Casistica e situazione attuale in ambito umano

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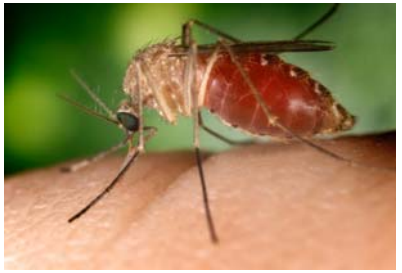
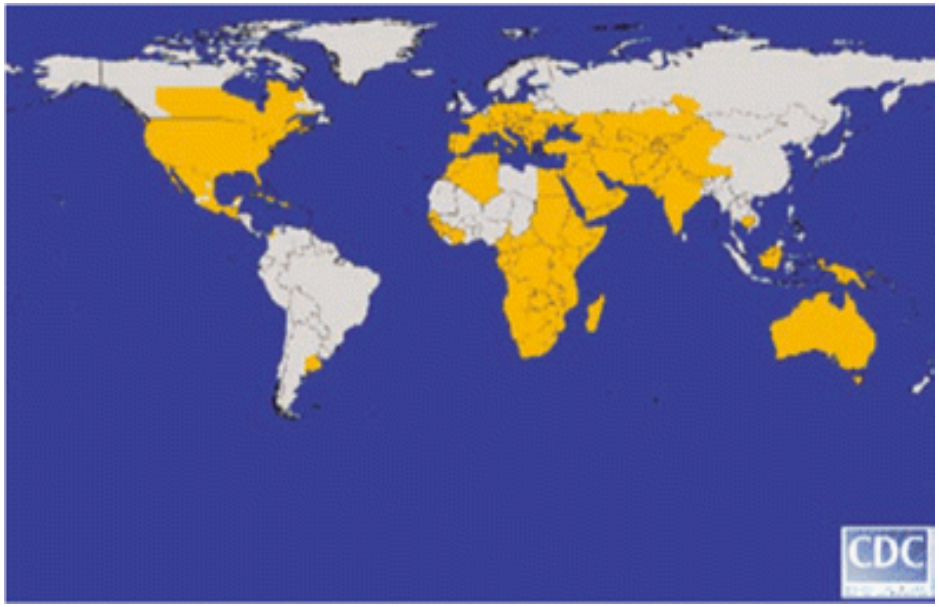
West Nile virus

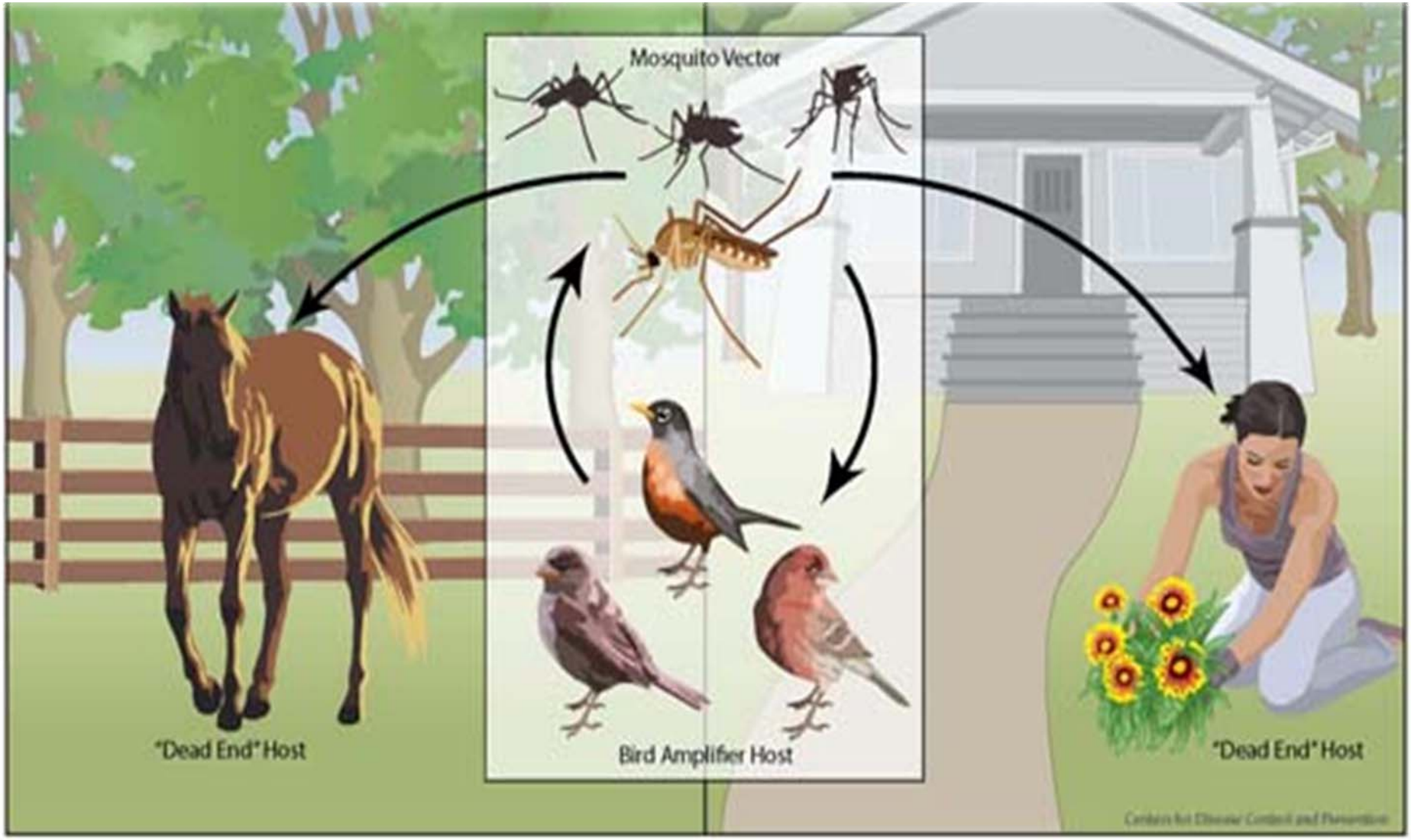


West Nile virus (WNV) is a mosquito-borne flavivirus.

WNV was first isolated from a woman in the West Nile district of Uganda in 1937.

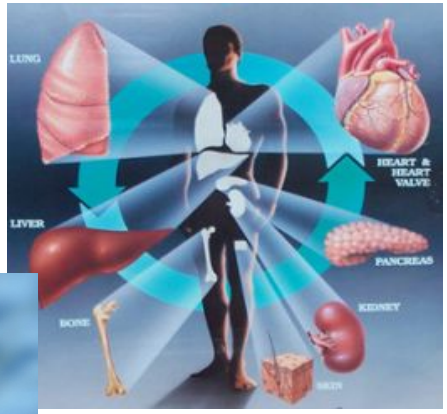
WNV is widely distributed in Africa, Western Asia, Europe, Australia and North America.




