Seroprevalence of West Nile virus antibodies in blood donors living in the metropolitan area of Milan, Italy, 2009-2011

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SUMMARY

A seroprevalence study for anti-West Nile virus-specific antibodies was carried out in healthy blood donors resident in the metropolitan area of Milan in two different years, 2009 and 2011. In 2009 no positive sera were found, whereas 5 positive sera were found in 2011, revealing viral circulation in this naive area. The seroprevalence rate identified in 2011 was 0.57%, suggesting that the area of WNV circulation in Italy is larger than that previously identified.

KEY WORDS: WNV, ELISA, IFA, MNTA, Seroprevalence, Italy.

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West Nile virus (WNV) is an arbovirus belonging to the *Flaviviridae* family and is transmitted with a natural cycle that involves birds and mosquitoes. In recent years, the spread of WNV has been observed worldwide and several outbreaks were also recorded in Europe (Lanciotti *et al.*, 1999; Zeller *et al.*, 2004). Since the first report of WNV infections in humans in north-east Italy in 2008, several human cases of WNV-related meningo-encephalitis were identified in Italy in the Emilia-Romagna and Veneto regions (Rossini *et al.*, 2011), indicating an endemic circulation of this virus in the wetland of the Po river valley. As a consequence of these findings, an integrated surveillance system was started in Emilia Romagna to monitor WNV circulation in the region. The results obtained indicated WNV circulation starting from 2009 and continuing afterwards (Calzolari *et al.*, 2010). Recently, WNV has been reported in animals in naive areas of Southern Italy suggesting an even wider virus circulation (Calistri *et al.*, 2010). In addition, WNV human infections have been recorded for more than 10 years in areas previously considered free from virus circulation (Bagnarelli *et al.*, 2011; Cusi *et al.*, 2011). One and five cases of WNV transmission with organ transplantation were detected in Italy in 2010 and in 2011, respectively (Morelli *et al.*, 2010; Nanni Costa *et al.*, 2011), despite active nucleic acids amplification testing (NAT) screening among the donor populations. All this evidence clearly suggests that WNV is spreading nationwide in Italy. On the basis on these observations, this study was undertaken to investigate the seroprevalence of WNV in a population of healthy blood donors living in

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an area where WNV activity was not previously reported. A seroprevalence study for the presence of anti WNV antibody was performed among healthy blood donors resident in the metropolitan area of Milan in two different years: 2009 and 2011. A total of 1266 sera were collected and evaluated between 1st May and 1st June 2009, while 864 specimens were collected and tested between 1st June and 1st August 2011. Samples were obtained from the blood banks of two hospitals, namely "San Carlo" and "Maggiore" in Milan. The mean age of the healthy blood donors of 2009 was 47 years, with a range of 18-65, and with a 1:2.4 male to female ratio, while for the healthy blood donors of 2011 the mean age was 46 years (ranging from 18 to 65) with a 1:3 male to female ratio. The presence of IgM and IgG antibodies against WNV was investigated using a commercial enzyme-linked immunosorbent assay (ELISA) (Anti-West Nile virus ELISA (IgG), Anti-West Nile virus ELISA (IgM), Euroimmun, Lübeck, Germany). Each positive sample was confirmed by an immunofluorescent assay (IFA) and tested to evaluate the IgG and/or IgM titers (Anti-West Nile virus IFA (IgG) and Anti-West Nile virus IFA (IgM), Euroimmun, Lübeck, Germany). All the ELISA and IFA positive samples were further evaluated by a micro neutralization assay (MNTA) performed against WNV and Usutu virus (USUV) as confirmatory testing, as previously described (Pierro *et al.*, 2011). In addition, to evaluate the time elapsed from the beginning of infection, the avidity of the IgG-WNV was investigated on all positive specimens as previously reported (Pierro *et al.*, 2011). Among the 1266 samples collected in 2009, only 11 gave an ELISA IgG positive result that were not confirmed by IFA and MNTA. No IgM positivity was detected. Among the 864 obtained in 2011, 10 samples gave a positive ELISA result for IgG, all being negative for IgM. When tested by IFA only 5 sera were confirmed, with IgG titers ranging from 1/400 to 1/6400 (Table 1). MNTA showed the presence of WNV neutralizing antibodies only in the same 5 samples positive by IFA, the anti-USUV neutralizing activity being completely absent. Details of the neutralizing titers are given in Table 1. To investigate the time elapsed from the beginning of the infection and the bleeding, the avidity of the IgG was investigated for all 5 positive specimens, showing values above 60% (see Table 1 for details). The absence of IgM response and the level of IgG avidity clearly indicate a presumable date of infection prior to spring 2011 (Table 1). All 5 positive BDs were interviewed for a retrospective clinical evaluation and none reported even a mild febrile syndrome: all BDs were completely asymptomatic up to one month prior to donation.

In order to compare the findings obtained for the two groups of specimens a Fisher exact test statistical analysis was performed. Our results showed that the seropositivity against WNV detected in specimens collected in 2011 ($P<0.05$) was statistically significant in comparison with the negativity identified for the samples obtained in 2009 in the same area. This fact clearly indicates a non casual exposure to WNV since 2010, suggesting a likely circulation of WNV among humans in the Milan area.

The results of this study indicate a possible recent activity of WNV in the highly populated area of Milan that was considered non endemic to

TABLE 1 - *Details of the serological findings for the 5 MNTA confirmed positive sera collected in 2011.*

Sample ID	*Elisa IgG-WNV*	*Avidity IgG-WNV(%)*	*IFA IgG-WNV Titer*	*WNV MNTA Titer*
1	Positive	75%*	1/6400	1/320
2	Positive	92%*	1/1600	1/80
3	Positive	94%*	1/400	1/20
4	Positive	78%*	1/6400	1/40
5	Positive	91%*	1/800	1/80

*High avidity (≥60%) indicate a time of infection rise over to 40 days since the index donation (10).

date. The deduced related risk factor calculated as previously reported was 1 infection in 173 persons (Pierro *et al.*, 2011). A previous study conducted between October 2008 and September 2009 on a population of blood donors living in a nearby region where WNV circulation has been endemic since 2008 (Pierro *et al.*, 2011) reported a similar seroprevalence rate (0.69%) for WNV and a related risk factor of 1/144.

In conclusion, since the first reports of human cases of WNV-related neuroinvasive diseases in the Veneto and Emilia-Romagna regions (Rossini *et al.*, 2011) local surveillance activities have been implemented in these affected areas. The results obtained showed a widespread diffusion of WNV (Calzolari *et al.*, 2010). Given the high environmental similarity between the area where WNV circulation has been reported in Northern Italy and the area of Milan, we investigated the seroprevalence of anti-WNV antibodies in this metropolitan area and found a rate of WNV positive citizens of 0.57%. Even though a weak point of the study could be the fact that the studied population does not include individuals under 18 years and older than 65 years, the great majority of seroprevalence studies are based on a similar population (Pierro *et al.*, 2011). Therefore the reported seroprevalence data strongly suggest that WNV is likely to have been circulating since at least 2010 among a population living in the Milan metropolitan area. Based on these serological data, the risk of WNV transmission by blood and organ donations should now be extended to a larger geographical area where the implementation of an integrated surveillance system to monitor the seasonal activity of the virus should be considered. A follow-up study will be performed to clarify the persistence of WNV IgG antibodies in asymptomatic patients.

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