

West Nile Virus outbreak in Lombardia region, Northern Italy, in summer 2013

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Introduction and Purpose: West Nile virus (WNV) is a positive-sense RNA virus belonging to the *Flaviviridae* family (genus *Flavivirus*). The virus is now endemic in many countries and has caused large outbreaks in recent years in Europe and North America [1]. WNV is maintained in nature, cycling between birds, the main WNV vertebrate hosts and mosquitoes. Humans and horses are incidentally infected and represent “dead-end” hosts [2]. Symptomatic infections are mostly characterized by a mild, self-limiting febrile illness [2], while WNV neuroinvasive disease (WNNND) develops in < 1% of WNV-infected persons.

In Italy, on summer 2008 the first human cases of WNNND and WNV fever (WNF) were diagnosed in an area of the Po river valley across the Emilia-Romagna and Veneto Regions [3-4]. From 2009 to 2012 human cases of WNV infections were detected in Emilia-Romagna, Veneto, Friuli-Venezia Giulia, Sardinia, Marche, and Basilicata Regions [5]. The Lombardy Region was only marginally involved in the WNV epidemics (two human cases) in province of Mantua in 2008 [6]. In this report, an outbreak of WNV involving mosquitoes, birds, horses and humans, in four different provinces of the Lombardy Region is described.

Methods: serum and cerebral spinal fluid (CSF) samples of patients with potential WNV infections were tested for the presence of specific IgM and IgG antibodies (WNV IgM Capture DxSelect and WNV IgG DxSelect by Focus Diagnostics, Cypress, USA). Furthermore, the presence of WNV specific antibodies was confirmed by a plaque-reduction neutralization test (PRNT) [7].

Serum, CSF and urine samples, collected during the acute phase, were examined for the presence of WNV RNA with three methods: a real-time reverse transcriptase-polymerase chain reaction (RT-PCR) targeting a conserved region of WNV lineages 1 and 2 [8] and two pan-Flavivirus nested RT-PCRs [9-10] followed by sequencing of amplicons. In addition, WNV RNA was searched for in urine samples as previously suggested [7]. Furthermore, 455 stored CSF samples collected in six previous spring-autumn seasons 2007-2012, which had been previously scored as negative for bacterial and virus infections, were investigated retrospectively to evaluate the potential unreported presence of WNV infection in Lombardy.

