Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2022 19(3): 1346-1350. **OPEN ACCESS**

Seroprevalence of equine herpes virus-1, 4 (EHV-1, 4) and west nile virus (WNV) the equid in Turkey

Metin Gürçay *

Bingol University, Veterinary Faculty, Department of Virology, 12100, Bingöl, **Türkiye**.

***Correspondence: drmgurcay@gmail.com** **Received 30-05-2022, Revised: 26-06-2022, Accepted: 04-07-2022 e-Published: 09-07-2022**

Equine herpes viruses (EHV) and west nile virus (WNV) are highly contagious pathogens of all members of the worldwide equidae family. It causes significant economic losses to members of the equi. Transmission of the EHV viruses occur by direct contact with the virus or contaminated materials. WNV infections in horses constitute a source for infections in humans in the chain of transmission. West nile virus is a mosquito-borne disease that can cause fatal infection in mammals. In this study, it was aimed to determine the presence of antibodies against herpesvirus type-1,4 (EHV-1,4) and WNV in local horses and donkeys used in the eastern and southeastern anatolia region of Turkey. For this purpose, blood serum was obtained from 83 horses and 395 donkeys who were not vaccinated against these viruses from the provinces of Elazığ, Tunceli, Siirt, Şırnak and Van. Blood samples were tested with commercial ELISA kits for antibodies againest these viruses. EHV 1,4 specific antibodies were detected in 5 (6.02%), 65 (78.31%) of the 83 tested horse sera, respectively. No positive antibody results were detected for WNV in horses. EHV-1,4 and WNV specific antibodies were detected in 40 (10.12%), 199 (50.37%) and 19 (4.81%) of the 395 tested donkeys sera, respectively. This study showed that EHV-1,4 and WNV infections are quite common in horses and donkeys in the eastern and southeastern Anatolian regions of Turkey. The fact that EHV-1,4 and WNV are more common especially in donkeys proves that donkeys are an important source for these viruses. Consequently, it suggests that the source of these infections in this region may be horses and donkeys.

Keywords: EquineHerpesvirus 1 (EHV-1), Equine Herpesvirus 4 (EHV-4), West Nile Virüs (WNV), equid, Turkey.

INTRODUCTION

The order *Herpesvirales* has since been made up of three families. The family *Herpesviridae* in this order includes the subfamilies Alpha, Beta, and *Gammaherpesvirinae*. Equine herpesviruses are members of the *herpesviridae* family. Equine herpesvirus type 1 (EHV-1) and Equine herpesvirus type 4 (EHV-4) belongs to the genus *Varicellovirus* in the subfamily *Alphaherpesvirinae.* Equine herpesviruses are DNA viruses that are found in most horses all over the world. Equine herpesvirus type 1 (EHV-1) is a very important pathogen that causes an infection characterized by neurological disorders such as respiratory system infection and myeloencephalitis in horses and causes economic damage as a result of sporadic and epidemic wastes it causes. They are pathogens with epidemiological, clinical and economic impact among equid (Derbal, 2021). The viral genomes are linear double-stranded DNA (dsDNA). The capsid with icosahedral symmetry, approximately 125 nm in diameter and 162 capsomeres, is surrounded by an amorphous tegument layer. The virus is enveloped and has peplomers in the glycoprotein structure of about 8 nm in length on the envelope (Yeşilbağ, 2021). EHV-1,4 are closely related to each other due to their genetic and antigenically important cross-reactivity properties (Crabb, 1993; Sabine, 1981). EHV-1 causes respiratory tract infection, abortion in pregnant horse, neonatal death in newborn foals and myeloencephalopathy. EHV-4 causes poor performance in running horses as a result of respiratory tract damage. EHV-4 causes abortion in mares (Pavulraj, 2021). The most common mode of transmission of the virus is the respiratory system tract. These viruses are usually transmitted through direct contact. It occurs directly as an aerosol or indirectly through contaminated materials from the nasal discharge and respiratory tract discharges of infected animals. Respiratory secretions, fetal and placental tissue and secretions are common sources of the virus. The virus is abundant in the placental fluids and aborted fetal tissues of infected mares. In addition, contaminated feed and feeders, equipment using water can also be a source of contamination (Yeşilbağ, 2021). Both cause EHV-1,4 latent infections. After primary infection, latency occurs in neural and lymphoid tissues in more than 50% of cases. Viruses can be reactivated by different stress factors such as animal migration, parasite

