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First seroprevalence study of West Nile Virus (WNV) infection in blood donors after the upsurge of West Nile Neuroinvasive Disease (WNND) cases in southern Italy in 2023

Francesca Centrone[1], Rossella Procacci[2], Raffaella Melilli[3], Valentina Annachiara Orlando[3], Vito Colella[3], Daniela Loconsole[4], Antonello Amendola[5], Giulietta Venturi[5], Angelo Ostuni[2], Maria Chironna[1,3*] and the Apulian Blood Donors Study Group

Abstract

Background Mosquito-borne diseases are an emerging threat in Europe. West Nile virus (WNV) is an arbovirus commonly transmitted in an enzootic cycle involving ornithophilic mosquitoes as major vectors. In recent years, global climate change has been identified as a significant driver of the increased spread of this virus. In Italy, outbreaks of WNV infection have been recorded every year in northern Italy. The Apulia region of southern Italy was not considered a high-risk region until 2023, when an unexpected increase in WNV infections occurred. The aim of this study was to evaluate the prevalence of anti-WNV antibodies in a wide sample of blood donors in the Apulia region of southern Italy enrolled between November 2023 and February 2024. In addition, the use of protective measures against mosquito bites was assessed.

Methods A retrospective cross-sectional study was conducted on a total of 1,579 blood donors. All sera were tested for anti-WNV IgG by ELISA. Reactive serum samples were also tested by CLIA and by the plaque reduction neutralization test (PRNT). All healthy donors answered a short anonymous questionnaire. Data analysis was performed using StataMP14.0® (StataCorp LLC, CollegeStation, TX77845-4512, USA).

Results The median age was 47 years (IQR: 37–53), and 75% were male. The questionnaires administered revealed that 68.6% of the subjects had not made any trips in the few weeks prior to blood sampling, and 30.5% remembered being bitten by mosquitoes in the previous 15 days or longer. While 17 samples tested by ELISA were positive for anti-WNV IgG, only six were also positive by CLIA testing. Analysis by PRNT for WNV confirmed 5 cases. The findings revealed a WNV seroprevalence of 0.32% (95% CI: 0.07–0.59). Among the subjects who tested positive, none recalled being bitten by mosquitoes or regularly using mosquito repellents.

Conclusions Our study suggests the circulation of WNV in Apulia and highlights the potential human health concerns associated with this emerging virus. Strengthening the integrated surveillance system and planning adequate preventive strategies are crucial next steps to address the potential massive spread of WNV in southern Italy.

*Correspondence:
Maria Chironna
maria.chironna@uniba.it
Full list of author information is available at the end of the article

Keywords Southern Italy, West Nile virus, Blood donors, Seroprevalence, Virus neutralization test, Vector-borne infections, Zoonoses

Background

Orthoflavivirus nilense (West Nile virus, WNV) is an emerging arbovirus belonging to the Japanese encephalitis serocomplex [1]. WNV is a single-stranded RNA virus of the *Orthoflavivirus* genus, *Flaviviridae* family, and is commonly transmitted in an enzootic cycle involving ornithophilic mosquitoes, especially *Culex* species, as major vectors and birds as major reservoirs [1, 2]. Infection can also occur in several mammalian species, including humans, who are considered dead-end hosts unable to contribute to virus transmission due to low levels of viraemia [1].

WNV in humans can also be transmitted through blood transfusions, organ transplants, and vertical transmission from mother to fetus, during delivery or through breastfeeding [3].

WNV can have a significant burden on human health [1]. WNV infection in humans can be asymptomatic (80% of cases) or present with a wide range of clinical manifestations (20% of cases), varying from influenza-like febrile illness to severe neuroinvasive forms such as acute flaccid paralysis, meningitis or encephalitis [2]. Severe forms of disease, also known as West Nile neuroinvasive disease (WNND), are associated with significant post-illness morbidity, especially in elderly individuals [2]. Severe manifestations of WNV infection are rare, occurring in less than 1% of infected subjects, leading to a risk of underestimation of the actual circulation of the virus [2].

In recent years, the number and geographical distribution of WNV epidemics have increased. In Europe, the first WNV epidemic occurred in 1996 in Romania, and cases of WNV infection were reported every year between 2010 and 2018 [4, 5]. Compared with that in previous years, the highest number of cases was recorded in 2018, and the number of affected countries also increased accordingly [5]. In recent years, outbreaks of WNV have occurred in several European countries, including France [6], Spain [7], Greece [8], Italy [9] and Hungary [10].

