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Original Article

Seroepidemiology of West Nile Virus in Blood Donors and Pediatric Patients from Central Anatolia, Türkiye: A Cross-Sectional Study, 2024

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Abstract

Background: West Nile virus (WNV) is a neurotropic pathogen transmitted by mosquito vectors. The present study was designed to determine the seroprevalence of WNV in Konya Province and to investigate potential associations between seropositivity and sociodemographic and socioeconomic characteristics.

Methods: The study was conducted at Necmettin Erbakan University Hospital from April to August 2024 and included the analysis of serum samples from 720 blood donors and 300 individuals recruited from paediatric outpatient clinics. WNV-specific antibodies were detected using Enzyme-Linked Immunosorbent Assay (ELISA) and Indirect Immunofluorescence Test (IIFT). The potential impact of sociodemographic, socioeconomic and environmental factors (including age, gender, residential area, education level, income, occupation, outdoor exposure, animal contact, travel history and underlying medical conditions) on seropositivity was investigated. Categorical variables were compared using the chi-square or Fisher's exact test and associated factors were assessed by multivariable logistic regression.

Results: ELISA-based serological analysis identified WNV IgM positivity in 50 samples (4.9%) and IgG positivity in 17 samples (1.7%). In total, 67 serum samples were classified as WNV-seropositive. Among 1,020 participants, ELISA screening identified 50 (4.9%) IgM-reactive samples, including 25 (2.45%) positive and 25 (2.45%) borderline results. ELISA IgG was positive in 17 participants (1.7%), including 16 (1.6%) positive and one (0.1%) borderline sample.

Conclusions: The findings provide the first comprehensive serological evidence of WNV circulation in Konya and its surrounding regions, suggesting that this area may represent a potential endemic zone.

Keywords: Arboviruses; Seroprevalence; Vector-borne diseases; Risk factors; Enzyme-Linked immunosorbent assay

Introduction

Arthropod-borne viruses (arboviruses) transmitted by vectors such as mosquitoes, sandflies and ticks continue to represent a major global public health challenge. Among these pathogens, West Nile virus (WNV), a neurotropic virus belonging to the family Flaviviridae, has drawn growing concern due to its widening geographic range and its capacity to cause serious neurological illnesses, such as meningitis and encephalitis (1, 2). In humans, the incubation period of WNV infection is generally 2–6 days, although incubation periods of up to 21 days have been reported in immunocompromised individuals (3). Approximately 80% of WNV infections remain asymptomatic, contributing substantially to underreporting, as these infections are often identified only through blood donor screening or seroepidemiological studies rather than clinical presentation (4).

Around 20% of infected individuals develop a symptomatic illness known as West Nile fever, which is typically characterized by a mild to moderate febrile syndrome. Common manifestations include fever, headache, fatigue, myalgia and arthralgia, while some patients may

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also experience transient maculopapular rash, gastrointestinal symptoms such as nausea, vomiting, or diarrhea, lymphadenopathy and ocular pain (5). Although the disease is generally self-limiting and resolves within days to weeks, prolonged fatigue and weakness may persist following the acute phase (5). Less than 1% of infected individuals develop neuroinvasive disease, including meningitis, encephalitis, or meningoencephalitis, with elderly and immunocompromised patients being at particularly high risk for severe clinical outcomes (6).

WNV was first isolated in 1937 from a febrile woman in the West Nile district of Uganda (7). Since then, the virus has spread widely across Africa, Europe, the Middle East, Asia and the Americas. Over recent decades, both the incidence and clinical impact of WNV infections have increased, with recurrent outbreaks of neuroinvasive disease and geographic expansion reported particularly in Europe and neighboring regions. Recent surveillance data indicate continued WNV circulation across Europe, with an increasing number of affected regions and countries, highlighting its growing public health importance and the need for coordinated surveillance strategies (8–11). In nature, WNV is maintained through an enzootic trans-mission cycle involving *Culex* mosquito species as vectors and birds as the primary amplifying hosts. Humans and other mammals are considered incidental, dead-end hosts and are most commonly infected through mosquito bites (12, 13). Although rare, non-vector transmission routes such as blood transfusion, organ transplantation, breastfeeding and vertical transmission from mother to child have also been described (14).

