

# ASSESSMENT OF WEST NILE VIRUS INFECTION IN TURKEY BASED ON TWO RECENT CASES

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**Abstract.** West Nile virus (WNV) is an arbovirus maintained in enzootic transmission cycles between mosquitoes and birds and is occasionally transmitted to mammals. This study evaluated WNV-infected patients and public health measures taken in Turkey. Upon notification of two cases of WNV neuroinvasive form in October 2016 serological and neutralization tests were performed and clinical and epidemiological surveillance studies conducted. A female patient in Izmir recovered but a male patient in Manisa died from the infection. The prevalence of suspected WNV-infected cases in the two provinces in 2016 varied between 0-22.1 per 100,000 population. However, patients diagnosed with viral encephalitis, arthropod-borne viral encephalitis or mosquito-borne viral encephalitis were not infected with WNV. From a “One Health, One World” perspective, collaboration among veterinary and health authorities, entomologists, environmental specialists, and biologists, provides the best strategy towards control and prevention of WNV disease in Turkey.

**Keywords:** West Nile virus, prevalence, Turkey

## INTRODUCTION

West Nile virus (WNV), a single-stranded RNA virus of family Flaviviridae, genus *Flavivirus* is the cause of a mosquito-borne zoonotic disease with a mortality rate of 4-14% (Rossi *et al*, 2010; Conde *et al*, 2017). The natural life-cycle of WNV is

through birds and mosquitoes, whereas humans and horses are considered dead-end hosts (Hubálek and Halouzka, 1999; Petrić *et al*, 2017). WNV was first isolated in 1937 from a female patient in the West Nile District of Uganda (Smithburn *et al*, 1940). WNV infection is a growing public health problem, especially in regions of Africa, Asia, Europe, and the Middle East. Europe has seen WNV fever epidemics since 1960's (Hayes *et al*, 2008). Turkey, a northeastern Mediterranean country, is at risk for arboviral diseases such as WNV because of the country's favorable ecological and climatic characteristics

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(Ergünay *et al*, 2011).

WNV is mostly transmitted to humans by mosquitoes of *Culex* genus, but also through virus contaminated blood transfusion and organ transplantation and from infected mother-to-child route during pregnancy, delivery or breastfeeding (Ergünay *et al*, 2017). The incubation period lasts 3-14 days. While it is estimated the majority of WNV infections are asymptomatic, 20-30% of patients develop acute systemic febrile illness, and mostly the elderly and immunocompromised patients develop a neuroinvasive form, *eg* encephalitis, meningitis or myelitis, which may lead to death (Winston *et al*, 2014); however, WNV infection is often self-limited. Currently, there is no specific treatment or vaccine for WNV fever (Rossi *et al*, 2010; Patel *et al*, 2015).

This study evaluated epidemiology and public health measures following reports of WNV-infected patients in 2016 from Izmir and Manisa Provinces, Turkey. The information obtained should be of value in the implementation of public health policies to control and prevent spread of this potentially life-threatening mosquito-borne viral disease.

## MATERIALS AND METHODS

### Study areas and surveillance procedures

Upon notification by the National Virology Reference Laboratories in Ankara of two cases of WNV neuroinvasive form in Izmir and Manisa Provinces in October 2016, clinical and epidemiological surveillance studies were conducted in the two provinces. Risk factors, such as animal feeding, bird feed-contact, blood/ plasma/blood product delivery; environmental factors, *eg* presence of mosquitos in living/working place, presence of water catchment area (lake/dam) near

the living/working place; and number of patients with meningitis/meningoencephalitis in the region were evaluated. Numbers of meningitis/meningoencephalitis patients were calculated according to 25 ICD-10 diagnostic codes for meningitis and encephalitis (Turkish Ministry of Health, 2016). Duplicate subjects were excluded from the study. Prevalence is calculated as the number of new cases/100,000 population.

### Laboratory assays

Specimens kept at 4°C were submitted to the National Virology Reference Laboratories, Arbovirus and Viral Zoonotic Diseases Unit and stored at -25°C until tested for presence of anti-West Nile virus IgG and Ig M using ELISA (Anti-West Nile virus ELISA IgG and IgM; Euroimmun, Lübeck, Germany) and IFA (IIFT Flavivirus Mosaic 1 IgG and IgM; Euroimmun) kits. Plaque-reduction neutralization test (PRNT) was carried out for detection of specific neutralizing antibodies according to case definitions by ECDC (2017a).

IFA test was also performed for detection of flavivirus mosaic 1 (Euroimmun), tick-borne encephalitis virus (TBEV), yellow fever virus (YFV), WNV, and Japanese encephalitis virus (JEV). A PRNT validation test was performed with WNV/NY99 strain [using 200 plaque forming units (PFU) per reaction] for specific differential diagnosis of WNV.

