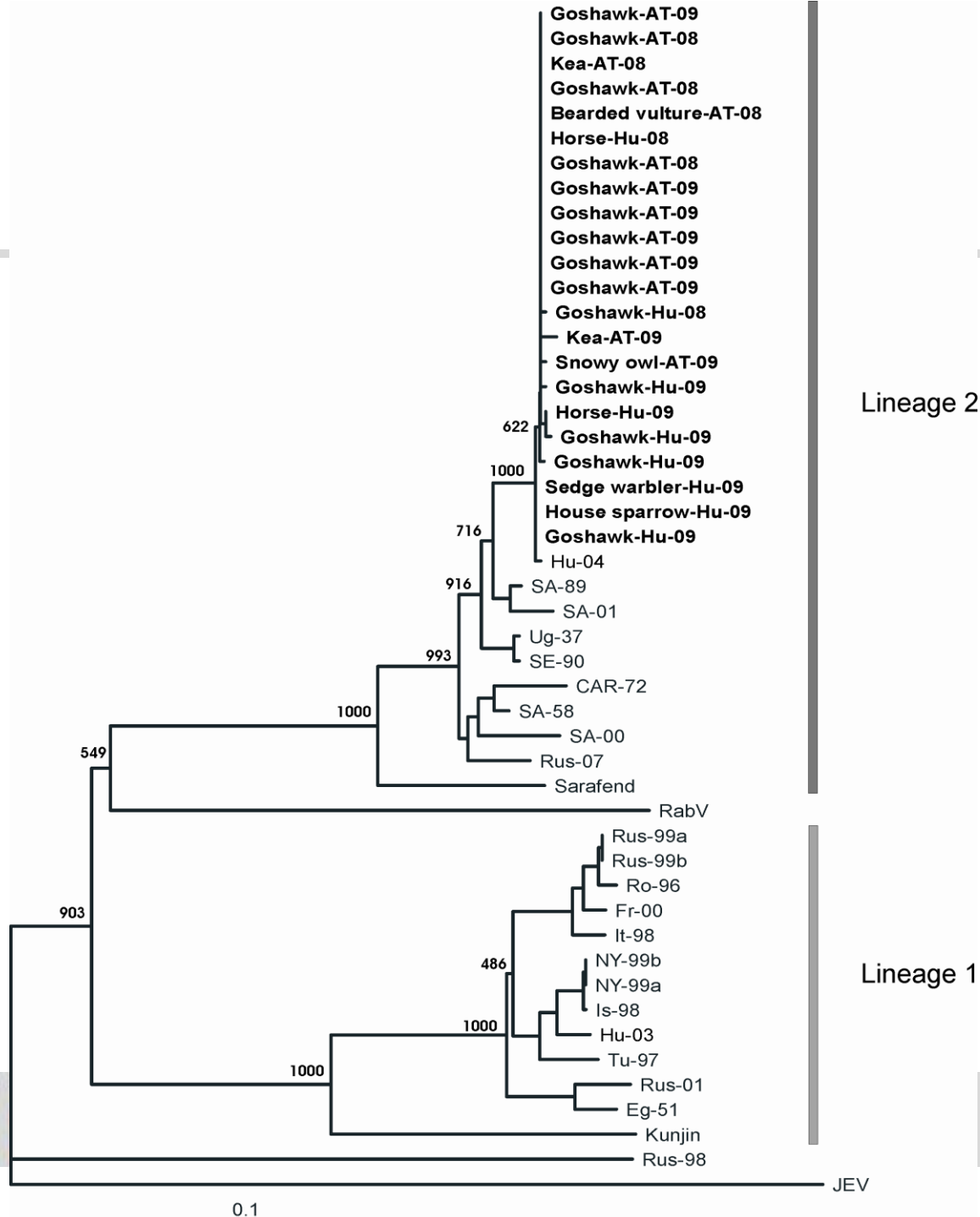
WNV cases 2004-2005-2007-2008-2009-2010

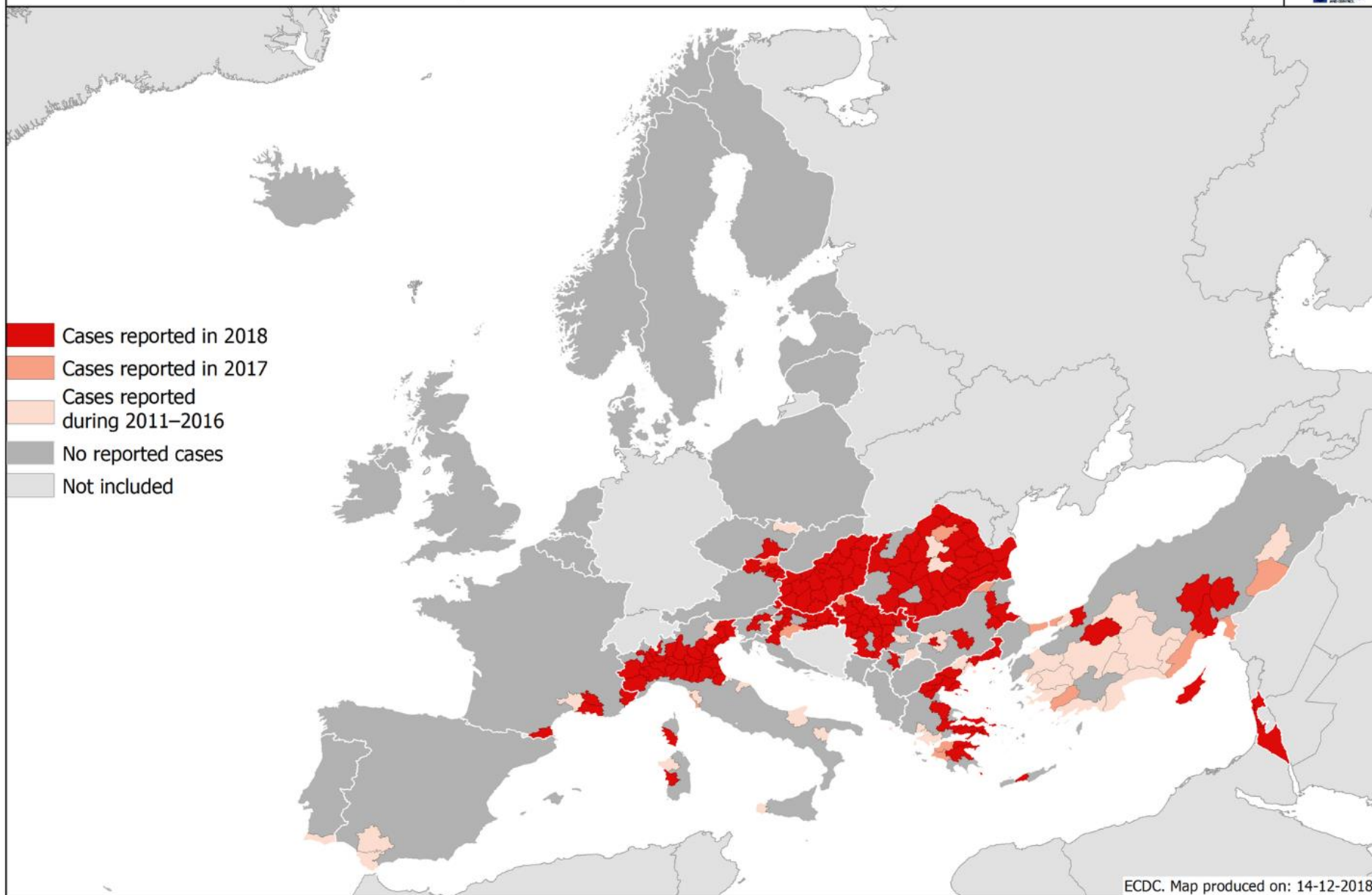




Limited mosquito
surveillance
2008/2009 >

WNV-carrying
mosquitoes have
been overwintering
in Austria





West Nile Virus Clinical Syndromes

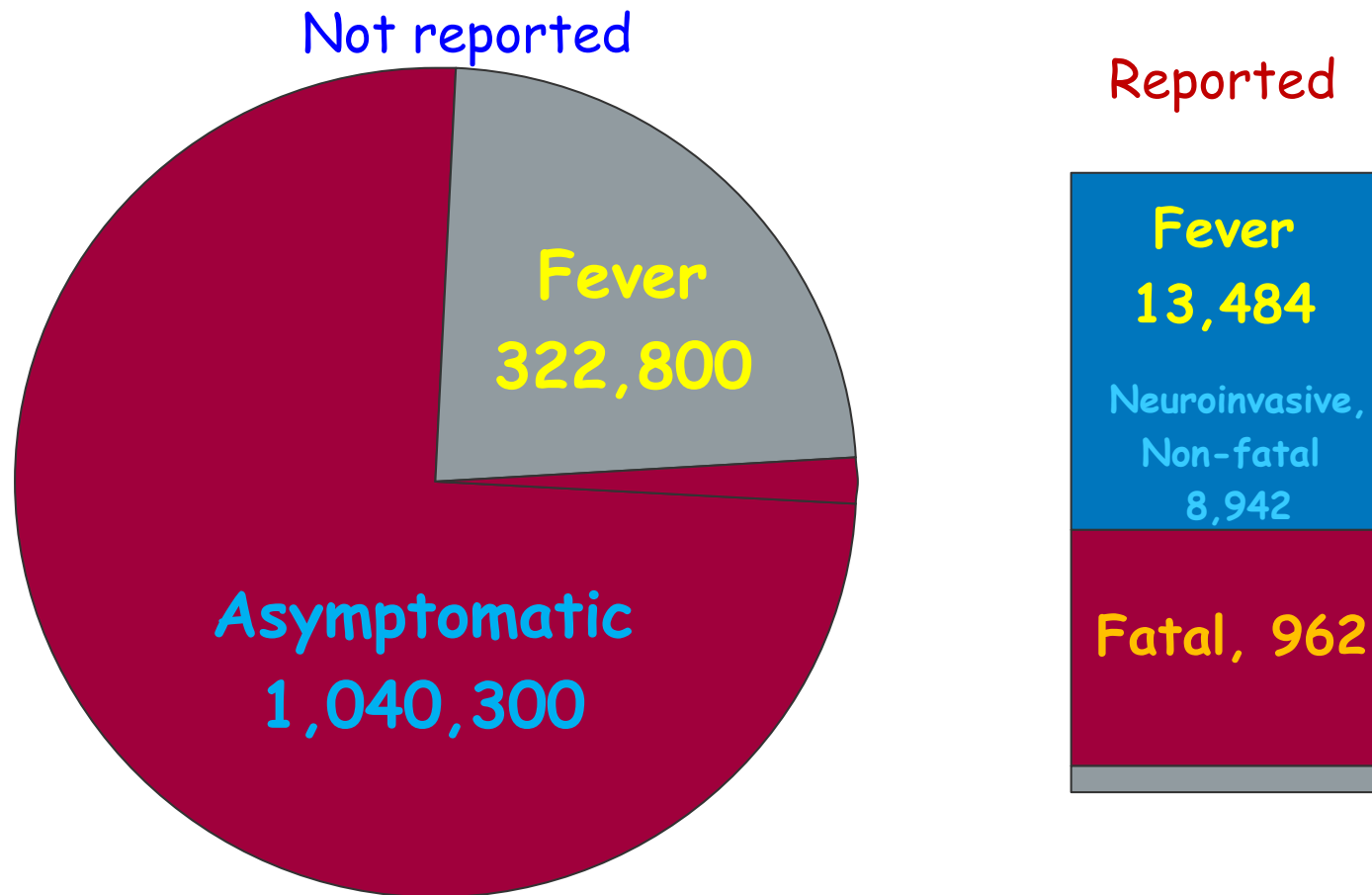
- Asymptomatic (approx. 75%)
- West Nile fever (approx. 25%)
- Neuroinvasive disease (approx. 1/140)
 - Encephalitis
 - Meningitis
 - Flaccid paralysis



Courtesy L. Petersen, CDC

The Hidden Epidemic

WNV Infections (n=1.4 million), 1999-2006



Courtesy L. Petersen, CDC

Blood transfusion !





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[PLOS One](#). 2015; 10(5): e0126381.

PMCID: PMC4427133

Published online 2015 May 11. doi: [10.1371/journal.pone.0126381](https://doi.org/10.1371/journal.pone.0126381)

West Nile Virus Positive Blood Donation and Subsequent Entomological Investigation, Austria, 2014

[Jolanta Kolodziejek](#),¹ [Bernhard Seidel](#),² [Christof Jungbauer](#),³ [Katharina Dimmel](#),¹ [Michael Kolodziejek](#),¹ [Ivo Rudolf](#),⁴ [Zdenek Hubálek](#),⁴ [Franz Allerberger](#),⁵ and [Norbert Nowotny](#)^{1,6,*}



Kolodziejek et al. *Emerging Microbes & Infections* (2018)7:25
DOI 10.1038/s41426-018-0021-5

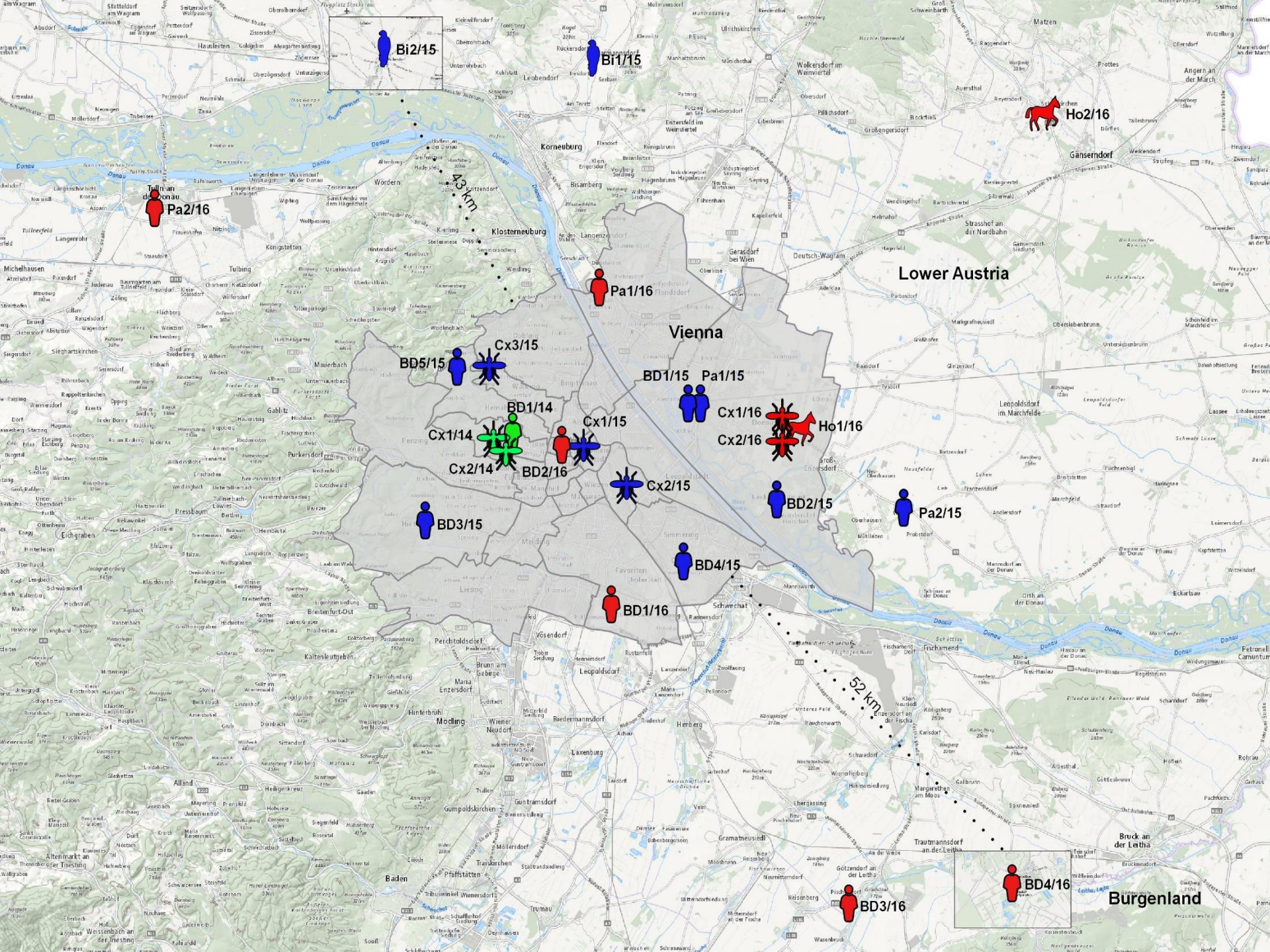