Mosquito Vector
"Dead End" Host
Bird Amplifier Host
"Dead End" Host

Person to Person transmission of WNV

- **Organ transplantation**
- **Blood transfusion**
- **Intrauterine**
- **Breastfeeding**



Who is at risk for infection with West Nile virus?

Anyone living in an area where West Nile virus is present in mosquitoes can get infected.

•US: West Nile virus has been detected in all lower 48 states (not in Hawaii or Alaska). Outbreaks have been occurring every summer since 1999.

•The risk of infection is highest for people who work **outside** or participate in **outdoor activities** because of greater exposure to mosquitoes.

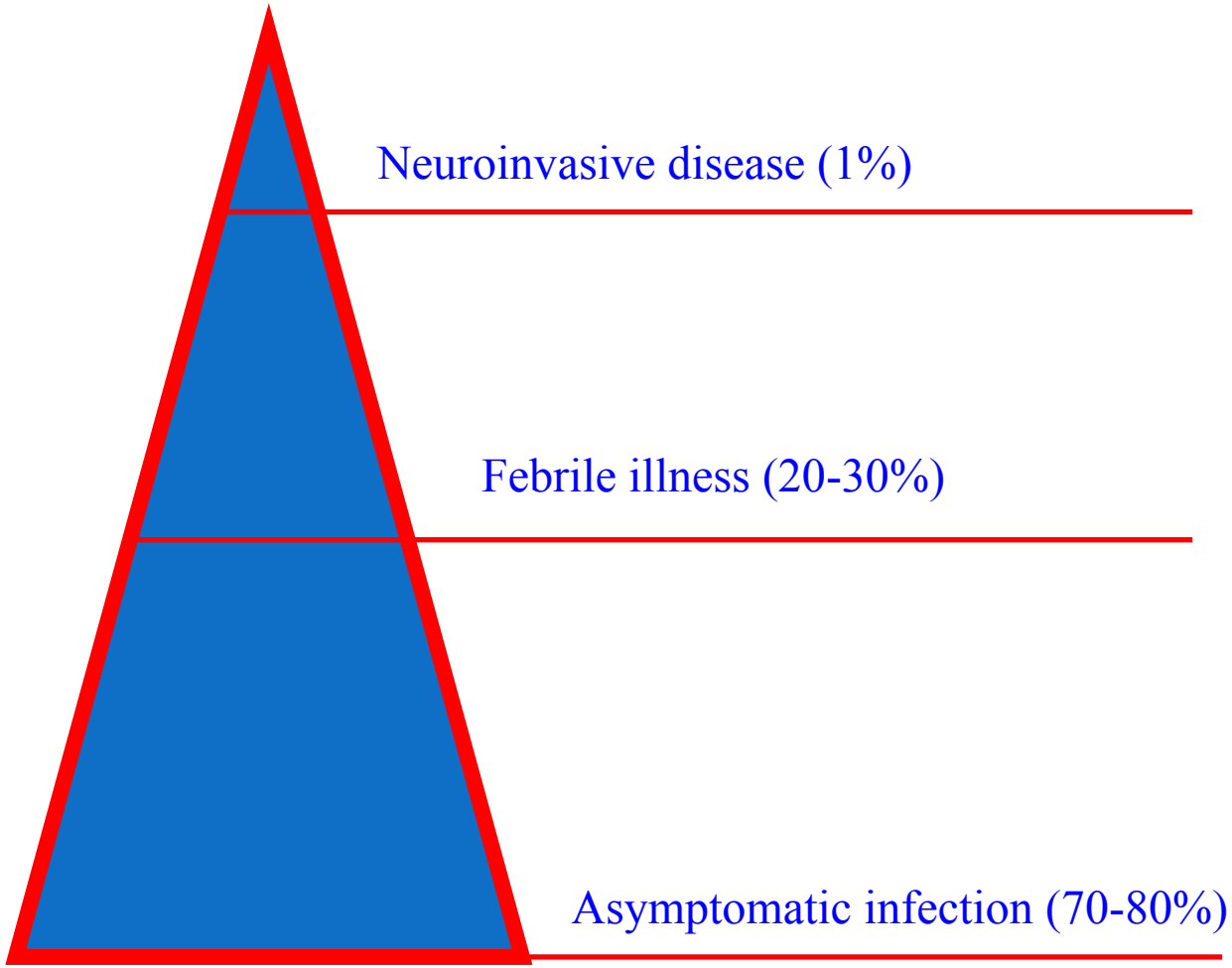
Is there a vaccine available to protect people from West Nile virus?

No. Many scientists are working on this issue, and there is hope that a vaccine will become available in the future.

How soon do people get sick after getting bitten by an infected mosquito?

The incubation period is usually 2 to 6 days but ranges from 2 to 14 days.
This period can be longer in immunocompromised patients.

Clinical presentation



Risk factors for severe disease

- Age >60 years
- Diabetes
- Hypertension
- Cancer history
- Chronic renal disease
- Chronic alcohol abuse

Lindsey NP, Am J Trop Mad Hyg. 2012; 87(1):179-184

How is West Nile virus disease diagnosed?

Diagnosis is based on a combination of:

clinical signs and symptoms and
specialized laboratory tests of blood, spinal fluid, urine.

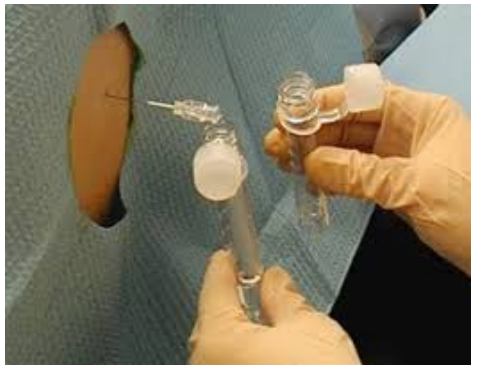
These tests typically detect **antibodies** that the immune system makes against the viral infection.

