UNEXPECTEDLY HIGH NUMBER OF WEST NILE NEUROINVASIVE DISEASES IN BULGARIA IN 2018

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ABSTRACT

West Nile virus (WNV) is an emerging arbovirus in Europe and America which disseminated widely in recent years. This study analysed epidemiological data and laboratory findings of probable and confirmed human WNV cases in Bulgaria in 2018. A total of 15 patients with WNV infection were detected in 2018, with 2 deaths among them. All patients were diagnosed in August or September. Four patients were from Sofia district, 4 from Burgas, 3 from Plovdiv and 1 from each of the following districts: Shumen, Yambol and Pazardzhik. Laboratory diagnosis for 7 of the patients was based solely on detection of specific antibodies in the serum samples. Eight patients met the criteria for confirmed WNV case. In 6 of them WNV genome was detected by RT-PCR in blood or urine sample and in 3 patients specific IgM antibodies were found in CSF samples which confirmed WNV neuroinvasive infection. The high number of diagnosed human WNV cases in 2018 in Bulgaria is a good sign of increasing recognition of the disease. More efforts are needed in proper transportation of clinical samples undergoing genetic detection of the viral genome. WNV neuroinvasive infection should be suspected and included in the differential diagnosis of viral encephalitis or meningoencephalitis especially in late summer and early autumn.

***KEYWORDS*:**

viral encephalitis, flavivirus, Bulgaria, West Nile virus

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INTRODUCTION

West Nile virus (WNV) is an emerging arbovirus in Europe and America which disseminated widely in recent years.

WNV is a member of genus *Flavivirus* of the *Flaviviridae* family. The virus is maintained in nature by enzootic cycle involving wild or domestic birds as hosts and *Culex* mosquitoes as vectors. Migratory birds contribute to the transfer of the virus over long distances.

The disease in people is most often asymptomatic. About 20% of WNV infections result in diseases that are usually presented only with non-specific febrile syndrome. Less than 1% of WNV infections involve central nervous system, mostly with encephalitis but also with meningitis or myelitis (1). The fact that mainly neuroinvasive infections are recognised means that the actual number of infected people is at least a hundred times higher. The first recognised WNV outbreak was described in southern France in 1962-1963. The first significant epidemic in humans was registered in Romania in 1996 with 393 WNV infection cases. The largest outbreak of WNV in Europe was recorded between 2010 and 2013 in Greece with a total of 609 laboratory-confirmed cases and 73 deaths (2). The outbreak was caused by WNV lineage 2. The first time this lineage caused disease outside Africa was in Hungary in 2004 (3). In subsequent years WNV lineage 2 appeared in many countries of Central, East and South Europe (Hungary, Russia, Romania, Italy, Albania, Serbia, Greece) and now is the predominating lineage in Europe.

In Bulgaria, human WNV cases are reported every year since 2015, when the first laboratory-confirmed neuroinvasive case appeared (4). However, in 2018 unusually high number of WNV cases were recorded in the country. The aim of this study was to analyse epidemiological data and laboratory findings of these cases.

MATERIAL AND METHODS

Patients and clinical samples

Serum, blood and CSF samples were drawn from suspected patients by physicians at the regional hospitals in Bulgaria. Laboratory investigations were performed at the National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria by ELISA and RT-PCR.

ELISA

Serum and CSF samples were tested for WNV-specific IgM and IgG antibodies using ELISA test (Euroimmun, Germany).

RT-PCR

Viral RNAs were extracted from blood and urine samples using QIAmp Viral Mini Kit (Qiagen, Hilde, Germany). WNV RNAs were detected by commercially available real-time RT-PCR kit (Sacace Biotechnologies, Italy).

RESULTS

A total of 15 patients with West Nile virus (WNV) infection were detected in 2018, with 2 deaths among them (case fatality ratio 13.3%). All except 4 were male (73.3%).

The age of the patients varied between 45 and 84 years. The most affected age groups were 70-79 (4 patients) and 50-59 (3 patients). Older age was a risk factor for more complications and even lethal outcome (as for the 84-year-old patient).

All patients were diagnosed in August or September with 6 in August and 9 in September. This fact coincides with the known high activity of *Culex* mosquitoes in the country.