Emerging Microbes & Infections

ARTICLE

Open Access

Integrated analysis of human-animal-vector surveillance: West Nile virus infections in Austria, 2015–2016

Jolanta Kolodziejek¹, Christof Jungbauer², Stephan W. Aberle³, Franz Allerberger⁴, Zoltán Bagó⁵, Jeremy V. Camp¹, Katharina Dimmel¹, Phebe de Heus⁶, Michael Kolodziejek¹, Peter Schiefer⁵, Bernhard Seidel⁷, Karin Stiasny³ and Norbert Nowotny^{1,8}



RAPID COMMUNICATIONS

Usutu virus infections among blood donors, Austria, July and August 2017 – Raising awareness for diagnostic challenges

Tamás Bakonyi^{1,2,3}, Christof Jungbauer^{3,4}, Stephan W. Aberle^{3,5}, Jolanta Kolodziejek¹, Katharina Dimmel¹, Karln Stiasny⁵, Franz Allerberger⁶, Norbert Nowotny^{1,3,7}

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7. Department of Basic Medical Sciences, College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, United Arab Emirates

Correspondence: Norbert Nowotny (norbert.nowotny@mbru.ac.ae)

Between July and August 2017, seven of 12,047 blood donations from eastern Austria, reacted positive to West Nile virus (WNV) in the cobas test (Roche). Follow-up investigations revealed Usutu virus (USUV) nucleic acid in six of these. Retrospective analyses of four blood donors diagnosed as WNV-infected in 2016 showed one USUV positive.