WNV diagnostic testing

Specimens:

- serum
  - Serology
  - NAT

- cerebrospinal fluid (csf)
  - Serology
  - NAT



- Urine
  - NAT

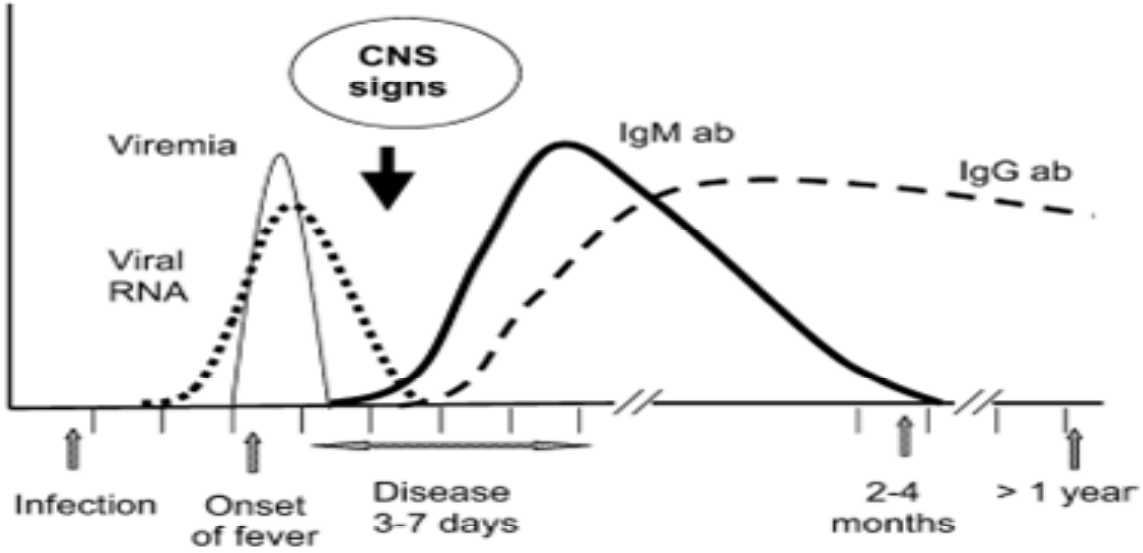


Fig. 2 Viremia and antibody kinetics in West Nile virus infection

Zeller HG et al., Eur J Clin Microbiol Infect Dis (2004) 23: 147-156

WNV antibody testing

IgM antibodies in serum or CSF

- performed by commercial ELISA or IFA assay.
- WNV-specific IgM antibodies are usually detectable 3 to 8 days after onset of illness and persist for 30 to 90 days (longer peristence has been documented).
- provides presumptive diagnosis of recent WNV infection but may also result from cross-reactive antibodies after infection with other flavivirus or from non-specific reactivity.

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www.mdpi.com/journal/ijerph

Review

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

Cécile Beck [1], Miguel Angel Jimenez-Clavero [2], Agnès Leblond [3,4], Benoît Durand [5], Norbert Nowotny [6,7], Isabelle Leparc-Goffart [8], Stéphan Zientara [1], Elsa Jourdain [4] and Sylvie Lecollinet [1,*]

IgG antibodies in serum and/or CSF

- performed by commercial ELISA or IFA assay.

- WNV IgG generally are detected shortly after IgM and persist for many years .

- the presence of IgG alone is only evidence of previous infection.

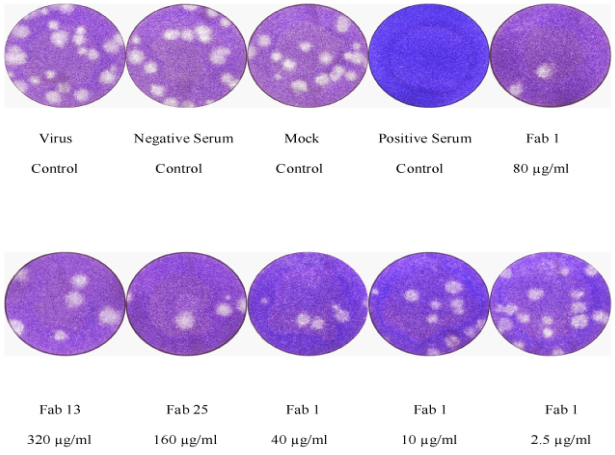
- Serum and CSF antibodies MUST be searched for in paired samples.

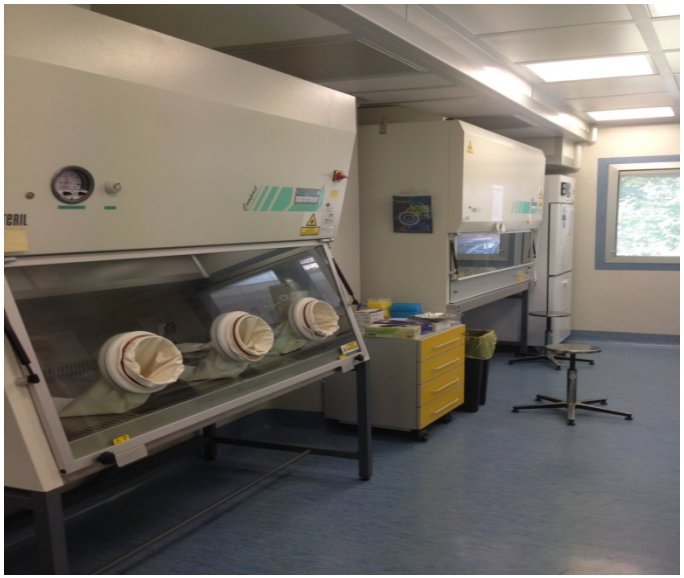
Neutralization assay

• mandatory for differentating WNV-specific from cross-reactive antibodies.

• can also confirm acute infection by demostrating a fourfold or greater change in WNV-specific neutralizing antibody titer between acute- and convalescent-phase serum samples collected 2 to 3 weeks apart.

• It requires culturing of WNV and must be performed in BSL3 reference laboratories by trained personnel.





WNV molecular testing

• Reverse transcriptase-polymerase chain reaction (RT-PCR) can be performed on serum, CSF and urine, collected early in the course of illness.

• Adoption of multiple PCR techniques is adviced.

West-Nile virus Real-time RT-PCR targeting a conserved region of West-Nile virus lineage 1 and 2 (Linke et al., Virol Methods 2007; 146: 355-358).

Pan-Flavivirus nested RT-PCR (Sánchez-Seco et al., J Virol Methods 2005;126: 101-109; Scaramozzino et al., J Clin Microbiol 2001;39: 1922-1927).

•Sequencing of PCR products is mandatory for:

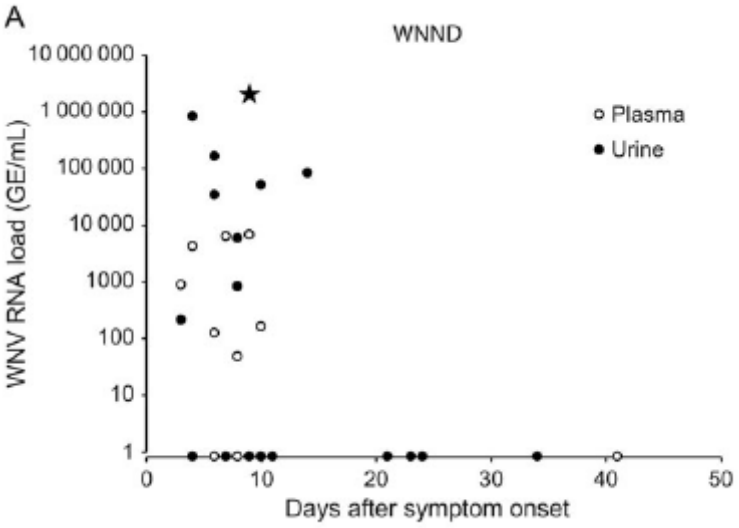
- Specificity confirmation
- Epidemiology of circulating WNV strains

