

# A Serological Investigation of West Nile Virus Infections in Various Animal Species and Humans in Western Turkey

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## ABSTRACT

West Nile Virus (WNV) infection, transmitted mainly through mosquito bites, is generally asymptomatic but may also result in mild fever, meningitis, encephalitis and death in various animals and humans. The presence of the WNV in Turkey has been reported previously. However, comparative epizootiological data on the recent prevalence of the WNV infection in various animal species and humans in western Turkey are limited. The objective of this study was to investigate the seroprevalence of WNV infection in animals and humans in western Turkey. Four hundred and forty serum samples were collected from 40 cats, 60 cattle, 90 humans and 50 sheep, goats, camels, horses, and broiler breeder chickens between the years 2009 and 2012. The serum samples were tested for antibodies against the envelope protein (E) of the virus using a commercial competitive Enzyme-Linked Immunosorbent Assay (cELISA). The human samples were re-tested using commercial Indirect Fluorescence Antibody Test (IFAT). No antibodies were detected in cats, sheep, goats, and chickens. Seropositivity rates of 44% (22/50) in camels, 32% (16/50) in horses, 20% (12/60) in cattle and 41.1% (37/90) in humans were found. The results of the seropositivity in humans obtained using cELISA and IFAT were identical. These data suggest that the WNV infection may be present subclinically in horses, camels, cattle and humans in western Turkey and may pose a threat to animal and human health in the region and surrounding areas.

**Keywords:** West Nile Virus; Antibody; ELISA; IFAT; Western Turkey; Camel; Cattle; Horse; Human.

## INTRODUCTION

West Nile virus infection (WNV) is a mosquito-borne zoonotic disease. It is classified within the family of *Flaviviridae*, genus *Flavivirus*, positive sense, single-stranded RNA viruses (1,2). Serologically, it is classified within the Japanese Encephalitis (JE) virus antigenic complex group of viruses that also includes Japanese encephalitis, Murray Valley encephalitis, St. Louis encephalitis, Usutu and Kunjin viruses

(1). Transmission of the WNV is mainly through *Culex spp.* of mosquitoes which serve as the “bridge” between birds and humans (3). Mosquitoes infect many bird species and infected birds may in turn infect mosquitoes thus serving as intensifying hosts that eventually infect humans and other mammalian species. The virus replicates in birds that serve as the natural reservoirs and may, in some cases may kill the infected bird (1).

In addition to mosquitoes, blood transfusion, organ transplantation, and intrauterine transmission are also important in WNV transmission in humans (4). Interestingly, the virus was not isolated in any of the 6,457 mosquito specimens collected in Turkey (5). Animal trading and wild life movements may also contribute to the spread of the virus (6).

Although many mammalian species can be infected experimentally, confirmed natural WNV cases have been reported only in humans and horses (1). The infection presents with symptoms ranging from mild fever to meningoencephalitis in 20-40% of humans whereas it is asymptomatic in the remaining 60-80% (7). The mortality rate is approximately 10% in humans whereas it may be as high as 43% in horses (1).

The infection has been endemic in sub-Saharan Africa where almost 90% of the adults and 50% of the children are seropositive (6). The infection has been reported in Europe and Australia as well and has spread to North America in 1999 (8). Turkey is located near European countries where the recent WNV cases in humans and animals have been reported (9-11). The climate and ecological conditions, mosquito fauna, and its location on the routes of migratory birds make it susceptible to the WNV infections. WNV infection has been reported in Greece, the neighbor of Turkey in the west as recently as 2012 (9).

Seropositivity for the virus has been reported in Turkey for over 30 years especially in humans (3,12-14). In August 2010, a WNV outbreak in the Manisa province of Western Turkey and its surrounding areas resulted in meningitis-related deaths (15). However, comparative studies investigating recent seroprevalence of WNV infection in western Turkey in humans and animals are very limited. The objective of this study was to determine the seroprevalence of the WNV infection in humans and various animal species in the Aegean region of western Turkey bordering the Aegean Sea and Eastern Europe.

