Molecular and serological investigation of West Nile virus (WNV) infection in donkeys, horses and native geese in Turkey

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SUMMARY

West Nile virus (WNV), an arthropod-borne viral pathogen of global importance, is considered to be the most widespread flavivirus in the world. Here we present a serological and virological study on WNV in horses, donkeys and Turkish native geese in the North-eastern Anatolian province. Blood sera were collected randomly from 118 horses, 70 donkeys and 378 geese, and tested for antibodies against WNV using a commercial competitive enzyme-linked immunosorbent assay (C-ELISA). The overall results revealed that 0.8% (1/118) of the horses, 20% (14/70) of the donkeys and 1.1% (4/378) of the geese were WNV seropositive. To determine the presence of WNV nucleic acid, positive blood sera were tested by the reverse transcription polymerase chain reaction (RT-PCR) technique. WNV nucleic acid was not found in horse and goose samples, while it was demonstrated in four donkey samples. The results suggest that the infection was spreading in private small-scale production units. This study is the first molecular and serological study to determine virus prevalence and seroprevalence of WNV infection in horses, donkeys and Turkish native geese in the North-eastern Anatolian province of Turkey. It is also the first to be conducted on Turkish native goose in Turkey.

Keywords: Donkey, Horse, Turkish native goose, West Nile virus, C-ELISA, RT-PCR, seroprevalence

RÉSUMÉ

Investigation moléculaire et sérologique de l’infection par le virus de West Nile chez les ânes, les chevaux et les oies indigènes en Turquie

Le virus de West Nile (VWN) est considéré comme le flavivirus le plus répandu dans le monde entier. Nous présentons ici une étude sérologique et virologique réalisée chez les chevaux, ânes et oies turques indigènes dans le nord-est de l’Anatolie en Turquie. Des sérums ont été prélevés au hasard sur 118 chevaux, 70 ânes et 378 oies, et testé pour les anticorps contre le VWN à l’aide d’un dosage immuno-enzymatique compétitif (C-ELISA). Les résultats globaux ont révélé que 0,8 % (1/118) des chevaux, 20 % (14/70) des ânes et 1,1 % (4/378) des oies étaient séropositifs. Les sérums positifs ont été testés par RT-PCR. L’acidé nucléique du VWN n’a pas été trouvé dans des échantillons de chevaux et oies, alors qu’il était présent dans quatre échantillons obtenus sur les ânes. Les résultats suggèrent que l’infection se propageait dans les petites unités de production privées. Cette étude est la première étude moléculaire et sérologique visant à déterminer la prévalence du virus et la séroprévalence de l’infection VWN chez les chevaux, les ânes et les oies indigènes turques dans la province d’Anatolie du nord-est de la Turquie.

Mots-clés : Ane, Cheval, Oie, Turquie, Virus de West Nile, C-ELISA, RT-PCR, séroprévalence

Introduction

West Nile virus (WNV) is an enveloped, single-stranded positive polarity-bearing RNA virus, which belongs to the genus Flavivirus in the family Flaviviridae. The agent is also part of the JE-serocomplex that contains the Japanese encephalitis virus (JEV), Saint Louis encephalitis virus (SLEV), Murray Valley encephalitis virus (MVEV) and Kunjin virus (KUNV). It causes infections characterized by mild inflammatory diseases, meningitis, encephalitis or deaths in various animals such as humans, horses and birds [3, 4, 28, 31].

The natural transmission cycle of WNV infection occurs between wild and domestic birds and mosquitoes, especially of the Culex species [23]. In this respect, presenting in this study the status of the infection in native species of free-range geese raised in small family-owned businesses, which share joint basins with wild birds [17] is important for the region. Migratory birds play in the epizootiology of WNV infection.

The study was performed in Kars, Ardahan and Iğdır provinces (43.05° E and 40. 36° N), which is undergoing ecological and socioeconomical changes and also has a rich mosquito fauna. It is located in the Northern Anatolian. Vector-borne viral diseases such as Bluetongue (BT) and Akabane (AKA) are endemic in this region, which is the most important livestock production area in Turkey.