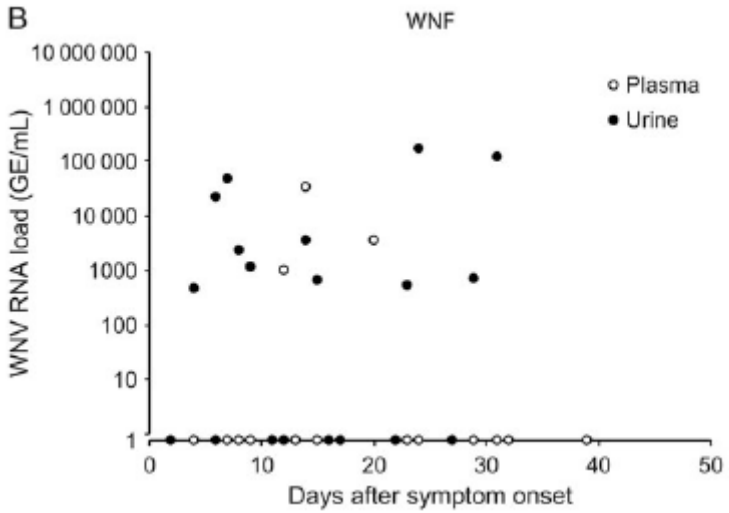
JID 2013: 208 (1 October)

Excretion of West Nile Virus in Urine During Acute Infection

Luisa Barzon,[1,2] Monia Pacenti,[2] Elisa Franchin,[1,2] Silvana Pagni,[1,2] Thomas Martello,[2] Margherita Cattai,[2] Riccardo Cusinato,[2] and Giorgio Palù[1,2]

[1]Department of Molecular Medicine, University of Padova, and [2]Regional Reference Laboratory for Infectious Diseases, Microbiology and Virology Unit, Padova University Hospital, Padova, Italy





JCM 2014 Sep;52(9):3411-3

JCM
Journals.ASM.org

Isolation of West Nile Virus from Urine Samples of Patients with Acute Infection

Luisa Barzon,[a,b] Monia Pacenti,[b] Elisa Franchin,[a,b] Laura Squarzon,[a,b] Alessandro Sinigaglia,[b] Sebastian Ulbert,[c] Riccardo Cusinato,[b] Giorgio Palù[a,b]

Department of Molecular Medicine, University of Padua, Padua, Italy[a]; Microbiology and Virology Unit, Padua University Hospital, Padua, Italy[b]; Department of Immunology, Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany[c]

TABLE 1 Clinical and laboratory findings in patients with WNV RNA detected in urine

Case no.	Diagnosis[a]	WNV lineage	Days since symptom onset	Serum anti-WNV antibodies	WNV load in plasma (copies/ml)	WNV load in urine (copies/ml)	Urine storage conditions	WNV isolated from urine	Cell line for virus isolation[b]	Day of CPE appearance[b]	Mean WNV RNA copies/ml in cell supernatant[b]
1	WN-ND	1	8	IgM^+/IgG^-	8,000	2,500,000	Frozen	Yes	Vero E6	3	8.0×10^8
									BHK21	5	2.4×10^9
2	WN-ND	1	8	IgM^+/IgG^+	Undetectable	2,300,000	Frozen	No			
3	WN-ND	2	3	IgM^+/IgG^-	1,300	8,300,000	Unfrozen	Yes	Vero E6	2	1.6×10^9
									BHK21	4	8.9×10^9
4	WN-ND	2	2	IgM^+/IgG^+	Undetectable	15,000,000	Unfrozen	Yes	Vero E6	2	2.0×10^9
									BHK21	3	1.4×10^{10}
5	WN-ND	2	4	IgM^+/IgG^+	100	1,300,000	Frozen	No	NA[c]	NA	NA
6	WN-ND	2	4	IgM^+/IgG^-	400	350,000	Frozen	No	NA	NA	NA
7	WN-ND	2	5	IgM^+/IgG^+	Undetectable	100	Unfrozen	No	NA	NA	NA
8	WN-F	2	8	IgM^+/IgG^-	Undetectable	180,000	Unfrozen	Yes	Vero E6	3	1.2×10^9
									BHK21	4	9.0×10^9
9	WN-F	2	10	IgM^+/IgG^+	Undetectable	1,200,000	Frozen	No	NA	NA	NA
10	WN-F	2	21	IgM^+/IgG^+	Undetectable	<100	Frozen	No	NA	NA	NA
11	WN-F	2	16	IgM^+/IgG^+	Undetectable	28,000	unfrozen	No	NA	NA	NA
12	WN-F	2	14	IgM^+/IgG^+	Undetectable	160	Unfrozen	No	NA	NA	NA
13	WN-F	2	3	IgM^-/IgG^-	Undetectable	28,000	Unfrozen	No	NA	NA	NA
14	Blood donor	1	5	IgM^-/IgG^-	3,900	1,000	Unfrozen	Yes	Vero E6	3	1.7×10^{10}
									BHK21	5	9.2×10^9
15	Blood donor	1	6	IgM^+/IgG^-	14,000	100,000	Frozen	No	NA	NA	NA
16	Blood donor	2	4	IgM^+/IgG^-	50,000	3,200	Unfrozen	Yes	Vero E6	3	1.6×10^8
									BHK21	5	2.0×10^9
17	Blood donor	2	4	IgM^-/IgG^-	2,500	37,000	Frozen	No	NA	NA	NA

[a] WN-ND, West Nile neuroinvasive disease; WN-F, West Nile fever.
[b] Data from WNV isolation in 6-well tissue culture plates.
[c] NA, not applicable.

Possible West Nile neuroinvasive disease (WNND)
pts in endemic or epidemic area presenting with:

- viral encephalitis
- viral meningitis
- polyradiculoneuritis
- acute flaccid paralysis

Possible West Nile fever (WNF)
Pts in endemic or epidemic area presenting with:

- fever ≥38°C
- absence of other concomitant diseases

Probable WNND and WNF
As above +

WNV-specific IgM and IgG or in serum with seroconversion or a 4-fold increase in IgG titers

Confirmed WNND and WNF
As above +

- WNV isolation from blood, CSF
- detection of WNV RNA in blood, CSF
- WNV-specific IgM in the CSF
- Confirm of WNV IgG-specificity by neutralization assay

Barzon L et al., JID 2013

Europe

Before 1996 WNV caused only sporadic infections in Europe.

1996: large outbreak of WNV neuroinvasive disease occured in Romania.