invasion, corticosteroid use. Scattering of reactivated latent virus from the nasal mucosa plays an important role in virus shedding in the epidemiology of EHV-1,4 (Borchers, 1999; Yeşilbağ, 2021).

Flaviviridae is a family of small enveloped viruses with positive-sense RNA genomes of approximately 9. 0-13 kb. Viruses with cubic symmetry are enveloped. The E2 glycoprotein on the envelope is the immunodominant protein of the virus. Naked RNA without capsid has infectious properties. Members of the Flavivirus genus are carried by arthropods and are transmitted to humans and animals by blood sucking. West nile virus is an important human pathogen. It causes a variety of diseases in humans, ranging from severe or fatal hemorrhagic fever to neurological disease. Mammals and poultry are the primary intermediate hosts for West Nile virus. Transmission to susceptible individuals is by infected mosquitoes (Blitvich, 2008; Yeşilbağ, 2021). Up to 80% of people infected with West Nile virus do not have any clinical symptoms. In other groups of people, headache, body aches, joint pains, vomiting, diarrhea symptoms are seen together with high fever. It causes central nervous system disorders in risky groups where the infection is severe. Mortality is 10% in infections that cause central nervous system disorders (Venter, 2009). The infection is zoonotic. In humans, west nile virus infections occur in the months of mosquito activation, beginning in the summer and continuing in the fall. There are no vaccines to prevent or medications to treat WNV in people. Studies have been conducted on the presence and prevalence of EHV-1,4 and WNV infections in different regions in Turkey (Albayrak and Ozan 2013; Ataseven et al. 2009; Ataseven et al. 2010; Avcı et al. 2014; Gür and Yapıcı 2008; Kale et al. 2017; Turan et al. 2012; Özkul et al. 2006; Yazıcı et al. 2012; Yıldırım et al. 2015; Yıldırım et al. 2018; Yıldırım et al. 2021). For effective diagnosis, control and eradication of the infection, its existence should be made and discussed in different regions.

The aim of this study was to determine the presence and prevalence of EHV-1,4 and WNV infections in horse and donkey populations in eastern and southeastern Turkey.

MATERIALS AND METHODS

The animals and area

The study was conducted in small family businesses with one or two local horses and/or donkeys aged 6-20 years. The study was carried out in the provinces of Tunceli, Elazig, Siirt, Şırnak and Van. Blood samples were taken from a total of 83 randomly selected horses and 395 donkeys, who were used to carry loads in rural areas, were not vaccinated against EHV-1, EHV-4, WNV and were clinically healthy. These blood samples were used as the research material. The provinces where blood samples were taken, their locations, animal species and sample numbers are shown in Table 1.

Table 1: Geographical positioning of the Turkish provinces in which the study was performed.

Province	Geographical position	Species	Number of serum samples
Elazığ	38^0.68' N- 39^0.22' E	Horse- Donkey	87
Tunceli	39^0.30' N- 39^0.43' E	Horse- Donkey	134
Siirt	37^0.93' N- 41^0.95' E	Donkey	102
Şırnak	37^0.41' N- 42^0.49' E	Donkey	70
Van	38^0.48' N- 43^0.40' E	Donkey	85

The Sera

Blood samples from animals were obtained from their vena jugularis. Blood samples taken from animals were placed in sterilized blood collection tubes and kept at +4 ^oC overnight to ensure clotting. The blood tubes were centrifuged at 1000 rpm for 10 minutes. The serum portion formed in the upper part was taken into sterilized tubes and stored by freezing until the tests were performed.

