

# SEROPREVALENCE OF WEST NILE VIRUS IN BULGARIA, 2018

**E. Panayotova, I. Christova,  
I. Trifonova, E. Taseva,  
T. Gladnishka, V. Ivanova**

National Centre of Infectious and Parasitic Diseases, Sofia

## ABSTRACT

In Bulgaria, the first laboratory-confirmed West Nile neuroinvasive disease (WNND) case occurred in 2015 and more cases have been reported ever since. The aim of our study was to track the current circulation of West Nile virus (WNV) in the country. We collected 1830 serum samples from healthy people of every district in Bulgaria. Commercially available ELISA kits were used to detect specific anti-WNV IgG antibodies. All positive samples were tested for specific IgM antibodies using the same method. Possible risk factors were identified by calculating odds ratio. Specific IgG antibodies were detected in 22 of the samples (1.2%, CI 0.8% to 1.8%). No IgM antibodies were detected in the positive samples. The highest seroprevalence rates were found in the districts of Pleven, Varna, Silistra and Yambol. This study showed the continuous circulation and spread of WNV in Bulgaria.

## KEYWORDS:

West Nile virus, seroprevalence, antibodies, neuroinvasive disease

## INTRODUCTION

West Nile virus (WNV) is a mosquito-borne flavivirus emerging in Europe and America. As many other flaviviruses, WNV is maintained in nature in a

## ADDRESS FOR CORRESPONDENCE:

Elitsa Panayotova, PhD  
National reference laboratory  
of vector-borne pathogens  
National Center of Infectious  
and Parasitic Diseases  
26 Yanko Sakazov Blvd.  
Sofia, Bulgaria  
email: elica.panayotova@gmail.com

cycle which involves mosquitoes and vertebrate hosts. WNV uses *Culex* mosquitoes as vectors, and domestic and wild birds as amplifying hosts. Humans and horses are an incidental end host of the virus.

Most human infections with WNV are asymptomatic (~80%). About 20% of WNV infections result in influenza-like fever, called West Nile fever, and less than 1% cause neuroinvasive diseases (West Nile neurologic disease, WNND). Clinicians mostly recognise neuroinvasive infections which represent less than 1% of WNV infections, but the actual number of infected people is a lot higher.

In Europe, WNV emerged in Romania in 1997 when 352 WNND cases were confirmed (6). In the following years, a number of WNV outbreaks occurred in Europe, including in the Balkans: 609 cases in Greece (2010-2013), 371 cases in Serbia (2012-2013) (5) and 47 cases in Romania (7).

In Bulgaria, the first laboratory-confirmed WNND case occurred in 2015 and more cases have been reported ever since. Following the first confirmed case, a nationwide seroprevalence study was conducted in 2015 (1). An overall seroprevalence of 1.5% was found, varying from 0 to 10% among districts. The aim of our study was to track the current circulation of WNV in Bulgaria.

## MATERIAL AND METHODS

Between February and March 2018, a total of 1830 serum samples were obtained from healthy people (57.7% females and 42.3% males) from each of the 28 districts of Bulgaria. The age of the people ranged between 15 and 93 years, with a median age of 57. The samples were transported and stored at -20°C before testing.

Commercially available ELISA kits were used for detection of specific anti-WNV IgG antibodies (Euroimmun, Germany), following the manufacturer's instructions. Samples resulting positive were further tested for the presence of specific IgM antibodies using ELISA kits from the same manufacturer.

In order to identify possible risk factors, univariate analysis was performed calculating the odds ratio (OR) and 95% confidence interval (CI).

**SEROPREVALENCE OF WEST NILE VIRUS IN BULGARIA, 2018**

**Table 1.** WNV seroprevalence rates by district (Bulgaria 2018).

<b>District</b>	<b>Positive (no)</b>	<b>Tested (no)</b>	<b>Prevalence rate</b>	<b>lower CI</b>	<b>upper CI</b>
<b>Blagoevgrad</b>	1	60	1.67%	0.29%	8.86%
<b>Burgas</b>	2	81	2.47%	0.68%	8.56%
<b>Dobrich</b>	0	70	0.00%	0.00%	0.00%
<b>Gabrovo</b>	1	60	1.67%	0.29%	8.86%
<b>Haskovo</b>	1	70	1.43%	0.25%	7.66%
<b>Kardzhali</b>	0	60	0.00%	0.00%	0.00%
<b>Kustendil</b>	0	60	0.00%	0.00%	0.00%
<b>Lovech</b>	0	60	0.00%	0.00%	0.00%
<b>Montana</b>	2	80	2.50%	2.50%	8.66%
<b>Pazardzhik</b>	0	60	0.00%	0.00%	0.00%
<b>Pernik</b>	0	60	0.00%	0.00%	0.00%
<b>Pleven</b>	3	70	4.29%	1.47%	11.86%
<b>Plovdiv</b>	1	70	1.43%	0.25%	7.66%
<b>Razgrad</b>	0	60	0.00%	0.00%	0.00%
<b>Ruse</b>	1	70	1.43%	0.25%	7.66%
<b>Shumen</b>	0	60	0.00%	0.00%	0.00%
<b>Silistra</b>	2	70	2.86%	0.79%	9.83%
<b>Sliven</b>	0	60	0.00%	0.00%	0.00%
<b>Smolyan</b>	0	60	0.00%	0.00%	0.00%
<b>Sofia city</b>	1	80	1.25%	0.22%	6.75%
<b>Sofia district</b>	1	80	1.25%	0.22%	6.75%
<b>Stara Zagora</b>	1	60	1.67%	0.29%	8.86%
<b>Targovishte</b>	0	60	0.00%	0.00%	0.00%
<b>V Tarnovo</b>	0	60	0.00%	0.00%	0.00%
<b>Varna</b>	2	60	3.33%	0.92%	11.36%
<b>Vidin</b>	0	49	0.00%	0.00%	0.00%
<b>Vratsa</b>	1	70	1.43%	0.25%	7.66%
<b>Yambol</b>	2	70	2.86%	0.79%	9.83%
<b>TOTAL</b>	22	1830	1.20%	0.80%	1.81%