1997: outbreaks in human population in the Czeck Republic (5 cases).

1999: 826 pts admitted to hospital in Russia: 183 confirmed cases, 40 deaths.

2003: 7 human cases in France.

Italy (since **2008**) and Greece (since **2010**) have been the most affected countries in Europe.

Italy

1998: outbreak in Toscana limited to 14 horses, of which 6 died (no human cases)

2008: first human cases of WNND and WNF were detected in the Po river area in northeastern Italy

FIGURE 1

West Nile virus outbreaks in Italy, 1998 (horses) and 2008 (horses and humans)

Gobbi et al., Eurosurveillance, 2009

Rapid communications

First human case of West Nile virus neuroinvasive infection in Italy, September 2008 – case report

G Rossini[1], F Cavrini[1], A Pierro[1], P Macini[2], A. C. Finarelli[2], C Po[2], G Peroni[3], A Di Caro[4], M Capobianchi[4], L Nicoletti[5], M P Landini[1], V Sambri (vittorio.sambri@unibo.it)[1]

1. Centro di Riferimento Regionale per le Emergenze Microbiologiche (Regional Reference Centre for Microbiological Emergencies - CRREM), Microbiology Unit, Azienda Ospedaliero-Universitaria di Bologna, Policlinico S.Orsola-Malpighi, Bologna, Italy
2. Servizio di Sanità Pubblica (Public Health Service), Regione Emilia Romagna, Bologna, Italy
3. Department of Public Health, Azienda Unità Sanitaria Locale di Imola, Imola, Italy
4. Istituto Nazionale Malattie Infettive (National Institute of Infectious Diseases) "L. Spallanzani", Rome, Italy
5. Istituto Superiore di Sanità (National Institute of Health, ISS), Rome, Italy

Rapid communications

West Nile virus transmission with human cases in Italy, August - September 2009

C Rizzo (caterina.rizzo@iss.it)[1], F Vescio2, S Declich[1], A C Finarelli[3], P Macini[3], A Mattivi[3], G Rossini[4], C Piovesan[5], L Barzon[6], G Palù[6], F Gobbi[7,8], L Macchi[9], A Pavan[9], F Magurano[2], M G Ciufolini[2], L Nicoletti[2], S Salmaso[1], G Rezza[2]

1. National Centre for Epidemiology, Surveillance and Health Promotion, National Institute of Health (Istituto Superiore di Sanità, ISS), Rome, Italy
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5. Direction of Prevention, Veneto region, Venice, Italy
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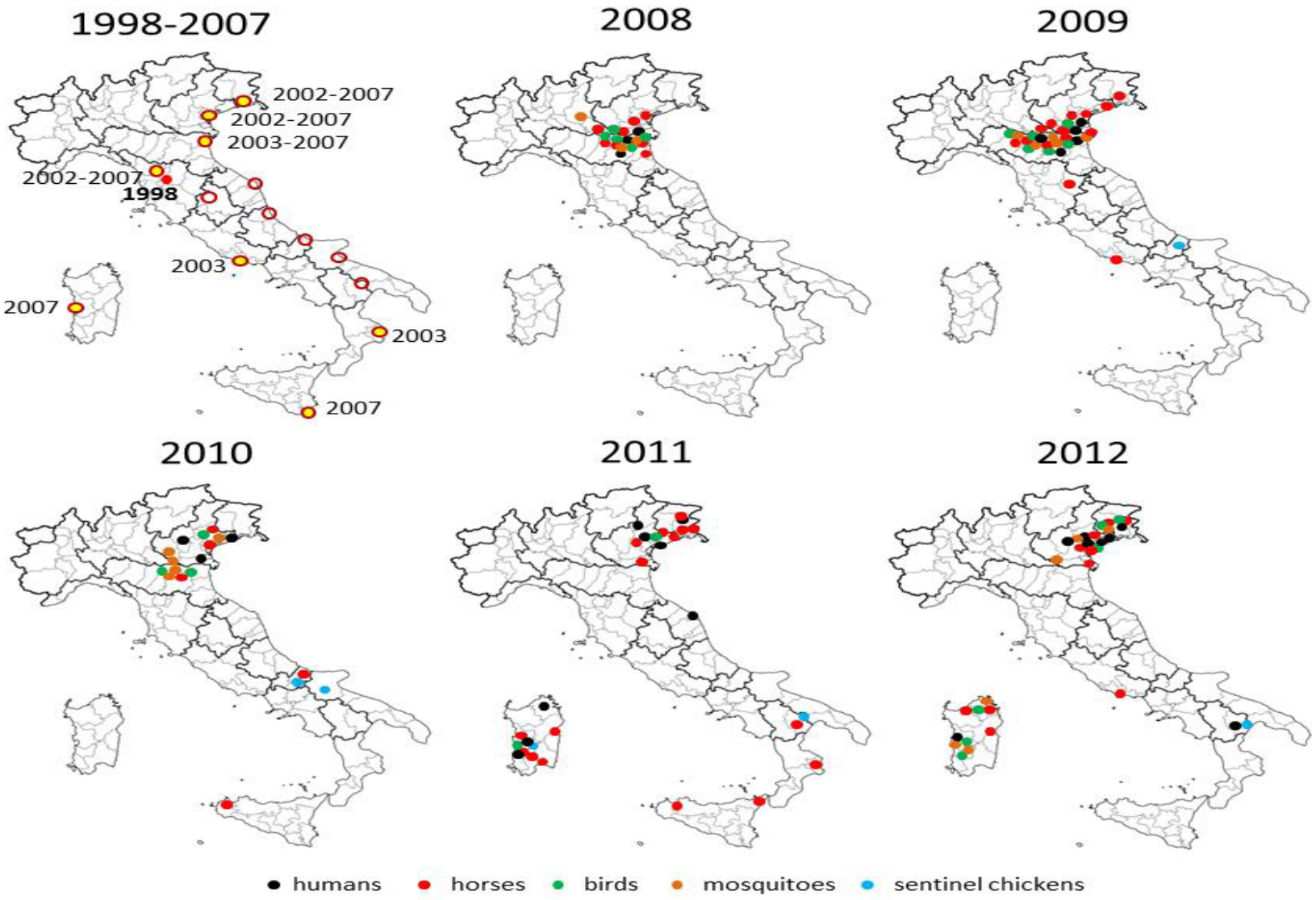
8. Department of Prevention, ULSS 20, Verona, Italy
9. Regional Health Authority of Lombardy, Milan, Italy



Barzon et al., *Int. J Environ. Res Public Health*, 2013

Figure 2. Map of Italy showing the areas where different WNV strains were detected in the period from September 2008 to August 2013. WNV lineage 1 strains are indicated in blue; WNV lineage 2 strains are indicated in red.