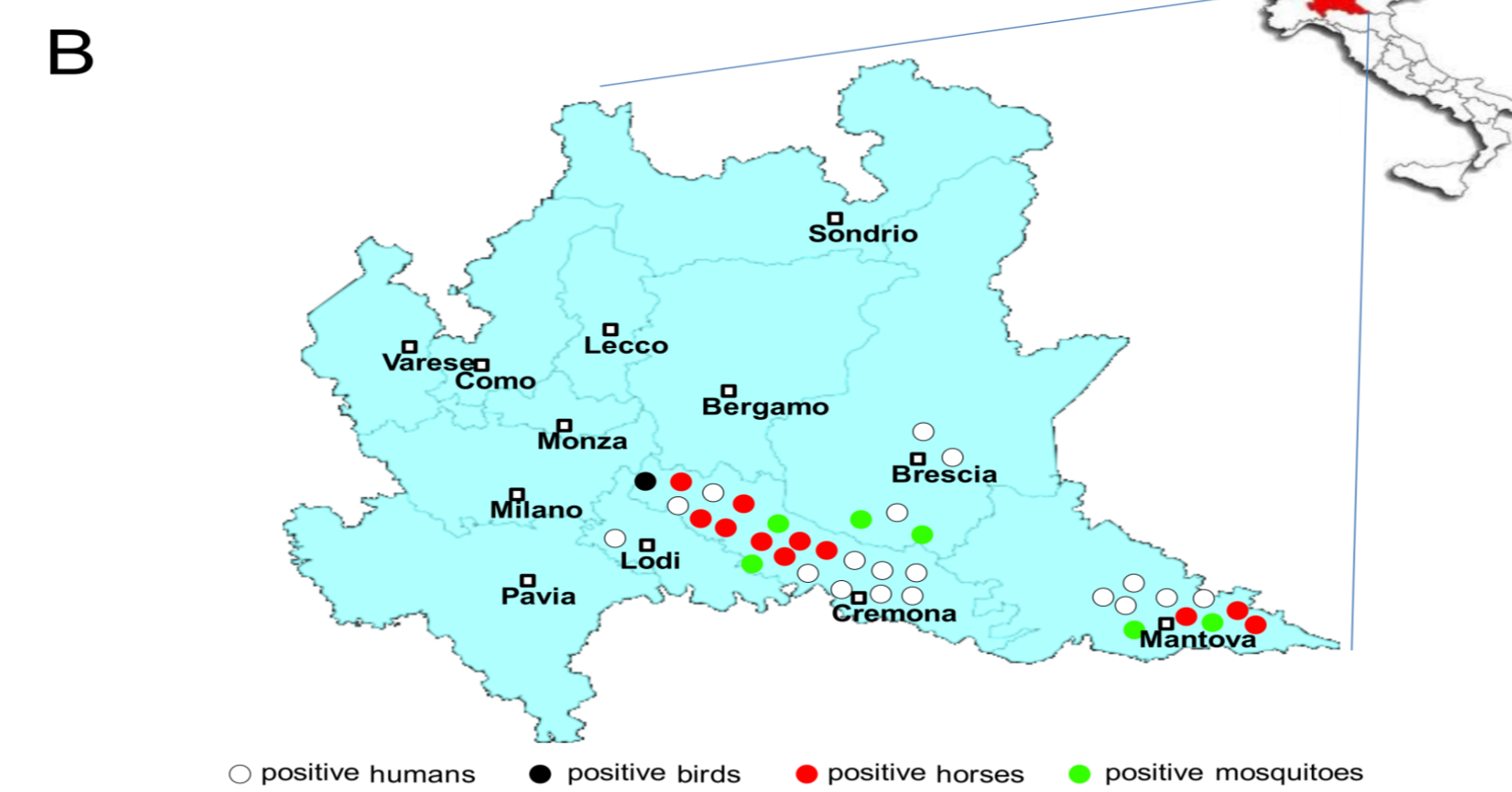
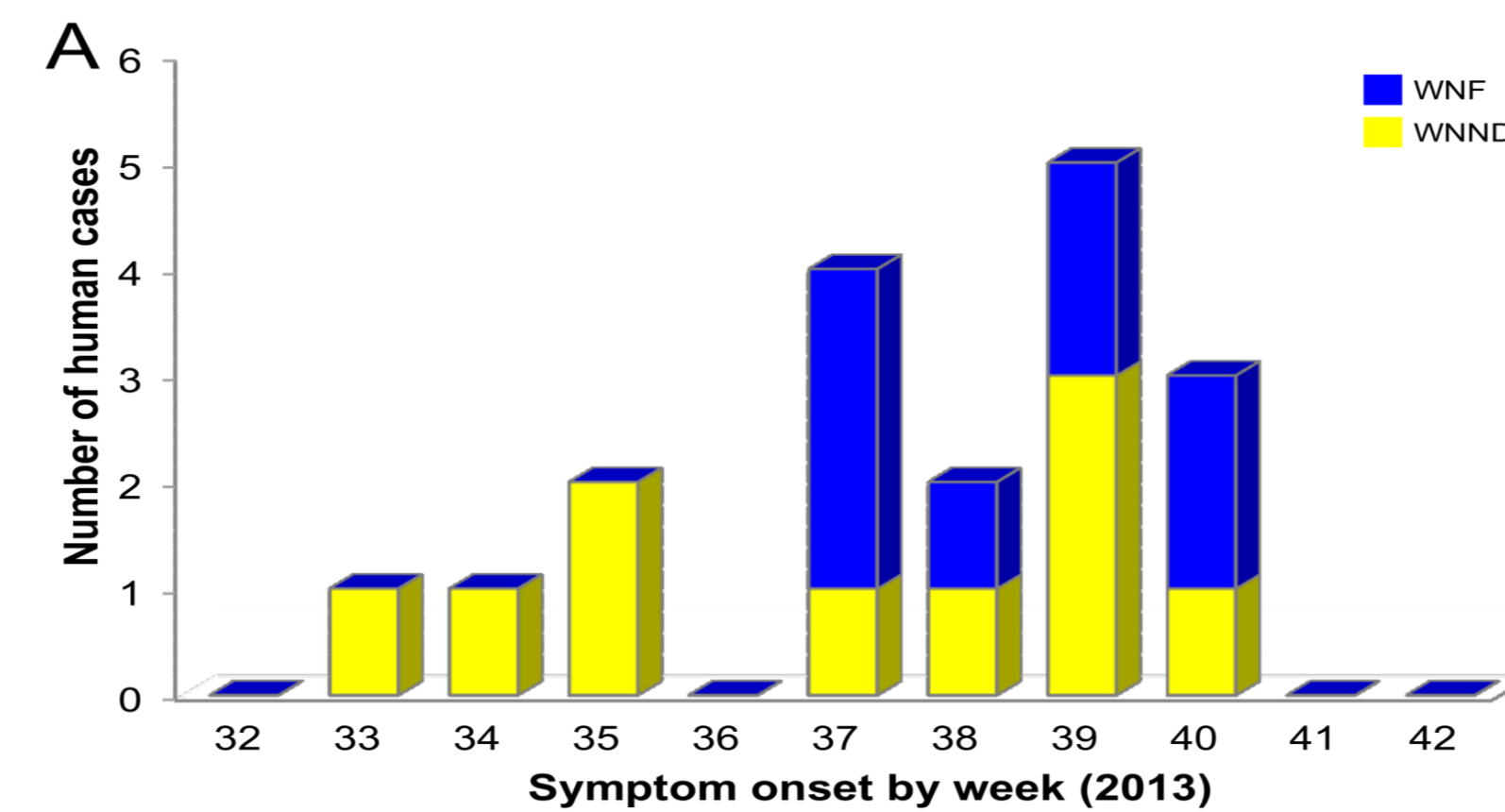
Results: in the period August 13 to October 4, 2013, ten confirmed cases of acute WNNND and eight cases of acute WNF (six confirmed and two probable) were diagnosed (Figure A). WNV cases were distributed in four different provinces: Cremona (n=9), Mantua (n=5), Brescia (n=3) and Lodi (n=1) in the southeastern area of the Lombardy Region (Figure B), in close proximity to areas of the Emilia Romagna and Veneto regions affected by WNV outbreaks in previous years. Among patients with WNNND, nine (90%) were males and one (10%) female, with a median-age of 75 years (range, 54-89). WNNND cases were detected in all the four provinces involved in the WNV outbreak. All WNNND patients required hospitalization: eight presented with encephalitis 3 of them died and two with meningoencephalitis. Of the eight patients with WNF, four (50%) were males and four (50%) females, with a median age of 58 years (range, 17-87). The cases were mostly detected in patients living in the province of Cremona (n=7) and one in the province of Mantua. Six were confirmed cases of WNF and two were probable. The most common clinical signs were fever (n=8) and rash (n=2). Sequencing of gene NS5 showed that all WNV strains belonged to lineage 2. Evidence of this outbreak during summer of 2013 is further strengthened by the finding that among the 455 CSF samples collected during the six previous summer seasons, only one was scored as IgG positive.

Conclusions: an outbreak of WNV in the summer of 2013 involving the southern-eastern provinces of the Lombardy Region is described, as a part of the larger outbreak involving the neighboring Emilia-Romagna and Veneto regions. This is the largest outbreak of WNV reported in Lombardy so far, showing a west bound spread of the virus after 5 years spread in a Northeastern direction.

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| 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 | 78/M | Mantua | encephalitis | alive | + | + | + | - | + | + | + | NA |
| 2 | 66/M | Mantua | meningoencephalitis | alive | + | + | + | + | + | - | - | NA |
| 3 | 89/M | Mantua | 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 | Mantua | West Nile fever | alive | + | NA | - | NA | ND | - | NA | + |
| 8 | 54/M | Cremona | encephalitis | alive | + | + | + | - | + | - | - | + |
| 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 | Mantua | 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 |

¹WNV IgM Capture DxSelect (Focus Diagnostics); ²WNV IgG DxSelect (Focus Diagnostics); ³real-time RT-PCR WNV L1-2 [15]; ⁴Nested RT-PCR pan-Flavivirus [16-17]; ⁵real-time RT-PCR Flavivirus [20]; ⁶WNV IgG/IgM IIFT (Euroimmun); * convalescent serum sample; ND: not done; NA: not available