Serosurvey of Blood Donors to Assess West Nile Virus Exposure, South-Central Spain

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We analyzed West Nile Virus (WNV) exposure from 1,222 blood donors during 2017–2018 from an area of south-central Spain. Results revealed WNV seroprevalence of 0.08% (95% CI 0.004%–0.4%) in this population. Our findings underscore the need for continued surveillance and research to manage WNV infection in this region.

West Nile virus (WNV), a member of the family Flaviviridae, genus *Orthoflavivirus*, is classified within the Japanese encephalitis virus (JEV) serocomplex (*1*). It is the most widespread arbovirus globally, primarily because of the abundance and broad distribution of its main competent vector, mosquitoes belonging to the genus *Culex* (*2*). During the past 2 decades, WNV has led to epidemic outbreaks with a substantial proportion of severe cases in Europe, emerging as a considerable threat to public and animal health in these regions. Nonetheless, very limited information exists on seroprevalence in the general population, hindering a comprehensive understanding of the virus' epidemiologic landscape.

In Spain, WNV is considered endemic because of conducive conditions for virus maintenance and circulation, including diverse bird reservoirs, geographic characteristics such as migratory bird routes, and specific climatic conditions. Since a notable outbreak reported in 2020, the virus has produced human cases annually (*3*), demonstrating the spread of the virus in the country (*4*). Therefore, vigilant surveillance in new risk areas is imperative to anticipate potential human health emergencies. Studies in vectors and animal hosts in south-central Spain have underscored the region's potential as a hotspot zone (*5*–*7*). Within this area, the province of Ciudad Real, where no human WNV cases have been reported to date, serves as an ideal scenario for assessing circulation of the virus in the general population. We conducted a serosurvey in blood donors to investigate WNV exposure in the general population of this region in Spain, shedding light on the transmission dynamics of this emergent virus.

We conducted a retrospective cross-sectional study to analyze the seroprevalence of WNV in serum samples collected from blood donors at the Transfusion Center of the Hospital General Universitario de Ciudad Real (south-central Spain) (Figure) during 2017–2018 (Appendix, https://wwwnc.cdc.gov/EID/article/30/7/24-0450-App1.pdf). We selected and analyzed blood from 1,222 donors (Appendix Table 1). Sex and age data were not available for 129 (10.5%) donors. Of the 1,093 donors for whom information was available, 571 (52.2%) were men and 522 (47.8%) women. The age of the donors was categorized into 3 classes: <30 years (21.8% of samples), 30–50 years (34.8%), and >50 years (32.7%). Nineteen (1.6%) of the samples reacted positively to the IgG WNV ELISA. We administered an epidemiologic survey to the 19 ELISA-positive donors; 16 donors responded (Appendix Table 2).

We analyzed all ELISA-positive samples by using a virus neutralization test (VNT) (Appendix Table 2). Regarding WNV, ELISA reactivity was only confirmed by VNT in 1 donor who showed a titer of 1/256, which indicated a seroprevalence of 0.08% (95% CI 0.04%–0.4%) for WNV. This donor declared that he had not traveled outside of Spain and therefore did not receive any vaccine against yellow fever virus, tick-borne encephalitis virus, or Japanese encephalitis virus.

In Europe, no seroepidemiologic studies have been conducted since 2013; therefore, our study would provide valuable insights into the current status of WNV exposure. Our study encompasses a vast region of south-central Spain and marks initial identification of seropositivity in humans in this specific region of Spain, indicating a broad spread of the virus. In Spain, recent serosurveys are lacking; 2 studies were conducted in Catalonia in 2001 (0.2%) (*8*) and 2011 (0.12%) (*9*), and another was conducted in the province of Sevilla in 2006 (0.6%)

