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Research Article

An investigation of West Nile virus (WNV) infection in local wild birds species

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ABSTRACT

The transmission of West Nile Virus (WNV) to new locations is mostly facilitated by migratory birds. Türkiye's domestic ducks, geese, and chickens have already tested positive for WNV by serology. This study was conducted to identify the seroprevalence of WNV in wild bird species because wild birds in the Western Mediterranean Region are found along migration routes from Africa to Europe, they are home to a wide variety of bird species due to the abundance of lakes and wetlands, the mild Mediterranean climate, and some areas that are suitable habitat for mosquitoes due to their low altitude. Serum samples were taken from 141 wild birds in Isparta (66), Burdur (42), and Antalya (33) for this study on birds in the wild. During serological studies, there was no evidence of WNV-specific antibodies.

INTRODUCTION

The RNA virus known as West Nile Virus (WNV) belongs to the Flaviviridae family. Antigenically, it is also very similar to the dengue and yellow fever viruses. The substance is membrane-bound, single-chained, icosahedral, positively polarized, and between 40 and 60 nm in size (Devine, 2003). WNV has ten serological subgroups. The RNA genome of WNV is coded by three structural and seven non-structural proteins. Virion creation requires structural proteins, but immunological infiltration and viral transcription and replication require non-structural proteins (Kramer et al., 2008; Kireçci et al., 2011). The West Nile region of Uganda is where WNV was initially discovered in 1937. In addition to Africa, this agent was found in Asia, the United States, the Southern and Eastern areas of Europe, Australia (Marfin & Gubler, 2001) and Türkiye (Kalaycioglu et al., 2012). Though bloodsucking flies of the *Culex and Aedes* species are the disease's primary carriers, tick infection has also been documented. The hosts on which the virus is reproduced are birds. The last two hosts are thought to be horses and people. The WNV titer is quite high in wild bird blood. Birds that migrate have a part in spreading the virus to new areas. The majority of hosts on whom the virus normally replicates are birds. One of the main causes of this is that reservoir birds may produce significant amounts of viremia (Hayes et al., 2005; Uyar & Bakır, 2016). This infectious agent is typically found in avian species and is spread by mosquitoes to people and domesticated mammals. As a result, however infrequently, domesticated creatures and humans may exhibit diseases and mortality cases. WNV infection is typically found in wetlands locations with high mosquito populations, warm and hot temperature zones, and migratory birds may also carry it. With this work, we sought to identify the serological evidence of WNV infection in a number of wild bird species found in the Western Mediterranean region.

MATERIAL and METHODS

Animals and Sample collection

In this study, 0.5-1 ml blood was collected from veins on the inner wing surfaces of wild birds in, Isparta SDU Faculty of Agriculture Research Farm (66) Antalya Zoo (33) and Burdur-Karakent village Lisinia Nature Rehabilitation Center (19). Isparta, Antalya, Burdur, coordinates, respectively (30 E 33, 37 N 46), (30 E 42, 36 N 54), (30 E 17, 37 N 43). The following tools were employed to achieve this goal: a disposable sterile tuberculin needle and injector, alcohol, betadine disinfectant, sterile adhesive bandage, and disposable sterile gloves. Throu-

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gh the use of a sterile injector, blood samples were obtained from the hearts of dead animals.

Figure 1. Locations of serum samples collected from Southwest region of Türkiye.

The collected blood samples were put into sterile blood tubes and sent to the lab with a +4 °C cold chain. Blood samples were centrifuged at 2000 rpm for 20 minutes. The collected serum samples were then placed in eppendorf tubes and stored at a low temperature (-20 °C) until testing.

Indirect ELISA

Blood samples were brought to room temperature after being solubilized in a bain marie. During testing, the WNV-ELISA (Cat No. WNC-2P) (antibody) test kit from ID-Vet (France) company was utilized. The testing was done in accordance with the company's protocol. The test findings were used in the calculation of the test kit and were either detected as positive, suspicious, or negative.

RESULTS

At the conclusion of this investigation, no local wild bird species in Türkiye's Western Mediterranean region (Burdur, Isparta, and Antalya) had been shown to have WNV antibodies (Table 1). During testing, no questionable samples were discovered.

