

New incursions of West Nile virus lineage 2 in Italy in 2013: the value of the entomological surveillance as early warning system

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Summary

West Nile virus (WNV) is one of the most serious public health threats that Europe and the Mediterranean countries are currently facing. In Italy, WNV emerged in 1998 and has been circulating since 2008. To tackle its continuous incursions, Italian national and regional institutions set up a surveillance program, which includes the serological screening of sentinel horses, sentinel-chickens and backyard poultry flocks and the surveillance on all equine neurological cases, resident captured and wild dead birds, and vectors. This communication aims to assess the importance of the entomological surveillance program as an early warning system for WNV circulation. In the province of Modena, the circulation of WNV lineage 2 strains was first detected in pools of *Culex pipiens* on July the 3rd, 42 days prior to the onset of the first 2013 human WNV neuroinvasive case reported in the same province. Similarly in Veneto, WNV was first detected on July 3rd in a pool of *Cx. pipiens* collected in the province of Venezia. The first human neuroinvasive case in this region occurred in the Rovigo province on July the 24th, seven days after the detection of WNV lineage 2 in a mosquito pool collected in the same province. Up to the end of July 2013, WNV circulation was further detected in several other pools of *Cx. pipiens* mosquitoes collected in Emilia-Romagna, Veneto and Lombardia. According to the NS3 partial sequence alignments including all recent European and Italian Lineage 2 strains, the new circulating WNV lineage 2 strains share high nt homology with the Hungarian and with the previous lineage 2 strains isolated in Veneto and Sardegna in 2011 and 2012. These data provide a clear and practical demonstration of the relevance of a reliable entomological surveillance program to early detect WNV in Italy.

L'importanza della sorveglianza entomologica nel rilevare precocemente la circolazione dei ceppi del virus della West Nile di linea genetica 2: l'esperienza italiana del 2013

Parole chiave

Culex pipiens,
Italia,
Sistema di allerta rapido,
Sorveglianza
entomologica,
Virus della West Nile
linea genetica 2.

Riassunto

Il virus della West Nile costituisce una delle emergenze sanitarie che attualmente minacciano la sanità pubblica europea e dei paesi del Mediterraneo. In Italia il virus è comparso per la prima volta nel 1998, è quindi riapparso nel 2008 e da allora non ha smesso di circolare. Per fronteggiare le continue incursioni virali, le Autorità nazionali e regionali hanno realizzato un piano di sorveglianza che implica l'utilizzo di polli e cavalli sentinella, l'esame di tutti i cavalli affetti da forme nervose, delle carcasse di uccelli selvatici e domestici recuperate nelle aree a rischio e dei vettori. Questa comunicazione vuole valutare l'importanza della sorveglianza entomologica come sistema di allerta rapido per rilevare la circolazione del virus della West Nile. Nel 2013, ceppi di WNV di linea genetica 2 sono stati rilevati per la prima volta in un pool di zanzare *Culex pipiens* catturate in provincia di Modena il 3 luglio, in anticipo di 42 giorni rispetto al primo caso umano della forma neuroinvasiva di West Nile riscontrato nella stessa provincia. Analogamente in Veneto, il virus è stato rilevato per la prima volta il 3 luglio 2013 in un pool di zanzare *Cx. pipiens* catturate in provincia di Venezia. In questa regione, il primo caso di encefalite umana da WNV è stato diagnosticato in provincia di Rovigo il 24 luglio, 7 giorni dopo il primo rilevamento del virus in un pool di zanzare *Cx. pipiens* catturate nella stessa provincia. Altre positività al WNV sono state rilevate in pool di zanzare *Cx. pipiens* prelevate in Lombardia, Emilia Romagna e Veneto. Il multiallineamento delle sequenze parziali del gene NS3 dei nuovi ceppi di WNV di linea genetica 2 rilevati in questo studio con quelli circolati di recente in Italia e in Europa, ha evidenziato un'elevata omologia tra i nuovi ceppi e quelli isolati in Ungheria nel 2004 e in Italia nel 2011 e 2012. Quanto riscontrato è una chiara dimostrazione dell'efficacia della sorveglianza entomologica come sistema di allerta rapido per il virus della West Nile.

West Nile virus (WNV) is today the most widespread arbovirus in the world and is one of the most serious public health threats that Europe and the Mediterranean countries are currently facing. WNV belongs to the family *Flaviviridae* of the genus *Flavivirus*. Serologically, it is included in the Japanese encephalitis group, while virologically it can be designated into 8 phylogenetic lineages (23). Only lineage 1 and 2 West Nile viruses have been associated with significant outbreaks in humans and horses, which act as incidental hosts in the natural enzootic bird-mosquito-bird cycle. Infections are usually subclinical and only part of them develops clinical signs as either West Nile fever (25%) or neuroinvasive disease (1%) (16).