In order to differentiate the agent from other flaviviruses and prevent cross-reactivity, specific tests such as the plaque reduction neutralization test (PRNT), enzyme linked
immunosorbent assay (ELISA), indirect fluorescent antibody test (IFAT), and especially PCR, in which WNV-specific RNA sequences are used. Serological tests detect the host immune response to WNV infection. Although viremia is detectable earlier than the immune response, serologic (IgG and IgM) assays are typically more sensitive for detecting active and convalescent WNV infection. IgM is typically detectable at the time of initial presentation. The IgM antibody capture enzyme-linked immunosorbent assay (MAC–ELISA) is the most conclusive laboratory method for diagnosis of WNV infection of the CNS. The method is high sensitivity and specificity >95%. RT-PCR has proven to be an effective method for detection of WNV nucleic acid from a variety of sample types. WNV viremia peaks at about the time of symptom onset and rapidly fades to undetectable levels. Thus, RT-PCR assays can detect WNV RNA in clinical samples as early as several days before symptom onset, prior to seroconversion. Although this assay has excellent analytical sensitivity, it lack the clinical sensitivity of antibody tests and is typically not used alone for screening or diagnosis.

The 2010 human cases (n=47) of WNV infections in Turkey were observed in several provinces mostly in the western part. Ten of the patients died. The WNV infections were included in the national notifiable diseases list as of April 2011.

This study aims to detect the WNV-seroprevalence in horses, donkeys and free-range Turkish native geese raised in three provinces (Kars, Ardahan and Igdır) located in the Northeast Turkey and make suggestions to help prevent economic losses. In addition, this survey is important in terms of acquiring initial data for the aforementioned infection in horses, donkeys and Turkish native geese and forming a basis for studies to be conducted in the future. Unlike previous studies, it is studied that in the region of Turkey which includes the migratory birds route. Considering the epidemiology of infection (the animals which use common water sources with migratory birds) are investigated the role of migratory birds for WNV infection in the transmission.

### Materials and methods

**THE STUDY AREA AND ANIMALS SAMPLED**

The study was carried out on small family-owned businesses located between February 2014 to January 2015 in the Northeast Anatolia in Turkey (Figure 1). In the region climate differences can be seen due to elevation. Some of the most important migration routes of the Western Palearctic region pass through Turkey. There are three migration routes in Turkey. These are the Istanbul Bosphorus, Belen Passage (Antakya) and Çoruh Valley.

![Figure 1: Geographical positioning of the Turkish provinces in which the study was performed.](image)

One of the important territorial features of the region is the wide use of odd-toed animals (e.g. horses and donkeys) for haulage, transportation and in agriculture, and thus, one or two horses are raised on every farm in the study area. The equines used in this study comprised healthy appearing horses or donkeys older than 1 year of age and unvaccinated against the aforementioned infection. Likewise, materials were obtained from regional native geese older than 3 months of age and from these family-owned small-scale businesses, which raise 20-30 geese on average for both food-supply and source of income purposes. In accordance with this, distribution of a total of 566 randomly selected animals in terms of province of origin and species were shown in Table I. All sampling were performed after the approval of local

<table>
<thead>
<tr>
<th>Location</th>
<th>Species</th>
<th>Number of samples</th>
<th>C-ELISA Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ardahan Province</td>
<td>Goose</td>
<td>129</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td></td>
<td>Horse</td>
<td>38</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>Donkey</td>
<td>24</td>
<td>2 (8.3%)</td>
</tr>
<tr>
<td>Igdır Province</td>
<td>Goose</td>
<td>124</td>
<td>2 (1.6%)</td>
</tr>
<tr>
<td></td>
<td>Horse</td>
<td>40</td>
<td>1 (2.5%)</td>
</tr>
<tr>
<td></td>
<td>Donkey</td>
<td>25</td>
<td>9 (36%)</td>
</tr>
<tr>
<td>Kars province</td>
<td>Goose</td>
<td>125</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td></td>
<td>Horse</td>
<td>40</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>Donkey</td>
<td>21</td>
<td>3 (14.3%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>566</td>
<td>19 (3.4%)</td>
</tr>
</tbody>
</table>

Table I: Distribution of sampled species according to regions and the West Nile virus seropositivity rate of the samples
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The main differences &.

