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SHORT COMMUNICATION

Serological Signs of West Nile Virus Infection in Horse Serum Samples Collected for Equine Infectious Anemia Virus screening in the Northeastern Turkey: Traces of Past

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A B S T R A C T

West Nile virus (WNV) is an important arthropod-borne pathogen and a leading cause of morbidity and mortality in human and animal populations in many parts of the world. The presence of WNV in Turkey has been reported in various studies during the last 15 years. In this study, 1500 horse serum samples collected from Ardahan Province in Northeastern Turkey were screened by using a virus neutralization test against WNV and then positives were confirmed by a plaque reduction test-90 ($PRNT_{90}$). The overall seropositivity was 0.13% (2/1500), with neutralizing antibody titers determined to be 1/40 and 1/80. These findings suggest that some of horses in Ardahan had been exposed to WNV and also indicates the evidence of WNV circulation in the Northeastern Turkey.

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INTRODUCTION

West Nile virus (WNV) is an important arthropod-borne pathogen transmitted by mosquitoes and can result in serious illness in both human and animal populations. The virus is a member of the Japanese encephalitis virus (JEV) antigenic serocomplex and is a member of the genus *flavirus* within the family *Flaviviridae* (McVey *et al*., 2015). WNV has become endemic in many countries of Europe, America, Africa, the Middle East and Asia following the first isolation of virus in Uganda in 1937 (David and Abraham, 2016).

Mosquitoes belonging to the genus *Culex* within the family *Culicodiea* are the primary vectors of WNV. The natural cycle of WNV is mosquito-bird-mosquito (Lafri *et al*., 2017). Birds are considered to be the primary reservoir host of WNV, with high levels of viremia all owing transmission to *Culex* mosquitoes. Both humans and horses represent dead-end hosts, which develop low-level viremia that precluding onward infection of feeding mosquitoes (Lafri *et al*., 2017).

Horses are more susceptible to WNV as compared to the other animal species. Fatal meningoencephalitis can occur in horses infected with WNV (Lafri *et al*., 2017). Historically, many WNV outbreaks in horses have previously been reported in numerous parts of the world e.g., Morocco, Israel, Northern Italy, France, USA, Canada and Tunisia (Di Sabatino *et al*., 2014), with several countries, such as Algeria, South France and Corsica, reporting outbreaks more recently (Di Sabatino *et al*., 2014). In Turkey, serological and virological evidence of WNV infection in humans and domestic animals has previously been reported (Albayrak and Ozan, 2013; Toplu *et al*., 2015). In light of the historical presence of WNV, the virus continues to represent a public health importance due to its epizootic and zoonotic potential (David and Abraham, 2016).

The aim of this study was to determine the presence of neutralizing antibodies to WNV in horse sera collected from Northeastern Turkey due to the proximity to countries where human cases have been reported e.g., Armenia, Georgia and Russia.

MATERIALS AND METHODS

This study was conducted in Ardahan province, located in the northeastern part of Turkey, using archived horse serum samples collected in 2009 as part of equine infectious anemia virus (EIAV) surveillance program with the approval of Turkish Ministry of Food, Agriculture and Livestock (Approved date/ no:27.12.2017/3336566). A total of 1500 horse serum samples were randomly selected

among archived sera. All horses were non-vaccinated against WNV and were EIAV seronegative. Serum samples were kept at -20°C until used and were inactivated at 56°C for 30 min prior to performing serological tests.

The Vero-E6 cell line was used in this study. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco, UK) supplemented with 10% fetal bovine serum (FBS) (Sigma, UK) and 1% antibiotic (Sigma, UK). The NY99 strain of WNV was kindly provided by Aykut OZKUL from Faculty of Veterinary Medicine Ankara University. Virus was propagated on Vero-E6 cells with DMEM supplemented with 3% FBS.

