RESEARCH ARTICLE

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Serological Investigation of West Nile Virus Infection in Feral Horses in Konya

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ABSTRACT: West Nile virus (WNV) is an Arboviral infection with encephalitis in humans, horses, birds, and various wild animals. It is a zoonotic cause of disease within the family Flaviviridae. In this study, it was aimed to investigate the West Nile virus infection serologically (serum neutralization) in feral horses in Konya. For this purpose, randomized sampling of blood serum samples from 36 horses from a farm with 250 feral horses in Konya was used. Samples were evaluated for the presence of antibodies by subjecting them to a microneutralization test. 7 of the samples (19.4%) were found to be seropositive and 29 were seronegative. These results obtained have shown the serological presence of WNV for the first time in feral horses in our country. Also, these data showed that the continued presence of WNV infection in Turkey.

Keywords: West Nil virus, Neutralization Test, Feral Horse

INTRODUCTION

West Nile virus (WNV) is a flavivirus transmitted by mosquitoes to horses, humans, and birds. WNV was first detected in Uganda in 1937. The virus is specific to Africa, Asia, Europe, and Australia and has recently caused major outbreaks in Romania, Russia, and Israel. Birds are natural reservoir (enhancer) hosts, and the WNV is kept in a mosquito-bird-mosquito transition cycle in nature mainly involving Culex sp (Chancey et al., 2015).

West Nile virion is an enveloped, single-stranded, positive-polar RNA virus. It shows icosahedral symmetry and is coated with a capsid protein of approximately 12 kDa, main envelope protein E (53 kDa), and membrane protein M (8 kDa) (Castillo Olivares and Wood, 2004). WNV is not resistant to environmental conditions. It is inactivated in a short time by heat, lipid solvents, or disinfectants (Diamond et al., 2003; Sampatlıkumar, 2003).

The incubation period in naturally infected horses is 1 to 6 days. Clinical symptoms may not be evident in many horses bitten by a WNV-infected mosquito. Clinical findings observed common are fever, ataxia, tremor, muscle weakness, teeth grinding, twitching, and blindness (Trock et al., 2001; Murgue et al., 2001; Ostlund et al., 2001; Blitvich et al., 2003). Encephalitis manifestation is frequently observed in horses and neurological symptoms remain in some animals that can recover (Ostlund et al., 2001).

The temperature dependence of both mosquito reproduction and viral replication reveals that seasons are important in the emergence and transmission of the disease. Most cases of encephalitis in the northern states of Europe, Canada, and America are observed in late summer or autumn when the number of insects is intense and temperatures are high (Castillo Olivares and Wood, 2004).

The factor reproduces in cell cultures by creating a cytopathic effect (CPE). For this purpose, primer cell cultures and continuous cell lines such as Vero and BHK-21 can be used. Tissue homogenates and cerebrospinal fluid (CSF) prepared from the brain, spinal cord samples can be used for virus isolation (Garmendia et al., 2000; Autorino et al., 2002; Wilson et al., 2017). Tests such as Polymerase Chain Reaction (PCR) (Briese et al. 2000), Immunofluorescence Test (Malan et al., 2003) and Immunohistochemistry (Toplu et al., 2015) are used for direct diagnosis. Detection of IgM and IgG class antibodies in serum samples, especially by ELISA technique, serum neutralization tests, and complement fixation test can be used serologically (Dupius et al., 2003; Turan et al., 2017). The biggest challenge in the serological diagnosis of flaviviruses is that they show 70% or higher antigenic affinity and cross-react with each other (Mansfield et al., 2011).

Turkey, located in the northeastern part of the Mediterranean region, is considered to be a potentially endemic region for viral infections from many arthropods, including WNV, due to favorable ecological and climatic conditions (Ergunay et al., 2011). Many studies are showing the existence and activity of WNV in Turkey. Serological surveillance data revealed that humans and animals were exposed to WNV in some cities and both sporadic cases and outbreaks were observed since 2009 (Kalaycioglu et al., 2012; Erdem et al., 2014).

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In this study, it was aimed to obtain data for the first time on the seroepidemiology of the West Nile Virus, which is economically important due to the underperformance and losses it may cause in horse breeding, in feral horses in the Konya region.

MATERIALS AND METHOD

Serum samples were taken from feral horses of different gender and age brought from Karadağ (Karaman province of Turkey) to Çumra (Konya province). Serum samples of 36 horses were collected by randomized sampling from a herd of 250 feral horses. After the collected blood samples were centrifuged, the serum portion was separated and stored at -20°C until analysis. The WNV (100DKIDso = ıo$^{-4.5}$/0.05 mL) used in the study was obtained from Selcuk University Faculty of Veterinary Medicine Virology Department. VERO (African Green Monkey Kidney) cell line continuous cell culture was used for the production and neutralization test of WNV.