Late August 2001:

Mass die-off of blackbirds
(*Turdus merula*) in Vienna
and surrounding villages
to the north and south
(7 birds examined)



male blackbird

Ausgabe 262/Dienstag, 9. April 2002, Erscheinungsort Wien

Express

DIE ERSTE WIENER GRATIS-TAGESZEITUNG

Wien: Virus tötet tausend Amseln!

Tiermediziner schlagen Alarm – Bericht Seite 7

GERETTET:
Israeli-Niere für
Palästinenserin
Rührende Hilfe – Seite 2

GEPLÜNDERT:
18.000 € Beute
bei Rififi-Coup
Seite 6

GEPLAUDERT:
Marianne Mendt
im Interview
Seite 13

TANZ
Neue Paarkurse
– auch mit Kinderbetreuung –
Kinder- & Boogie Kurse
MÜHLSIEGL
12, Schönbrunner Str. 249, 015 55 88
<http://www.muehsiegl.at>

ANZEIGE

ihre vier Enkelsöhne – im Bild Prinz Charles – hielten Montagabend in London Totenwache am Sarg von Queen Mum. Das Begräbnis wird heute live im TV übertragen (ORF 2, ab 10.15 Uhr).

Photo: BFA

A photograph showing a blackbird (Turdus merula) feeding its young in a nest. The adult bird is on the right, holding a piece of food in its beak. Several young birds are on the left, with their beaks open, waiting to be fed. The nest is made of twigs and is situated in a wooded area.

Late August 2001:

Death of all 5 great gray owls (*Strix nebulosa*) in the Vienna Zoo





Credit: Jean Meyer, Carinthia



RESEARCH

Emergence of *Usutu virus*, an African Mosquito-Borne *Flavivirus* of the Japanese Encephalitis Virus Group, Central Europe

Herbert Weissenböck,* Jolanta Kolodziejek,† Angelika Url,* Helga Lussy,†
Barbara Rebel-Bauder,* and Norbert Nowotny†‡

During late summer 2001 in Austria, a series of deaths in several species of birds occurred, similar to the beginning of the *West Nile virus* (WNV) epidemic in the United States. We necropsied the dead birds and examined them by various methods; pathologic and immunohistologic investigations suggested a WNV infection. Subsequently, the virus was isolated, identified, partially sequenced, and subjected to phylogenetic analysis. The isolates exhibited 97% identity to *Usutu virus* (USUV), a mosquito-borne *Flavivirus* of the Japanese encephalitis virus group; USUV has never previously been observed outside Africa nor associated with fatal disease in animals or humans. If established in central Europe, this virus may have considerable effects on avian populations; whether USUV has the potential to cause severe human disease is unknown

Emerging Infectious Diseases • Vol. 8, No. 7, July 2002



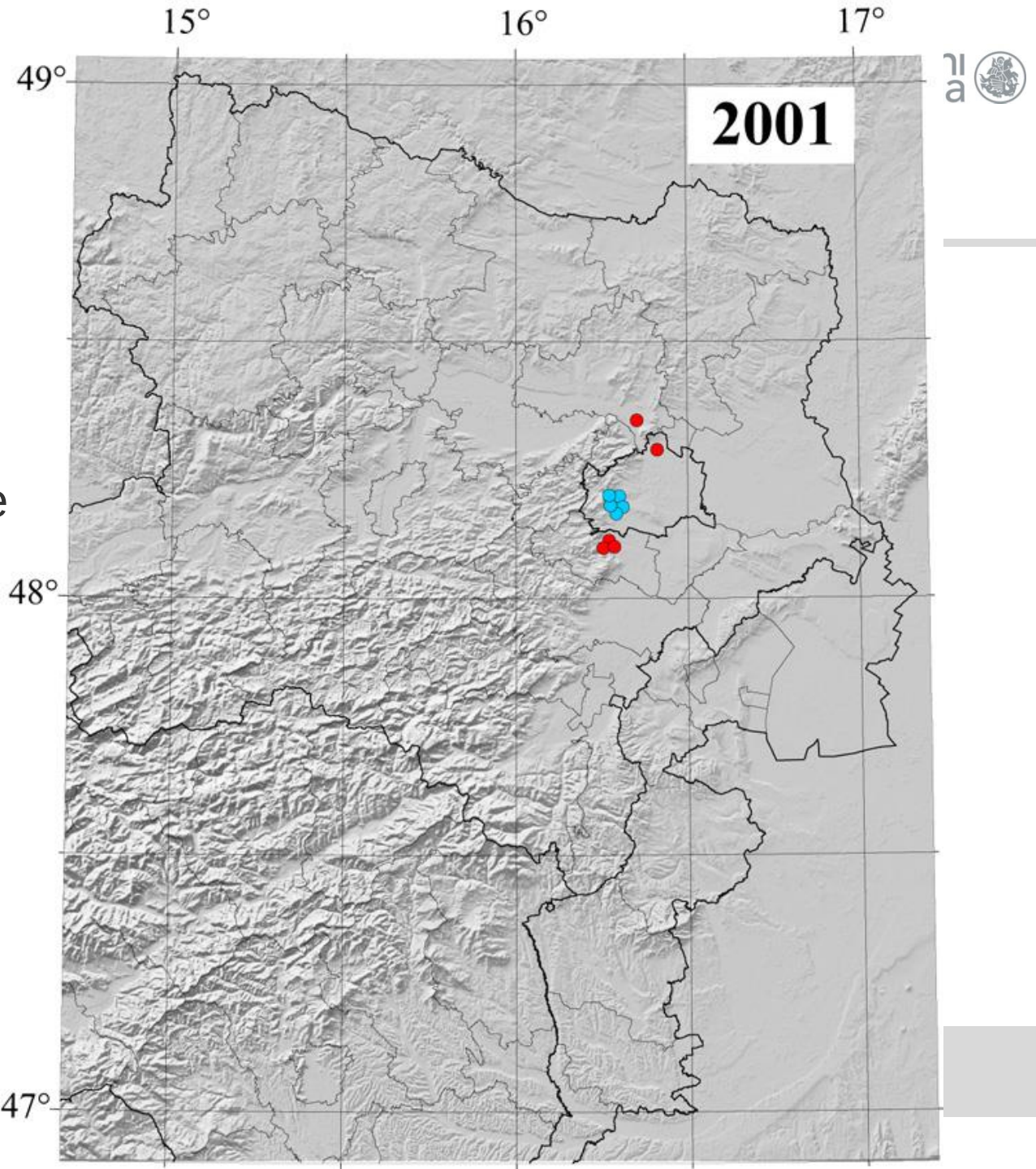
- ***Usutu virus (USUV, Flaviviridae, Flavivirus)***
- **First isolated in South Africa in 1959**
- **The name is derived from the **Usutu river in Swasiland****
- **Closely related to West Nile virus**
- **Emerged in Austria in 2001**