ELİSA

To detect the presence of EHV1/EHV4 and WNV antibodies, the EHV1/EHV4-Ab ELISA kit (SVANOVIR® EHV1/EHV4-Ab, Swedish Veterinary Institute, INDICAL Sweden AB) and The ID Screen West Nile Competition Multispecies ELISA kit (Veterinary ID Innovative Diagnostics, Grabels) , France) were obtained. The kits were applied in accordance with the recommendations of the companies it was prepared for. The test plates were evaluated at 450 nm in the ELISA optical concentration marker.

Statistical Analysis

Statistical Package for Social Sciences Software (IBM Corp.) was used for statistical studies. p value <0.05 was considered as remarkable value. The chi-square ($\chi 2$) test was used to evaluate the relationship between the seroprevalence values of the provinces.

RESULTS

Blood serum samples obtained from 83 horses and 395 donkeys who were not vaccinated against EHV-1,4 and WNV from the provinces of Elazığ, Tunceli, Siirt, Şırnak and Van were tested with the commercial Ab ELISA kit. Antibodies against EHV-1 were detected in 6.02% of horses and against EHV-4 in 78.31% of them. The presence of antibodies against WNV could not be determined of horses. Antibodies were detected against EHV-1 in 10.12%, against EHV-4 in 50.37% and against WNV in 4.81% of blood sera taken from donkeys. No statistical difference was found between provinces. These datas were shown in Table 2,3.

Presence of antibodies against EHV-1 and WNV could not be determined in 14 blood sera obtained from horses bred in Elazig, the presence of antibodies against EHV-4 was detected in 12 (85.71%) of them. These datas

were shown in Table 2.

Table 2: Distribution of EHV-1, EHV-4 and West Nile Virus specific antibodies in horses by province*.

Province:	**Tested Sera**	**EHV-1(+) %**	**EHV-4 (+) %**	**EHV-1, 4 (+) %**	**WNV (+) %**
Elazığ	14	0	12 (%85.71)	0	0
Tunceli	69	5 (%7.24)	53 (%76.81)	5 (%7.24)	0
Totally	**83**	**5 (%6.02)**	**65 (%78.31)**	**5 (%6.02)**	**0**

*No statistical difference was found between provinces.

Table 3: Distribution of EHV-1, EHV-4 and West Nile Virus specific antibodies in donkeys by province*.

Province:	**Tested sera no**	**EHV-1 (+) %**	**EHV-4 (+) %**	**EHV-1 and 4 (+)**	**WNV (+) %**
Elazığ	73	6 (%8.21)^{b}	23 (%31.50)^{b}	3 (%4.10)^{b}	2 (%2.73)^{b}
Tunceli	65	6 (%9.23)^{b}	28 (%43.07)^{e}	4 (%6.15)^{b}	3 (%4.61)^{b}
Siirt	102	19 (%18.62)^{a}	60 (%58.82)^{d}	18 (%17.64)^{a}	1 (%0.98)^{b}
Şırnak	70	5 (%7.14)^{b}	54 (%77.14)^{a}	4 (%5.71)^{b}	10 (%14.28)^{a}
Van	85	4 (%4.70)^{b}	34 (%40.00)^{c}	3 (%3.52)^{b}	3 (%3.52)^{b}
Totally	**395**	**40 (%10.12)**	**199(%50.37)**	**32 (%8.10)**	**19 (%4.81)**

*The superscripts in the common columns (a-e) show the differences in positivity rates between provinces. The difference in the amount of P< 0.05 was evaluated.

Although the presence of antibodies against WNV could not be detected in the blood serum obtained from 69 horses raised in Tunceli, the presence of antibodies against EHV-1,4 was detected in 5 (7.24%) and 53 (76.81%) of them, respectively. These datas were shown in Table 2.