Serum samples with >70% neutralization were accepted as seropositive. A confirmed case is defined as a patient with at least one of the following laboratory criteria: isolation of WNV from blood, detection of WNV RNA by RT-PCR in blood, detection of increasing levels of IgM and IgG against WNV using ELISA, and positive PRNT (ECDC, 2017a). Clinical criteria of confirmed WNV cases were based on a

modified European case definition, which includes neurological symptoms (ECDC, 2017a).

## RESULTS

### Geographical features

Selendi District, Manisa Province, situated at an altitude of 390 m above sea level, and Kemalpaşa District, İzmir Province, at an altitude of 240 m surrounded by mountains covered with pine trees, lie in western Turkey. The two villages lie among highly irrigated fields with access to water from river channels. Weather is usually warm in these regions and mosquitoes are present nearly all year round.

### Cases

In 2016 two cases of WNV infection were reported, a female (18 years of age) from Selendi District, Manisa Province and a male (63 years of age) from Kemalpaşa District, İzmir Province. Both patients presented high fever ( $\geq 38.5^{\circ}\text{C}$ ), nausea, vomiting, weakness, headache and lack of appetite. The patients were not vaccinated against flaviviruses (yellow fever, tick-borne encephalitis or Japanese encephalitis virus) and had a history of contact with animals and mosquitoes. The female patient was working as an agricultural worker in the vineyard and her family had sheep. The male patient was a guard and living with six dogs. Clinicopathological findings [high fever ( $\geq 38.5^{\circ}\text{C}$ ), nausea, vomiting, weakness, headache and lack of appetite] were suggestive of WNV neuroinvasive form. Clinical samples [cerebrospinal fluid (CSF) and blood] sent to the National Virology Reference Laboratories in Ankara, showed positive serological and neutralization tests for blood (increasing levels of IgM and IgG against WNV using ELISA, and positive PRNT) but CSF samples were

negative for WNV (including tests after two weeks of admission) and other neurotrophic viruses [herpes simplex viruses (1 and 2), varicella zoster virus, Epstein-Barr virus, cytomegalovirus, human herpes viruses (6, 7 and 8) and enterovirus] and arboviruses using RT-PCR. Gram and fungal stains of blood and CSF cultures were negative as were autoimmune tests. Negative serological tests were obtained for brucellosis, Lyme disease, leptospirosis, syphilis and tularemia were negative. Computed tomography and magnetic resonance imaging of the brain were unremarkable. The patients were given supportive treatment including intravenous fluids and antipyretics, the female patient was discharged in a healthy condition after day 21 of hospitalization but the male patient died on day 39 after admission.

### Surveillance

In 2015 and 2016, İzmir and Manisa Provinces reported 628 (143 cases in Manisa Province) hospitalized cases of meningitis/meningoencephalitis in 2015 and 528 (221 cases in Manisa Province) hospitalized cases in 2016. In Manisa Province the number of cases rose in September, October and November, 2015 and in July, August and September, 2016. Until October, 2016 when WNV infection case was reported, 135 cases with meningitis/meningoencephalitis cases had been admitted.

Attention was focused on Manisa Province, given the 1.5-fold increase in meningitis/meningoencephalitis cases from 2015 to 2016. When the numbers of suspicious cases of meningitis/meningoencephalitis in all districts in Manisa Province from January to October, 2016 were examined, prevalence varied from 4.7 to 22.1 per 100,000 population, with 9.8 per 100,000 in the whole Manisa Province

(Table 1). The prevalence of meningitis/meningoencephalitis during this period in Selendi District, from where the WNV infection case came, was 4.8 per 100,000, the 2<sup>nd</sup> lowest prevalence in Manisa Province. None of the other patients were diagnosed with WNV infection, including eight patients with viral encephalitis, arthropod-borne viral encephalitis and mosquito-borne viral encephalitis.

Following the reports of WNV infected cases, public health authorities of Manisa and Izmir Provinces conducted a search for *Culex* mosquito breeding habitats and reported negative results; nevertheless insecticidal spraying was

carried out in both provinces (unpublished). In addition, the Ministry of Food, Agriculture and Livestock and the Veterinary Central Research Institute of Ankara, which conduct annual routine screening of race horses and horse ranches for WNV, reported absence of the virus in horses and birds in 2016 (Turkish Ministry of Food, Agriculture and Livestock, 2016).

## DISCUSSION

In 2016, 214 cases of West Nile fever were reported in Austria, Bulgaria, Cyprus, Egypt, Hungary, Israel, Italy, Romania, Russia, Serbia, Spain, Syria, Tunisia, and Ukraine (ECDC, 2017c), countries

Table 1  
Distribution of meningitis/meningoencephalitis cases in Manisa Province, Turkey, January-October, 2016.