In Italy, WNV is currently endemic in the northern regions after the first human case of WNND was detected in 2008 in the Emilia Romagna region [11]. Since then, outbreaks of WNV infection have been recorded every year in northern Italy. In more recent years, cases of WNV infection have also been recorded in central and southern Italy [12]. In light of the emerging circulation of WNV in Italy, an integrated system of WNV and USUV (Usutu virus), which is based on human, animal and entomological surveillance, has been implemented nationwide and is active throughout the year, intensifying from May to November, when vector activity peaks [13]. Integrated surveillance identifies and monitors WNV circulation both in vectors and in target animal hosts with the aim of evaluating the risk of transmission to humans and ensuring timely activation of adequate preventive measures, including vector control and screening of blood and organ donors [14]. There are currently no available vaccines or treatments against WNV for humans [14, 15].

In the Apulia region, eight autochthonous cases of WNV infection were identified between July and October 2023. Of these, six were WNND, and two were identified through blood donor screening. The unexpected increase in WNV infections, occurring ten years after the unique autochthonous case reported in 2013, changed the classification of the Apulia region from low risk to high risk [16].

To date, in Italy, few studies have analyzed the seroprevalence of WNV to estimate the real circulation of the virus and the burden of infection on the population. Some studies were conducted in northern Italy [17–19] and focused on the prevalence of antibodies against WNV in the local population. However, no data are available from southern Italian regions. Therefore, prompted by the recent outbreak of WNV infections in 2023 in Apulia, updated evidence is needed to estimate the real circulation of WNV in the area in view of the potential health risks associated with the infection.

The aim of this study was to evaluate the prevalence of anti-WNV antibodies in a wide sample of blood donors in the Apulia region of southern Italy to achieve a more accurate estimation of the circulation of the virus in this region and to plan effective preventive strategies to contain the spread of the virus. In addition, the tendency to use protective measures against mosquito bites was explored.

Materials and methods

Study population

A retrospective cross-sectional study was conducted on blood donors (aged 18–65 years) who attended one of the seven blood donation centers located in five of the six provinces in the Apulia region (Foggia, Barletta-Andria-Trani, Bari, Taranto and Lecce provinces) between November 2023 and February 2024. All healthy donors answered a short anonymous questionnaire

(Supplementary 1). An anonymized serum sample was collected from each subject. Data collected from the anonymized questionnaires (age, sex, blood type, travel to endemic areas in the past six months, recollection of recent bites, and habitual use of mosquito repellents) were collected and analyzed using StataMP12.0® (StataCorp LLC, CollegeStation, TX77845-4512, USA). Data were evaluated in relation to Gaussian distribution. Median and interquartile ranges (IQR) were used. Confidence intervals for proportions were calculated using the exact confidence interval (95% CI) of the binomial distribution. Data on animal and entomological surveillance data were retrieved from the National Bulletin from 2013–2023 (https://www.epicentro.iss.it/arbovirosi/bollettini).

ELISA and CLIA assays

All collected sera were centrifuged and sent to the Laboratory of Molecular Epidemiology and Public Health of the U.O.C. Igiene – A.O.U.C. Policlinico in Bari, the regional reference laboratory for arboviruses human infections. All the samples were tested for anti-WNV IgG by ELISA (EUROIMMUN, Lübeck, Germany) following the manufacturers' instructions. To confirm the results, all sera positive for anti-WNV IgG by ELISA were also tested for anti-WNV IgG by chemiluminescent immunoassay (CLIA, VIRCELL, Granada, Spain), which combine chemiluminescence technique with immunochemical reactions.

Plaque reduction neutralization assays

All samples reactive by ELISA were sent to the National Reference Laboratory for Arboviruses (NRLA) at the Italian National Institute of Health to confirm positive results via the plaque reduction neutralization test (PRNT) for WNV. All sera that were reactive for WNV IgG antibodies were also tested by the PRNT for USUV due to the high cross-reactivity that can lead to misinterpretation of serological results. The assay was performed as previously described [20]. The viruses used were serotype 2 Dengue virus, Chikungunya virus and Zika virus [20]. Neutralizing antibody titers were calculated as the reciprocal of the serum dilution that resulted in a 50% or 80% reduction in the number of plaques (PRNT50/PRNT80) compared with the virus control. PRNT80s $\geq$ 10 were considered positive, whereas PRNT50s $\geq$ 10 were considered borderline.

For the purposes of the study, patients with PRNT-confirmed anti-WNV IgG were considered positive.