## MATERIALS AND METHODS

### Samples

Antibodies against WNV were investigated in 440 blood serum samples collected randomly from 40 cats, 60 cattle, 90 humans and 50 sheep, goats, camels, horses, and broiler breeder chickens in the Aydin, Denizli, Izmir and Manisa provinces of western Turkey (Table 1). The samples were collected between 2009 and 2012 and archived for serosurveys

for different diseases. The serum samples were obtained by centrifugation of the blood for 30 minutes at room temperature and stored at -20°C until analyzed. No clinical signs of the infection were observed in any of the sampled animals or humans. Informed and free consent was obtained from the participants or legal guardians of the minors involved. This study was approved by the Animal Ethics and Medical Ethics Committees of the Adnan Menderes University (Approval No: B.30.2.ADU.0.00.00.00/050.04/2012/029 and 2013/311, respectively).

### Enzyme-Linked Immunosorbent Assay (ELISA)

The serum samples were tested for antibodies against the viral membrane protein (E) using a commercially available competitive Enzyme-Linked Immunosorbent Assay (cELISA) (ID Screen West Nile Competition Multi-Species from IDVET Innovative Diagnostics, Montpellier, France). Samples and controls were added into the wells of the ELISA plates coated with purified extracts of the WNV antigen. After incubation and washing of the microplates, peroxidase-conjugated anti-WNV antibody was added into each well. After incubation and washing to eliminate the excess conjugate, the substrate

**Table 1:** Number of samples analyzed and the seropositivity rates obtained using antibodies against West Nile virus in animals and humans in the Aydin, Denizli, Izmir and Manisa provinces of western Turkey.

Species	Status	Province				Total	Positivity
		Aydin	Denizli	Izmir	Manisa		
Cat	Tested	20	-	20	-	40	0%
	Positive	0	-	0	-	0	
Sheep	Tested	25	-	25	-	50	0%
	Positive	0	-	0	-	0	
Goat	Tested	25	25	-	-	50	0%
	Positive	0	0	-	-	0	
Camel	Tested	50	-	-	-	50	44%
	Positive	22	-	-	-	22	
Horse	Tested	-	-	50	-	50	32%
	Positive	-	-	16	-	16	
Chicken	Tested	-	-	-	50	50	0%
	Positive	-	-	-	0	0	
Cattle	Tested	30	30	-	-	60	20%
	Positive	12	0	-	-	12	
Human <sup>a</sup>	Tested	90	-	-	-	90	41.1%
	Positive	37	-	-	-	37	

<sup>a</sup>Samples taken from humans were re-tested using an Indirect Fluorescence Antibody Test (IFAT) and identical results were obtained.

solution TMB (3,3',5,5'-tetramethylbenzidine) was added to the wells. The optical density (OD) was measured at 450 nm using an ELISA reader (BioTek Absorbance Microplate Reader, ELx808, USA). Optical density for each sample was divided by the OD for the negative control and the result was multiplied by 100 to obtain the S/N percentages. S/N percentages less than or equal to 40% were considered positive and greater than 50% were considered negative. The S/N percentages greater than 40% but less or equal to 50% were considered equivocal. All equivocal samples were repeated and valid results were obtained.

### Indirect Fluorescent Antibody Test

The serum samples from humans diagnosed using ELISA were re-tested using a commercially available Indirect Fluorescent Antibody Test (IFAT) (Euroimmun, Lübeck, Germany). The test was designed exclusively for the *in vitro* detection of IgG and IgM classes of antibodies against WNV in human serum and plasma. WNV-infected cells on the test slides were incubated with human serum samples diluted 1:100. After incubation and washing, a secondary rabbit anti-human antibody was added on the slides. The fluorescence was detected using a fluorescence microscope (Olympus, Japan).

## RESULTS

The results obtained using cELISA are shown in Table 1. No antibodies were found in cats, sheep, goats and chickens. Seropositivity rates of 44% (22/50) in camels, 32% (16/50) in horses, 20% (12/60) in cattle, and 41.1% (37/90) in humans were detected. Twelve of the 30 (40%) cattle in the Aydin province were seropositive whereas there were none among the cattle in the nearby Denizli (0/30) province. The 37 positive and 53 negative human samples initially diagnosed using cELISA were re-tested using IFAT and identical results were obtained.

## DISCUSSION

Our results show the presence of antibodies against WNV in camels, horses, cattle and humans in western Turkey. The reason for this relatively high seroprevalence among animals and humans in western Turkey could be due to Turkey's geographical location on the routes of migratory birds that play an important role in the epidemiology of the virus. The birds may carry the virus from endemic areas to regions of sporadic outbreaks (16).