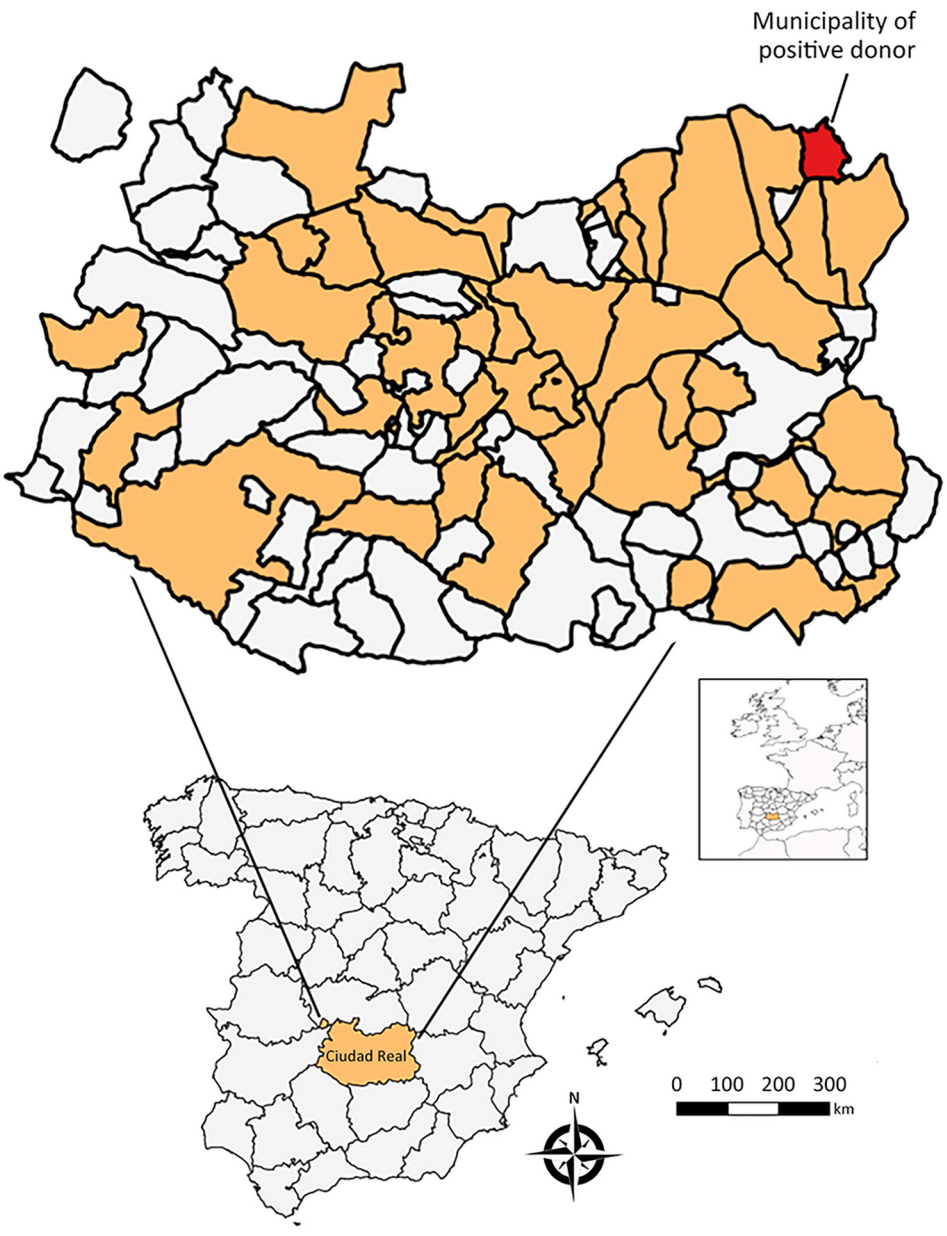

Figure. Locations sampled in serosurvey of blood donors to assess West Nile virus exposure in the general population, Spain, 2017–2018. Inset maps show location of study area in Spain and of Spain in Europe.

(*10*). In the past 3 years, the regions of those studies have experienced large WNV outbreaks, similar to that which occurred in summer of 2020 (*3*) or the first description of clinical cases in Catalonia in 2022 and 2023 (*4*). This development suggests greater exposure to the virus than in the previous decade and highlights the need to carry out new serosurveys in the general population that enable collection of updated data.

The observed seroprevalence among blood donors from south-central Spain in our study suggests a low exposure (0.08%) to WNV in the general population within this spatiotemporal context. Of note, the number of WNV cases in Spain has been on the rise in recent years, being detected even in areas where previously no evidence of WNV circulation existed, suggesting that WNV has been expanding during recent years and that outbreaks can be expected in areas not currently considered endemic for WNV.

In our study, and in line with other studies (*9*), a high percentage (94.7%) of ELISA-positive WNV samples could not be confirmed as positive for specific antibodies. This finding highlights the need to perform additional neutralization tests against other flaviviruses in the serosurvey studies. The absence

of an ELISA test with high sensitivity and, more crucially, specificity for WNV, limits the design of large-scale population serosurvey studies. Urgent efforts are required to address this limitation.