Microneutralization Test

The presence of neutralizing antibodies in horse serums against WNV was investigated by the microneutralization test in the study. The test was conducted according to the method reported by Frey and Liess (1971). Sera to be controlled was diluted in the ratio of 1/5. 50 µL of each serum were put into 4 wells in a microplate. After this sera, 50 µL of virus with known titre ($100DKID_{50} = 10^{-4.5}$ / 0.1 mL) was added. It was left for incubation for 1 hours in a 37°C CO_2 incubator. At the end of the period, 50 µl VERO cells (with 2% FBS) $3x10^5$ were added to the wells. It was left in a 37°C CO_2 incubator. WNV was evaluated at the end of the 5th day with daily controls performed in a tissue culture microscope, and the resulting cytopathological changes were evaluated according to cell control and virus controls (Figure 1 and Figure 2).

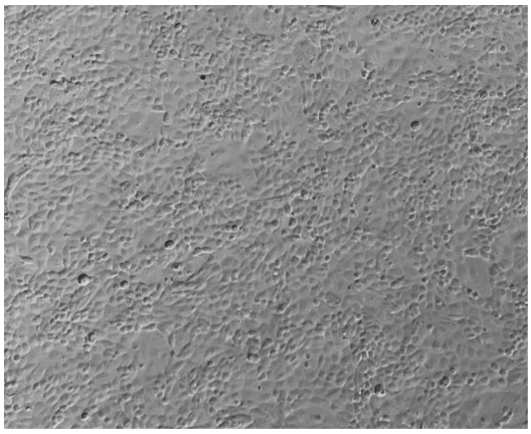

Figure 1. *VERO Cell Control (100)*

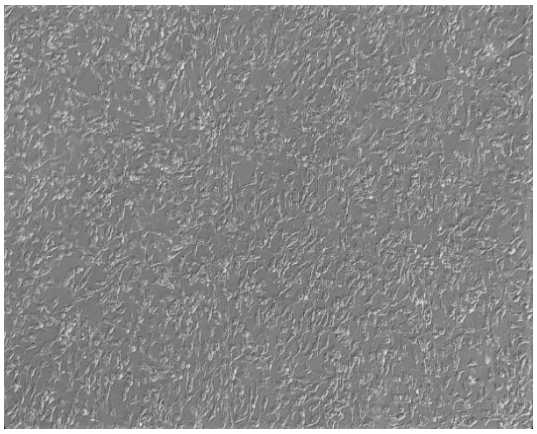

Figure 2. *West Nile Virus (WNV) Virus Control (40)*

RESULTS

As a result of the neutralization test applied to serum samples taken from 36 feral horses, 7 (19.4%) antibodies against serum WNV were found positive (Table 1).

Table 1. *WNV/Ab results of the samples used in the study*

Number	WNV Ab Microneutralization Test Result	Number	WNV Ab Microneutralization Test Result	Number	WNV Ab Microneutralization Test Result
1	-	**13**	-	**25**	-
2	+	**14**	-	**26**	-
3	-	**15**	-	**27**	-
4	+	**16**	-	**28**	-

Table 1. cont.

Number	WNV Ab Microneutralization Test Result	Number	WNV Ab Microneutralization Test Result	Number	WNV Ab Microneutralization Test Result
5	-	**17**	-	**29**	-
6	-	**18**	-	**30**	-
7	-	**19**	-	**31**	-
8	+	**20**	-	**32**	-
9	-	**21**	+	**33**	+
10	-	**22**	-	**34**	+
11	-	**23**	-	**35**	+
12	-	**24**	-	**36**	-

DISCUSSION AND CONCLUSION

Horse encephalitis cases caused by the West Nile Virus were first reported in Egypt and France in the early 1960s (Chambers and Monath, 2003). Later, at various times, it has been reported to cause epidemics in horses and other mammals in Asia, Africa, the Middle East, the Balkans, the east, and south of Europe (Campbell et al., 2002). In our country, it has emerged as an important infection, especially in horse breeding enterprises in the last 15 years. It is observed that the infection increases with the warming of the weather, especially in the spring and summer seasons, and it spreads in the stalls of the migratory bird populations and in regions where the rate of wetlands is high (Duyum and Karaoğlu, 2019).

ELISA test may not be reliable because West Nile Virus cross-reacts with other members of the Flavivirus genus. For this reason, it is recommended to perform the serum neutralization test (SN) in which neutralizing antibodies specific to WNV are detected and the plaque reduction neutralization test (PRNT) for serological definitive diagnosis (Ostlund et al., 2001). Gennaro et al. reported in their study in 2014 that they examined a large number of serum samples to examine the feasibility of the SN test as an alternative to the PRNT test, and that there was no statistically significant difference between the two methods. They suggested that the SN test be used in the routine diagnosis of WNV because it is faster and requires less effort.