Two of the most important migratory

2 2

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Statistical analysis was carried out via the Statistical Package for Social Sciences software (IBM SPSS Statistics 20.0, SPSS, Inc., Chicago, IL, USA) [15]. The main differences between species and regions were evaluated using the Chi-Square (χ²) test. At the end of the study, the data from which the value of P<0.05 was derived was accepted as significant.

The study was carried out in the regions of Igdir Valley, Kars Plateau and Ardahan Plateau, which are located in the Northeast Anatolia and have different climatic and geographical features. In addition, the area chosen for the study provides suitable habitats for mosquito larvae with a variety of wetlands, and because of the extensive amount of animal-farming done in the area, there is a variety of host types on which mature insects are able to feed. When all the areas conditions are considered, it can be said that it is a potentially risky area for WNV infection.

Discussion

Arthropod-borne viruses are transmitted by blood-sucking arthropods such as mosquitoes, ticks and sand flies. Because the actual cycle of the West Nile virus is between birds and mosquitoes, many studies have been done to research the link between virus transmission and migratory birds [9, 11, 13, 21]. Two of the most important migratory routes enter Turkey from the northeast and northwest, meet in Antakya, and continue down through continental Africa. Millions of birds flying to the Middle East and Africa from Russia and the Caucasus in autumn and returning in the spring travel over the Northeast Anatolia region of Turkey from end to end. The serological identification of the existence of WNV infection in domestic geese sharing wetlands used by migratory birds to take breaks along their migratory routes, as well as other equid species, is important for this study in terms of the role of migratory birds play in the epizootiology of WNV infection.

Results

WNV nucleic acid was not found in horse and geese samples by RT-PCR. In contrast, WNV nucleic acid originated amplicon (408 bp in length) was detected in 4 donkey seropositive samples.

In this study, 566 samples were examined in terms of the presence of WNV-specific IgG using the C-ELISA method. WNV-specific antibodies were detected in 20% (14/70) of tested donkey sera, 0.8% (1/118) of horse sera, and 1.1% (4/378) of goose sera (Table I).

According to the statistical analyses, the difference in seropositivity rate confirmed in donkeys statistically compared with the seropositivity rate confirmed in horses and Turkish native geese was determined to be very significant (p<0.001).

Distribution of seroprevalence ratios according to the provinces in which the study was carried out is as follows: 1.6% (3/191) in Ardahan, 6.3% (12/189) in Igdir, and 2.2% (4/186) in Kars (Table I). According to the statistical analyses, the antibody positivity rate in the Igdir region statistically compared with the seropositivity rate in the Kars and Ardahan regions was noted to be significant (p<0.05).

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Arthropod-borne viruses are transmitted by blood-sucking arthropods such as mosquitoes, ticks and sand flies. Because the actual cycle of the West Nile virus is between birds and mosquitoes, many studies have been done to research the link between virus transmission and migratory birds [9, 11, 13, 21]. Two of the most important migratory routes enter Turkey from the northeast and northwest, meet in Antakya, and continue down through continental Africa. Millions of birds flying to the Middle East and Africa from Russia and the Caucasus in autumn and returning in the spring travel over the Northeast Anatolia region of Turkey from end to end. The serological identification of the existence of WNV infection in domestic geese sharing wetlands used by migratory birds to take breaks along their migratory routes, as well as other equid species, is important for this study in terms of the role of migratory birds play in the epizootiology of WNV infection.