Ecological and environmental factors play a crucial role in the transmission dynamics and persistence of WNV infection. The virus is primarily maintained in an enzootic cycle involving birds and ornithophilic mosquitoes, especially *Culex pipiens*. Human exposure to infected mosquitoes is influenced by several interrelated factors, including vector abundance and competence, climatic conditions, land-use characteristics, occupational exposure and socioeconomic determinants (15). Environmental changes such as rising temperatures and altered precipitation patterns may promote mosquito survival and viral replication, thereby increasing the risk of WNV transmission. The unusually warm summer recorded in Europe in 2018 was associated with an extended transmission season and a substantial rise in reported WNV cases (6, 16). Furthermore, climate change is expected to facilitate the expansion of mosquito habitats, prolong vector activity periods and alter the geographical distribution of both mosquito vectors and arboviruses (17). Recent European surveillance data further highlight the continued geographic expansion and seasonal circulation of WNV, emphasizing the importance of integrated human, veterinary, entomological and environmental surveillance (9, 10).

Most WNV infections are asymptomatic or result in mild, self-limiting illness; however, approximately one in every 150 infected individuals develops neuroinvasive disease, manifesting as meningitis, encephalitis, or acute flaccid paralysis. The risk of severe clinical outcomes is notably higher in individuals aged over 50 years and in those with underlying immuno-suppressive conditions (1, 18). Currently, no specific antiviral treatment or licensed human vaccine is available for WNV, emphasizing the necessity of robust surveillance systems and timely diagnostic approaches.

Türkiye's marked ecological and geographical diversity, combined with its location along major migratory bird routes, places the country at heightened risk for vector-borne infections. Türkiye, located at the crossroads of Asia, Europe and Africa, possesses diverse ecological and climatic conditions that provide suitable habitats for numerous mosquito species capable of transmitting arboviruses. Among mosquito-borne flaviviruses, WNV is the most extensively studied in Türkiye, with reports demonstrating its widespread circulation in

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mosquitoes, various vertebrate hosts and human and equine populations (19, 20). The largest documented outbreak occurred in 2010 and was notable for a high proportion of neuroinvasive clinical presentations (21). Despite these findings, data on WNV epidemiology in Central Anatolia remain scarce, and information specific to Konya Province is especially limited. Given Türkiye's strategic location along major migratory bird flyways connecting Europe, Asia and Africa, strengthening integrated One Health surveillance involving humans, animals, mosquito vectors and environmental monitoring is increasingly important for the early detection and prevention of WNV transmission (8, 18, 20).

Given that approximately 80% of WNV infections are asymptomatic, blood donors represent an important sentinel population for detecting silent WNV circulation and assessing the potential risk of transfusion-transmitted infections (4, 14, 15). Recent international recommendations emphasize that effective WNV surveillance should adopt a One Health approach integrating human, animal, entomological and environmental surveillance to improve early detection and outbreak preparedness (8, 10).

Serological cross-reactivity among flaviviruses may reduce the specificity of many diagnostic assays. Therefore, the Plaque Reduction Neutralization Test (PRNT) is considered a highly specific serological method for differentiating flaviviral infections. Nevertheless, PRNT is labor-intensive and time-consuming and requires specialized laboratory facilities and appropriate biosafety procedures, limiting its availability to specialized reference laboratories (22, 23).

Konya, situated in the heart of Central Anatolia, encompasses broad rural areas, agricultural lands, wetlands and environmental conditions that are conducive to the breeding and persistence of *Culex* mosquito species. Nevertheless, no large-scale seroepidemiological study has yet been conducted to assess the extent of WNV exposure among the local population in this region.

Accordingly, the present study aimed to determine the seroprevalence of WNV antibodies in Konya Province using ELISA and IIFT. In addition, potential associations between WNV seropositivity and sociodemographic, environmental and exposure-related factors were evaluated. The findings of this study are expected to contribute valuable epidemiological insights to support regional surveillance efforts and inform public health planning.

Materials and Methods

Study design and population

This cross-sectional study was conducted between April and August 2024 in Konya Province. Konya Province is located in Central Anatolia, Türkiye (approximately 37.87°N, 32.48°E), characterized by continental climatic conditions, extensive agricultural activity and ecological features favorable for mosquito breeding and migratory bird movement. Participants were recruited voluntarily from the Blood Centre and the Paediatrics Outpatient Clinic of Necmettin Erbakan University Hospital during the study period.