Analysis of case distribution according to the district of origin showed that 4 patients were from Sofia district, 4 from Burgas, 3 from Plovdiv and 1 from each of the following districts: Shumen, Yambol and Pazardzhik.

Serological diagnosis of serum samples was carried out in all except one case. However, this finding alone is suggestive only of a probable case. In our study, laboratory diagnosis in 7 patients was based solely on detection of specific antibodies in the serum samples.

Eight patients met the criteria for confirmed WNV case. In 6 of them WNV genome was detected by RT-PCR in blood or urine sample and in 3 patients specific IgM antibodies were found in CSF samples which confirmed WNV neuroinvasive infection. In one of these patients, antibodies in CSF and viral genome in the blood sample were detected simultaneously.

Detailed characteristics of the patients are presented in Table 1.

Table 1. Patients diagnosed with WNV infection in 2018.

No	Age	Sex	Month	District	Laboratory findings			Case category
					ELISA serum	ELISA CSF	RT-PCR	
1.	49	M	August	Sofia	IgM+ IgG+	n.p.	n.p.	Probable
2.	55	M	August	Plovdiv	IgM+	IgM+	+ blood	Confirmed
3.	57	M	August	Sofia	n.p.	IgM+	n.p.	Confirmed
4.	75	F	August	Burgas	IgM+ IgG+	n.p.	n.p.	Probable
5.	72	M	August	Pazardzhik	IgM+ IgG+	n.p.	n.p.	Probable
6.	-	M	August	Plovdiv	n.p.	n.p.	+ blood + urine	Confirmed
7.	51	M	September	Burgas	IgG+	n.p.	n.p.	Probable
8.	69	M	September	Shumen	IgM+ IgG+	n.p.	n.p.	Probable
9.	45	M	September	Plovdiv	IgM+ IgG+	n.p.	n.p.	Probable
10.	-	F	September	Sofia	IgM+ IgG+	IgM+ IgG+	n.p.	Confirmed
11.	84	M	September	Yambol	IgM+ IgG+	n.p.	n.p.	Probable

No	Age	Sex	Month	District	Laboratory findings			Case category
					ELISA serum	ELISA CSF	RT-PCR	
12.	70	F	September	Burgas	IgM+ IgG+	n.p.	+ blood + urine	Confirmed
13.	70	F	September	Burgas	IgM+/- IgG+/-	n.p.	+ blood + urine	Confirmed
14.	-	M	September	Sofia	IgM+ IgG+	n.p.	+ blood + urine	Confirmed
15.	66	M	September	Sofia	IgM+	n.p.	+ blood + urine	Confirmed

*n.p. – not performed

DISCUSSION

In 2018, WNV caused unusually high number of infections across Europe. According to ECDC data, the total number of confirmed and probable WNV infections reported in 2018 exceeds the total number in the previous seven years (5). A total of 2 083 autochthonous WNV infections in 2018 were reported in Europe – 576 in Italy, 415 in Serbia, 311 in Greece, 277 in Romania, 215 in Hungary, 53 in Croatia, 27 in France, 20 in Austria. Except Italy, the 3 most affected countries are Bulgaria's neighbours. This fact should be taken into account when analysing the situation in Bulgaria. Even though 15 patients in one year is much more than the number of cases reported in the last seven years in the country (a total of 7 cases: 2012-2, 2015-2, 2016-2, 2017-1), this number is much lower compared with the neighbouring countries. This means that the vast majority of the WNV infections were not recognised by physicians. Moreover, again according to ECDC data, 68% of WNV infections in 2018 were neuroinvasive (5), indicating that about one third of the recognised infections were with non-specific febrile syndrome. For comparison, all recognised WNV cases in Bulgaria were neuroinvasive. Therefore, more effort is needed to add WNV infection in differential diagnosis of viral encephalitis or meningoencephalitis.

More effort is also needed to ensure appropriate transportation of clinical samples. They should be sent on ice to the Reference laboratory as this is the only way to preserve the virus during transportation. It should be kept in mind that blood is a more suitable clinical sample for diagnosis early in the course of the disease and urine is preferable later and in case of neuroinvasive infection. Detection of specific antibodies in CSF is also suggestive of confirmed WNV infections. For this reason blood, urine and CSF are appropriate clinical samples for reliable laboratory diagnosis of WNV infection.