The WNV titre was established in Vero-E6 using the conventional $TCID_{50}$ method and were calculated as $\log_{10}$ $TCID_{50}$/ml. The presence of neutralizing antibodies against the WNV-NY99 strain was investigated by using a conventional virus neutralization test (VNT). Positive serum samples were confirmed with plaque reduction neutralization test (PRNT) using the NY99 strain of WNV as described before [15]. A four-fold greater reciprocal 90% neutralization titer is required for a sample to be considered positive for WNV infection due to the fact that high levels of cross-reaction are observed among *flaviviruses.*

RESULTS AND DISCUSSION

Out of the 1500 serum samples tested, 2 (0.13%) were found to be seropositive for WNV as assessed using VNT. These serum samples were also confirmed using $PRNT_{90}$ as being WNV seropositive. The positive serum samples had 1/40 and 1/80 mean neutralizing antibody titers.

WNV is an important arthropod-borne pathogen that is considered to be a public health concern. Like humans, horses can become infected with WNV and neurological disorders including fetal meningoencephalitis in severe infections can develop in 8% of exposed horses (Lafri *et al*., 2017). However, WNV infection is subclinical in the vast majority of horses following exposure to WNV. Transmission is directly related to environmental conditions; so, there are many factors that strongly affect WNV infection, including humidity, wetlands, an abundance of avifauna and mosquitoes etc (Lafri *et al.*, 2017).

In Turkey, Ozkul *et al.* (2006) first serologically reported WNV in horse serum samples collected from the Marmara and Mediterranean Regions with a rate of 13.5%. In subsequent years, seropositivity rates of WNV in horses ranged from 4.1% to 31.6% (Ozkul *et al.*, 2013; Kale *et al*., 2017). Despite the previous studies indicating WNV circulation in Turkey, there was no previously reported WNV seropositivity in human and horses as well as any notification concerning the WNV identification from mosquitoes, human and horses in Northeast Turkey (Yazici *et al*., 2012; Albayrak and Ozan, 2013). The only report of WNV in this region of Turkey was reported in goats, with a seroprevalence of 2.85% (Albayrak and Ozan, 2013). Furthermore, there have been no reports of WNV in human, horse or mosquito populations from Ardahan. Georgia, Armenia and Russia, which are considered to be endemic for WNV and border the north-east Turkey and the current study is the first report concerning the seroprevalence of WNV in horses in the Northeast Turkey. After screening 1500 horse sera we observed a seropositivity rate of 0.13%. This rate could be considered remarkably low, both compared to previous WNV studies in Turkey, and given the abundant breeding habitats for mosquitoes in the Ardahan region. However, this result may have been affected by some factors including the use of archived sera and difference in climate. For example, these serum samples had been collected and stored for an extended period of time. In this context, it cannot be ruled out that the antibody titer in some samples may have dropped depending as a result of any freezing and thawing processes. Furthermore, the climatic condition of a region, such as amount of rainfall, wind status and insecticide applications are particularly important factors in vector biology. In Ardahan Province, yearly averages of temperature, humidity and rain are reported as 3.8ºC, 72.6%, 543mm, respectively. When compared, these values are lower than the Western Anatolia region of Turkey where the disease is seen frequently, reportedly 10.3ºC, 63%, 2, 950 mm respectively. These data are important factors affecting vector abundance. In addition, the existence of *Culicoides* spp was far less in the Northeast Turkey than in the South and West parts of Turkey.

Despite the low seroprevalence, the results reported here indicate that WNV was in circulation in the Northeastern regions of Turkey. Moreover, it could get a warning for WNV infection for human beings living Ardahan and its neighbor areas due to the fact that horses might be one of the sentinel animals for WNV.

Conclusions: The determination of neutralizing antibodies in the horse, human, chicken, and dogs may be thought to have pointed toward a sustained arboviral infection. In this context, the results of this study should be taken into consideration even though the seroprevalence rate is lower than previous reports considering the fact that WNV may have impacts on public health together with its zoonotic potential. It is also recommended that the new serological screening on humans and animals with more recent and /or actual samples should be planned in order to realize dynamic of the virus in the region.

Authors contribution: This study was conducted by ZY, CT and SG, EO helped in sample collection analysis. All authors have approved the final version of the manuscript.

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