Phylogenetic analysis of West Nile virus isolated in Italy in 2008

G Savini[1], F Monaco[1], P Calistri (p.calistri@izs.it)[1], R Lelli[1]
1. Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise "G. Caporale" (Institute of Experimental Zooprophylaxis of Abruzzo and Molise), Teramo, Italy

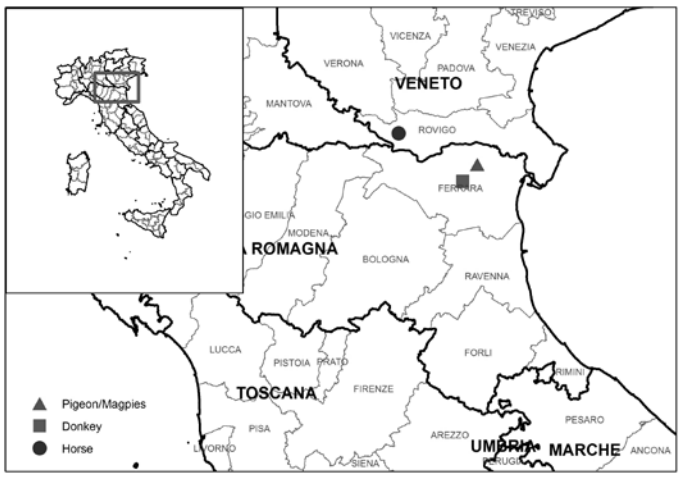
In Italy the first occurrence of West Nile virus (WNV) infection was reported in Tuscany region during the late summer of 1998. In August 2008, the WNV infection re-emerged in Italy, in areas surrounding the Po river delta, and involving three regions Lombardy, Emilia Romagna and Veneto. WNV was isolated from blood and organs samples of one horse, one donkey, one pigeon (*Columba livia*) and three magpies (*Pica pica*). The phylogenetic analysis of the isolates, conducted on 255 bp in the region coding for the E protein, indicates that these isolates belong to the lineage I among the European strains. According to the analysis, both the 1998 and 2008 Italian strains as well as isolates from Romania, Russia, Senegal and Kenya fell in the same sub-cluster.

Introduction

In Italy the first occurrence of West Nile virus (WNV) infection was reported in Tuscany region during the late summer of 1998 [1]. Since 2001, a national surveillance system has been in place in Italy, based on the periodical testing of sentinel-chicken flocks and sentinel horses [2]. Apart from sporadic seroconversions occurring in few horses and in few sentinel chickens, no further WNV outbreaks had been reported before 2008 either in horses or in humans.

Figure 1

Location of equine stables (horse and donkey) and sites (pigeon and magpies) were the animals were found from which West Nile virus (WNV) was isolated; Italy, 2008



In August 2008, the WNV infection re-emerged in Italy, in areas surrounding the Po river delta, involving three regions Lombardy, Emilia Romagna and Veneto. Following the evidence of first outbreaks in equines [3], extensive monitoring was carried out including syndromic surveillance in horses as well as laboratory analysis of samples collected in horse stables and from wild and domestic birds. In the infected stables insect traps were placed, and mosquitoes collected and identified. Mosquitoes, blood, serum and tissue samples from horses and birds within and around the outbreak area were collected and tested both serologically and virologically.

As a result, West Nile virus strains have been isolated from blood samples of one horse of the Rovigo Province and one donkey kept in a stable in the Ferrara Province, and from pools of brain, kidneys, heart and spleen of one pigeon (*Columba livia*) and three magpies (*Pica pica*) caught in the same territory (Figure 1). All these isolates were sequenced and found to be identical considering the 255 bp in the region coding for E protein. Full genome sequencing is ongoing.

Virus isolation and phylogenetic analysis

Virus isolation was performed on Vero E6 cell monolayers, RK13 or C6/36 followed by Vero E6 passages. The growth of WNV was confirmed either by reverse transcription polymerase chain reaction (RT-PCR) or by immunofluorescence (IFA).