In conclusion, our study indicated seropositivity in the south-central region of Spain. In this way, reporting cases in Spain may be plausible even in areas not at high risk, highlighting the importance of ongoing surveillance and research to manage WNV infection in this region.

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Funding information was missing in Novel Highly Pathogenic Avian Influenza A(H5N1) Clade 2.3.4.4b Virus in Wild Birds, South Korea (S.-h. Lee et al.). The article has been corrected online (https://wwwnc.cdc.gov/eid/article/29/7/22-1893_article).

Correction: Vol. 28, No. 6

An affiliation for the first author was missing in Lyme Disease, Anaplasmosis, and Babesiosis, Atlantic Canada (Z.O. Allehebi et al.). The article has been corrected online (https://wwwnc.cdc.gov/eid/article/28/6/22-0443_article).

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Appendix

Selection of Blood Donors and Sample Size Calculation

The blood donor samples comprised 10,232 serum samples, providing extensive coverage across the Ciudad Real province (42 municipalities) (Figure, https://wwwnc.cdc.gov/EID/article/30/7/24-0450-F1.htm). In a cross-sectional epidemiologic approach, we sought to determine the overall exposure rate of the human population in the study area by employing a representative subset. To achieve this, we calculated the minimum sample size necessary to estimate the prevalence of WNV exposure at the finest spatial resolution available for blood donor information, namely the municipality. Appendix Table 1 shows the 2020 human census and sample size per municipality. The required sample size for estimating the prevalence of exposure at the municipal level, considering an expected seroprevalence of 1.7% (based on findings a in Greek seroprevalence study [*1*]), was determined to be 26 donors per municipality, with a 5% of precision and 95% confidence interval. Sample selection was conducted in a balanced manner, according to the age (three classes: <30 years old, 30–50 years old, and >50 years old) and sex of donors per municipality.

Serologic Methods

To detect IgG antibodies against WNV, the commercial ELISA West Nile Virus IgG DxSelect (Focus Diagnostics Inc., Cypress, California) were used. ELISA-positive samples were subjected to additional testing using a virus neutralization test (VNT) for WNV lineage 1 (WNV-1), Usutu (USUV) and Tick-borne encephalitis (TBEV). VNT is described in detail elsewhere

(*2*). We selected these viruses because are endemic in Europe, in fact, in recent years, the areas in Europe reporting flavivirus infections and specifically WNV, USUV, or TBEV have significantly increased (*3*). WNV and USUV are endemic in Spain and although no autochthonous TBEV human cases have been detected, recent animal seroprevalence studies indicate the possibility of TBEV circulation in horses and dogs from several areas in Spain (*4*,*5*). The titers of neutralizing antibodies were defined as the highest serum dilution that showed >90% neutralization of the virus challenge. Neutralizing antibody titers ≤1:8 were considered negative.

VNT used Vero E6 cells and the virus strains HU6365/08 (WNV-1, GenBank: JF707789.1), Neudorfl (TBE) and HU10279/09 (USUV, GenBank accession no. KX268472.1). A sample was considered WNV seropositive when it was reactive via ELISA in the VNT assay the antibody titer obtained against WNV was 4 times higher than the antibody titer obtained against another of the three tested flavivirus.

Epidemiologic Survey

ELISA-positive donors underwent a telephone interview conducted by the blood bank staff, which an epidemiologic survey was performed to obtain information about their country of birth, WNV-endemic countries visited, vaccination history, and specific vaccinations against other flaviviruses, such as yellow fever virus (YFV) and Japanese encephalitis virus (JEV), due to the potential for cross-reaction in the ELISA results. Only the WNV-seropositive donor confirmed by VNT was informed of the results.

Ethics Considerations

This study was designed and performed according to the Helsinki Declaration. All samples were integrated into 'Biobanco del Sistema Sanitario Pública de Andalucía (Nodo Hospital Universitario Reina Sofía-IMIBIC)'. All donors signed an informed consent form. The CEIC (Clinical Research Ethics Committee) of Hospital de Ciudad Real approved the collection of samples.

Supplementary Results

Two donors with positive results in the IgG WNV ELISA assay and not confirmed by VNT against any of the three tested flaviviruses, were natives from countries where the virus is endemic (Colombia and Ecuador) and frequently traveled to their countries of origin. Therefore, the positive results from the WNV IgG ELISA are due to a previous flavivirus infection endemic to this area, such as dengue and/or Zika viruses. None of the ELISA-positive donors showed neutralizing antibodies against USUV or TBEV.