Table 1 Demographic, anamnestic characteristics and serological results of the enrolled subjects

			N.	%	CI 95%
Total			1.579	100,0%	
Sex	Female		395	25,0%	
	Male		1.184	75,0%	
Median age, years (IQR)			47 (37–53)		
Blood type	A	+	366	23,2%	21,1-25,3
		-	53	3,4%	2,5-4,2
	B	+	172	10,9%	9,4-12,4
		-	21	1,3%	0,8-1,9
	AB	+	62	3,9%	3,0-4,9
		-	9	0,6%	0,2-0,9
	0	+	561	35,5%	33,2-37,9
		-	96	6,1%	4,9-7,3
	NA		239	15,1%	-
Recent travel history	No		1.083	68,6%	66,3-70,9
Recent mosquito bites	Yes		481	30,5%	28,2-32,7
	No		782	49,5%	47,1-52,0
	Not sure		316	20,0%	18,0-22,0
Habitual use of mosquito repellents	Yes		271	17,2%	15,3-19,0
Serological results	ELISA +		17	1,1%	0,6-1,6
	CLIA +		6	0,4%	0,1-0,7
	PRNT80≥10 +		5	0,3%	0,04-0,6

N number, *CI* confidence interval, *IQR* interquartile range, *NA* not available, *ELISA* enzyme-linked immunosorbent assay, *CLIA* chemiluminescent immunoassay, *PRNT* plaque reduction neutralization test

Results

A total of 1,579 blood donors were enrolled. The median age was 47 years (IQR: 37–53), and 75% were male (Table 1). The analyzed blood samples, 862 (54.5%) were collected in Bari Province, 315 (20.0%) were collected in Foggia Province, 206 (13.0%) were collected in Lecce Province, 110 (7.0%) were collected in Taranto Province, and 86 (5.4%) were collected in Barletta-Andria-Trani (BAT) Province.

The questionnaires administered revealed that 68.6% of the subjects had not made any trips in the few weeks prior to blood sampling, and 30.5% remembered being bitten by mosquitoes in the previous 15 days or longer (Table 1).

While 17 samples tested by ELISA were positive for anti-WNV IgG, only six were also positive when tested by CLIA. Analysis by PRNT for WNV confirmed 5 cases (PRNT80 $\geq$ 10), representing 0.32% (95% CI: 0.07–0.59) of the sera (Table 1).

The median age of the patients in the PRNT positive group was 56 years (IQR: 40–66), and 80% were

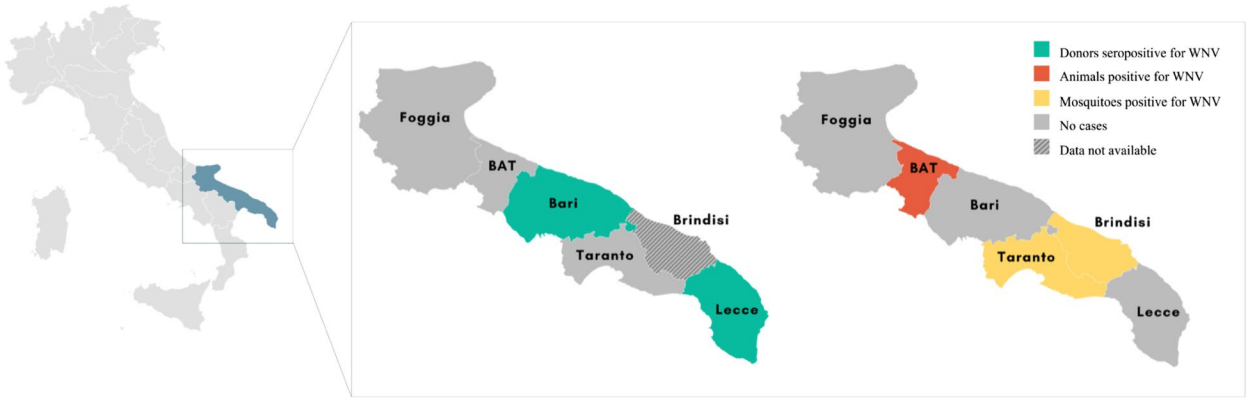

Fig. 1 Geographic distribution of enrolled subjects who were seropositive for WNV, as determined by ELISA, CLIA, and PRNT assays (1a) and of animal cases of infection identified by entomological and veterinary surveillance in 2023 (1b). BAT: Barletta-Andria-Trani province

male. Among the subjects who tested positive, only one reported having traveled in the previous week to the Veneto region in Italy. None of the participants recalled being bitten by mosquitoes or using mosquito repellents regularly. All the blood samples that tested positive by PRNT (PRNT80 ≥ 10) were collected in October and December 2023.

Among the positive individuals confirmed by PRNT ($N=5$), four lived in the province of Bari, whereas only one lived in the province of Lecce (Fig. 1).

Of all the reactive sera tested for USUV by PRNT analysis, two were positive (PRNT80 ≥ 10). Both serum samples were also positive for WNV by PRNT (PRNT80 ≥ 10), indicating either infection with both viruses (simultaneously or at different times) or cross-reactivity.