Pediatric outpatients (n=300) were recruited from the Paediatrics Outpatient Clinic. Inclusion criteria were age 2–17 years; blood sampling performed for routine outpatient evaluation (well-child visits or mild non-specific complaints); absence of clinical signs suggestive of meningitis, encephalitis, or acute febrile illness at the time of enrollment; and provision of parental informed consent. Exclusion criteria included suspected neuroinvasive disease, history of confirmed WNV infection, or insufficient serum sample volume (22).

The sample size was calculated to estimate the seroprevalence of WNV antibodies in the study population. An expected seroprevalence of 2.5% was assumed based on previous seroprevalence studies conducted in Turkey (24, 25).

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With a 95% confidence level (Z=1.96) and an absolute precision (margin of error) of ±1.0%, the minimum required sample size was calculated using the standard formula for estimating a single population proportion, $n=Z^2P(1-P)/d^2$ (26). Based on these assumptions, the minimum required sample size was approximately 937 participants. A total of 1,020 participants were included in the study, exceeding the calculated minimum and thereby providing the desired precision for the prevalence estimate.

Participants who consented to take part completed a structured questionnaire designed to collect information on age, occupation, permanent residence and potential risk factors for arboviral infect-ions. All responses were systematically recorded.

Study variables

Dependent variable

The dependent variable was defined as serological evidence of exposure to WNV. Individuals aged ≥2 years were classified as seropositive if WNV-specific IgG and/or IgM antibodies were detected in serum samples and seronegative if no antibodies were identified.

Independent variables

Independent variables included demographic and environmental factors such as age, gender, place of residence, income level, occupation, education status, toilet location within the household, daily duration of outdoor activity, use of insect repellents, presence of chronic diseases (renal failure, hypertension, diabetes mellitus, cardiovascular disease, organ transplantation), pet ownership and history of travel abroad.

Laboratory procedures

Venous blood samples (3–5 mL) were collected from each participant via venipuncture into EDTA-containing Vacutainer tubes. Following serum separation, samples were transported to the laboratory under refrigerated conditions and stored according to standard biosafety and laboratory protocols until analysis.

Enzyme-linked immunosorbent assay (ELISA)

Serological screening for WNV-specific antibodies was performed using commercial ELISA kits for IgM and IgG (Anti-West Nile Virus IgM and IgG ELISA; Euroimmun, Lübeck, Germany), which utilize virus-infected cell lysates as antigen substrates. Serum samples were diluted 1:101 in accordance with the manufacturer's instructions.

For IgM antibodies, results were interpreted as negative (<0.8), borderline (≥0.8–<1.1), or positive (≥1.1). IgG antibody levels were considered negative (<16 RU/mL), borderline (≥16–<22 RU/mL), or positive (≥22 RU/mL). According to the manufacturer, the reported sensitivity and specificity of the ELISA assay are 99.5% and 96.9%, respectively.

Indirect immunofluorescence test (IIFT)

ELISA-positive and borderline samples for both WNV IgM and IgG were further evaluated by IIFT, according to the manufacturer's instructions. The Euroimmun IIFT kit (Lübeck, Germany), based on virus-infected cells, was employed. Serum samples were tested at dilutions of 1:10 for IgM and 1:100 for IgG.

Fluorescence intensity was assessed microscopically and categorized qualitatively as weak (+), moderate (++), or strong (+++) by comparison with positive and negative control sera. The manufacturer reports a sensitivity of 100% and specificity of 98% for the WNV IgG IIFT assay.

All serological analyses were performed using commercially validated diagnostic kits according to manufacturer instructions. Internal positive and negative controls supplied with the kits were included in each assay run to ensure analytical validity and reliability. In addition, all positive or borderline ELISA results were further evaluated using IIFT to improve diagnostic consistency.

Confirmatory neutralization assays such as PRNT were not included in the present study because the study was designed primarily as a large-scale seroepidemiological screening investigation based on commercially available

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serological as-says. In addition, the technical complexity, biosafety requirements and limited availability of specialized neutralization testing restricted the use of PRNT in routine analysis. Therefore, although ELISA-positive samples were additionally evaluated by IIFT, potential serological cross-reactivity with other flaviviruses cannot be entirely excluded.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). Continuous variables were summarized as mean ± standard deviation (SD) or median with interquartile range (IQR), as appropriate according to the distribution of the data. Categorical variables were presented as frequencies and percentages.