A total of 395 serum samples obtained Elazığ, Tunceli, Siirt, Şırnak and Van were tested for EHV1, EHV-4 and WNV specific antibodies using ELISA. Overall results revealed that 10.12% (40/394) of the donkeys sampled were EHV-1 seropositive, while antibodies specific to EHV-4 were detected in 50.37% (199/395) of sera samples. Specific antibodies against WNV were found in 4.81% (19/395) of the donkeys. Differences in EHV-1, EHV-4 and WNV antibody prevalences between the provinces (Şırnak and Tunceli) and other locations were found to be statistically significant. These datas were shown in Table 3.

DISCUSSION

Equine herpesviruses are very common DNA viruses in horse populations worldwide. There are two important strains that affect the health of equidae. EHV-1 causes respiratory disease, abortion, and neurologic disease. EHV-4 primarily causes respiratory disease and only occasionally can cause abortion or neurologic disease. EHV-1,4 are viruses that are of importance in veterinary medicine, which have a latent period and can spread out among susceptible horses with scatter during the reactivation period. (Derbal, 2021). WNV is an important infection in the chain of transmission with many mosquito species, many bird species and equidae. It is a virus that has an increasing importance in terms of human health with this spread of vectors, which increased due to the global climate change that developed after 2010 (Yıldırım et al. 2021). For these reasons, it is important to determine the presence and prevalence of viruses in different regions. In this study, the presence and prevalence of equidae in eastern and southeastern Anatolia were revealed.

Various serological methods are used to detect specific antibodies produced against EHV-1,4. The development of type-specific ELISA tests with the use of monoclonal antibodies (mAbs) is accepted as a reliable and preferred method due to its increased test sensitivity, high specificity and sensitivity, and practical advantages over other serological tests (Ataseven et al. 2009; Gür and Yapıcı 2008; Kale et al. 2017; Yesilbag, 2021; Yıldırım et al.2018). In the detection of WNV antibodies, ELISA and Plaque Reduction neutralization test (PRNT) are often used to diagnose infection (Blitvich, 2003). In experimental studies, screening tests were performed using ELISA and PRNT on blood samples from WNV-infected horses, cats and pigs. At the end of the scan, it was concluded that the diagnostic efficiency of both techniques was similar. They also stated that ELISA gives faster results and is much cheaper than PRNT (Blitvich et al. 2003; Gür and Kale et al. 2017; Yapıcı 2008). For these reasons, ELISA test was preferred to determine the presence and prevalence of EHV-1,4 and WNV in Eastern and Southeastern Anatolia.

Horses and donkeys still play an important role in human life in the production of agricultural products in the provinces of Elazığ, Tunceli, Siirt, Şırnak and Van, which are located in the Eastern and Southeastern Anatolia

Regions. Considering the importance of the subject, 478 blood serum samples were obtained from horses and donkeys raised in small family businesses in the region. Specific antibodies against EHV-1,4 and WNV were detected in these samples. Infections caused by these diseases in horses and donkeys cause economic losses. Therefore, the presence and prevalence of EHV-1,4 infections have been reported in the range of 8-95% in countries such as Germany, Korea, Egypt, Serbia, Sarajevo, Nigeria and Croatia (Derbal, 2021). (Derbal, 2021). Ataseven, Gür, Avcı and Yıldırım found seropositivity between 3.7% and 52.25% against EHV-1 and between 56.9% and 81.7% against EHV-4 in blood serum samples taken from horses from different regions in Turkey (Ataseven et al. 2009; Ataseven et al. 2010; Avcı et al. 2014; Yıldırım et al. 2015) In donkeys, in the Kars and Ardahan regions, Yıldırım determined the seropositivity against EHV-1 as 51.85% and against EHV-4 as 64.20%. In this study, the seropositive rate of horses against EHV-1 was 6.02% and 78.31% against EHV-4 in Elazig and Tunceli regions. Seropositivity was detected against EHV-1,4 at the rates of 10.12% and 50.37%, respectively, in blood sera taken from donkeys taken from the provinces of Elazig, Tunceli, Siirt, Şırnak and Van. Although the seroprevalence of EHV 1,4 infections in donkeys is high in Siirt and Şırnak provinces, the seroprevalence of EHV 1.4 in horses and donkeys is in line with the results obtained from studies conducted in the world and in Turkey, according to the results across the region. The reason why these results show parallelism is the global product mobility due to the increase in world trade of infections spread by contact contamination.

