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RESEARCH ARTICLE

Serological Investigation of West Nile Virus Infection in Domestic Horses and Donkeys in Turkey

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A B S T R A C T

West Nile Virus (WNV) antibody presence was studied in domestic horse (*Equus caballus*) and donkeys (*Equus asinus*) of various age and types owned by public in different regions of Turkey. In the study, serum samples were collected from 650 domestic horses and 234 domestic donkeys seeming to be in good health. In order to detect WNV antibody presence in these samples, commercial c-ELISA (Competitive Enzyme Linked Immunosorbent Assay) kit (ID Screen® West Nile Competition-IgG- ELISA kit, Montpellier, France) was used. At the end of the test, WNV seropositivity was detected at a level of 4.15% (27/650) for horse serum samples and 1.28% (3/234) for donkey serum samples. Seropositivity was found for horses in Aegean and Central Anatolia and for donkeys in Eastern Anatolia regions. WNV seropositivity was detected only for Native Anatolia type horse races. It was concluded that WNV presence continued its existence among equidae in our country.

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INTRODUCTION

West Nile Virus (WNV) infection has arboviral and zoonosis characteristics. It takes place within Flavivirus strain in *Flaviviridae* family (Surhone *et al.,* 2010). The natural way of contamination of WNV takes form among birds and mosquitoes. Within the disease, mosquitoes act as vectors and birds as reservoir hosts. Vector mosquitoes transmit WNV from one animal to the other. Mosquitoes often feed themselves with bird blood especially towards the end of summer. Healthy birds are infected by bloodsucking of infected mosquitoes. WNV is replicated in infected birds and newly reproduced virus mixes with blood. Virus is transmitted by way of blood when another mosquito bites the newly-infected bird. This cycle between birds and mosquitoes continues during mosquito feeding season and the virus is spread through a vast geographical area (Sfakianos and Hecht, 2009).

Humans and horses are usually infected as incidental hosts. They both are infected after being bitten by a mosquito infected by WNV. WNV generally doesn't transit to another host as it doesn't centralize in host's blood. However, host might be infected by the virus and display symptoms of disease (neurologic disorders etc.). Mosquitoes usually feed themselves with bird blood and rarely with human and horse blood. When they sometimes go out of normal feeding routine, they are thought to feed with human and horse hosts as incidental or last host (Sfakianos and Hecht, 2009). In this study, we aimed to detect WNV seroprevalence among domestic horse and donkeys of various age and types owned by public and reveal the reasons.

MATERIALS AND METHODS

Animals and sera samples: This study was performed on healthy looking 650 domestic horses (*Equus caballus*) and 234 domestic donkeys (*Equus asinus*) aged between 3-13, owned by public and used as draught animals in different regions of Turkey (Fig. 1). In managements where samplings were done, number of horses and donkeys had a population between 1-3. No classification based on gender was done for horses and donkeys in the study.

In the study, horse races of Kabardin, Native Anatolian and Native Uzunyayla type were searched (Table 1). Kabardin is a mountain race. Their homeland is Northern Caucasia region. They are generally used as draught animals and for riding. It is claimed that this race was created by using and chasting Turkmen and Arab horses. Native Anatolian race types are small and well-built. Legs are strong, shinbones and ankles are middle-length and hooves are quite strong. It is a type that is adapted for Anatolia conditions and that has been used by Turkish farmers for centuries. Native Uzunyayla horse has

a higher and more massive body structure than Turkish native types. It is claimed that its origin is a mixture of Cherkes, Hungarian, Arab and Akhal-Tekehorses. It has been more suitable for agriculture and draught than other native horses (Arpacık, 1994).

Table 1: Distribution of sampled horse races according to regions.

Regions	Races (Types)		
	Kabardin	Native Anatolia	Native Uzunyayla
Aegean	-	24	-
Western Mediterranean	-	32	5
Central Anatolia	12	376	8
Eastern Anatolia	10	181	2
Total	22	613	15

It was detected that no protective vaccination against any viral disease was performed on sampled horses and donkeys in the study in accordance with the information from animal owners and veterinarians of Ministry of Food, Agriculture and Livestock Animal Health Department. Blood samples of horses and donkeys taken into vacuumed tubes from their vena jugularis was centrifuged for 20 min at 3000 rpm in the laboratory and after the obtained serum was inactivated in water bath for 30 min at 56ºC, they were taken into Eppendorf tubes, backed up and stored at -80ºC until testing.

