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2009 West Nile disease epidemic in Italy: First evidence of overwintering in Western Europe?

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ABSTRACT

For the second consecutive year a West Nile disease (WND) epidemic has affected Italy causing disease in horses and humans. The infection re-occurred in the same places of the 2008 and moved westerly and southerly involving new areas and regions. The whole genome sequence of the Italian 2009 West Nile disease isolate (WNDV) was compared with those responsible for the 2008 WND outbreaks. The epidemiological findings of the two years of epidemic were compared as well. The high identity between 2008 and 2009 WNV strains (>99%), the earlier virus circulation in 2009 and the re-occurrence of the disease starting from the bordering infected areas reached by the infection in the previous year, strongly support the hypothesis of the overwintering of the virus and the endemisation to local host populations.

1. Introduction

West Nile is a mosquito-borne disease (WND) caused by a single strand RNA virus of the *Flavivirus* genus within the *Flaviviridae* family. West Nile virus (WNV) is included in the Japanese encephalitis sero complex (Calisher et al., 1989) and, as the other members of the group, the epidemiology of the infection involves birds and mosquitoes, particularly *Culex* spp. and *Aedes/Ochlerotatus* spp., to complete its natural cycle (Hubalek and Halouzka, 1999; Kulasekera et al., 2001). Birds represent the vertebrate amplifying hosts (Komar et al., 2001) responsible for the virus maintenance in the environment while migratory birds probably account for the virus dispersal. Humans, horses and other mammals are regarded as incidental or dead-end hosts. West Nile viruses have been reported in all continents. In line with their genome sequences, WNV strains are grouped into 5 lineages (Bondre et al., 2007). The majority of the strains which caused outbreaks in Europe and the Mediterranean Basin was within the lineage 1. Isolates of this lineage 1 could be included into either European Mediterranean/Kenyan or Israeli/American clusters (Schuffenecker et al., 2005). Strains included in the European Mediterranean/Kenyan cluster are able to cause mild encephalitis in humans and horses while in birds infection is generally sub clinical. Conversely, deaths and, at least in the United States, high rates of illness are reported in birds, humans and horses affected by strains of the Israeli/American cluster (Lanciotti et al., 2002). In the past, lineage 2 was thought to include low pathogenic strains endemic to sub Saharian Africa. Recently, strains belonging to lineage 2 have been correlated to clinical disease in South African horses (Venter et al., 2009) and also reported in Hungary and Austria affecting birds and horses (Bakonyi et al., 2006).

In 2008, 10 years after the first outbreak, WNV reoccurred in Italy causing death and clinical signs in horses and humans (Savini et al., 2008; Barzon et al., 2009; Calistri et al., 2010b). WND outbreaks were also reported in 2009 (Angelini et al., 2010). As in the previous year, the virus (WNV) has been able to cause disease in horses and humans and, similarly, no birds fatalities were recorded. Following these events, a comprehensive monitoring was put in place. Samples from sick and healthy horses and wild birds were collected and tested virologically and serologically. Mosquito traps were also placed in the infected barns and mosquitoes were caught, identified and tested for the presence of WNV. The whole genome of WNV strain responsible for the 2009 outbreak was sequenced and the sequence compared with those which caused the 2008 WND outbreaks. This paper reports the results of the WND surveillance carried out in Italy in 2009 and compares the results of 2 years monitoring.

2. Materials and methods

2.1. Surveillance program

Based on the viral circulation detected during 2008, the national surveillance activities in place since 2001 (Calistri et al., 2010b) were updated and three different epidemiological areas were identified (Fig. 1):