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Appendix Table 1. Demographic characteristics

Municipality	Census persons (year 2020)	No. donations available	No. donors included
Agudo	1,657	72	41
Alcázar San Juan	30,766	951	28
Aldea del Rey	1,631	30	29
Almadén	5,200	91	33
Almagro	8,905	448	31
Almodóvar	5,983	63	31
Argamasilla de Alba	6,955	216	31
Bolaños de Calatrava	12,019	273	27
Calzada de Calatrava	3,630	141	31
Campo de Criptana	13,312	122	30
Carrión de Calatrava	3,099	21	21
Castellar de Santiago	1,862	78	31
Ciudad Real (Capital province)	75,504	643	30
Corral de Calatrava	1,117	35	31
Daimiel	17,916	687	31
Fuente El Fresno	3,208	137	31
Herencia	8,456	230	31
Malagón	7,881	209	29
Manzanares	17,962	599	31
Membrilla	5,942	161	30
Miguelturra	15,498	189	28
Montiel	1,294	20	19
Moral de Calatrava	5,208	163	31
Pedro Muñoz	7,285	137	31
Picón	668	26	26
Piedrabuena	4,379	183	31
Pozuelo de Calatrava	3,586	105	28
Puerto Lápice	891	30	30
Puertollano	46,607	1154	26
Retuerta del Bullaque	927	22	22
Robledo	1,053	20	20
San Carlos del Valle	1,109	63	31
Santa Cruz de Mudela	4,085	92	26
La Solana	15,419	226	31
Tomelloso	36,168	1,019	30
Torralba de Calatrava	2,966	48	30
Valdepeñas	30,252	829	28
Villahermosa	1,790	39	31
Villamanrique	1,128	22	22
Villanueva de los infantes	4,869	128	31
Villarrubia de los ojos	9,762	459	31
Villarta de San Juan	2,739	51	31
Total	430,688	10,232	1,222

Appendix Table 2. Serology and epidemiology of ELISA-positive donors

ID	Donation date	Age (years)	Sex	Country of origin	Travel in WNV-endemic area	Vaccines	IgG ELISA	VNT WNV1	VNT USUV	VNT TBEV
WNV01	Feb-18	30–50	Male	Spain	No	No	Positive	Negative	Negative	Negative
WNV02	Feb-18	30–50	Female	Ecuador	Ecuador	NA	Positive	Negative	8-Jan	Negative
WNV03	Feb-18	30–50	Male	Spain	No	No	Positive	Negative	Negative	Negative
WNV04	Feb-18	30–50	Male	Colombia	Colombia	No	Positive	Negative	Negative	Negative
WNV05	Feb-18	>50	Male	Spain	No	No	Positive	Negative	Negative	Negative
WNV06	Jan-18	30–50	Female	Spain	No	No	Positive	Negative	Negative	Negative
WNV07	Jan-18	<30	Male	Spain	No	No	Positive	1/256	1/8_1/16	Negative
WNV08	Jan-18	<30	Male	Spain	NA	NA	Positive	Negative	Negative	Negative
WNV09	Jan-18	>50	Female	Spain	No	No	Positive	Negative	Negative	Negative
WNV10	Jan-18	<30	Male	Spain	No	No	Positive	Negative	Negative	Negative
WNV11	Feb-18	<30	Male	Spain	NA	NA	Positive	Negative	Negative	Negative
WNV12	Jan-18	<30	Male	Spain	No	No	Positive	Negative	Negative	Negative
WNV13	Feb-18	>50	Male	Spain	No	No	Positive	Negative	Negative	Negative
WNV14	Feb-18	>50	Female	Spain	No	No	Positive	Negative	Negative	Negative
WNV15	Feb-18	<30	Female	Spain	No	No	Positive	Negative	Negative	Negative
WNV16	Feb-18	<30	Male	Spain	No	No	Positive	Negative	Negative	Negative
WNV17	Apr-18	>50	Male	Spain	No	No	Positive	Negative	Negative	Negative
WNV18	Apr-18	<30	Female	Spain	No	No	Positive	Negative	Negative	Negative
WNV19	Apr-18	30–50	Female	Romania	Romania	No	Positive	Negative	Negative	Negative

NA, unavailable information.