Competitive Enzyme Linked Immunosorbent Assay (c-ELISA): In order to detect WNV antibody presence in blood serum samples taken from 650 domestic horses (*Equus caballus*) and 234 domestic donkeys (*Equus asinus*), commercial ELISA kit of IDvet firm (ID Screen® West Nile Competition-IgG/IgM-ELISA kit, Montpellier, France) was used. Using the samples during tests and evaluating the results was applied according to kit procedure.

RESULTS

In this study, WNV seropositivity was detected in 27 out of 650 domestic horses (*Equus caballus*) at a rate of 4.15%. According to regions where horse samplings were done, the lowest seropositivity was found around Afyon (Aegean) province at a rate of 4.17%, and the highest around Ankara (Central Anatolia) province at a rate of 10.67%. Seropositivity was detected in horses in Kayseri (Central Anatolia) at a rate of 6.12%, and 5.51% in horses in Konya (Central Anatolia). Horses in Burdur (Western Mediterranean), Elazıg-Center (Eastern Anatolia), Elazıg-Palu (Eastern Anatolia), Tunceli-Hozat (Eastern Anatolia) and Tunceli-Pertek (Eastern Anatolia) were also found seronegative in terms of WNV (Table 2). The horse type with WNV seropositivity in Aegean and Central Anatolia regions was detected as Native Anatolian. Kabardin and Native Uzunyayla horses were found WNV seronegative.
In the other sampling of the study, WNV seropositivity was detected in 3 out of 234 domestic donkeys (*Equus asinus*) at a rate of 1.28%. According to regions where donkey samplings were done, the lowest seropositivity was found around Elazıg-Palu (Eastern Anatolia) at a rate of 2.27%, and the highest around Elazıg-Center (Eastern Anatolia) at a rate of 2.44%. Also, while donkeys in Elazıg-Palu (Eastern Anatolia) were found seropositive at a rate of 2.27%, those in Afyon (Aegean), Elazıg-Maden (Eastern Anatolia) and Tunceli-Pertek (Eastern Anatolia) were detected seronegative in terms of WNV (Table 2).

DISCUSSION

In this study, WNV seropositivity was detected at a rate of 4.15% healthy looking domestic horses owned by public and kept for draught. Infection rates were found between minimal 4.17% and maximal 10.67%. It was reported that among horses in Russia, France, Croatia, Spain and Romania, WNV infection prevelance proceeded between 1-15% (Jimenez-Clavero *et al.*, 2007; Madic *et al.*, 2007). Pauvolid-Correa *et al.* (2011) 3% in horses in Pantanol region of Brazil, Hubalek *et al.* (2013) 4.8% in local cases in horses in Slovakia, Barbic *et al.* (2012) 3.43% in horses in Croatia, and they found minimal-maximal distribution rates between 1.41-9.09% according to regions. However, there are also studies that found lower WNV seropositivity values [Kuwahara *et al.*, 2012 (0.425%); Burgueno *et al.*, 2013 (1.88%); Ziegler *et al.*,