Associations between demographic, clinical and exposure-related variables and WNV IgM or IgG seropositivity were initially assessed using univariable analyses. Pearson's chi-square test was used for categorical variables when the assumptions of the test were met. Fisher's exact test was applied to 2×2 contingency tables with small expected cell frequencies, whereas the Fisher–Freeman–Halton exact test was used for contingency tables larger than 2×2 when more than 20% of the expected cell counts were less than five.

Variables with a p-value< 0.10 in the univariable analyses, together with clinically relevant covariates, were considered for inclusion in separate multivariable binary logistic regression models for WNV IgM and IgG seropositivity. The enter method was used for model construction. For categorical independent variables, the last category was designated as the reference category. During preliminary model assessment, complete or quasi-complete separation was observed in models including the original four-category educational level and three-category age variables, as indicated by markedly inflated standard errors and excessively wide confidence intervals for some parameter estimates. Therefore, these variables were recoded into categories with adequate cell frequencies before the final regression analyses. Age was categorized as <18 years versus ≥18 years, while educational level was categorized into three groups: pre-school-age children, low/intermediate education and high school or higher.

Adjusted odds ratios (aORs) with corresponding 95% confidence intervals (95% CIs) were calculated to assess independent associations with WNV seropositivity. Model calibration was assessed using the Hosmer-Lemeshow goodness-of-fit test. All statistical tests were two-tailed, and a p-value< 0.05 was considered statistically significant.

Results

A total of 1,020 individuals were included in the study, comprising 720 voluntary blood donors and 300 paediatric patients who presented without clinical symptoms suggestive of fever, meningitis, or suspected encephalitis.

Serological screening by ELISA demonstrated that 25 participants (2.5%) were positive for WNV IgM antibodies (≥1.1), while an additional 25 participants (2.5%) yielded borderline IgM results (≥0.8–<1.1). Regarding WNV-specific IgG antibodies, 16 participants (1.6%) were seropositive (≥22 RU/mL) and one participant (0.09%) exhibited a borderline IgG result (≥16–<22 RU/mL). Three participants (0.29%) were positive for both WNV IgM and IgG antibodies.

Among the 50 IgM-positive or borderline samples identified by ELISA, confirmation by IIFT revealed 29 strongly positive, 13 moderately positive and eight weakly positive results. Similarly, of the 17 IgG-positive samples, 13 demonstrated strong, three moderate and one weak fluorescence intensity by IIFT.

Among the 1,020 participants included in the study, 789 (77.4%) were male and 231 (22.6%) were female. Participants ranged in age from 2 to 65 years. The age distribution was as follows: 162 (15.9%) aged 2–9 years, 138 (13.5%) aged 10–17 years, 238 (23.3%) aged 18–29

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years, 238 (23.3%) aged 30–39 years, 167 (16.4%) aged 40–49 years, 75 (7.4%) aged 50–59 years and two (0.2%) aged 60–69 years. A majority of participants (n=787; 77.2%) resided in the urban centre of Konya, while 233 participants (22.8%) lived in surrounding districts. Detailed demographic characteristics are presented in Table 1.

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Univariate analysis revealed a statistically significant association between WNV IgM seropositivity and age group (p=0.012) as well as educational level (p=0.008). In addition, WNV IgG seropositivity was significantly associated with age (p=0.043), gender (p=0.034) and educational attainment (p<0.05) (Table 1).

No statistically significant associations were observed between occupational category and WNV seropositivity (IgM: p=0.871, IgG: p= 0.628), nor between income level and antibody positivity (IgM: p=0.655, IgG: p=0.551).

The location of household toilets, assessed as a socioeconomic and environmental indicator, was not significantly associated with either WNV IgM or IgG seroprevalence (p= 0.623 and p=1.000, respectively). Although prolonged outdoor exposure was hypothesised to increase mosquito contact, time spent outdoors was not significantly associated with WNV seropositivity (IgM: p=0.430, IgG: p= 0.418).

Similarly, the use of mosquito repellents, evaluated as a personal protective measure, showed no statistically significant association with WNV IgM or IgG sero-prevalence (p= 0.424 and p=1.000, respectively) (Table 2).