USUV-associated avian mortality **2001**

red: USUV- positive
blackbirds

blue: USUV- positive
great grey owls

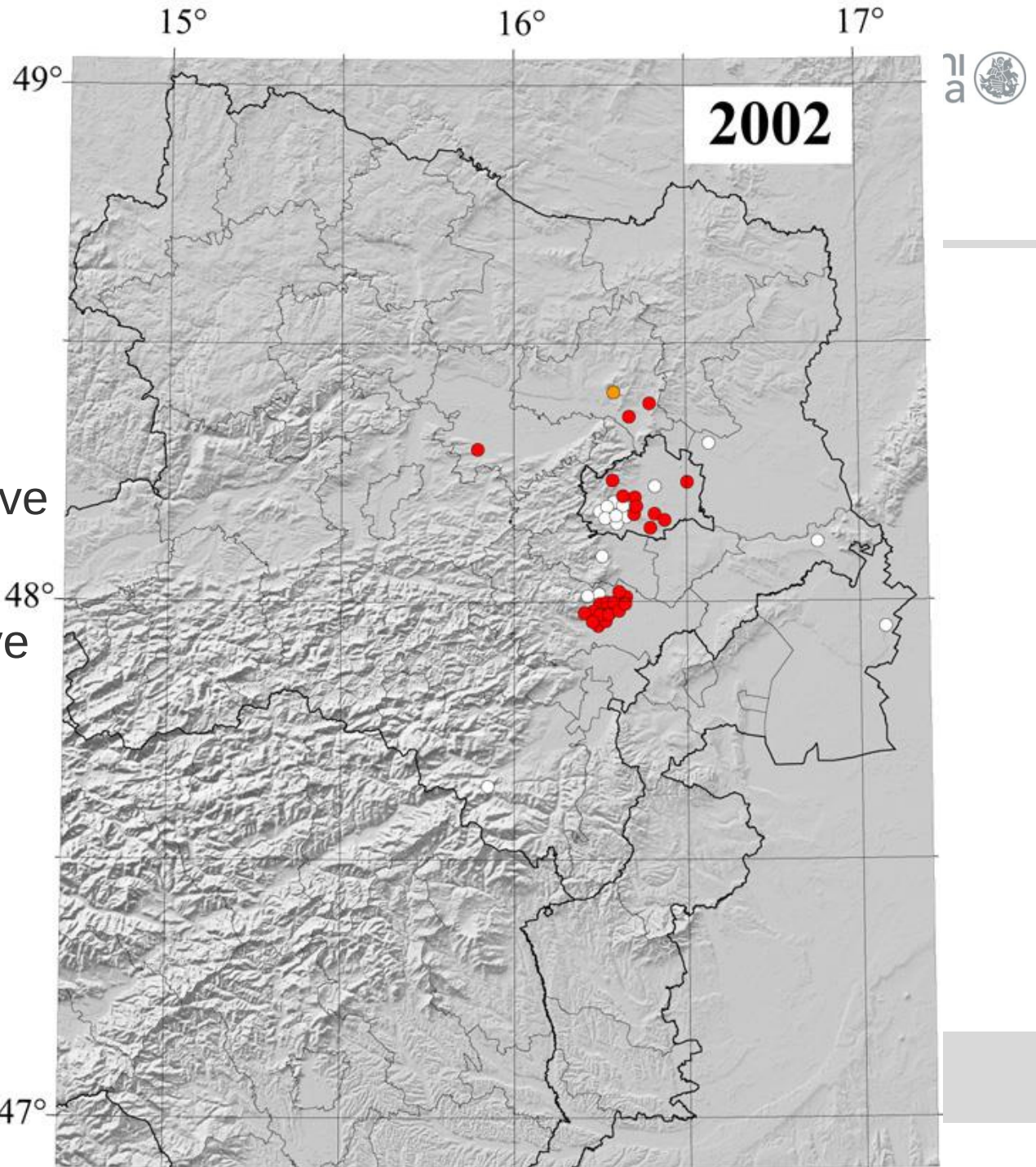


USUV-associated avian mortality **2002**

red: USUV-positive
blackbirds

orange: USUV-positive
house sparrow

white: USUV-negative
cases



USUV surveillance program **2003**

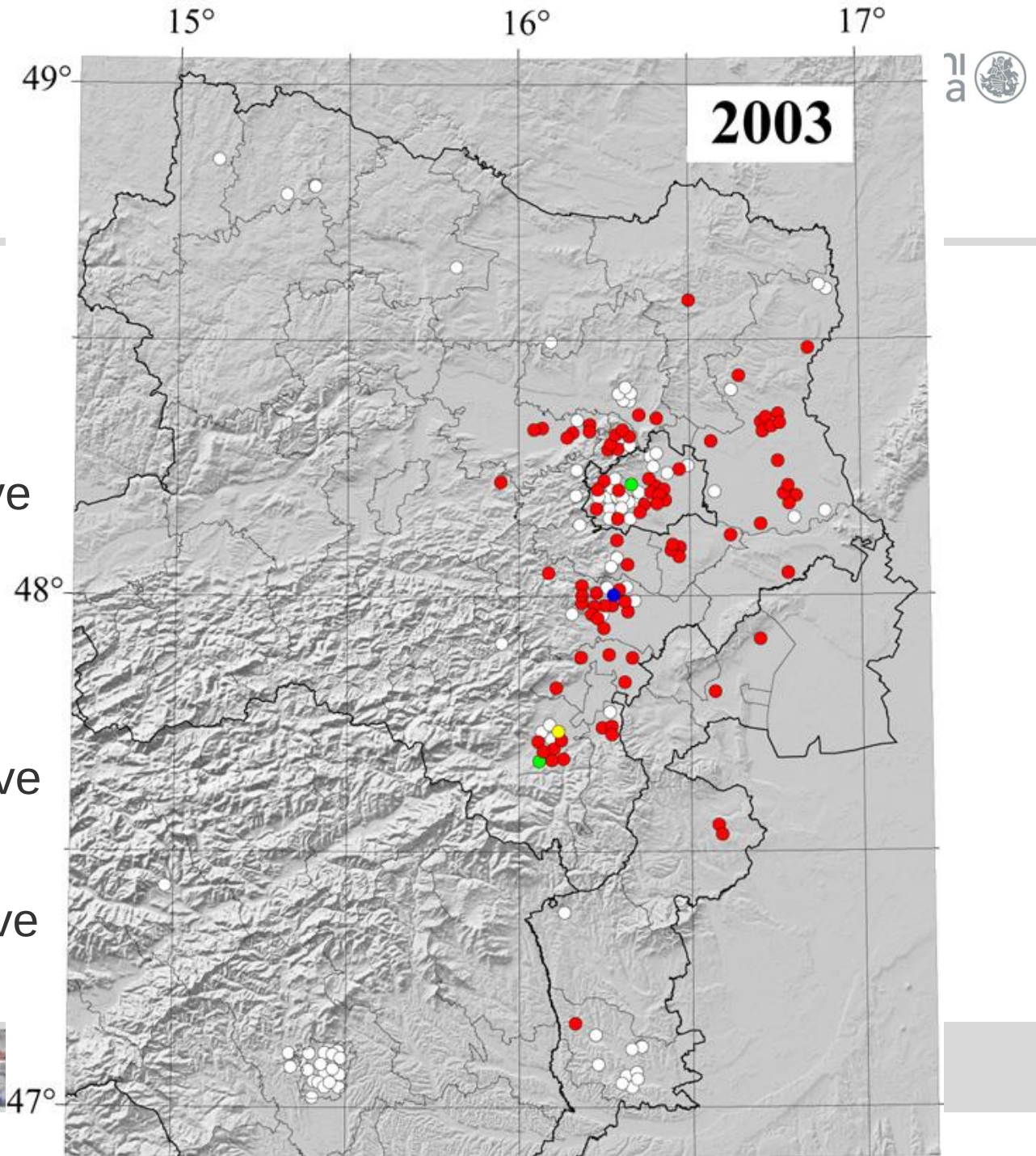
red: USUV-positive
blackbirds

green: USUV-positive
great tits

blue: USUV-positive
robin

yellow: USUV-positive
nuthatch

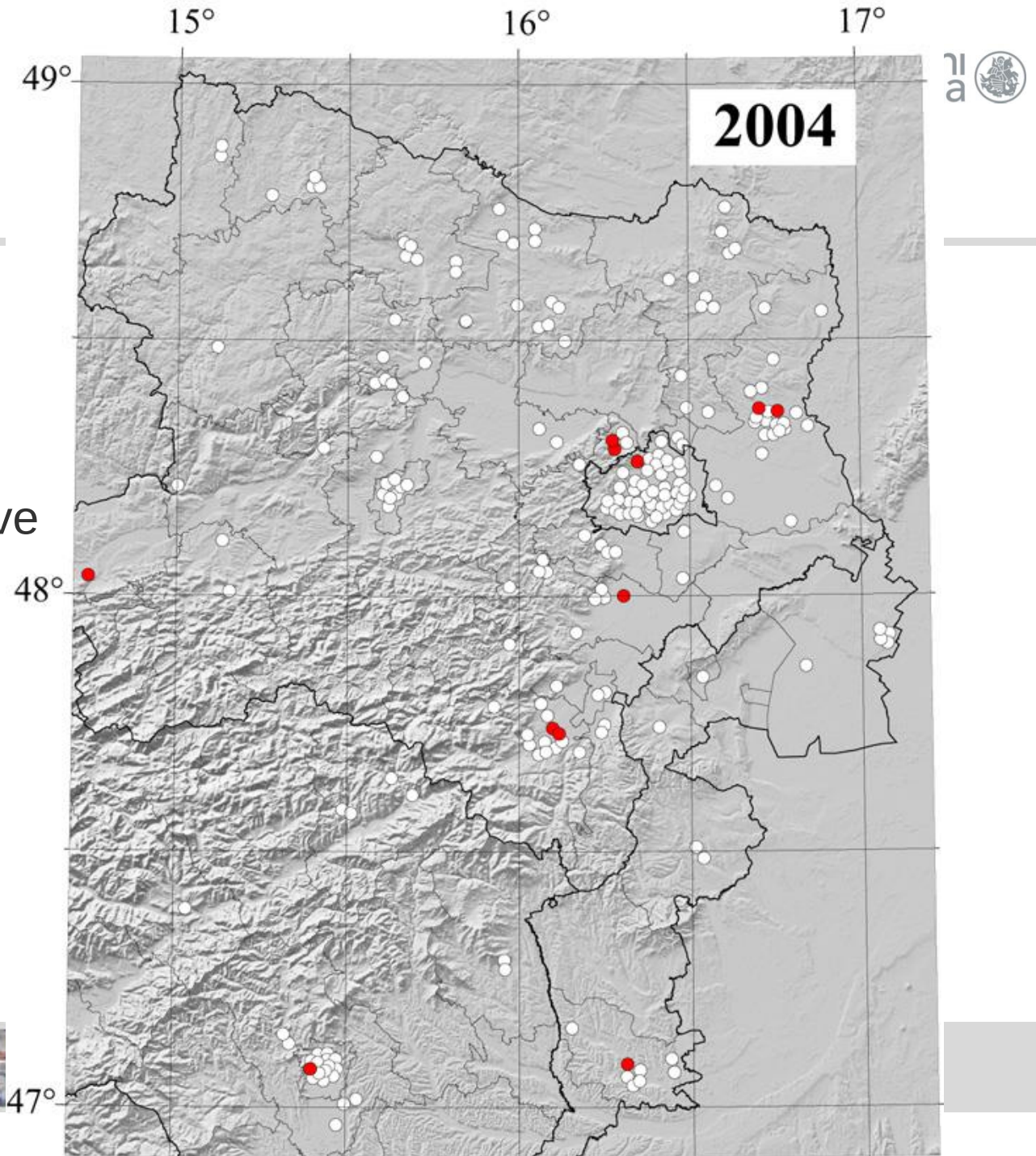
white: USUV-negative