Analysis of epidemiological data of our patients revealed that they were mostly men above 44 years, some of them with chronic diseases and other complications (like alcoholism). These could be identified as risk factors for occurrence of WNV infection and especially neuroinvasive form of the illness.

Regarding the location of the WNV cases, it should be noted that the disease is found where it is searched for. The territory around Sofia is an endemic area as shown by the seroprevalence study (6) and confirmed by detection of WNV genome in patients (4). The first and nationwide seroprevalence study revealed the highest seroprevalence rate of WNV-specific IgG antibodies in Sofia Province (10%). The study also showed high seroprevalence rates in districts along the river Danube (4-7.5%), low rates in the districts of Yambol and Plovdiv (2% and 1.6%, respectively) and 0% in the districts of Burgas, Shumen and Pazardzhik, where human WNV cases were detected in 2018. Obviously, much more cases could be found in districts along the river Danube provided that are properly and accurately diagnosed with the assumption that in late summer and early autumn WNV is not uncommon cause of febrile illnesses and viral encephalitis.

Etiology of the vast majority of neuroinfections in the country remains unknown (7). WNV infection of CNS should be suspected among cases presenting with clinical manifestation of viral encephalitis. Significant increase in the number

of WNV infections this year may lead to an outbreak next year (8).

In conclusion, the high number of diagnosed human WNV cases in 2018 in Bulgaria is a good sign of increasing recognition of the disease, a tendency that should be kept in the future. More efforts are needed in proper transportation of clinical samples undergoing genetic detection of the viral genome. WNV neuroinvasive infection should be suspected and included in the differential diagnosis of viral encephalitis or meningoencephalitis especially in late summer and early autumn.

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REFERENCES

1. Mostashari F, Bunning ML, Kitsutani PT, Singer DA, Nash D, Cooper MJ, Katz N, Liljebjelke KA, Biggerstaff BJ, Fine AD, Layton MC, Mullin SM, Johnson AJ, Martin DA, Hayes EB, Campbell GL.. *Epidemic West Nile encephalitis. New York. 1999: results of a household-based seroepidemiological survey*. The Lancet. 2001; 358(9278):261–264.
2. Hadjichristodoulou C, Pournaras S, Mavrouli M, Marka A, Tserkezou P, Baka A, Billinis C, Katsioulis A, Psaroulaki A, Papa A, Papadopoulos N, Mamuris Z, Tsakris A,
Kremastinou J. *West Nile virus seroprevalence in the Greek population in 2013: A nationwide cross-sectional survey*. PLoS One. 2015; 10(11):e0143803.
3. Bakonyi T, Ivanics E, Erdélyi K, Ursu K, Ferenczi E, Weissenböck H, Nowotny N. *Lineage 1 and 2 strains of encephalitic West Nile virus, central Europe*. Emerg Infect Dis. 2006; 12(4): 618–623.
4. Baymakova M, Trifonova I, Panayotova E, Dakova S, Pacenti M, Barzon L, Lavezzo E, Hristov Y, Ramshev K, Plochev K, Palu G, Christova I. *Fatal case of West Nile neuroinvasive disease in Bulgaria*. Emerg Infect Dis. 2016; 22(12):2203-2204.
5. ECDC. Epidemiological update: West Nile virus transmission season in Europe, 2018. https://ecdc.europa.eu/en/news-events/epidemiological-update-west-nile-virus-transmission-season-europe-2018.
6. 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.
7. Argirova P, Boev I, Baltadzhiev I, Venchev Ch, Murdzheva M, Stoycheva M. *Characteristics of current bacterial neuroinfections in Plovdiv region*. Probl Infect Parasit Dis. 2017; 45(1):31-36.
8. Kurchatova A, Krumova S, Vladimirova N, Nikolaeva-Glomb L, Stoyanova A, Kantardjiev T, Gatcheva N. *Preliminary findings indicate nosocomial transmission and Roma population as most affected group in ongoing measles B3 genotype outbreak in Bulgaria, March to August 2017*. Euro Surveill. 2017; 22(36): 30611.