In the study conducted by Ozkul et al. (2006), they examined blood serum of different species with PRN T and found WNV neutralizing antibodies in 1 of 40 mule samples (2.5%), 4 (4%) of 100 cattle samples, 43 (13.5%) of 114 dog samples, 35 (%) of 259 horse samples. 13.5), 18 of 88 human samples (20.4%), and 1 (1%) of 100 sheep samples. They interpreted the results they obtained that a wide range of mammals was exposed to the virus in Turkey. In the study of Ergunay et al. (2014), they detected seropositivity with PRNA in 42 of 423 duck blood serum (9.9%), 48 of 389 horse blood serum (12.3%) and 2 of 102 sheep blood serum (1.9%), respectively, from Kars, Van and Şanlıurfa and Mersin provinces. In the study conducted by Ergunay et al. (2015), they have studied the full-genome nucleotide sequence and amino acid sequence of the T2 WNV strains isolated from Turkey. They revealed that this strain has a genetic similarity with the ArB310/67 strain isolated from Africa in 1967. They revealed that these two viruses form a separate genetic cluster within the WNV lineage 1a. They stated that the strains circulating in Turkey are genetically different from the strains in Eastern Europe, the Mediterranean region, and the Middle East. In the study conducted by Kale et al. (2017), they collected blood samples from 650 domestic horses that seem to be in good health and 234 domestic donkeys from different provinces of Turkey and examined them with the West Nile Competition-IgG/IgM-ELISA kit. As a result, they detected seropositivity in 27 horses (4.15%) and 3 donkeys (1.28). In the study of Yıldırım et al. (2018), conducted on serum samples collected from 118 horses, 70 donkeys, and 378 geese in the Northeast Anatolia region of Turkey, when they examined the presence of antibodies against WNV by ELISA test, they detected 0.8% (1/118) of horses, 20% (14/70) of donkeys and 1.1% (4/378) of geese as seropositive. To determine the presence of nucleic acid, they examined the blood samples of all seropositive animals with the RT-PCR technique and reported that while they did not find any positive in horse and goose samples, they found positive in 4 donkeys. In the study of Duyum and Karaoğlu (2019), they evaluated blood serum samples taken from 277 breeding horses in the Şanlıurfa province of Turkey for the presence of antibodies by subjecting them to ELISA test in terms of WNV Ab. 42 of the samples (15.16%) were considered as seropositive, 16 (5.77%) as suspicious, and 219 (79.06%) as seronegative. They exposed existing seropositive and suspicious samples to PRNT and found that 11 of the 42 seropositive samples (26.19%) carried the specific WNV antibody. They did not find PRNT seropositivity in any of the suspicious samples. They found specific WNV antibodies in 11 (3.97%) of 277 breeding horses sampled. They stated that they

did not detect viral RNA by Real-Time Reverse Transcriptase-Polymerase Chain Reaction (rtRT-PCR) in any of the 42 samples detected as seropositive by WNV Ab ELISA test and 16 suspicious samples.

In this study, it was aimed to serologically investigate WNV infection in feral horses residing in the Konya region and seem to be in good health. For this purpose, 36 feral horse blood serum samples were used. The samples mentioned were subjected to a microneutralization test against WNV, and antibodies were found in 7 (19.44%) of the blood serum samples of 36 horses tested. When the results obtained were compared with other studies conducted in Turkey, the seropositivity rates obtained from horses were determined to be similar. This condition revealed that WNV infection has continued to exist in different regions of Turkey in the last 15 years.

As a result, it is a known fact that the viremia period lasts quite short in equines exposed to infection (Bunning et al., 2002). Hence, this result was considered normal due to the absence of a defined long-term viremia like the virus. The fact that our country is among the important migration routes of birds is important in terms of the spread of infection. Also, the fact that our country is at a very advanced point in terms of horse breeding makes WNV infection economically important due to the low performance and losses that the infection may cause in horse breeding. In this sense, larger field studies are needed to determine the circulation of the virus in our country. Besides, considering that WNV, which is in zoonotic character, may cause possible human cases, it should be kept in mind that especially the mosquito populations that play a role in the transmission of the disease can survive longer, and the infection always poses a greater risk in seasonally warmer regions.

ETHICAL CLEARANCE

All procedures and animal care complied with the guidelines of the Selcuk University Veterinary Faculty Ethics Committee (Ethical approval number 2020/111 on 01/12/2020).

CONFLICT OF INTEREST

No conflict of interest was declared by the authors

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