No significant associations were identified between WNV antibody positivity and the presence of chronic kidney disease (IgM: p=1.000, IgG: p=1.000), hypertension (IgM: p=1.000; IgG: p=1.000), diabetes mellitus (IgM: p= 1.000, IgG: p=1.000), or cardiovascular disease (IgM: p=1.000, IgG: p=1.000).

Additionally, alcohol consumption, presence of animals in the household, and history of international travel were not significantly associated with WNV IgM or IgG seropositivity (Table 3).

Multivariate analysis

Multivariate logistic regression analysis was conducted to assess independent predictors of WNV seropositivity. Variables found to be significant in univariate analyses (namely age group and educational level for WNV IgM and age, gender and educational level for WNV IgG) did not retain statistical significance in the multivariate models (Table 4).

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Multivariate analysis

Multivariate logistic regression analysis was conducted to assess independent predictors of WNV seropositivity. In the multivariable logistic regression analysis, education level was independently associated with WNV IgM seropositivity (overall p=0.012). Participants in education category 1 had significantly higher odds of IgM seropositivity than those in the reference education category (adjusted OR [aOR]=3.093, 95% CI: 1.467–6.522, p=0.003). Education category 2 was not significantly associated with IgM seropositivity (aOR=1.810, 95% CI: 0.878–3.733, p=0.108). Age ≥18 years compared with <18 years (aOR=2.368, 95% CI: 0.892–6.290, p=0.084) was independently associated with IgM seropositivity (Table 4).

In the multivariable logistic regression analysis, the overall association between education level and WNV IgG seropositivity did not reach statistical significance (p=0.059). However, participants in education category 2 had significantly lower odds of IgG sero-positivity than those in the reference category (aOR= 0.196, 95% CI: 0.051–0.755, p=0.018), whereas education category 1 was not significantly associated with IgG seropositivity (aOR=0.321, 95% CI: 0.071–1.442, p=0.138). Gender was not independently associated with IgG seropositivity (aOR=0.599, 95% CI: 0.218–1.644, p=0.320). The age coefficient showed numerical instability in the model and the adjusted OR and 95% CI could not be reliably estimated (Table 5).

Table 1. Distribution of WNV IgM and IgG seropositivity according to demographic characteristics, Konya Province, Türkiye, 2024

Variables		IgM Positive (n)	IgM Negative (n)	p value	IgG Positive (n)	IgG Negative (n)	p value
Gender	Male	34	755	0.118	9	780	0.034*
	Female	16	215		8	223	
Age	2-9	17	145	0.008*	7	155	0.016*
	10-17	10	128		5	133	
	18-29	11	227		2	236	
	30-39	7	231		3	235	
	40-49	4	163		0	167	
	50-59	1	74		0	75	
	60-69	0	2		0	2	
Living area	Urban (central district)	34	753	0.121	10	777	0.081
	Rural (surrounding)	16	217		7	226	
Educational status	Not of school age	11	78	0.003*	0	89	0.018*
	Illiterate	1	7		0	8	
	Primary school student	7	79		5	81	
	Primary school graduate	8	222		6	224	
	Secondary school graduate	7	70		3	74	
	High school graduate	8	196		1	203	
	University graduate	8	284		2	290	
	Postgraduate	0	34		0	34	

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Table 2. Association between antibody positivity and occupation, income, toilet location, time outdoors and mosquito repellent, Konya Province, Türkiye, 2024

Variables		IgM Positive (n)	IgM Negative (n)	p value	IgG Positive (n)	IgG Negative (n)	p value
Occupation	Unemployed	2	36	0.791	0	38	0.809
	Housewife	3	31		0	34	
	White-collar	12	241		7	246	
	Blue-collar	23	475		8	490	
	Farmer	2	37		0	39	
	Student	1	35		0	36	
	Employer	3	28		1	30	
	Freelancer	4	87		1	90	
Annual equivalized income	High income	20	441	0.685	5	456	0.643
	Low income	30	529		12	547	
Toilet location	Inside	50	947	0.623	17	980	0.676
	Both inside and outside	0	23		0	23	
Daily outdoor exposure	<6 hours	49	904	0.284	17	936	0.626
	6–12 hours	1	65		0	66	
	>12 hours	0	1		0	1	
Use of mosquito repellent	Never	48	889	0.424	16	921	0.591
	Occasionally	2	81		1	82	
	Daily	-	-		0	0	

