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Serological Investigation of West Nile Virus (WNV) Infection in Cats and Dogs

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

West Nile Virus, whose natural life cycle continues between birds and mosquitoes, causes neuropathic diseases in horses, cats, dogs, humans and other mammal animals. Particularly in recent years, as a result of the fact that the number of dam reservoirs have increased and areas where irrigated farming is applied have become widespread, depending on the increase in the population of stinger flies, the increase in various human and animal infections transmitted by these has reached remarkable levels. In this study, the presence/prevalence of WNV in cats and dogs around Burdur province was serologically searched using C-ELISA method. For this purpose, blood samples from 82 cats and 246 dogs of different race, gender and age that were not vaccinated against the so-called disease were taken into coagulant tubes. Besides, if there were any animals showing symptoms of disease among the sampled ones, the kind of the clinical symptoms and the housing/life conditions of the animals was broadly questioned. In the study, WNV specific antibody presence was detected in 0.41% of the tested dog blood serum (1/246) and in 1.22% of the cat blood serum (1/82). From the research log, the cat detected as positive turned out to be a two-year old, female, non-vaccinated Tekir stray cat and the dog was an owned, four-year old, female, regularly vaccinated hound dog. Both positive animals showed no clinical findings. Consequently, in this study, WNV presence was revealed in cats and dogs the Burdur region even though it was at low rates.

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1. INTRODUCTION

West Nile Virus (WNV), which is a neuropathic arbovirus, is classified within the group that is considered as the active Japanese encephalitis (JE) complex found in flavivirus genus of *Flaviviridae* family. There are 10 serological subgroups in the genus which includes the factor carrying single chain pozitive polarity RNA [1-3]. Since it has a virion envelope, the heat is rapidly inactivated in lipid solvents or disinfectants containing detergents [4-7]. Within the structure of WNV, there are a total of ten proteins, seven of which are non-structural (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5) and three of which are structural (capsid [C], envelope [E] and premembrane [prM]/ membrane [M]) and they participate in viral replication [8,9]. The pathogenic varieties seen between the isolates of the virus are thought to be caused by the changes in nucleotide sequencing in E, prM and non-structural proteins [10-12]. WNV isolates have displayed 5 different genetic sources according to phylogenetic analysis, amino acid sequencing changes in envelope proteins and consisting deletions [8,9,13].

Wild and domestic birds and especially Culex type mosquitoes (*Cx. antennatus, Cx. univittatus* ve *Cx. pipiens*) are the main vectors in the transmission cycle of WNV, and contamination with *Aedes* and *Anopheles* has also been reported. Transovarial transmission is seen in mosquito species [14,15]. Bird species are considered as the primary main host where WNV is replicated. Resident and migratory birds play an important role in spreading of WNV during long-range or local migrations [16,17]. Humans, cats, dogs and especially horses may be alternative hosts for WNV and the principal contamination means of the infection factor is by mosquito bites [18,19]. The factor causes mild inflammatory diseases, encephalitis, meningitis or infections characterized by death [2,20,21]. Especially when central nervous system is affected, clinical findings such as twitching on face and muscle, depression and trembling of legs as well as ataxia, weakness of feet, walking defects, trembling of head, tremor, bruxism, blindness, internal organ lesions, neural necrosis, non-suppurative encephalomyelitis in brain and spinal cord have been reported [22,23].

During lab diagnosis of the infection, PRNT, IFAT, enzyme linked immunosorbent assay (ELISA) and molecular techniques are used in order to prevent cross reactions and to distinguish it from other flaviviruses within JE-serocomplex [24].

In this study, we aimed to detect WNV infection presence / seroprevalence in owned (kept in homes or gardens) or stray cats and dogs of different race, gender and age with clinical symptoms or not and to suggest some ways to control and combat the disease. Besides, the data from this study could be useful as a source for the future studies.

2. MATERIALS AND METHODS

2.1 The Sampled Animals

In this study, 328 blood samples were collected from 82 cats and 246 dogs that were owned (kept in homes or gardens) or stray animals of different race, gender and age with clinical symptoms or not. Samplings were applied for animals over three months of age not vaccinated against the infection. The study was carried out in the Burdur region in Turkey (Fig. 1).

Due to epidemiological features of WNV, the owners were also given a survey while blood samples were being collected from cats and dogs in order to contribute to evaluation and discussion of the data to be collected at the end of the study. In the survey, information about cats and dogs (age, race, way of feeding, frequency of trimming of the animal, state of clinical symptoms and vaccination), their life conditions, time periods and frequency of their walking and presence/state of walking areas were questioned.