Before 1990s, human and horse cases were sporadic and mainly confined to Africa. Since the mid 1990s new viral strains, with likely African origin, invaded Western countries causing disease in humans, horses and birds. Large outbreaks of increased clinical severity have been reported in parts of Russia, Southern and Eastern Europe, Northern Africa and North and South America (12). Until 2010, strains of WNV lineage 1 were responsible for most of these outbreaks. After 2010, in a sort of baton passing, lineage 2 replaced lineage 1 strains as the major virus responsible for European WNV outbreaks. Between 2011-2012, 1,276 human cases were recorded in

Europe, Russian Federation and Mediterranean basin (10). New human cases have been already reported in Greece, Macedonia, Russian Federation and Israel in 2013 (10). Two neuroinvasive cases have also been described in Italy where the WNV situation is quite complex due to the concurrent circulation of both lineages (17, 18).

In Italy, WNV lineage 1 first appeared in 1998 (2). It then reappeared in the North-Eastern part of Italy in 2008 (5) where it became endemic (4, 11, 14). Unrelated new foci were also reported in Central and Southern Italy (6) and, more recently, lineage 2 strains were detected in Central (3) and Northern-Eastern Italy as well as in Sardegna (8, 19, 20). Following the first outbreak of West Nile, the Italian government put in place a multi-species national surveillance program including birds, chicken, horses, mosquitoes, and humans. The aim of this program is to detect early incursions of new viruses and monitor the possible spread of infection. The national program includes the serological screening of sentinel horses, sentinel-chickens and backyard poultry flocks and the surveillance on all equine neurological cases, resident captured and wild dead birds, and vectors (13, 22). Ten high-risk areas have been identified on the basis of the presence of significant numbers of waterfowl and species of migratory birds. The surveillance plan

has been updated annually in line with changes in the WNV epidemiological situation. Besides the entomological activities supported by the Ministry of Health at national level, more comprehensive surveillance programs are also carried out at Regional level in WNV affected areas (1, 7, 15).

The entomological surveillance is based on a range of collection sites placed either in at-risk areas or in areas with virus circulation and aims both at identifying possible WNV vector species and at determining their abundance and distribution. Mosquitoes were collected by attractive traps, grouped in pools (50 or 200 of specimens) according to species, data and sites of sampling and screened by biomolecular analysis for the presence of WNV (20).

The purpose of this rapid communication is to emphasize the importance of having in place a functional entomological surveillance program to early detect the WNV circulation. The 2013 entomological plan started on May the 6th in Veneto and on June the 4th in Emilia-Romagna. By the end of July, 247,914 mosquitoes were classified and tested in Veneto, and 173,009 in Emilia-Romagna. Most of these mosquitoes (86.7%) were identified as *Culex pipiens*, the main vector of WNV in Italy. On July the 3rd, 42 days before the occurrence of the first 2013 human neuroinvasive case in the province

of Modena (17), two *Cx. pipiens* mosquito pools were found positive to WNV lineage 2. The positive mosquitoes were from Finale Emilia (Modena province) and Jesolo (Venezia province). In contrast to the province of Modena, no human or horse cases have been reported in the Venezia province after the positive catch; in Veneto the first human neuroinvasive case occurred in the Rovigo province on July the 24th (18), seven days after a mosquito pool collected in the same province was first found positive to the WNV lineage 2.

In the following weeks, the circulation of the virus was further detected in several other pools of *Cx. pipiens* mosquitoes collected in Emilia-Romagna and Veneto (Figure 1). Furthermore, WNV was also detected in a pool of mosquitoes sampled for experimental purposes in Lombardia region (Figure 1).