Table 3. The relationship between antibody positivity and chronic disease, alcohol use, the presence of animals in the living area and travel abroad, Konya Province, Türkiye, 2024

Variables		IgM Positive (n)	IgM Negative (n)	p value	IgG Positive (n)	IgG Negative (n)	p value
Chronic disease	Present	0	30	0.395	1	29	0.400
	Absent	50	940		16	974	
Renal failure	Present	0	5	1.000	1	4	0.081
	Absent	50	965		16	999	
Hypertension	Present	0	10	1.000	0	10	1.000
	Absent	50	960		17	993	
Diabetes mellitus	Present	0	11	1.000	0	11	1.000
	Absent	50	959		17	992	
Cardiovascular disease	Present	0	4	1.000	0	4	1.000
	Absent	50	966		17	999	
Alcohol consumption	Present	1	83	0.116	1	83	1.000
	Absent	49	887		16	920	
Presence of animals (pets/livestock/poultry)	Present	9	191	0.857	2	198	0.549
	Absent	41	779		15	805	
	Absent	50	919		17	952	
Traveling history	Within the past 6 months	0	31	0.473	0	31	1.000
	12 months ago	0	20		0	20	

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Table 4. Multivariable regression analysis of factors affecting WNV IgM positivity, Konya Province, Türkiye, 2024

Predictor	Significance level (p-value)	Adjusted Odds Ratio	95% confidence interval	
			Lower	Upper
Gender	0.944	1.024	0.529	1.980
Education (overall effect)	0.012	-	-	-
Education category 1 vs reference	0.003	3.093	1.467	6.522
Education category 2 vs reference	0.108	1.810	0.878	3.733

Notes: Adjusted odds ratios (adjusted ORs) were obtained from separate multivariable binary logistic regression models using the ENTER method. Gender and education age were entered simultaneously into each model

Table 5. Multivariable regression analysis of factors affecting WNV IgG positivity, Konya Province, Türkiye, 2024

Predictor	Significance level (p-value)	Adjusted Odds Ratio	95% confidence interval	
			Lower	Upper
Age		Not estimable		
≥18 vs <18	0.994		-	-
Gender	0.320	0.599	0.218	1.644
Male vs female				
Education (overall effect)	0.059	-	-	-
Education category 1 vs reference	0.138	0.321	0.071	1.442
Education category 2 vs reference	0.018	0.196	0.051	0.755

Notes: Adjusted odds ratios (adjusted ORs) were obtained from separate multivariable binary logistic regression models using the ENTER method. Gender, education and age were entered simultaneously into each model. Education was entered as a three-category variable using indicator coding, with education category 0 as the reference category. For IgG, the age coefficient showed numerical instability in the SPSS output (B=−17.268; SE= 2485.072, p= 0.994); therefore, the adjusted OR and 95% CI were not reliably estimable

Discussion

WNV, one of the most prevalent mosquito-borne zoonotic pathogens worldwide, was initially endemic to Africa, Australia, West Asia, and parts of Europe. Following its first detection in the United States in 1999, the virus rapidly expanded throughout North and South America, with confirmed circulation from Canada to Venezuela. Since its initial isolation in Uganda in 1937, numerous outbreaks involving large numbers of human cases have been reported, particularly in Israel, Greece, Romania, Russia and the United States. In the Mediterranean Basin and Central Europe, WNV infections typically occur between May and October, coinciding with increased mosquito activity and peak bird migration periods (27).

The ecological and climatic characteristics of Türkiye provide favorable conditions for the maintenance and transmission of mosquito-borne arboviruses, particularly WNV. Previous studies have demonstrated widespread circulation of WNV in humans, horses, birds and mosquito populations across several regions of Türkiye, including Central Anatolia and neighboring Mediterranean areas. In addition, the documented distribution of compe-

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tent *Culex* mosquito species supports the ongoing risk of endemic WNV transmission in the region (28–32).