Out of 82 sampled cats, 48 were female and 34 were male and 63 of these were owned and 19 were stray. The youngest of these cats was three months old and the oldest was eight years old. In the study, out of 246 sampled dogs, 137 were female and 109 were male. The youngest was three months old and the oldest was 13 years old and 159 were owned while 87 were stray.

Within the study, among the sampled animals were 222 owned, 58 stray and 48 in the animal shelter. The classification of owning ways of these animals was stated as from pet shops (n=47), from shelters (n=23), from streets (n=36), private order (n=25), from friends (n=77) and other (n=14) if none of these. When vaccination states of sampled animals were controlled, 222

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

animals were found fully vaccinated (rabies, parvo, combination vaccines), 48 had lack of vaccines and 58 were non-vaccinated. While 184 of animals provided with materials showed no clinical symptoms, 21 had nervous system, 26 digestive system, 43 respiratory system and 54 other clinical symptoms. Sampling was done randomly and the living conditions of the sampled animals were rated as good (n=222), moderate (n=48) and bad (n=58). The sampled 241 animals were proved to be able to walk in open areas, parks/gardens and along lake/ stream/river banks while 48 had no walking areas (animal shelter).

2.2 Serum Samples

Samples were collected between May 2016 and October 2017. Blood sera samples were collected by cephalica antebrachii vein or saphenaparva vein puncture into vacum tubes with clot activator from animals. After clotting at room temperature for 15-20 minutes and centrifugation at 3000 g, at 4°C for 10-15 minutes, sera were carefully harvested and stored at –20°C until analysis.

2.3 Competitive Enzyme Linked Immunosorbent Assay (C-ELISA)

Anti-prE antibody presence of WNV against structural envelope protein (prE) was searched in serums collected from blood samples of cats and dogs using competition-ELISA method. For this purpose, ID Screen® West Nile Competition Multi Species ELISA test kit produced by ID.vet company was used (Product code: WNC-2P, Lot no: A76, IDvet, Grabels, France). This method functions within solid phase indirect C-ELISA principle.

The test was performed according to the procedure stated by the producing company. The OD of each well was read using an ELISA reader at a wavelength of 450 nm.

2.4 Statistical Analysis

Statistical analysis was carried out via the Statistical Package for Social Sciences software (IBM SPSS Statistics 20.0, SPSS inc., Chicago, IL, USA). The main differences between species was evaluated using the Chi-Square ($\chi 2$) test. At the end of the study, the data from which the value of $P<0.05$ was derived was accepted as significant.

3. RESULTS

In the study, at the end of C-ELISA to detect WNV antibodies, seropositivity rates for WNV infection were found as 1.22% (1/82) for cats, 0.41% (1/246) for dogs and 0,61% (2/328) in total (Table 1).

The seropositive cat turned out to be over two-year of age, female and stray. It had no protective vaccination against any infection and was a stray tabby cat while the seropositive dog was an owned four-year old female hound dog with routine vaccination. Both positive animals showed no clinical symptoms.

At the end of the statistical analysis, differences between seropositivity rates detected for cats and dogs were considered insignificant ($P>0.05$) when statistically compared.

Table 1. WNV seropositivity rates of sampled species

Species	Number of samples	WNV	
		Antibody (+)	%
Dog	246	1	0.41
Cat	82	1	1.22
Total	**328**	**2**	**0.61**

4. DISCUSSION

Arboviral diseases (WNV, Crimean-Congo hemorrhagic fever, tick encephalitis virus, sand fly fever etc.) are transmitted by blood sucking arthropods such as mosquitoes, ticks and sand flies. The diseases caused by these viruses are amongst the most vital public health problems confronted by the world of this millennium because of global warming, demographic changes, developing modern transportation systems, destruction of natural ecological borders due to urbanization and new fields to improve the communication between vector species and hosts.

Since the main cycle of WNV is between birds and mosquitoes, numerous studies have been carried out on the connection between convection of the virus and migratory birds [25-28]. Migration routes of migratory birds go along from Europe, Middle and Northern Asia to wetlands, rivers and coastlines of Northern and Eastern Africa. When considered from this aspect, it has a prominent state and international significance in terms of the fact that it builds a bridge for migratory birds between Turkey, Africa, Asia and Europe continents and hosts more than 400 migratory bird species. Two of the most important wild bird migration routes of the world start from northwest and northeast of Turkey, merge in south and then go down to Africa. Millions of birds, coming from Russia and Caucasus and travelling to Middle East and Africa in autumn and going back in spring, fly across Turkey. Therefore, infected migratory birds constitute a potential risk. Especially venues, rivers and coastlines located on or near the flying routes of migratory birds and areas of wetlands should be considered as special risk areas.