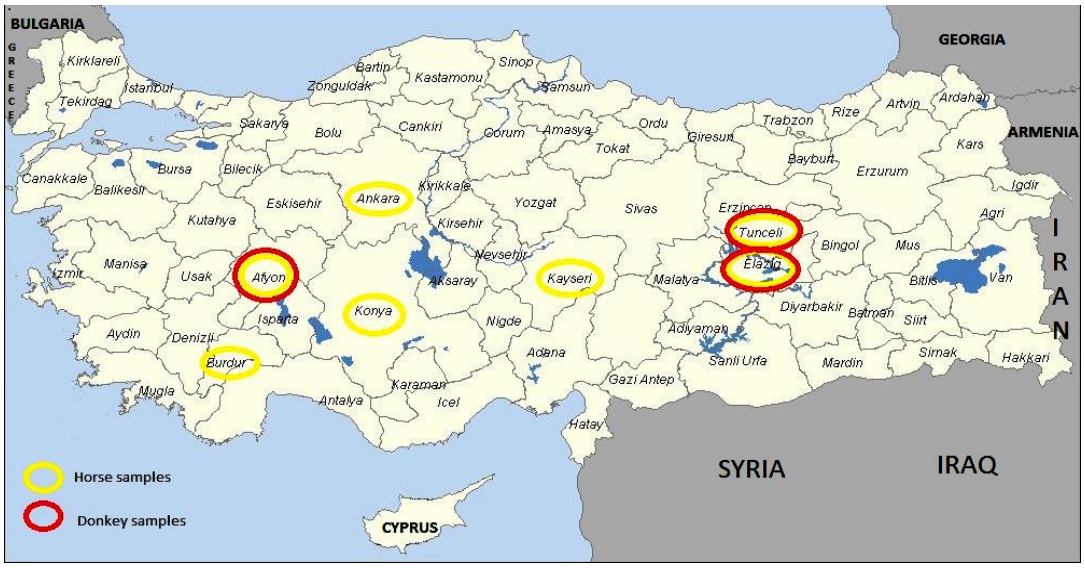

Fig. 1: Cities where samplings were performed.

Table 2: Distribution of West Nile Virus infection seroprevalence in the horses and donkeys

City (Region)	Horses		Donkeys	
	Tested samples	Seropositive samples	Tested samples	Seropositive samples
Afyon (Aegean)	24	1 (4.17)	7	-
Burdur (Western Mediterranean)	37	-	-	-
Kayseri (Central Anatolia)	49	3 (6.12)	-	-
Ankara (Central Anatolia)	75	8 (10.67)	-	-
Konya (Central Anatolia)	272	15 (5.51)	-	-
Elazıg-Center (Eastern Anatolia)	69	-	41	1 (2.44)
Elazıg-Maden (Eastern Anatolia)	-	-	46	-
Elazıg-Palu (Eastern Anatolia)	22	-	44	1 (2.27)
Tunceli-Hozat (Eastern Anatolia)	69	-	42	1 (2.38)
Tunceli-Pertek (Eastern Anatolia)	33	-	54	-
Total	650	27 (4.15)	234	3 (1.28)

Values in parenthesis indicate percentage.

2013 (1.17%)] and higher values [Alonso-Padilla *et al.*, 2009 (31.6%); Ahmadnejad *et al.*, 2011 (23.7%); Ibarra-Juarez *et al.*, 2012 (26%, 45%); Abutarbush and Al-Majali, 2014 (24.9%)] than our results. In our country, while Ozkul *et al.* (2006) detected WNV seropositivity in 35 out of 259 horses at a rate of 13.5% and Ergunay *et al.* (2014) 10.5% (18/171) in horses in Eastern Anatolia region (Van) and 13.8% (30/218) in horses in South-East Anatolia region (Şanlıurfa), Albayrak and Ozan (2013) found WNV seronegativity in 70 horses in Northern region.

In this study, WNV seropositivity wasn't detected in horses in some regions (Western Mediterranean and Eastern Anatolia). And some studies (Lefrançois *et al.*, 2006; Lan *et al.*, 2013) reported similar results. When Yazici *et al.* (2012) studied WNV-RNA presence in horses in Northern region of Turkey with real-time RT-PCR, they found no positivity. That they couldn't detect anything was attributed to negative conditions of the environment (freezing winters and mosquitoes having no suitable weather conditions) although this part of the country was available for WNV infection. We presume that WNV infection did not appear especially because horses sampled in Western Mediterranean and Eastern Anatolia were settled around mountainside and highlands with high altitudes. Horses used for sampling belonged to nomads. In our country, nomads take their sheep and goats to high-altitude uplands for grazing before flights of mosquitoes every year. Horses here are used for draught and stay here until the end of autumn.