This study is the first evidence of WNV infection in camels in Turkey. The 44% seropositivity detected in this study is higher than 3% reported in Canary Islands, Spain (17). The Aydin province where the seropositive camels were located is one of the largest centers of camel breeding in Turkey. The 41.1% seropositivity rate detected in humans in this study is similar to the 40% seropositivity rate obtained by Olaleye *et al.* (18) in Nigeria. However, the seropositivity rates in our study were lower in sheep (0% vs. 20%) and goats (0% vs. 18%) but higher in camels (44% vs. 26%) and cattle (20% vs. 6%) compared to the rates reported by Olaleye *et al.* (18). These findings do not agree with a study by Albayrak *et al.* (19) who reported that only two of the 350 serum samples taken from 70 of each of cattle, horse, sheep, goat and water buffalo in northern Turkey were positive using cELISA. These results suggest that a large variation may exist in seropositivity rates among different regions of Turkey.

Data on the seroprevalence rates in cats, sheep, goats and cattle are available in more than one province in Turkey. Interestingly, 12 of the 30 (40%) cattle in the Aydin but none of the cattle in the nearby Denizli (0/30) province were seropositive. The seropositivity rates in the remaining species of the animals were same in the remaining provinces.

In this study, 32% seropositivity was detected in horses that are considered the "dead-end host" for the virus because they do not infect other animals or mosquitoes (1). Similar seroprevalence rates have been reported in horses in Central Anatolia by Ozkul *et al.* (20) who reported 31.6% seropositivity in 2010. The authors (Ozkul *et al.*) detected nucleic acid of the virus in two of these horses using RT-PCR. In northern Turkey, however, WNV nucleic acid was not detected in any of the 120 horse serum samples collected from the middle Black Sea region of Turkey (21).

In the present study, 41.1% of humans were seropositive for WNV antibodies. This result suggests that WNV infection in humans may be more common in the west than the other regions of Turkey. A reason for the high seroprevalence in our study could be that humans travelling to the different parts of the world and/or Turkey could be bringing infection to the Western Turkey which is fashionable among both indigenous and foreign tourists. Recent seroprevalence of WNV in southeast Turkey is only 16% (3). In central Anatolia, Ozkul *et al.* (14) found antibodies against WNV in 20.4% (18/88) of the human samples collected from 10 representative provinces of Turkey.

In 2010, 47 human cases of West Nile virus (WNV) infection were reported in several regions of Turkey (15). The infection was most frequent in the Sakarya province (0.19/100,000 population) located in the northwest of Turkey where 10 patients died. Deaths mostly occurred in the month of August although they were reported throughout the summer of 2010. Deaths due to WNV were also reported in Greece and Romania in the same year, suggesting that the infection may have spread to the nearby countries. As of 2012, 161 cases of WNV infection in Greece and 621 in neighboring countries have been reported (9).

No antibodies against WNV were detected in the cats, sheep, goats and chickens included in our study. The likely cause of the absence of WNV infections in chickens is the good practice of hygiene in the poultry farms in western Turkey that appears to successfully limit the transmission of infection with biological vectors.

The possible cross-reactivity of the WNV with the viruses in the JE virus serocomplex and other flaviviruses should be taken into consideration when interpreting the results obtained in this study. Serological cross-reactions between members of the JE group of flaviviruses and WNV have been reported previously (22). Although the cELISA kit we used in this study recognizes the antibodies against envelope protein (pE) containing an epitope common to JE virus antigen complex, we are not aware of any cases of JE group infections reported in Turkey.

A limitation of this study was the use of cELISA for detection of the WNV. Although the plaque reduction neutralisation test (PRNT) is considered the “gold standard” for the detection of WNV antibodies (23), ELISA has also been routinely used for diagnosis (24). The cELISA has a specificity of 99.4% and a sensitivity of 84.9% for detecting WNV antibodies (25). The ELISAs is considered to be suitable for serological WNV surveillance programs and valid for a wide range of species (26). In the present study, results from samples taken from humans were confirmed using a commercial IFAT test developed for the routine detection of human IgG and IgM antibodies.

## CONCLUSIONS

Data obtained in this study suggest that the WNV antibodies are present in camels, horses, cattle, and humans in western Turkey and, thus, the virus may pose a threat to animal and human health in the region. The sampled animals and hu-

mans did not show any signs of infection suggesting that the infection may have been subclinical. It is, thus, necessary to conduct further research on isolation, molecular characterization, and epidemiology of the virus in this region. It is especially important to determine which species of the mosquitoes spread the disease, map their breeding sites, prevent production of the larvae through water reduction and chemical control, and educate the public to combat the WNV infection.

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