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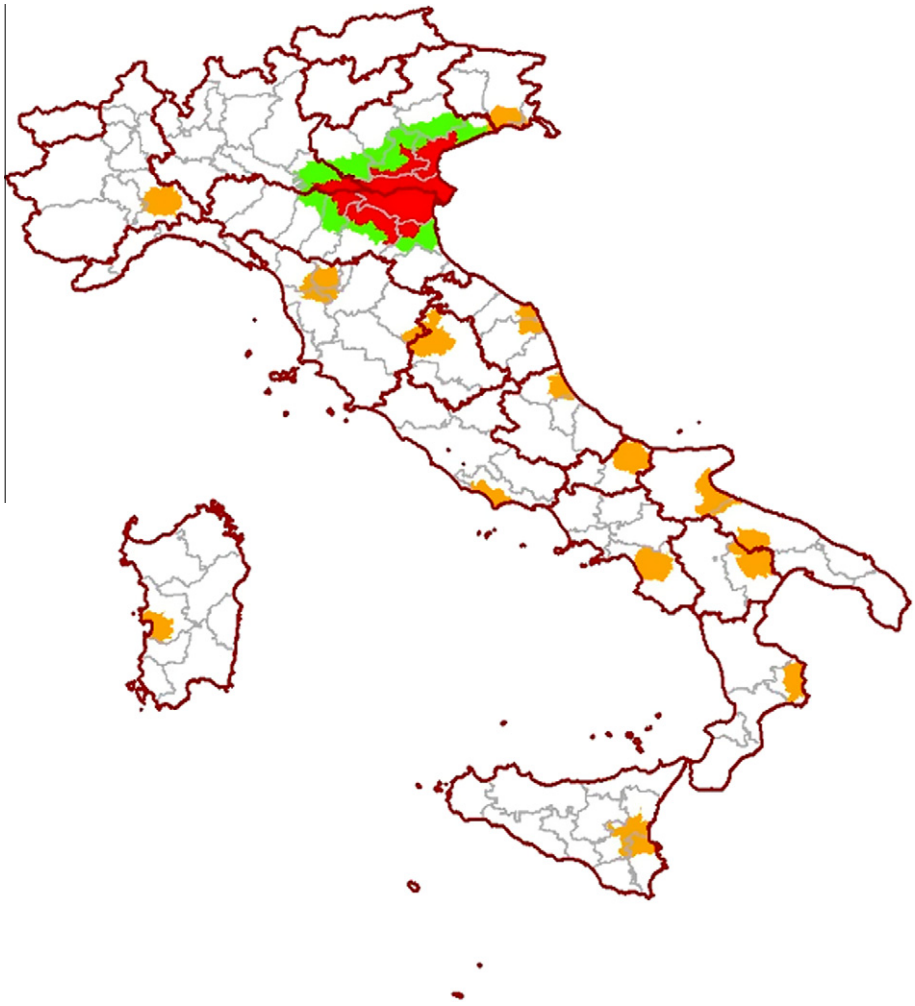

Fig. 1. The map represents the three different epidemiological areas identified in the territories with WNV circulation in 2008. Red: area with virus circulation (AVC); green: surveillance zone (SZ); yellow: areas at risk (AR). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

- area with virus circulation (AVC): areas of Northern Italy where WNV circulated during 2008;
- surveillance zone (SZ): the territories surrounding the AVC for an extension of 20 km;
- areas at risk (AR): fourteen wetlands characterised by the presence of a significant number of water fowls including species of migratory birds.

The surveillance program comprised the surveillance of all neurological cases occurring in equines as described in Calistri et al., 2010b and the serological monitoring program in sentinel horses located in the AR and SZ in May, August and September. Regular serological testing of sentinel-chickens (Monaco et al., 2010) and backyard poultry flocks were also implemented. Ten horse stables in the AVC and three in the SZ were selected for the fortnightly mosquito trapping, while in the AR catches were performed once a month. A detailed description of the objectives and activities included in the surveillance program has been summarised in Table 1.

2.2. Laboratory testing and diagnostic protocol

All collected samples (blood, sera, mosquitoes and tissue samples) were sent to the WND Italian Reference Centre laboratory (CESME) for serological and virological analyses as described by Monaco et al. (2010). To determine the presence of recent virus circulation and the spreading of the infection, serum samples from horses included in the National surveillance program and from those showing nervous symptoms were also tested by commercial IgM ELISA (Immunospec, CA, USA).

2.3. Outbreak data

Results of the surveillance activities were analysed. Both the attack and the case fatality rates of the 2009 Italian strains were determined and compared with the ones of the previous year (Calistri et al., 2010b). The attack rate was calculated as the number of clinical cases on the number of infected animals (positive to serology) whereas the case fatality rate was the number of fatalities due to the WNV infection on the number of confirmed clinical cases. For each proportion obtained in this study 95% confidence intervals were calculated using the Bayesian approach through the beta distribution (Sivia, 1996). All the results of the surveillance activity are recorded and regularly updated in a dedicated national information system, managed by the WND Italian Reference Centre (http://sorveglianza.izs.it/emergenze/west_nile/emergenze.htm).

2.4. Sequencing

Total RNA was extracted from 200 μl of cell culture supernatant from a WNV isolate of the 2009 epidemic season with High Pure Viral Nucleic Acid kit (Roche, Nutley, NJ, USA) and eluted with