Barzon et al., *Int. J Environ. Res Public Health*, 2013

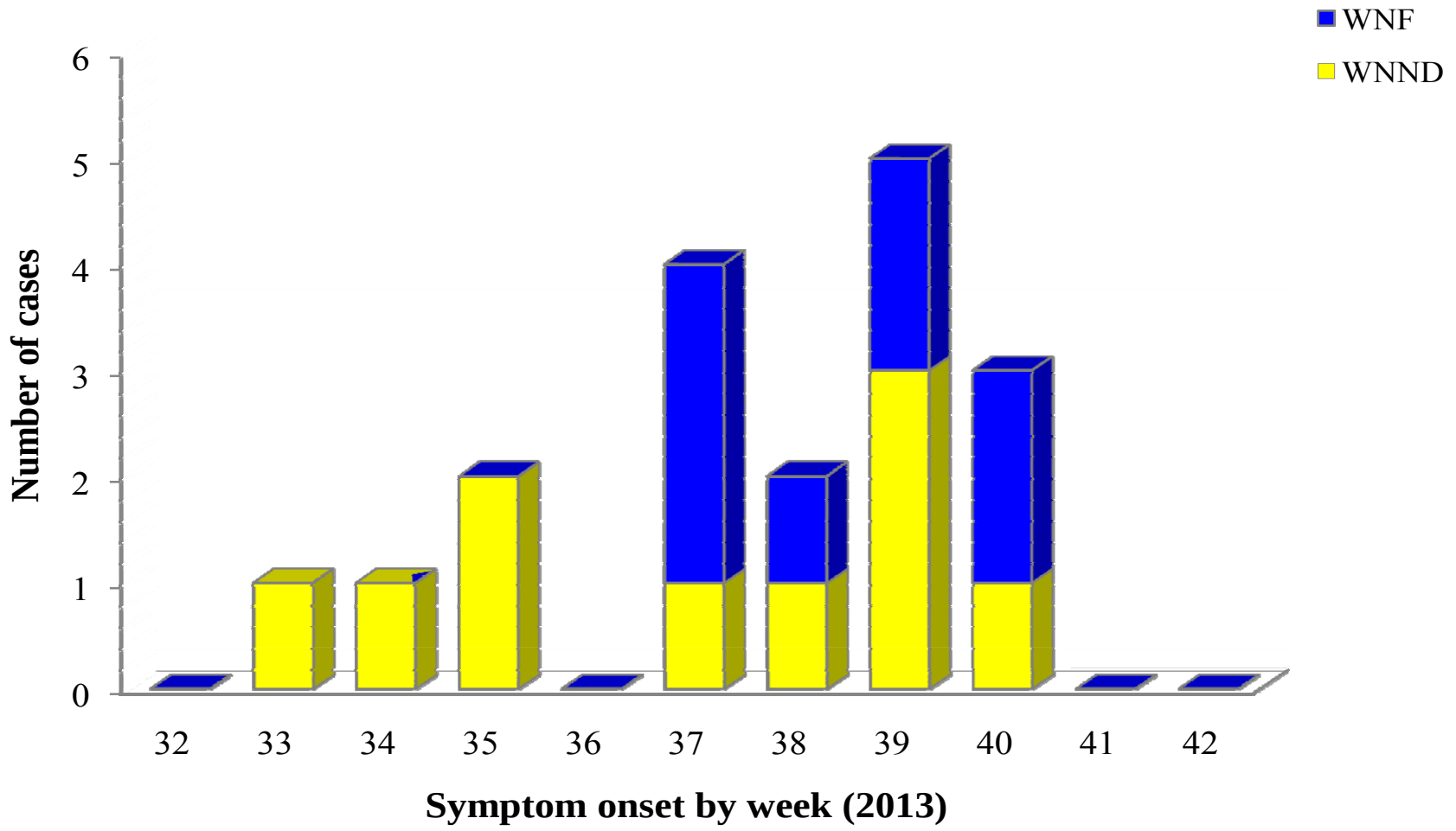
West Nile Virus in Lombardia region

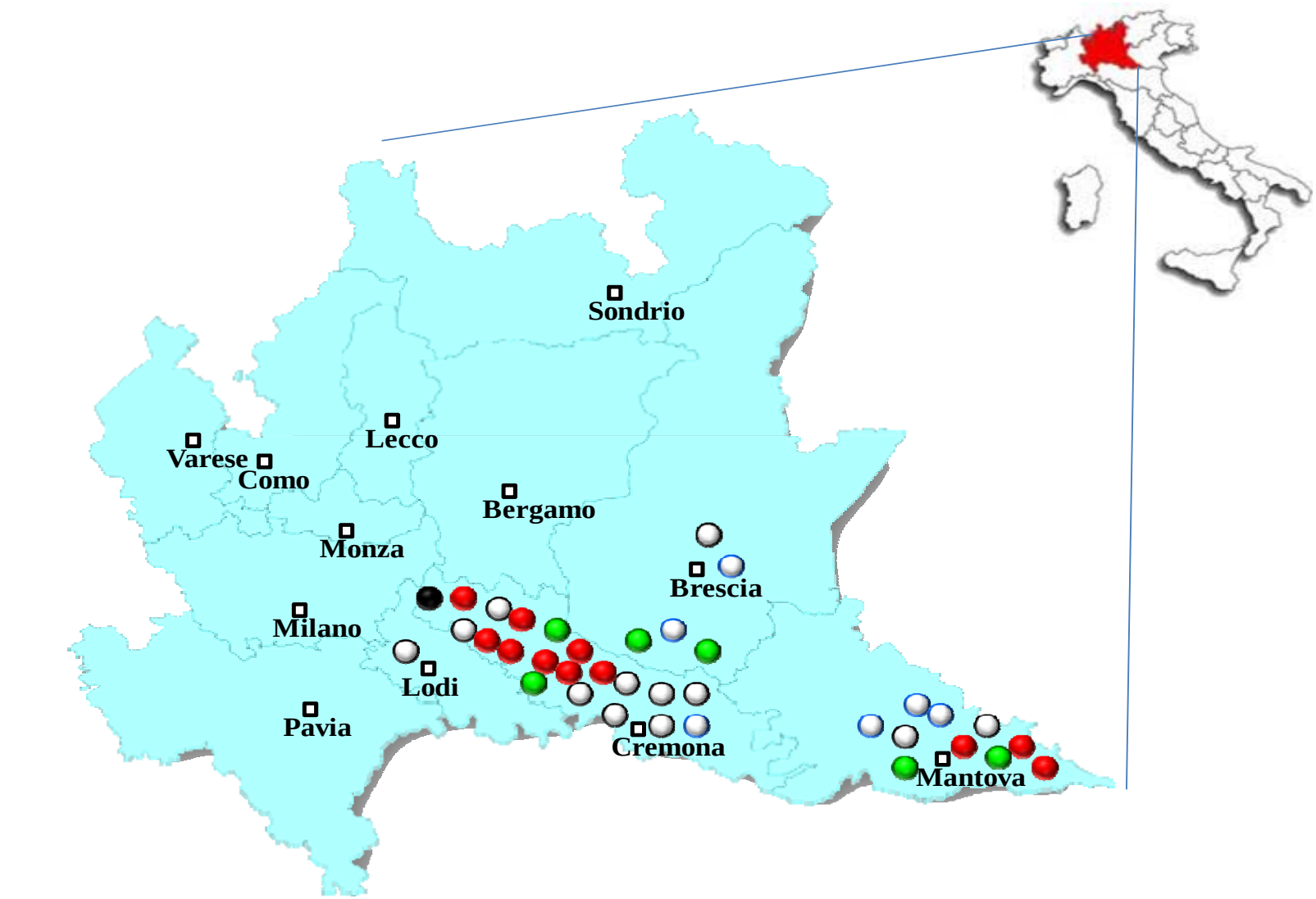
- **2007-2012,** 455 patients with potential WNV disease were investigated, but no WNV cases were found.