The seroprevalence of WNV in horses in Russia, Croatia, Spain and Croatia was found to be between 1-15% (Kale et. al. 2017). The presence of West Nile virus antibodies in donkey mules, cattle, dogs, horses, sheep and humans in Turkey was investigated in sera obtained from 10 different regions and their presence was first reported by Özkul et al. introduced by (Özkul et al.2006). Albayrak and Kale investigated the presence of WNV antibodies in blood serum obtained from different regions and different animal species, and they revealed their presence at different rates in different animal species in different regions (Albayrak and Ozan 2013; Kale et. al. 2017). In these study, although there was no antibody against WNV in horses, a seropositivity rate of 4.81% was found in donkeys. The results of this study are in line with other studies conducted in Turkey. The fact that WNV is transmitted by mosquitoes makes the virus, reservoir and climate trio important in the epidemiology of the disease. Therefore, detection of WNV antibodies in donkeys is an important finding.

CONCLUSION

WNV' antibodies were not found in horses bred in eastern and southeastern anatolia. Antibodies against EHV-1,4 have been demonstrated in horses. Antibodies against EHV-1,4 and WNV have been demonstrated in donkeys. The presence of WNV infections in donkeys is an important factor in virus shedding in WNV and viremic mosquito transmission. At the same time, donkeys play a major role in the transmission of persistent EHV-1,4 infections. More work should be done to know the regional-based profile for persistent and vector-born infections, and to determine the interactions between the strains exposed to infections and the risk factors for transmission.

CONFLICT OF INTEREST

Control Institute for their contributions to this study

AUTHOR CONTRIBUTIONS

The author declared that present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT

I would like to thank the staff of Elazig Veterinary Control Instute.The article is produced as a single name. Metin Gürçay conceived the idea, planned the work and wrote the article. However, I would like to thank my friends who contributed to the execution of the study, especially Assoc. Prof. Dr. Thank you very much Turhan Turan.

REFERENCES

Albayrak H & Ozan E, 2013. Seroepidemiological study of West Nile virus and Rift Valley fever virus in some of mammalian species (Herbivores) in northern Turkey. Journal of arthropod-borne diseases, *7*(1): 90.

Ataseven VS, Dağalp SB, Başaran Z & Keskin S, 2010. Seroepidemiological studies of equine herpesviruses 1 (EHV-1) and 4 (EHV-4) infections in working horses from the eastern Turkey. Ankara Üniversitesi Veteriner Fakültesi Dergisi, *57*(1): 39-42.

Ataseven VS, Dağalp SB, Güzel M, Başaran Z, Tan MT & Geraghty B 2009. Prevalence of equine herpesvirus-1 and equine herpesvirus-4 infections in equidae species in Turkey as determined by ELISA and multiplex nested PCR. Research in veterinary science, 86(2):339-344.

Avci O, Yavru S, Tokgoz S & Kale M, 2014. Detection of Antibodies against Equine Herpes Virus-1 and Equine Herpes Virus-4 in Horses in Southeast

Anatolia by Indirect Elisa. Acta Scientiae Veterinariae, *42*(1): 1-5.

Blitvich BJ, 2008. Transmission dynamics and changing epidemiology of West Nile virus. Animal Health Research Reviews, 9(1):71–86.