cases



USUV surveillance program **2004**

red: USUV-positive
blackbirds

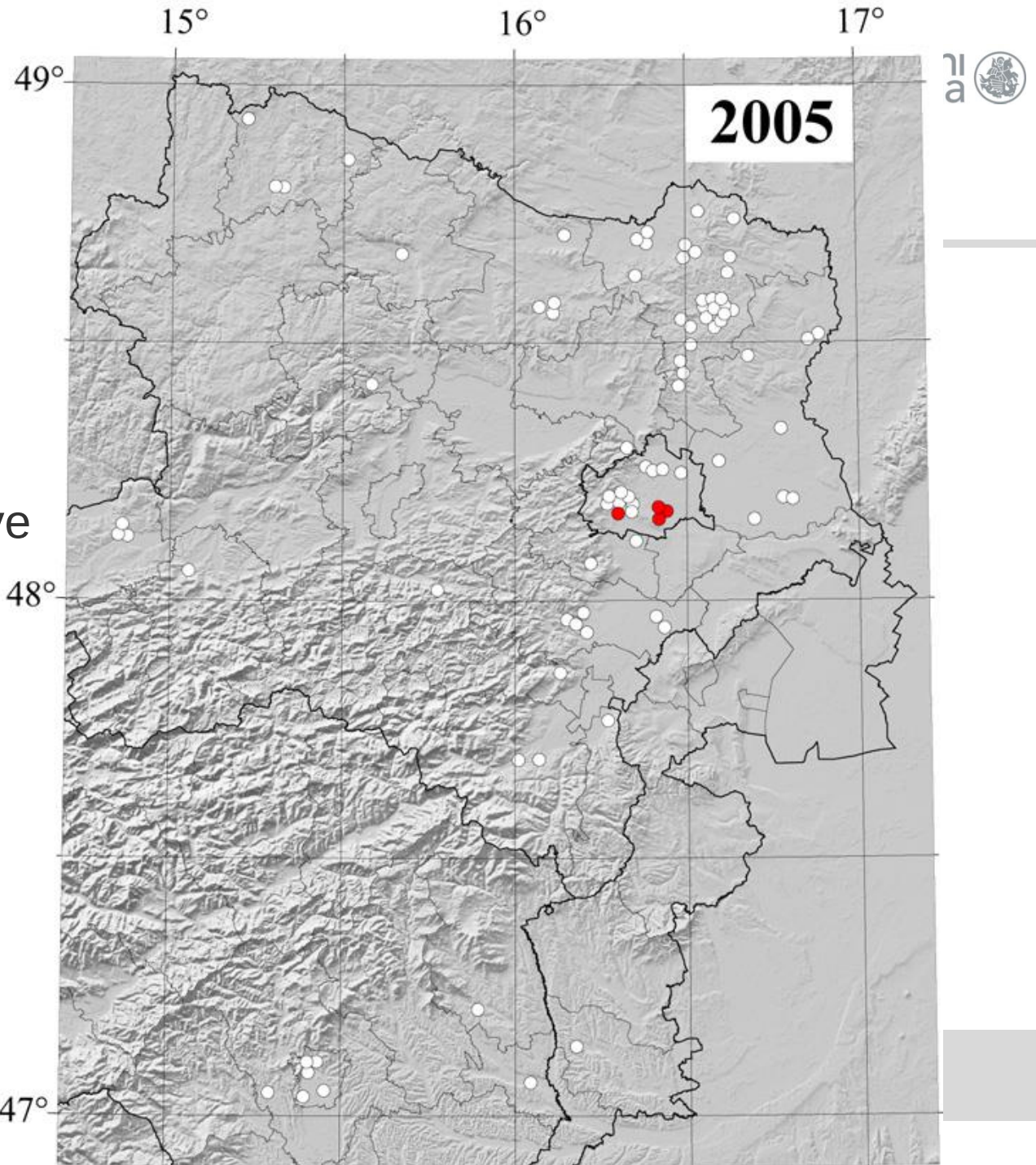
white: USUV-negative
cases



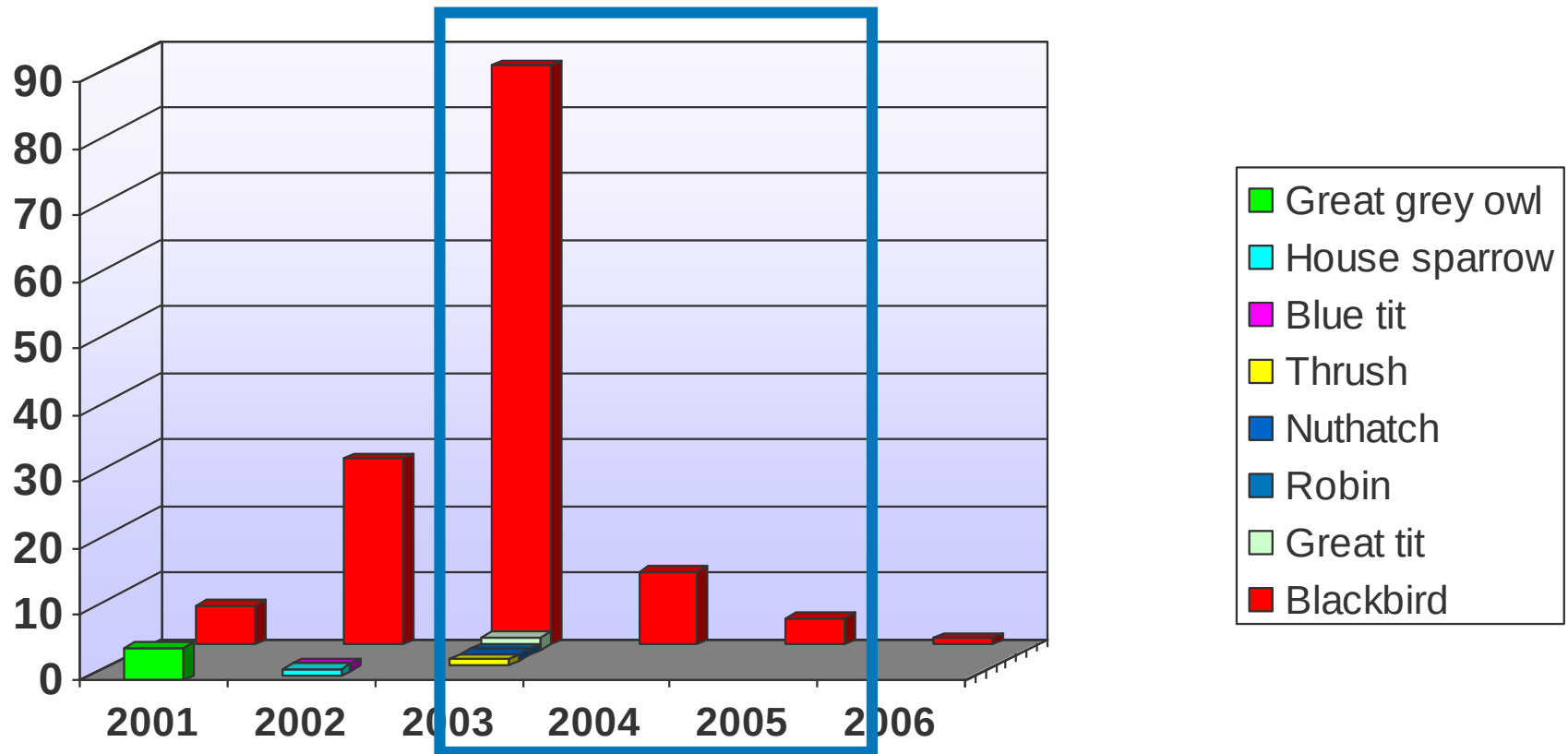
USUV surveillance program **2005**

red: USUV-positive
blackbirds

white: USUV-negative
cases

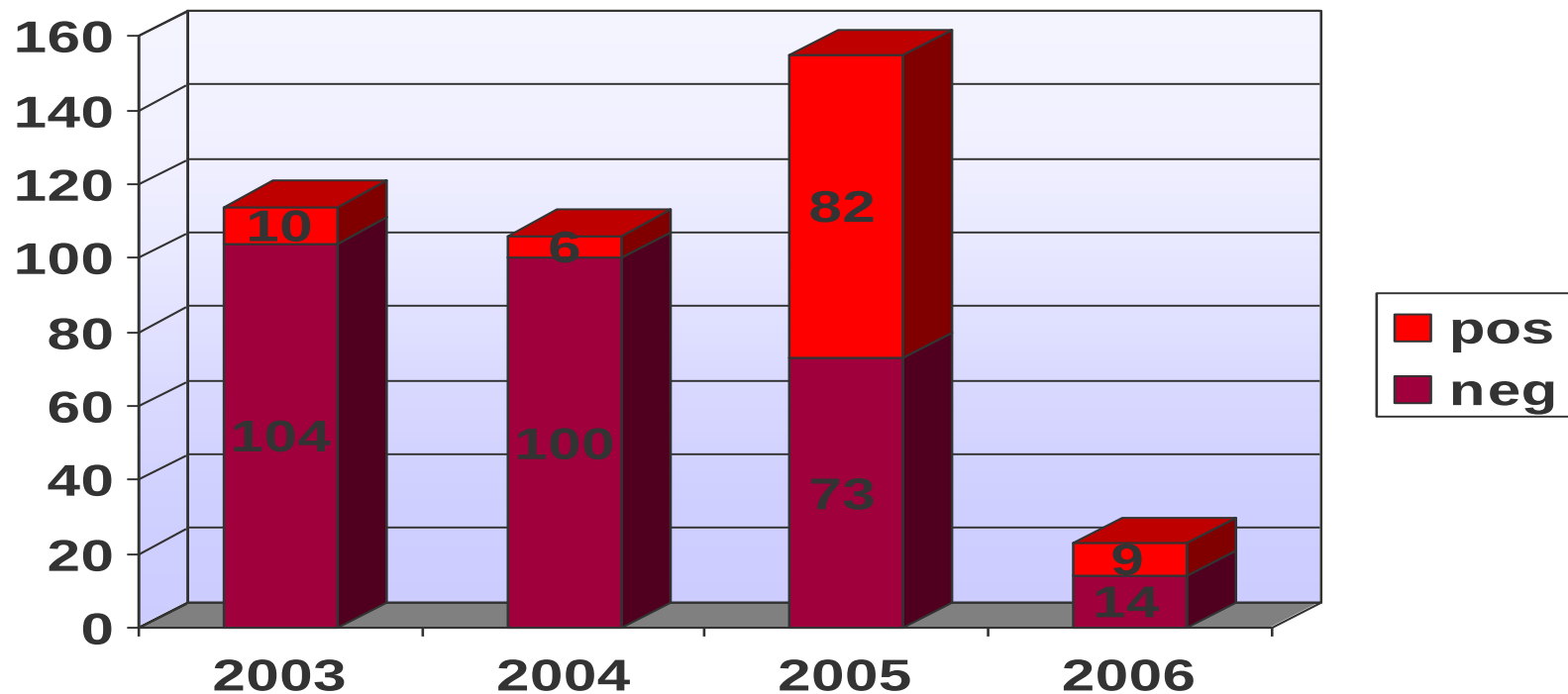


Cases of USUV infection diagnosed in Austria 2001 - 2006



Serological data on wild bird sera (n=398)

(Hemagglutination inhibition test; positives confirmed by
Plaque reduction neutralization test)



The spread of USUV in Europe

- **Austria: 2001**
- **Hungary: 2005 (sporadic)**
- **Italy (Milan): 2006 (mass-mortality)**
 - retrospective: 1996, Tuscany, mass-mortality
- **Switzerland (Zürich): 2006 (mass-mortality)**
- **Spain: 2006 (in mosquitoes, different genetic lineage)**
- **Germany: 2011 (mass-mortality)**
- **Czech Republic: 2011 (sporadic)**
- **Belgium: 2012 (sporadic)**
- **France: 2015 (increased mortality)**
- **Widespread: 2016 (new: Netherlands)**



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 • www.cdc.gov/eid • Vol. 19, No. 2, February 2013