- **13 Aug. 2013-7 Oct. 2013**, 18 cases of WNV infection were diagnosed.

18 cases of WNV infection

- 10 confirmed cases of acute WNV neuroinvasive disease (3 of them died)
- 8 cases of acute WNV fever (7 confirmed and 1 probable)


WNF
WNND
Number of cases
0
1
2
3
4
5
6
32
33
34
35
36
37
38
39
40
41
42
Symptom onset by week (2013)


Sondrio
Lecco
Varese
Como
Bergamo
Monza
Brescia
Milano
Lodi
Pavia
Cremona
Mantova
positive humans
positive birds
positive horses
positive mosquitoes

Patients with WNV neuroinvasive disease

- 9 (90%) males and 1 (10%) females
- 4 Mantova, 2 Cremona, 3 Brescia and 1 Lodi
- median age 75 years (range, 54-89)
- 33% (3/9) case -fatality rate

Patients with WNV fever

- 4 (50%) males and 4 (50%) females
- 1 Mantova and 7 Cremona
- median age 58 years (range, 17-87)

Characteristics of human WNV infections, in the Lombardia Region, August-October 2013.

					Elisa IgM[1]		Elisa IgG[2]			RT-PCR[3,4]		
	Age/Sex	Origin	Clinical presentation	Outcome	serum	CSF	serum	CSF	Neutralization	serum	CSF	urine
1	78/M	Mantova	encephalitis	alive	+	+	+	-	+	+	+	NA
2	66/M	Mantova	meningoencephalitis	alive	+	+	+	+	+	-	-	NA
3	89/M	Mantova	encephalitis	dead	+	+	+	-	+	-	-	NA
4	49/M	Cremona	West Nile fever	alive	+	NA	+	NA	+	-	NA	NA
5	55/F	Cremona	West Nile fever	alive	+	NA	-	NA	ND	-	NA	NA
6	75/F	Cremona	encephalitis	alive	+	+	+	+	+	-	-	NA
7	61/M	Mantova	West Nile fever	alive	+	NA	-	NA	ND	-	NA	+
9	17/M	Cremona	West Nile fever	alive	+	NA	+	NA	+	-	NA	-
10	71/F	Cremona	West Nile fever	alive	+	NA	+	NA	+	-	NA	-
11	63/M	Cremona	West Nile fever	alive	+	NA	+	NA	+	-	NA	-
12	27/F	Cremona	West Nile fever	alive	+	NA	+	NA	+	-	NA	-
13	57/M	Lodi	encephalitis	dead	+	NA	+	NA	+	-	NA	-
14	78/M	Brescia	encephalitis	alive	+	+	+	+	+	-	-	-
15	76/M	Brescia	meningoencephalitis	alive	+	NA	+	NA	+	-	NA	NA
16	79/M	Mantova	encephalitis	dead	+	+	+	+	+	-	-	NA
17	87/F	Cremona	West Nile fever	alive	+	NA	+	NA	+	-	NA	-
18	54/M	Brescia	encephalitis	alive	+*[6]	-[6]	+*[6]	-[6]	ND	-[5]	+[5]	+[5]

[1] WNV IgM Capture DxSelect (Focus Diagnostics); [2] WNV IgG DxSelect (Focus Diagnostics);
[3] real-time RT-PCR WNV L1-2 [14]; [4] Nested RT-PCR pan-Flavivirus [15-16]; [5] real-time RT-PCR Flavivirus [19]; [6] WNV IgG/IgM IIFT (Euroimmun);
* convalescent serum sample;
NA, not available; ND, not done

Sequencing of NS5 gene

Partial sequencing of the NS5 gene performed for positive urine samples demonstrated that the virus was a WNV lineage 2 strain, with > 95% sequence identity to WNV Rovigo strains circulating in Veneto Region in 2013 (GenBank: KF647251.1; GenBank: KF5883365.1).

West Nile Virus in Lombardia region in 2014

- **21 Aug. 2014-6 Oct. 2014**, 15 cases of WNV infection were diagnosed.

15 cases of WNV infection

- 13 confirmed cases of acute WNV neuroinvasive disease
- 2 confirmed cases of acute WNV fever

Patients with WNV neuroinvasive disease

- 11 (85%) males and 2 (15%) females
- 2 Mantova, 4 Cremona, 4 Pavia, 1 Lodi and 1 Piacenza
- median age 76 years (range, 12-86)

Patients with WNV fever

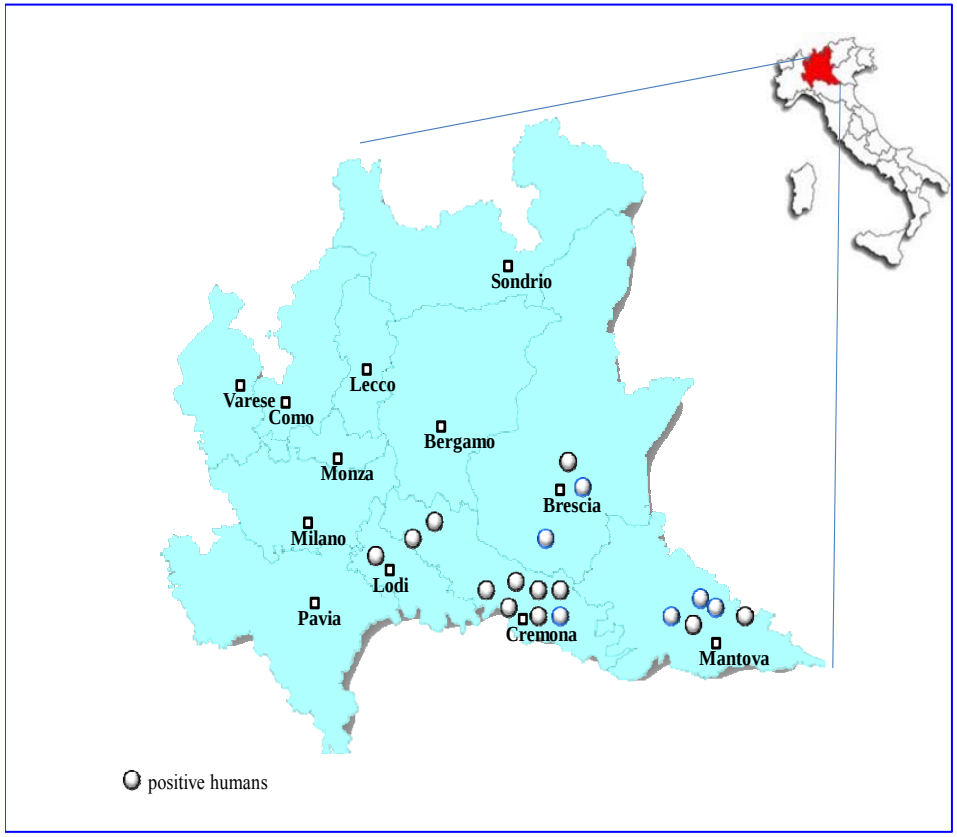
- 1 (50%) male and 1 (50%) female
- 1 Pavia and 1 Cremona
- median age 54 years (range, 39-70)