District	Number (%) of meningitis/ meningoencephalitis cases, <i>n</i> =135	Prevalence (per 100,000 population)
Ahmetli	2 (1)	12.2
Akhisar	9 (7)	5.5
Alasehir	7 (5)	7.0
Demirci	2 (1)	4.7
Gordes	5 (4)	17.2
Kirkagac	5 (4)	11.6
Koprubasi	3 (2)	22.1
Kula	3 (2)	6.6
Salihli	16 (12)	10.1
Sarigol	4 (3)	11.1
Saruhanli	5 (4)	9.3
Selendi	1 (1)	4.8
Soma	8 (6)	7.5
Sehzadeler	19 (14)	11.3
Turgutlu	14 (10)	9.1
Yunusemre	32 (24)	15.1
Total	135 (100)	9.8

which are migration routes of birds to Turkey. Most epidemics occur in August and September when both bird migrations and mosquito numbers are high (Conte *et al*, 2015).

WNV disease has been reported in Turkey since 1970 (Meço, 1977) and the first positivity with neutralization test was demonstrated in 1980 (Serter, 1980). The first WNV infection epidemic in the country occurred in 2010. Cases reported were sporadic (Turkish Ministry of Health,

2016), and studies on WNV patients in Turkey showed different seropositivity depending on the method used (Table 2). In a large study of 6,457 patients, seropositivity rate was found to be 16% using ELISA (Ozer *et al*, 2007).

Although viral RNA detection is the gold standard in WNV infection, viral RNA may be low or absent at the beginning of the disease in symptomatic patients (Busch *et al*, 2008) accounting for negative RT-PCR results in CSF of

Table 2  
Serological studies on West Nile virus infection in humans, Turkey, 1964-2015.

Reference	Study area	Number of serum samples	Percent positivity	Study method
Heperkan and Ari (1964)	Adana, Diyarbakır, Erzurum, and İzmir	559	1-57	HI
Serter <i>et al</i> (1968)	İzmir	20	21	PRNT
Serter <i>et al</i> (1980)	West Anatolia	1,074	8	HI
Meço (1977)	Southeast Anatolia	937	>40	HI
Ozkul <i>et al</i> (2006)	Ankara	48	14	PRNT
Ozkul <i>et al</i> (2006)	Antalya	40	4	PRNT
Ergunay <i>et al</i> (2017)	Southeast Anatolia	181	16; 9.5	IIFT; PRNT
Ergunay <i>et al</i> (2011)	Middle Anatolia	2,516	9.2	ELISA
Ergunay <i>et al</i> (2011)	Ankara	87 (CSF)	3.4	ELISA
Ozer <i>et al</i> (2007)	Sanliurfa	6,457	16	ELISA
Ayturan <i>et al</i> (2011)	Ankara	1,200	0.8	PRNT
Hızıl <i>et al</i> (2010)	Central Anatolia	2,821	0; 0	ELISA; PCR
Karakoç <i>et al</i> (2013)	Mardin	307	17	MNTA
Erdem <i>et al</i> (2014)	Northwest Anatolia	296	1.7	ELISA
Tezcan <i>et al</i> (2014)	Mersin	920	14.9	PRNT
Ergunay (2014)	Mersin	266	12.1; 0	PRNT; PCR
Biçeroglu <i>et al</i> (2015)	West Anatolia	438	2.5; 0	ELISA; PCR
Bakir <i>et al</i> (2015)	Edirne, İstanbul, Kocaeli, and Sakarya	226	0.9; 0	ELISA; PCR

ELISA, enzyme-linked immunosorbent assay; HI, hemagglutination inhibition test; IIFT, indirect immunofluorescence test; MNTA, micro neutralization assay; PRNT, plaque reduction neutralization test.

the patients in our study. According to case definitions by ECDC (2017a), an immunofluorescence test should be verified by a neutralization test. In the two cases, immunofluorescence positivity was confirmed with PRNT. At the same time, this verification test was conducted to exclude cross-reaction with other flaviviruses, a phenomenon due to antibody response against E protein present in all flaviviruses as the major epitope at DII end of the E protein of West Nile virus is a conserved region among flaviviruses (Sánchez *et al*, 2005; Sambri *et al*, 2013).

Therefore, control measures against vectors constitute the most important strategy in prevention of WNV infections and epidemics (ECDC, 2017b). In addition, surveillance of WNV cases, implementation of personal protective measures such as using mosquito repellents, updated knowledge of mosquito breeding sites and their elimination, and monitor of WNV infection in horses and birds, all must be utilized. WNV should be considered as an etiology in neuroinvasive diseases, *viz* acute central or peripheral neurological dysfunction, acute flaccid paralysis, encephalitis, meningitis, and myelitis, especially in elderly and immune-compromised patients.

WNV disease is a prime example of the need for a “One Health, One World” perspective, requiring collaboration among veterinary and health authorities, entomologists, environmental specialist and biologists, so as to provide the best strategy towards control and prevention of WNV infection in humans and animals alike.

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#### CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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