Discussion

Mosquito-borne diseases are an emerging threat in Europe. An outbreak of eight autochthonous WNV infections was recorded for the first time in 2023 in the Apulia region [16]. Therefore, while the literature focuses on the seroprevalence of WNV in the endemic regions of northern Italy, up-to-date data are needed to estimate the current prevalence of WNV in southern Italy.

The majority of WNV infections tend to be asymptomatic [2], leading to a risk of underestimation of the real circulation of the virus. Moreover, a reduced tendency toward laboratory diagnosis may be partially responsible for the underdiagnosis of WNV infections.

Our study screened asymptomatic blood donors for anti-WNV antibodies and revealed that at least 5 individuals had been unknowingly exposed to the virus.

Considering the significant false-positive rate of immunoassays (ELISA and CLIA), cross-reactivity issues, and the discrepancy between ELISA and CLIA, we use two screening tests and PRNT to confirm IgG-positive sera.

On the basis of the prevalence data in our blood donor sample (0.32%) and considering the Apulian population 18–65 years of age (approximately 2.3 million), it could be estimated that thousands undiagnosed infections may have occurred in adults.

In our study, four out of five positive subjects lived in Bari Province, suggesting that the circulation of WNV within the region may not be uniform and may be greater in this area; however, further data on the geographical distribution of the virus are needed to confirm this finding. Since a greater number of blood samples were collected from Bari Province alone (54.5%) than from the other provinces together (45.5%), our results may have been affected by the difference in sample size among the provinces within the region.

Anti-WNV IgG-positive subjects lived in the Bari and Lecce Provinces, where Entomological and Animal Surveillance of WNV 2023 did not detect cases of WNV infection in animals in 2023. Analysis of WNV entomological and animal surveillance bulletins from 2013–2022 revealed only two outbreaks over 10 years (https://www.epicentro.iss.it/arbovirosi/bollettini). The first case in 2015 in the province of Lecce involved a horse that tested positive for WNV. The second outbreak occurred in 2018 in the province of BAT, where two outbreaks were identified for a total of 3 WNV-positive horses. This might suggest the need to implement an integrated surveillance system.

None of the positive individuals reported the regular use of mosquito repellents, suggesting that there is a low tendency toward the use of protective measures against mosquito bites. This could be reflective of a lack of awareness and knowledge of WNV among the general

population. Therefore, more informative campaigns are needed.

However, the degree of real circulation of the virus is difficult to estimate due to the heterogeneity of clinical manifestations, high number of asymptomatic infections and low tendency toward diagnosis, likely resulting in a number of cases being underdiagnosed.

In recent years, global climate change has been identified as a significant driver of the increased spread of the virus. In particular, increased temperatures and precipitation have played a crucial role in the spread of WNV in horses and humans. The average temperature in the Apulia region in 2023 was higher than in previous years [16], and this may have been a contributing factor in the epidemiological change of WNV infection in the region.

Conclusions

Our study suggests the circulation of WNV in Apulia and highlights the potential human health concerns associated with this emerging virus. Therefore, strengthening the integrated surveillance system and planning adequate preventive strategies are crucial next steps to address the potential massive spread of WNV in southern Italy. From this perspective, vector control, screening of blood and organ donors, regular use of protective measures against mosquitoes by the general population and early identification of symptomatic infections through greater tendency toward laboratory diagnosis could be reinforced to control the spread of WNV in the region.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-10603-4.

Supplementary Material 1. Questionnaire.

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Authors' contributions
M.C., A.O. and F.C. conceived the idea for the study. R.P., R.M. and V.A.O. obtained the data. F.C., R.M. and V.C.O. cleared up the datasets; F.C. and D.L. performed the data analyses. V.C., A.A., G.V., D.L. interpreted the results of the data analyses. F.C. and M.C. wrote the manuscript. A.O. and R.P. coordinated the Apulian Blood Donors Study Group. All authors read and approved the final manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Ethics approval was obtained from the Ethics Committee of the IRCCS Istituto Oncologico Giovanni Paolo II, Bari, Italy (Prot. N. 594, 24/10/2023 – Studio 1408/CEL – WNV Pug.23). Written informed consent was obtained from the participants. This study was conducted according to the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Hygiene Unit, Azienda Ospedaliero-Universitaria Consorziale Policlinico Di Bari, 70124 Bari, Italy. [2]Immunohematology and Transfusion Medicine Service, Azienda Ospedaliero-Universitaria Consorziale Policlinico Di Bari, 70124 Bari, Italy. [3]Department of Interdisciplinary Medicine, Hygiene Section, "Aldo Moro" University of Bari, Piazza G. Cesare 11, 70124 Bari, Italy. [4]Policlinico Hospital Sanitary Direction, Bari Policlinico University Hospital, 70124 Bari, Italy. [5]Department of Infectious Diseases, Istituto Superiore Di Sanità, Viale Regina Elena 299, 00161 Rome, Italy.

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