OPEN

Emerging Microbes & Infections (2017) 6, e85; doi:10.1038/emi.2017.72

www.nature.com/emi

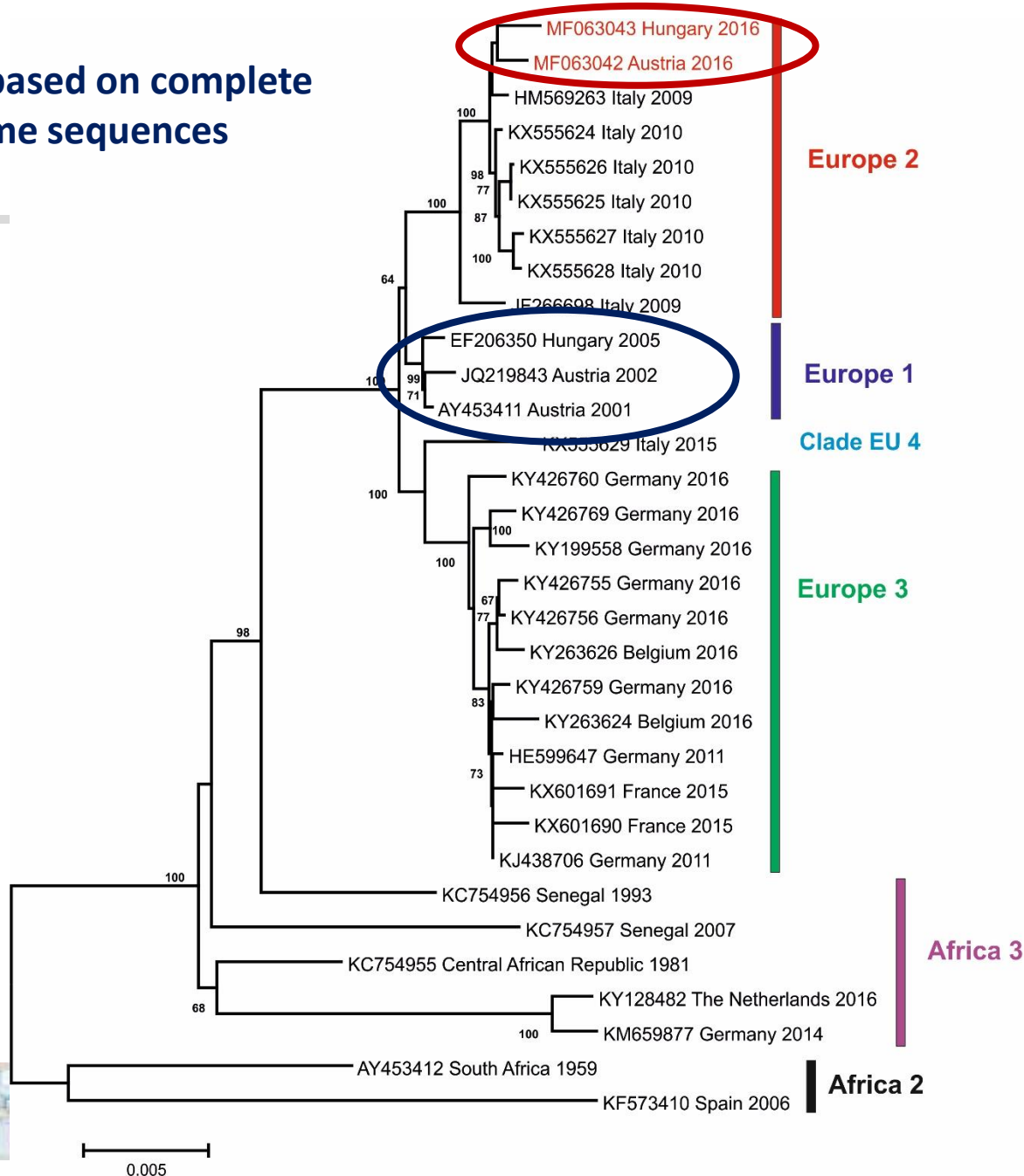
ORIGINAL ARTICLE

Usutu virus, Austria and Hungary, 2010–2016

Tamás Bakonyi^{1,2}, Károly Erdélyi³, René Brunthaler⁴, Ádám Dán³, Herbert Weissenböck⁴
and Norbert Nowotny^{1,5}



Phylogram based on complete USUV genome sequences



Has global warming been responsible for the introduction of WNV, USUV and other exotic viruses to Europe?

- **NO** - most likely viraemic migratory birds brought the virus from Africa to Europe, or infected ticks on migratory birds
- Also, the competent vectors for virus propagation and transmission have already been present in Europe (*Culex* sp.)
- **BUT:** Higher temperatures in spring, summer and autumn allow mosquitoes more reproduction cycles and thus contribute significantly to the establishment of a new infection in an area and facilitate the spread of the infection



Has global warming been responsible for the introduction of WNV, USUV and other exotic viruses to Europe?

Positive proof of global warming.



**18th
Century 1900 1950 1970 1980 1990 2006**

RAPID COMMUNICATION

Increase in human West Nile and Usutu virus infections, Austria, 2018

Stephan W. Aberle^{1,2}, Jolanta Kolodziejek^{2,3}, Christof Jungbauer^{2,4}, Karin Stiasny¹, Judith H. Aberle¹, Alexander Zoufaly⁵, Michael Kai Hourfar⁶, Lisa Weidner⁴, Norbert Nowotny^{2,3,7}

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6. German Red Cross, Blood Donor Service Baden-Württemberg-Hessen, Institute for Transfusion Medicine and Immunohaematology, Johann Wolfgang Goethe University, Frankfurt am Main, Germany
7. Department of Basic Medical Sciences, College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, United Arab Emirates

Correspondence: Norbert Nowotny (Norbert.Nowotny@vetmeduni.ac.at)



Number of diagnosed human West Nile and Usutu virus infections, Austria, 2009–2018

Year	West Nile virus infections						Reference	Usutu virus infections	Reference
	Autochthonous				Imported	Total		Autochthonous	
	WNND	WNF	Asympt WNV	Blood donors				Blood donors	
2009	1	1	0	ND	0	2	[5]	ND	NA
2010	1	0	0	ND	0	1		ND	NA
2011	0	0	0	ND	0	0	NA	ND	NA
2012	0	0	0	ND	3	3	NA	ND	NA
2013	0	0	0	ND	0	0	NA	ND	NA
2014	1	0	0	1	0	2	[7,8]	0	NA
2015	1	0	1	5	0	7	[6]	0	NA
2016	1	1	0	3	0	5		1	[9]
2017	2	2	1	1	1	7		6	
2018	4	11	0	6	6	27	NA	18	NA
Total	11	15	2	16	10	54	NA	25	NA

Asympt: asymptomatic; NA: not applicable; ND: not done; WNF: West Nile fever; WNND: West Nile neuroinvasive disease; WNV: West Nile virus.



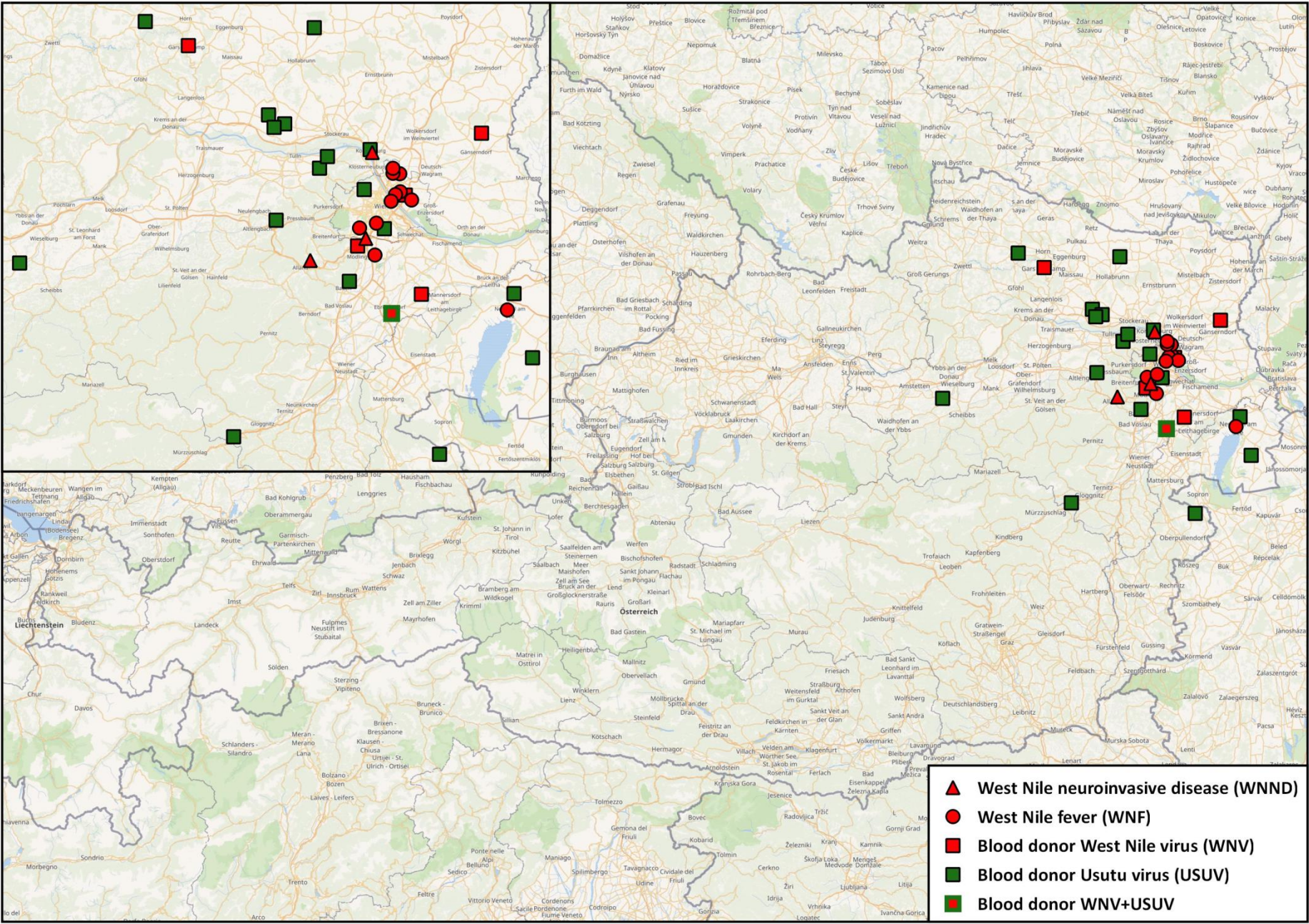
Details of autochthonous human West Nile and Usutu virus infections, Austria, 2018