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Wernery et al. [30] detected WNV seropositivity in the blood serum samples they took from equid species to be 20% in the study they conducted in the United Arab Emirates.

When Turkey’s geographic location and the geographical distribution of the infection are considered, the country is susceptible to many arboviral infections. The first data regarding WNV infection in Turkey dates back to 1970. According to a plaque infection assay (HA) done on human and sheep serum collected from the West Anatolia region, seropositivity in humans was detected to be 6%, and 1-5% in sheep [1, 29]. Five years later, human serum samples collected from the Southeast Anatolia region using HA were checked in terms of the existence of WNV antibodies, and infection seroprevalence was found to be 40% in human serum samples [24]. According to a plaque reduction neutralization test (PRNT) done on 764 blood serum samples belonging to mules, cats, cattle, dogs, horses, humans and sheep from various provinces in Turkey (Hatay, Muğla, Şanlıurfa, İzmir, Adana, Bursa, Ankara, Antalya), WNV seroprevalence was reported to be 2-5% in mules, 4% in cattle, 7-37% in dogs, 5-13% in horses, 4-20% in humans, and 1% in sheep, however, seropositivity was not found in cats [26]. In another study, mosquito samples (Culex pipiens, Ochlerotatus caspius and Aedes species) and human blood serum samples were collected in the province of Şanlıurfa. While RT-PCR, VecTest and virus isolation attempts on vero cell culture done on mosquito samples produced no positive results, an indirect immunofluorescence assay (IFA) done one 181 human blood serum samples detected WNV positive results, while RT-PCR, VecTest and virus isolation attempts on 256 horse and 266 human in Mersin, Adana and Mugla provinces for the presence of WNV RNA using nested and rRT-PCR and WNV RNA was detected in a total of 31 samples in the study. In another study of Ergunay et al. [7] found close relationships to WNV lineage 1 strain ArB310/67 from the Central African Republic, distinct from other WNVs circulating in the Mediterranean Basin, the Middle East, and Eastern Europe.

In this study, 566 samples were examined in terms of the presence of WNV-specific antibodies using the C-ELISA method. In 20% (14/70) of tested donkey sera, 0.8% (1/118) of horse sera, and 1.1% (4/378) of goose sera WNV-specific antibodies were detected. Distribution of seropositivity according to the regions in which the study was carried out is as follows: 1.6% (3/191) in Ardahan, 6.3% (12/189) in İlgdir, and 2.2% (4/186) in Kars.

In the molecular part of the study, WNV nucleic acid presence in serum samples was examined using the reverse transcriptase polymerase chain reaction method. WNV nucleic acid was only detected in four of the positive blood serum samples belonging to donkeys.

In parallel with the study performed by Ledermann et al. [20], detection of WN nucleic acid presence by RT-PCR method in serum samples detected as antibody positive by ELISA technique set us thinking that donkeys whose serum we collected were subclinically infected and samplings might have been done at the end of viremia, that is at the stage where the animals became seroconversion. Because detecting nucleic acid presence in serum samples is a prominent evidence of WN infection existence [12, 20].

It is thought that the reason seropositivity was high in donkeys is due to the fact that because the donkeys in the village and plateaus where the study was conducted are used especially for carrying water, they have a higher risk of being exposed to vectors carrying infectious agents in wetland areas. It was decided that the reason seropositivity was higher in the İlgdir region compared to the other two regions is due to the fact that it has a hotter climate and vector population could be more widespread.

In conclusion, this study has shown that WNV infection is found at different rates in horses, donkeys and domestically-bred geese. In order to avoid the aforementioned disease, reducing the contact between humans and vector mosquitoes and ticks is a helpful way to drop the rate of mortality, morbidity and infection, and this can be achieved through control activities of mosquitoes, ticks, and winged insects or vectors. In addition, the importance of vaccination should not be forgotten and more effort than ever should be made in this area. Also, the necessity of informing animal breeders about infectious diseases and the preparation of animal shelters in modern conditions gains importance.

Acknowledgement

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References

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