Blitvich BJ, Bowen RA, Marlenee NL, *et al.,* 2003. Epitope-blocking enzyme-linked immunosorbent assays for detection of West Nile virus antibodies in domestic mammals. J Clin Microbiol 41: 2676-9.

Borchers K, Wolfinger U & Ludwig H. 1999. Latency-associated transcripts of equine herpesvirus type 4 in trigeminal ganglia of naturally infected horses. J. Gen. Virol., 80: 2165–2171.

Crabb BS & Studdert MJ, 1993. Epitopes of glycoprotein G of equine herpesviruses 4 and 1 located near the C termini elicit type-specific antibody responses in the natural host. J. Virol. 67, 6332–6338.

Derbal S, 2021. Epidemiology of EHV-1 and EHV-4 infections: A review. International Journal of Veterinary Science and Research, 7(2): 108-112.

Gür S & Yapici, O. (2008). Equine herpesvirus type 1 and 4 in individually reared horses in central and western Turkey. Acta Veterinaria Brno, *77*(4): 609-613.

Kale M, Gür S, Yapıcı O, Nuri M, Yavru S, Hasırcıklıoğlu, S., ... & Gürçay M, 2017. Serological investigation of West Nile virus infection in domestic horses and donkeys in Turkey. Pak. Vet. J, *37*, 51-54.

Sabine M, Robertson GR & Whalley JM, 1981. Differentiation of sub-types of equine herpesvirus I by restriction endonuclease analysis. Aust. Vet. J., 57, 148–149.

Ozkul A, Yildirim Y, Pinar D, Akcali A, Yilmaz V & Colak D, 2006. Serological evidence of West Nile Virus (WNV) in mammalian species in Turkey. Epidemiology & Infection, 134(4); 826-829.

Pavulraj S, Eschke K, Theisen J, Westhoff S, Reimers G, Andreotti S & Azab W, 2021. Equine Herpesvirus Type 4 (EHV-4) Outbreak in Germany: Virological, Serological, and Molecular Investigations. Pathogens, *10*(7): 810.

Turan N, Yildirim F, Altan E, Sennazli G, Gurel A, Diallo I & Yilmaz H, 2012. Molecular and pathological investigations of EHV-1 and EHV-4 infections in horses in Turkey. Research in veterinary science, *93*(3): 1504-1507.

Venter M, Human S, Zaayman D, Gerdes GH, Williams J, Steyl J, Leman PA, Paweska JT, Setzkorn H, Rous G, Murray S, Parker R, Donnellan C & Swanepoel R, 2009. Lineage 2 West Nile virus as cause of fatal neurological disease in horses, South Africa. Emerging Infectious Diseases, 15: 877–84.

Yazici Z, Albayrak H, Ozan E & Gumusova S, 2012. The first investigation of West Nile virus in horses using real time RT-PCR in Middle Black Sea Region in Turkey. Journal of Arthropod-Borne Diseases, 6(2): 151.

Yeşilbağ K, 2021. Veteriner Viroloji, Hayvanların Viral hastalıkları. Medipres Matbaacılık Yayıncılık, Malatya, Türkiye.

Yıldırım Y, Yılmaz V, Öziç C, Ozkul A & Çağırgan AA, 2021. Phylogenetic analysis of West Nile virus: first report of lineage 1 in donkey in Turkey. Tropical Animal Health and Production, 53(5): 1-6.

Yildirim Y, Yilmaz V & Kirmizigul AH, 2015. Equine herpes virus type 1 (EHV-1) and 4 (EHV-4) infections in horses and donkeys in northeastern Turkey. Iranian journal of veterinary research, 16(4):341

Yildirim Y, Yilmaz V, Yazici K, Ozic C & Ozkul A, 2018. Molecular and serological investigation of West Nile virus (WNV) infection in donkeys, horses and native geese in Turkey. Revue Méd. Vét, *169*(4-6): 87-92.