Cases	Main symptoms	Month of symptom onset/ blood donation	Cobas WNV assay ^a	WNV RT-PCR ^b	USUV RT-PCR ^b	WNV NT	USUV NT
WNND							
1	Disorientation, aphasia	August	ND	Pos	Neg	240	40
2	Disorientation, coma	September	ND	Pos	Neg	640	60
3	Fever, stiff neck	August	ND	Pos	Neg	160	20
4	Dysarthria, limb weakness	September	ND	Neg	Neg	240	60
WNF							
1	Fever, rash, fatigue, headache	August	ND	Pos	Neg	960	160
2	Fever, rash, diarrhoea	August	ND	Pos	ND	320	80
3	Fever, rash	August	ND	Pos	Neg	640	60
4	Rash	August	ND	Pos	ND	ND	ND
5	Fever, rash	August	ND	Pos	ND	40	30
6	Fever, rash	August	ND	Pos	ND	320	120
7	Rash, ocular pain	August	ND	Pos	ND	320	40
8	Fever, fatigue, muscle aches	August	ND	Pos	ND	480	60
9	Rash, fatigue	August	ND	Neg	Neg	240	60
10	Fever, rash	August	ND	Pos	ND	120	< 20
11	Fever, fatigue	August	ND	Pos	Neg	320	< 20

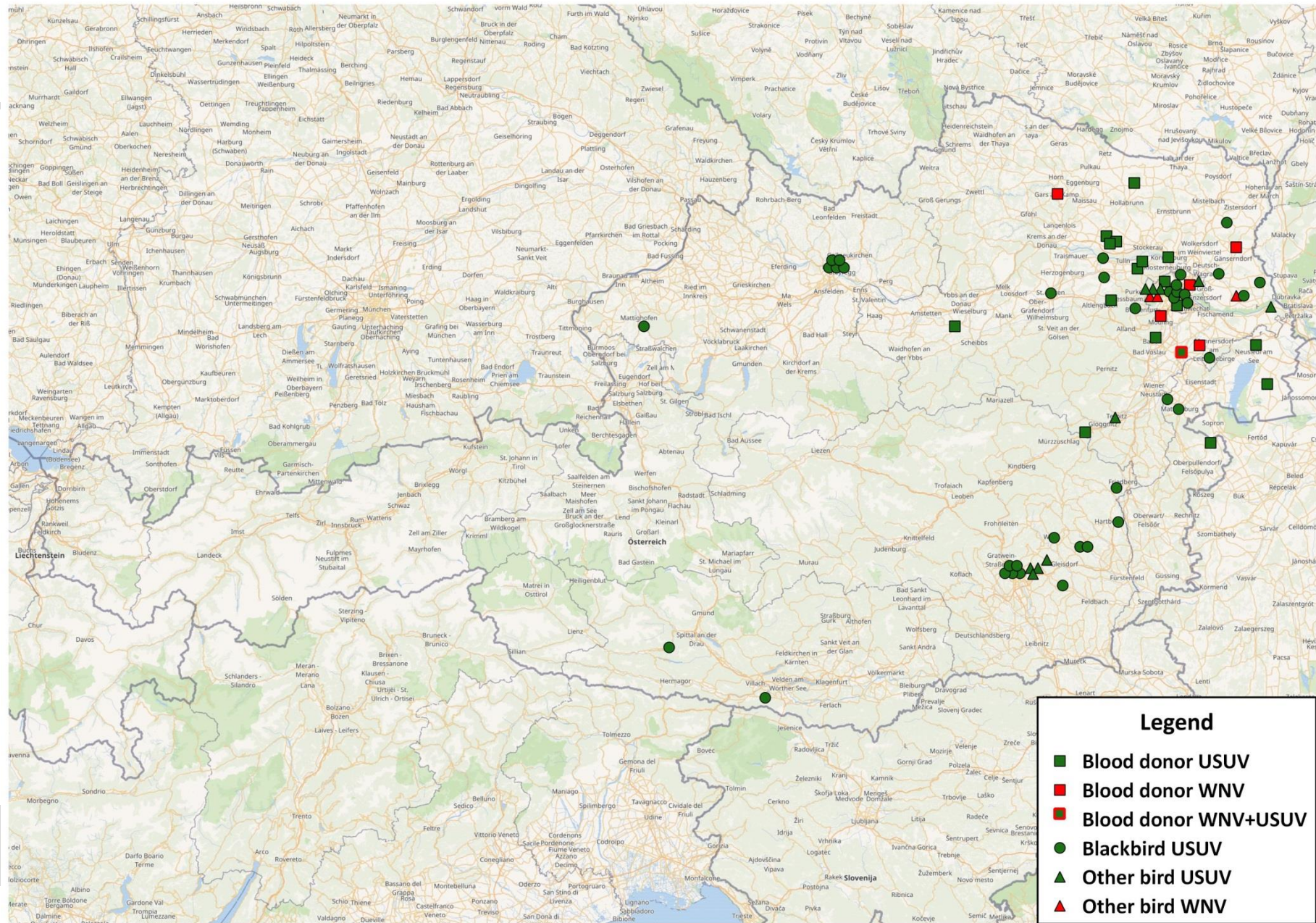


Blood donors							
WNV							
BD2	Rash, fatigue, headache ^c	August	Pos (30.4)	Pos	Neg	480	30
BD4	Rash, fatigue ^d	August	Pos (40.0)	Pos	Neg	1,920	160
BD9	Fatigue, joint aches ^c	August	Pos (30.8)	Pos	Neg	240	80
BD17	Asympt	August	Pos (30.0)	Pos	Neg	160	60
BD18	Asympt	August	Pos (39.4)	Pos	Neg	240	160
WNV/USUV							
BD7	Asympt	August	Pos (34.1)	Pos	Pos	960	120
USUV							
BD1	Asympt	June	Pos (38.7)	Neg	Pos	< 20	40
BD3	Rash ^c	August	Pos (37.6)	Neg	Pos	60	160
BD5	Asympt	August	Pos (39.4)	Neg	Pos	ND	ND
BD6	Asympt	August	Pos (37.1)	Neg	Pos	80	240
BD8	Asympt	August	Pos (44.0)	Neg	Pos	< 20	80
BD10	Asympt	August	Pos (35.3)	Neg	Pos	ND	ND
BD11	Asympt	August	Pos (38.8)	Neg	Pos	30	120
BD12	Asympt	August	Pos (40.7)	Neg	Pos	< 20	160
BD13	Asympt	August	Pos (38.4)	Neg	Pos	< 20	40
BD14	No information	August	Pos (39.3)	Neg	Pos	ND	ND
BD15	Asympt	August	Pos (36.5)	Neg	Pos	ND	ND
BD16	Asympt	August	Pos (36.8)	Neg	Pos	20	40
BD19	Asympt	August	Pos (36.6)	Neg	Pos	20	120
BD20	Asympt	August	Pos (42.1)	Neg	Neg	< 20	80
BD21	Asympt	August	Pos (41.7)	Neg	Neg	< 20	80
BD22	Asympt	September	Pos (40.0)	Neg	Pos	ND	ND
BD23	Asympt	September	Pos (38.6)	Neg	Pos	40	240

Human West Nile and Usutu virus infections, Austria, 2018



West Nile and Usutu virus infections in birds and human blood donors, Austria, 2018



WNV Infections among Blood Donors in the EU

During **2010–2017**, blood banks in the affected areas of 7 EU countries (Austria, France, Greece, Italy, Portugal, Romania, and Spain) detected **152 WNV RNA–reactive donations among 2,636,653 donated blood units.**



Transfusion-Transmitted WNV Infection in the EU

One report (Pervanidou et al., Euro Surveill. 2014;19:20758).

In **2012**, the **Greek** Haemovigilance Centre reported **2 TT-WNV infections**:

1 patient received a **platelet transfusion** and developed **WNND**, and another patient received **fresh frozen plasma** and became positive for WNV but remained **asymptomatic**. Both blood components, including nontransfused erythrocytes, were prepared from a single whole blood donation and tested positive for WNV RNA after notification.

The implicated donor, who retrospectively received a diagnosis of WNV infection, donated blood 8 days before a case of WNV infection in Greece was reported and preventive measures initiated.



West Nile and Usutu Virus Infections and Challenges to Blood Safety in the European Union

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Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 25, No. 6, June 2019





Thank you for your attention!