

## **Low seroprevalence of West Nile virus in blood donors from Catalonia (Spain)**

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## **Introduction**

West Nile Virus (WNV) is an emerging flavivirus maintained in nature in an enzootic cycle between mosquitoes of the genus *Culex* and birds. Humans are infected mainly by the bites of infected mosquitoes, although other routes of transmission such as blood transfusion and organ transplantation have been reported (Iwamoto et al. 2003). WNV is commonly found in Africa, Europe, the Middle East, North America and West Asia. Serological studies suggest that WNV may have circulated in Europe earlier than the first documented outbreak in France in 1962. Since then, a number of human outbreaks have been described in European countries over the last two decades (Zeller et al. 2004). Greece and Italy have reported human WNV infections for the last three and five consecutive years respectively (ECDC 2013). In Spain, early studies using hemagglutinin inhibition techniques suggested that WNV or closely related flaviviruses circulated in some parts of the country during the 1970's (Gonzalez et al. 1977; Lozano et al. 1998). Clear evidence of WNV activity in Spain has been accumulating since the year 2000, including detection of neutralizing antibodies in birds, horses, detection of WNV RNA and virus isolation in eagles (Jimenez-Clavero et al. 2010), and the description of a single human case of WNV neurologic disease in 2004 (Kaptoul et al. 2007). In 2010, the first outbreak of WNV was reported in Southern Spain, affecting more than 50 horses and 2 humans with WNV neurologic disease (Garcia-Bocanegra et al. 2011). In Catalonia, (Northeastern Spain) three main wetlands that serve as breeding sites for wild birds as well as mosquito vectors are present (Ebro river delta, Lobregat river delta and the and the Empordà wetlands). The region is considered a high-risk area for WNV and other arboviruses transmission. We conducted a WNV seroprevalence study in Catalonia to investigate if WNV had been circulating to a significant extent and to provide baseline seroprevalence data for this region for future studies

## **Materials and Methods**

Between November 2010 and February 2011 we collected samples from blood donors in 14 municipalities of the 4 catalonian provinces, selected according to the proximity of the wetlands and to *Culex* mosquitoes presence (Table 1). Basic demographic information of the donors was collected before anonymization of the samples with study numbers. The samples were screened by a commercial Enzyme-linked Immunosorbent Assay (ELISA) (Focus Diagnostics) for the presence of IgG antibodies against WNV. A microneutralization protocol using two-fold sample dilutions (1:10-1:640) and 100 TCID<sub>50</sub> of WNV strain NY99 in Vero cells was used as a confirmatory assay. As only WNV lineage 1 has been detected in humans, horses and birds in Spain, this lineage was selected for the neutralization protocol. Serum samples from 800 blood donors were collected and stored frozen until testing.

## **Results and Discussion**

The median age of the blood donors was 45 years, ranging from 18 to 69 years; 45% were female. Most donors (97%) were born in Spain. Fifty-two samples were positive and twelve showed equivocal results by ELISA. Among these samples, only one resulted positive in the microneutralization assay (titer 1/40), and was negative for IgM antibodies by capture ELISA (Diagnostics). This sole positive sample was obtained from a blood donor native of Pakistan, a country where WNV and other flaviviruses such as Japanese encephalitis or dengue circulate (Hayes et al. 1982, Igarashi et al. 1994, Rafique et al. 2015). Given the low neutralization titer and the country of origin of the donor, the serological results suggest past infection by WNV or a closely related flavivirus acquired not necessarily in our region. Cross reactivity with other flaviviruses infection or vaccination (Hogrefe et al. 2004) and even with other unrelated viruses (Focus Diagnostics 2011) has been reported to occur with WNV ELISA's and therefore seroneutralization is needed to be performed as a confirmatory assay. More than 75% of the samples analyzed by neutralization showed

low (<2) ELISA values (cut off: 1.1), whereas the sample with a neutralization titer of 1/40 exhibited the highest ELISA value (5.7) of all samples.

Similar WNV seroprevalence studies in blood donors have been performed in other European countries such as France (Charrel et al. 2001) or Italy (Pezzotti et al. 2011, Pierro et al. 2013), detecting 0.76 and 0.78% samples with neutralizing antibodies respectively, although these studies were conducted in areas with previous documented circulation of the virus. The last WNV seroprevalence study in Catalonia was performed in 2001, focussing on one of the three wetlands in the region (Ebro river delta). From 992 participants, 2 samples were positive by seroneutralization (Bofill et al. 2006). An ecological surveillance for WNV in Catalonia has been recently described (Alba et al. 2014). Horses, sentinel chickens, wild birds and mosquitoes were analyzed from 2007 to 2011. No evidence of WNV infection was found in domestic animals or mosquitoes. However, positive serological tests were obtained in wild birds during 2010 and 2011, suggesting that WNV or a closely related flavivirus may have circulated in the region. Our seroprevalence results correlate with the absence of acute WNV infections in the animal surveillance and the absence of reported human cases in Catalonia. Moreover, no evidence of WNV infection was detected in 50 human cases of meningoencephalitis of unknown aetiology tested at our laboratory during the last three years (unpublished data). Taken together, it seems that transmission of WNV and human infections in Catalonia are rare. However, WNV activity has remained in Southern Spain since the 2010 outbreak and the factors driving the emergence of WNV are not completely understood. Therefore, surveillance and preparedness in our region should be continued and strengthened. Our seroprevalence study may be useful as a baseline reference data for future monitoring of WNV presence in the Catalonia region.

#### **Author Disclosure Statement**

No competing financial interests exist.

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**Table1. Distribution of samples tested by location**

<b>Province (wetland)</b>	<b>Municipality</b>	<b>N° of samples</b>
Barcelona (Llobregat river delta)	El Prat de Llobregat	70
	Viladecans	70
	Gavà	50
	Sant Boi de Llobregat	90
Girona (Empordá wetlands)	Sils	39
	Castelló d'Empúries	15
	L'Escala	20
	Roses	50
Tarragona (Ebro river delta)	Deltebre	98
	L'Ampolla	24
	Sant Jaume d'Enveja	44
	Camarles	33
	L'Aldea	63
Lleida (none)	Lleida	134
<b>Total</b>		<b>800</b>