Horses with seropositivity were found in bordering Aegean and Central Anatolia regions. Horses in these regions were kept in savana premises, wetlands and green areas. The fact that Ergunay *et al.* (2010) and Kalaycıoglu *et al.* (2012) detected WNV seropositivity also in humans around Aegean and Central Anatolia regions (Ankara, Konya, Yozgat, Sivas, Afyon) and that they had WNV distribution at individual level and that there was viral activity was quite interesting. Hence, Ozkul *et al.* (2013) stated that they had human and horse clinical cases in Central Anatolia region (Ankara and Eskişehir), they performed their isolation and they found human and horse cases related with meningoencephalitis within two locations close to each other (150 km). Besides the horse type with WNV seropositivity in these two regions was stated as Native Anatolian. There are various horse types bred with a native name in Turkey. These native types are hardly distinguished since they don't have stable race typology. Therefore, it is not possible to call them a 'race'. Because horses in Turkey had become a way of migration due to historical and geographical position of the country and they were mixed with various horse types these immigrants brought with them. That's why it is more logical to search native horses classifying them into groups and types. Native horse types in Turkey are studied within four main groups (Anatolia, Çukurova, Uzunyayla, Midilli (=Pony) and other regional types (i.e. Canik horse) (Arpacık, 1994).

While Garcia-Bocanegra *et al.* (2012) detected seropositivity in clinically healthy looking donkeys around Southern Spain region at a rate of 4.9%, only in donkeys 47% from 436 blood samples of horses, donkeys and mules in Egypt, Baba *et al.* (2014) 8.6% from blood samples of 58 donkeys in Nigeria, and Alonso-Padilla *et al.* (2009) 50% in donkeys, Chikweto *et al.* (2008) detected WNV seronegativity in blood samples of 45 donkeys on Grenada and Carriacou islands. There are also cases in which some clinical symptoms (hepatitis, neurological disorders, encephalitis with/without fever etc.) were seen depending on WNV infection (Salazar *et al.*, 2004). In this study, WNV seropositivity was detected at a rate of 1.28%in 3 out of 234 healthy looking domestic donkeys owned by public and kept for draught. Prevalence distribution was found as minimal 2.27% and maximal 2.44%.

In this study, no WNV infection was seen in donkeys in Aegean region, whereas it was detected rather in donkeys in Eastern Anatolia (Elazıg-Tunceli) region. Donkeys in this region were used for draught, were located in mountainsides, were carrying heavy cargo and were kept in places where no health applications about equidae were performed. Both settlements in these regions are high places with over 1000 m altitudes in Elazıg and Tunceli surrounded by dam lakes, Toros mountains and natural borders located in Fırat reservoir (TUIK, 2014). Typical continental climate is dominant in both provinces, where summers are dry and hot and winters are cold and snowy. It rains in autumn and spring, and snows in winter. Due to physical geographical features, climate variations, and biovariation depending on quite rich water sources, all showing quite different data in both provinces (TUIK, 2014), we estimate that there are WNV seropositive donkeys even at low levels. In our country, donkeys aren't sold, disposed etc., however, they are kept for draught for long years. This shows that the infection doesn't travel from one place to another.

To diagnose WNV infection, ELISA and Plaque reduction neutralization test (PRNT) is generally used. Blitvich *et al.* (2003) performed periodic scanning using

ELISA and PRNT on blood samples of horses, cats and swines infected by WNV experimentally. At the end of scanning, they realized that the diagnostic effectiveness of both techniques was similar. They also stated that ELISA provided faster results compared to PRNT and was much cheaper. So we preferred ELISA in our study as well.

In conclusion, WNV seropositivity was detected in horses in Aegean and Central Anatolia regions of our country, and donkeys in Eastern Anatolia region. Horses proved to have higher seropositivity than donkeys. WNV seropositivity was detected only in Native Anatolian horse types. Since WNV can be seen among equidae in our country, we strongly advise to eliminate contact of flies and ticks with horses, donkeys and mules, to destroy sources that can increase fly growth, not to walk or work horses, donkeys and mules during night and sunset, to install swatters on doors and windows of stables, not to use insect repellents, to extend vaccine applications on horses, donkeys and mules and to keep all equidae away from the other types showing neural system symptoms (dogs, poultry etc.).

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