## RESULTS

Specific WNV IgG antibodies were detected in 22 of the 1830 samples (Table 1) (1.2%, CI 0.8% to 1.8%). No anti-WNV IgM antibodies were detected in the positive samples. The districts with the highest seroprevalence rates were Pleven (4.29%, CI 1.47% to 11.86%), Varna (3.33%, CI 0.92% to 11.36%), Silistra (2.86%, CI 0.79% to 9.83%) and Yambol (2.86%, CI 0.79% to 9.83%).

There were no significant differences between the positive female (1.42%) and male (0.9%) groups: OR 1.58 (CI 0.64 to 3.9, *p* value=0.32). Age (40 and above) also did not show any influence on WNV infection (OR 1.54, CI 0.62 to 3.81, *p* value=0.35).

## DISCUSSION

The current WNV seroprevalence rate of 1.2% is consistent with the previous study of ours, which showed an overall rate of 1.5%. Interestingly, there are differences regarding the spread of the viral circulation between the current and the previous nationwide seroprevalence study. Three years ago, the highest seroprevalence rates were found in the districts of Sofia province and Vidin (1). In the current study the prevalence rates for these districts were 1.25% and 0%, respectively, which might be showing better mosquito management and control. On the other hand, the highest seroprevalence rates were in the districts of Pleven, Varna, Silistra and Yambol, which is consistent with the previous results, demonstrating high WNV rates in the districts of Pleven, Silistra and Yambol (2%, 6% and 2%, respectively). This is also expected, since Pleven and Silistra are bordering the river of Danube with favourable conditions for mosquito populations;

Varna is bordering the Black Sea, as well as a couple of lakes (Lake Varna and Lake Beloslav); and Yambol is bordering the Tundzha river and is close to the neighbouring countries of Greece and Turkey, where there were numerous WNV cases in the recent years (3,4). We found high seroprevalence rate in the district of Burgas as well – 2.47% (CI 0.68% to 8.56%), where a total of 4 WNV cases were detected in 2018, representing almost 1/3 of the reported WNV cases that year (2).

The present country-wide WNV seroprevalence study shows that the virus continues to circulate and spread to new foci. Moreover, in 2018 there were an unexpectedly high number of WNV cases in Bulgaria. This suggests improved disease recognition by clinicians, but nevertheless further studies should be performed examining the circulation of the virus in reservoir hosts and vectors as well.

## REFERENCES

1. Christova I, Panayotova E, Tchakarova S, Taseva E, Trifonova I, Gladnishka T. A nationwide seroprevalence screening for West Nile virus and Tick-borne encephalitis virus in the population of Bulgaria. *J Med Virol.* 2017; 89(10):1875-1878.
2. Christova I, Panayotova E, Trifonova I, Taseva E, Gladnishka T. Unexpectedly high number of West Nile neuroinvasive diseases in Bulgaria in 2018. *Probl Infect Parasit Dis.* 2018; 46(2):16-19.
3. Ergunay K, Bakonyi T, Nowotny N, Ozkul A. Close relationship between West Nile virus from Turkey and lineage 1 strain from Central African Republic. *Emerg Infect Dis.* 2015; 21(2):352-355.
4. Lustig Y, Sofer D, Bucris ED, Mendelson E. Surveillance and Diagnosis of West Nile Virus in the Face of Flavivirus Cross-Reactivity. *Front Microbiol.* 2018; 9:2421.
5. Petrović T, Šekler M, Petrić D, Lazić S, Debeljak Z, Vidanović D, et al. Methodology and results of integrated WNV surveillance programmes in Serbia. *PLoS ONE.* 2018; 13(4):e0195439.
6. Tsai TF, Popovici F, Cernescu C, Campbell GL, Nedelcu NI. West Nile encephalitis epidemic in southeastern Romania. *Lancet.* 1998; 352(9130):767-771.
7. Popescu CP, Florescu SA, Badescu D, Ceianu CS, Zaharia M, Tardei G, Codreanu D, Ceausu E, Ruta SM. Re-emergence of severe West Nile virus neuroinvasive disease in humans in Romania, 2012 to 2017 – implications for travel medicine. *Travel Med Infect Dis.* 2018; 22:30-35.

## ACKNOWLEDGEMENTS

This work was supported by the Bulgarian Fund for scientific investigations, research project contract ДН03/15 from 19.12.2016.