Türkiye remains under continuous threat of WNV transmission due to its geographical position, diverse ecological conditions and location along major migratory bird routes (29). Since the first documentation of WNV in Türkiye in 1970, multiple studies have evaluated human exposure, reporting seroprevalence rates ranging from 0.9% to 57%, depending on study population and diagnostic methodology (34). Recent surveillance data from the European Union further highlight the public health burden of WNV, with reported fatalities of 92/1,133 cases in 2022, 67/728 cases in 2023 and 125/1,436 cases in 2024. The detection of WNV cases in countries bordering Türkiye, including Greece, Bulgaria, Hungary, Romania and Cyprus, reinforces the ongoing regional transmission risk (32, 35).

In the present study, serum samples from 1,020 individuals, comprising 720 blood donors and 300 pediatric patients, were analysed for WNV-specific anti-bodies using ELISA. Definite WNV IgM positivity was detected in 25 samples (2.45%), with an additional 25 samples (2.45%) yielding borderline results. WNV IgG antibodies were definitively detected in 16 samples (1.6%), with one borderline-positive sample (0.1%). All ELISA-positive or borderline samples were subsequently further evaluated by indirect immunofluorescence testing (IIFT). Among the 50 IgM-positive or borderline samples evaluated by IIFT, eight were weakly positive, 13 moderately positive and 29 strongly positive. Among the 17 IgG-positive or borderline samples, one was weakly positive, three moderately positive and 13 strongly positive.

An important highlight of this study is the demonstration of silent WNV circulation in an understudied region of Central Anatolia despite the absence of clinically recognized outbreaks. The strengths of this study are its relatively large sample size, the inclusion of both blood donors and pediatric participants, the use of complementary serological methods (ELISA and IIFT), and a comprehensive assessment of the demographic, environmental and socioeconomic risk factors linked to WNV seropositivity.

Advanced age, male sex and underlying chronic conditions, including diabetes, cardiovascular disease, malignancy and immunosuppression, are frequently reported as major risk factors for WNV infection. Additionally, residence in rural areas and occupational or environmental exposure associated with vector contact, such as agriculture and animal husbandry, have been identified as contributing factors (14). Numerous epidemiological studies have demonstrated increasing seroprevalence with advancing age (36–39), although some investigations have reported inconsistent findings (40). In this study, while the seropositivity of both IgM and IgG rose with age, these relationships lost statistical significance in the multivariate regression analysis, indicating that age by itself may not constitute an independent risk factor.

Male predominance in WNV seroprevalence has been consistently documented in population-based studies (35, 41). Similarly, our findings demonstrated significantly higher IgG seropositivity among male participants (p=0.034), supporting existing evidence of sex-related differences in exposure or susceptibility.

Although WNV outbreaks have historically been associated with rural environments, major epidemics in Romania (1996) and New York (2002) illustrated the virus's capacity to spread efficiently in urban settings under favourable ecological conditions (12, 42). While urbanisation has been linked to increased vector density and infection risk (43, 44). Our study did not identify a statistically significant association between area of residence (urban vs. rural) and WNV IgM or IgG seropositivity.

Socioeconomic variables were also examined as potential determinants of WNV exposure. Previous studies have reported higher se-

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roprevalence and neuro-invasive disease incidence in socioeconomically disadvantaged regions (44–47). However, in the present study, income level and household sanitation characteristics were not significantly associated with WNV seropositivity. Although higher positivity rates were observed among individuals with indoor sanitation facilities, this difference was not statistically significant.

Educational level demonstrated a significant relationship with WNV seroprevalence ($p< 0.05$). IgM positivity was more common among individuals with high school or university education, whereas IgG positivity was higher among those with primary education. These results are partly consistent with earlier research indicating that education level affects awareness, behavior and exposure risk, though the nature of this association can differ across populations (44, 45, 48).

Time spent outdoors and personal protective measures are considered important modifiers of WNV infection risk (49). While previous outbreak investigations have shown higher exposure among individuals spending prolonged periods outdoors (36, 50), no statistically significant association was identified in our cohort. The effect of occupation on WNV infection has been investigated in various studies (41, 51). Similarly, occupational classification did not significantly influence seroprevalence, possibly due to comparable exposure levels among different working groups and the inability to fully quantify environmental risk factors.

Contrary to reports linking alcohol consumption to increased WNV risk (52), no association was observed in the present study. Likewise, chronic conditions such as diabetes, hypertension, chronic kidney disease and cardiovascular disease, previously implicated as risk factors for infection or severe disease (43, 51–55), did not demonstrate statistically significant associations with WNV seropositivity. The predominance of healthy blood donors in the study population likely limited the statistical power to detect such relationships.