In this regard, geographical localization of the area where the study is carried out is highly significant. The study area is located on 37°43' North and 30°17' East coordinates and the altitude is 950 meters. The sampling area is in the Mediterranean region of Turkey and is located in a geography, also called as region of lakes, where there are rich water sources and rivers. The seventh largest lake of Turkey, Burdur Lake, is also found here within 37°45' North and 30°12' East coordinates with a surface area of 250 kilometer square, and it hosts nearly a hundred wild bird species. Many large and small dam lakes that are natural or built for irrigation are seen in the area where research samples were collected. In terms of its geographical settled situation, the study area which is located on the route of migratory birds flying over Turkey is highly rich in wetlands where these birds stop over along the migration routes they use. The annual average heat value of the area of study (13.2°C) and number of wet days [29] is quite high. Apart from these, agriculture based on irrigation is performed actively and commonly as well as stockbreeding in the area. When all these conditions are evaluated and the epidemiological cycle of WNV is considered, the sampling area is thought to be under potential risk for living areas of wild birds and vector mosquitoes.

Studies have confirmed that WNV infection increases in animals and humans especially in autumn and hot, arid summers. Epidemics have been encountered in America, Asia, Africa, Europe and many countries on the Mediterranean coast during many periods since 1937, when the first original isolation of the factor was performed, to our day [2,6,30,31].

There are numerous studies around the world for animals and humans on WNV using various lab techniques. In these studies on many animal species, WNV antibody positivity was stated as between 2.9-38% [29,32-40]. The positivity was between 9-14.9% for cats [37,38,40] while it was 4.9% [38] in China and 26 % [37] in America for dogs.

The first data on WNV infection in Turkey was seen in 1970. As a result of Hemagglutination Inhibition test (HI) applied on human and sheep serum collected from Western Anatolia region, the seropositivity was detected as 6% for humans and 1.5% for sheep [41,42]. Following this, in 1975, human serum samples collected from Southeast Anatolia region in Turkey were controlled in terms of WNV antibody presence using HI test and the infection seroprevalence was found at rates around 40% [43]. In the study by Ozkul et al. [44] in 2006 using PRNT test, WNV seroprevalence was detected as 4% for cattle, 1% for sheep, 2.5% for mules, 5-13% for horses, 7-37% for dogs and 4-20% for humans but no seropositivity was found for cats. In

another study, no positivity was seen at the end of PCR, VecTest and Vero cell culture tests applied on vector mosquito samples (*Culex pipiens Linnaeus, Ochlerotatus cospius ve Aedes types*) while WNV seropositivity was found at a rate of 16 % by indirect immunofluorescence test in 181 human blood serum samples [45]. Apart from these studies, in the research applied on humans about WNV infection using many different lab techniques in Turkey, WNV seropositivity was stated as between 0.9% and 47.8% [27,31,37,41,46-48]. WNV antibody positivity was found as between 1% and 37% for animals [48-51]. The latest research on WNV in Turkey was performed for horses, donkeys and domestic geese by Yıldırım et al. [51] In this study, WNV antibody positivity was detected as 0.8% for horses, 20% for donkeys and 1.1% for domestic geese.

In our study, WNV specific antibody presence in 328 blood samples was searched by C-ELISA and positivity was found as 0,61% (2/328). WNV specific antibody presence was detected in 0.41% (1/246) of dog blood serum and 1.22% (1/82) of cat blood serum.

When living conditions of WNV antibody positive cat and dog were considered in our study, it was revealed that both had free walking areas and were in constant contact with open areas, therefore, they were more likely to meet vectors carrying the infection factor most probably found in wetlands and green areas. Similarly, once the usage area of the antibody positive hound dog and the hunting season was considered, the dog was believed to have had the infection depending on summer and autumn seasons when vector mosquito populations were the highest.

In our study, the situated seropositivity rate was parallel to the previous ones in Turkey. Besides, our study happens to be the first specific one in which a positivity was found for cats.

5. CONCLUSION

Consequently, in this study, the presence/ prevalence of WNV infection for cats and dogs was serologically revealed. In order to combat and avoid the so-called disease, decreasing the contact between vectors and hosts is a helping way to decrease morbidity and mortality rates of WNV infection and this can only be achieved by vector combating programmers. To prevent the infection from spreading around wide geographical areas, carrying out intense follow-up studies on breeds of WNV and information sharing and cooperation between countries will be beneficial. Monitoring the applied measures in all affected countries and countries under risk and collecting more information about epidemiological and public health outcomes of WNV infection as well as entire analysis of the present epidemics must also be of great importance. Informing animal owners about infectious diseases and the importance of protective vaccine applications should be taken into consideration as well.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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