Table 1. Characteristics of human WNV infections, August-October 2014.

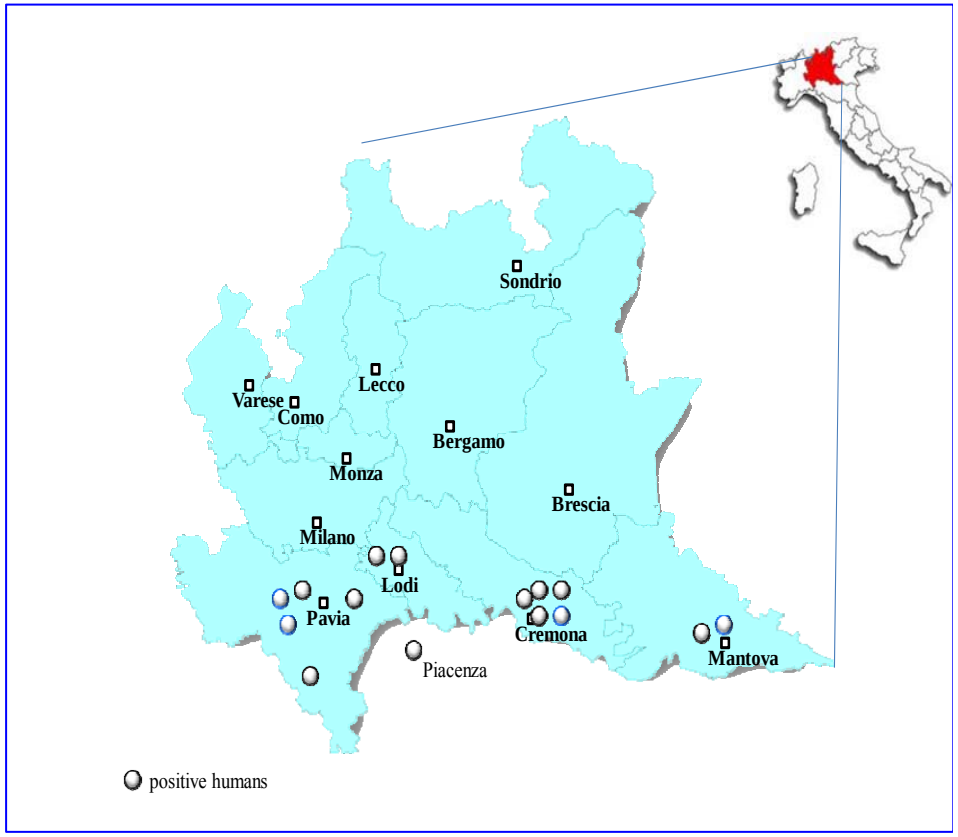
Patient	Age/Sex	Origin	Clinical presentation	Outcome	Elisa IgM[1]		Elisa IgG[2]		Neutralization	RT-PCR[3,4]		
					serum	CSF	serum	CSF		serum	CSF	urine
1	76/M	Pavia	WNND	alive	+	+	+	-	+	-	-	+
2	86/M	Cremona	WNND	dead	+	+	-	-	-	-	-	+
3	56/M	Cremona	WNND	alive	+	+	+	-	+	-	-	+
4	84/M	Pavia	WNND	alive	+	+	+	-	+	-	-	-
5	39/F	Cremona	WNF	alive	+	NA	+	NA	+	-	NA	-
6	72/M	Mantova	WNND	alive	+	NA	+	NA	+	+	NA	-
7	12/M	Pavia	WNND	alive	+	+	+	-	+	-	-	-
8	77/M	Lodi	WNND	alive	+	+	+	-		-	-	NA
9	77/F	Lodi	WNND	alive	+	+	+	+	+	-	-	-
10	78/M	Piacenza	WNND	?	+	+	+	+	+	-	-	+
11	81/F	Pavia	WNND	dead	+	NA	-	NA	+	-	-	+
12	65/M	Mantova	WNND	alive	+	+	+	-	+	-	-	-
13	52/M	Cremona	WNND	alive	+	NA	+	NA	+	-	NA	+
14	70/M	Pavia	WNF	alive	+	+	+	+	+	-	-	-
15	55/M	Cremona	WNND	alive	+	+	+	+	+	-	-	-

[1] WNV IgM Capture DxSelect (Focus Diagnostics); [2] WNV IgG DxSelect (Focus Diagnostics);
[3] real-time RT-PCR WNV L1-2 [14]; [4] Nested RT-PCR pan-Flavivirus [15-16];
NA, not available; ND, not done

2013


Sondrio
Lecco
Varese
Como
Bergamo
Monza
Brescia
Milano
Lodi
Pavia
Cremona
Mantova
positive humans

2014


Sondrio
Lecco
Varese
Como
Bergamo
Monza
Brescia
Milano
Lodi
Pavia
Cremona
Mantova
Piacenza
positive humans

Molecular Diagnosis on CSFs
(01-06-2013/30-11-2013)

Enterovirus	3
Herpes Simplex Virus	3
Varicella zoster Virus	2
West NileVirus	10

Molecular Diagnosis on CSFs
(01-06-2014/30-11-2014)

Enterovirus	2
Herpes Simplex Virus	3
Varicella zoster Virus	2
West NileVirus	12

Conclusions

•The veterinary and entomological surveillance program of WNV confirmed the presence of WNV infection in mosquitoes, horses and birds in Lombardia in summer 2013 and 2014.

•The occurrence of human cases in two consecutive years suggest the establishment of WNV in Lombardy region.

• WNV infection is presently a Major cause of neurological disorders in Lombardy during the summer period.

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