Total RNA was extracted from WNV isolates. Viral RNA was reverse transcribed and amplified by using the one step RT-PCR kit (Qiagen, Germany). Available sequences were collected from Genbank and aligned to determining highly conserved genomic sequences flanking the genome region coding for the envelope (E) protein. Based on the alignment, a pair of primers was designed to amplify a 1,058 bp region. PCR products were purified with the Qiaquick PCR Purification kit (Qiagen, Germany) and used for direct sequencing in both directions using the following primers: WN_E_484F: 5'-actcaggcagggagattca -3', WN_E_622R: 5'-ttccgacagtcatcacgtagta -3', WN_E_634F: 5'-ttggtccatcgtgagtggt -3', WN_E_768R: 5'-gcccaatgctatcacagact-3'.

Raw sequence data were assembled using Contig Express (Vector NTI suite 9.1, Invitrogen, USA) and consensus sequence (Genbank FJ471491) aligned with the corresponding sequences deposited in the Genbank database (D00246, AF260967, AY033389, AF317203, AF001570, AF146082, AF130362, AF260969,

AF404757, AF260968, AF001567) with ClustalW [4]. The aligned partial E gene sequences were used to generate a table of pairwise distances to evaluate the variation within the strains and translated into amino acid sequences using Vector NTI suite 9.1 (Invitrogen, USA) and aligned with ClustalW.

The phylogenetic analyses were conducted on 255 bp in the region coding for the E protein by using Phylogeny Interference Program Package (PHYLIP, version 3.6a, [5]).

Results

The 255 bp sequence of the genome region coding for the envelope (E) protein of the WNV isolates showed a 98.8% nucleotide similarity with the strain isolated in Tuscany during the 1998 and a complete similarity (100%) of the deduced amino acid sequence. According to the partial sequences of protein E, the 2008 Italian strain was similar to the Romanian 1996-7, Volvograd 1999, Senegal 1993 and Kenyan 1998 strains. However it showed 3.5% of divergence with the United States and Israeli strains which had almost identical E sequences.

The phylogenetic analysis of the 255 bp of the E gene of the WNV isolates included, with high bootstrap support, the Italian isolate in the lineage I among the European strains. According to the analysis, both the 1998 and 2008 Italian strains as well as isolates from Romania, Russia, Senegal and Kenya fell in the same sub-cluster (Figure 2).

Discussion

Although additional observations on the outbreaks and investigations of the cases are still in progress, the information available allows the assumption that a new epidemic of West Nile disease is occurring in Italy after 10 years of apparent silence. The results of phylogenetic analysis indicate that the current epidemic is caused by a strain of WNV included in the lineage I which showed high nucleotide and amino-acid similarity to the strain responsible for the 1998 outbreak in Tuscany. Up to now, however, there is no evidence of any direct epidemiological link between the two Italian outbreaks. The fact that in both outbreaks the areas where the infection took place were close to wildlife nature reserves, in which a consistent population of wild migratory birds rests, might support the hypothesis of a new introduction of the virus by migratory birds. Additional epidemiological investigations are currently ongoing.

References

1. Autorino GL, Battisti A, Deubel V, Ferrari G, Forletta R, Giovannini A, et al. West Nile virus epidemic in horses, Tuscany region, Italy. Emerg Infect Dis. 2002;8(12):1372-8.
2. Filipponi, G, Lelli R, Savini G, Giovannini A, Guberti V, Santucci U, et al.. West Nile Virus surveillance in Italy: results of three years activities. 2005 National Conference on West Nile Virus in the United States, San Jose, California, 8-9 February 2005. Abstract available from: http://www.izs.it/index.php?requestedAction=showDetailOfPublicationByID&currentResultsPageNumber=&selectedRootCategoryID=&selectedPageOfResults=&lang=it&option=com_izs_insertions_manager&Itemid=562&organizationID=&autorID=&projectID=&publicationID=507&categoryID=&lastScanningType=
3. Macini P, Squintani G, Finarelli AC, Angelini P, Martini E, Tamba M, et al. Detection of West Nile virus infection in horses, Italy, September 2008. Euro Surveill. 2008;13(39):pii=18990. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18990
4. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994;22(22):4673-80.
5. Felsenstein J. PHYLIP (Phylogeny Inference Package) 3.5c Manual (computer program). Seattle, United States: University of Washington, Department of Genetics; 1993.

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Figure 2

Phylogenetic analysis of West Nile virus (WNV) based on 255 bp partial nucleotide sequence of the E gene; isolates from animal samples, Italy, 2008