The NS3 423-bp fragment of the detected WNVs were amplified by nested RT-PCR (9), purified and used for direct sequencing in both directions using internal primers as described previously (19). Raw sequence data were assembled and translated into amino-acid sequences (Vector NTI software, Life Technology, Grand Island, NY, USA). Consensus sequences were aligned with other WNV lineage 2 NS3 sequences by using ClustalW program (21). The included sequences were those detected in Hungary in 2004

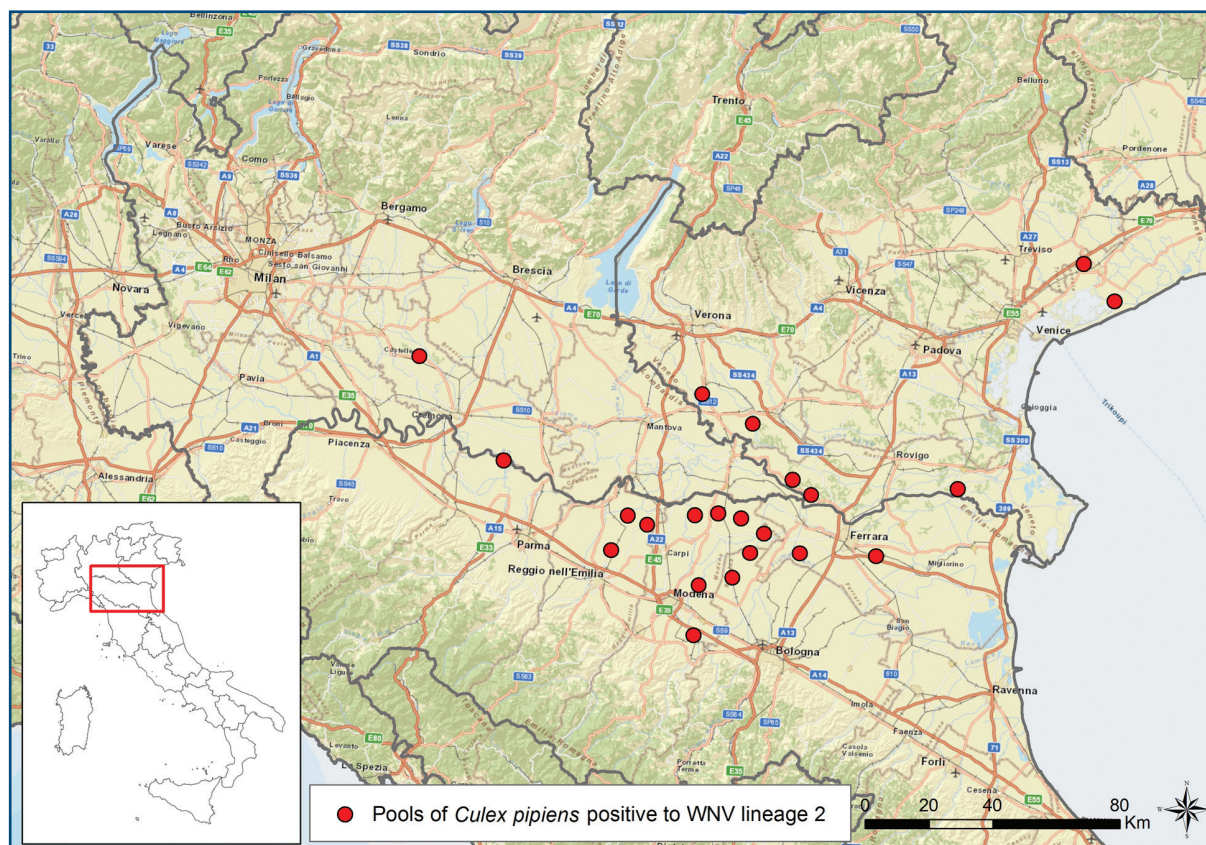


Figure 1. Geographic location of the *Culex pipiens* pools found positive to West Nile virus lineage 2 by specific RT-PCR (last update: 31st of July 2013).

(accession no. DQ116961), in Greece (accession no. HQ537483) and Italy (19) in 2010 and again in Italy in 2011 and 2012 (8, 19, 20). According to the partial NS3 sequence analyses, the 2013 North-Eastern strains share a high nt homology with the Hungarian and with the previous lineage 2 strains isolated in Veneto and Sardegna in 2011 and 2012, respectively (19, 20). Comparison of the deduced amino-acid sequences highlights a complete homology between these strains. Compared to the Greek isolate and to the strain isolated in the province of Rovigo in 2012 (8), all the 2013 detected strains showed histidine instead of proline at position 249.

These data provide a clear and practical demonstration of the relevance of a reliable entomological surveillance program to early detect WNV in Italy. The plan allowed for detecting WNV circulation much earlier than the occurrence of the human cases and provided important epidemiological information on the strains circulating in the surveyed area, which could infect humans. Having such a rapid health alarm system in place is of extreme value as it enables national and regional infrastructures to prevent/control infections as well as to be prepared to effectively face new infection waves.

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