Finally, pet ownership and international travel history were evaluated as potential exposure-related factors. Although birds serve as key reservoir hosts for WNV (56), no significant association was observed between pet ownership and antibody positivity. Similarly, recent international travel did not influence WNV IgM or IgG seroprevalence.

Because pediatric participants were enrolled primarily for seroepidemiological assessment and did not present with suspected neuroinvasive disease, the clinical relevance of detected WNV antibodies in relation to observed symptoms could not be definitively established.

The ecological suitability of Central Anatolia for mosquito breeding, together with previous reports demonstrating WNV activity in humans, animals and mosquito populations in Türkiye and neighboring Mediterranean regions, suggests that the seropositivity identified in the present study is more likely associated with long-standing silent endemic circulation rather than recent viral emergence in Konya Province. Moreover, the absence of recognized large-scale outbreaks despite measurable seroprevalence supports the presence of ongoing low-level viral transmission within the region.

This study has several limitations. First, the cross-sectional design limits causal interpretation of the identified associations. Second, although all ELISA-reactive samples were further evaluated using IIFT, the absence of plaque reduction neutralization testing (PRNT), the reference method for confirming WNV-specific neutralizing antibodies, remains an important limitation. Therefore, serological cross-reactivity with other flaviviruses cannot be completely excluded. Consequently, some seropositive results may reflect cross-reactive antibodies rather than true WNV-specific antibodies, potentially leading to an overestimation of the true WNV seroprevalence. Third, the study was conducted in a single geographical region, which may limit the generalizability of the findings to other parts of Türkiye. Fourth, no concurrent

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entomological investigation or mosquito surveillance was performed in Konya during the study period. Therefore, the presence, density, and infection status of competent mosquito vectors could not be directly evaluated. Although previous studies from Central Anatolia and neighboring regions have demonstrated the circulation of *Culex* mosquito species and WNV activity, the absence of local vector data remains an important limitation of the present study. Additionally, the predominance of healthy blood donors as the main study population may limit the representativeness of the findings for the general population and restrict the generalizability of the results to the wider community. Future multicenter longitudinal studies incorporating molecular, entomological and ecological surveillance are needed to better understand WNV transmission dynamics and outbreak risk in Türkiye.

Conclusion

This study provides novel data on WNV seroprevalence in a large mixed population from Türkiye, including both blood donors and pediatric patients. Unlike previous studies, it simultaneously evaluates multiple demographic and environmental risk factors, offering a broader epidemiological perspective. The use of combined serological methods further strengthens the reliability of the findings.

The results provide evidence of ongoing WNV circulation in Konya Province, Central Anatolia, Türkiye. Although several established risk factors did not show independent associations in multivariate analyses, the observed seroprevalence indicates continued silent transmission within the population. These findings highlight the multifactorial nature of WNV exposure, driven by complex interactions among ecological, demographic, environmental and behavioral determinants.

Given the absence of specific antiviral therapy and a licensed human vaccine, sustained surveillance is essential for early detection and prevention of potential outbreaks. Region-specific seroepidemiological studies such as this are important for understanding local transmission dynamics and informing evidence-based public health strategies.

To fill existing gaps, it is recommended to enhance mosquito surveillance and vector monitoring systems, especially in ecologically favorable areas like Central Anatolia. Integrated vector management strategies, including environmental control and reduction of breeding sites, should be expanded alongside community education on personal protective measures. A One Health–based surveillance framework integrating human, animal and environmental data is also crucial. Furthermore, enhanced monitoring of high-risk populations, migratory bird routes and vector reservoirs, together with multicenter molecular and entomological studies, is needed to better elucidate WNV transmission dynamics in Türkiye.

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Ethical consideration

This study was approved by the Drug and Non-Medical Device Research Ethics Committee of the Faculty of Medicine, Necmettin Erbakan University (Approval No: 2022/4100; Date: December 16, 2022). Written informed consent was obtained from all adult participants before enrollment. For participants under 18 years of age, written informed consent was obtained from parents or legal guardians. Assent was additionally obtained from minors whenever appropriate, according to age and comprehension level.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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