DISCUSSION

According to a report by the OIE (2018), WNV was thought to have been a disease discovered in humans in Africa around the 1950s (2018). Prior to 1996, encephalitis-progressing WNV cases were noted in a number of residents of France, Greece, Israel, Italy, North America, Romania, Russia, and Tunisia. In the 1960s, WNV infection was initially discovered in horses in Egypt and France (Schmidt & Mansoury, 1963). The infection was discovered in horses in the following years in the USA, Spain, Italy, France, Israel, Morocco, Canada, and Argentina (Frost et al., 2012). Antibodies against WNV have been found in humans, animals, and livestock in the Mediterranean and Aegean regions (Ozkul et al., 2006). These include sheep, cattle, dogs, horses, donkeys, and mules. Albayrak & Ozan (2013) were unable to find specific antibodies against WNV in sera of horses, sheep, cattle, and buffaloes gathered from various places in the Black Sea region.

Birds and mosquitoes of the Culex sp. species, which are involved in the virus's biological cycle, are important in the transmission of WNV infection. There was a significant incidence among birds, including geese, chickens, pigeons, and swallows. However, it was discovered that the virus had spread without the aid of vectors in a study on migratory geese. In both horses and people, the virus does not replicate (Erdem & Pahsa, 2003; Austin et al., 2004). Within their habitats, mosquitoes deposit their eggs in quiet, muddy waterways. They also use the blood of migrating birds that live in these locations to sustain themselves. They spread a virus through their saliva to the birds they feed on while they are doing so, which results in a protracted period of viremia. Migratory birds are a crucial reservoir for spreading the virus from one region to another (Hayes et al., 2005).

In particular, epidemics were observed as migrating birds flew from Africa to Europe. Because they travel greater distances, viremic migratory birds spread the agent more widely than domestic birds do. When birds are migrating and there are a lot of mosquitoes, which is towards the conclusion of the summer season and the beginning of fall, WNV epidemics appear (Hayes et al., 2005; Malkinson et al., 2002). Türkiye is situated along the bird migration pathways that connect Africa and Europe. The Western Mediterranean region, sometimes referred to as the "Lake District" is one of the most significant stops for migrating birds. Four separate locations in the Western Mediterranean region saw wild bird samplings. Migrational birds that had been injured while being hunted provided samples from the province of Burdur to the veterinary faculty clinics. In terms of species diversity, the zoo in Antalya that houses wild birds is significant to us. These gathered samples were thought to be crucial in identifying WNV seroprevalence.

ELISA, such as hemoagglutinin inhibition (HI), Virus Neutralization (VN), and Plaque Reduction Neutralization assays are used to determine the seroprevalence of WNV in birds (OIE, 2018). Yapici et al. (2012) used the ELISA test to identify serological signs of WNV infection in birds and thought it was a quick, easy, and accurate method. According to Padilla et al. (2009), the ELISA test's specificity was 99.4% and its sensitivity was 84.9%. Domestic chickens in the Konya province were not seropositive for WNV, according to Yapici

Table 1. WNV antibody results for wild birds in Western Mediterranean region.

Species	Numbers	WNV ELISA (Ab)
Gold Phoenix (*Phoenix*)	1	Negative
Ruddy shelduck (*Tadorna ferruginea*)	1	Negative
Brahma (*Gallus gallus*)	3	Negative
Pekin cochin (*Gallus gallus*)	3	Negative
Giant cochin (*Gallus gallus*)	3	Negative
Common pochard (*Aythya ferina*)	2	Negative
American flamingo (*Phoenicopterus ruber*)	1	Negative
Polish chicken (*Gallus gallus*)	1	Negative
Silver pheasant (*Lophura nycthemera*)	1	Negative
Common pheasant (Phasianus colchicus)	17	Negative
Lady Amherst's Pheasant (*Chrysolopus amherstiae*)	1	Negative
Japanase chicken (*Gallus gallus*)	1	Negative
Lesser black-backed gull (*Larus fuscus*)	1	Negative
Rook (*Corvus frugilegus*)	1	Negative
Rock dove (*Columba livia*)	7	Negative
Chukar partridge (*Alectoris chukar*)	61	Negative
Long-legged buzzard (*Buteo rufinus*)	2	Negative
Reeves's pheasant (*Syrmaticus reevesii*)	1	Negative
Little owl (*Athena noctua*)	2	Negative
Eurasian collared dove (*Streptopelia decaocto*)	1	Negative
White stork (*Ciconia ciconia*)	8	Negative
Budgerigar (*Melopsittacus undulatus*)	1	Negative
Muscovy duck (*Cairina moschata*)	1	Negative
Pekin duck (*Anas platyrhynchos*)	1	Negative
Great white pelican (*Pelecanus onocrotalus*)	1	Negative
Common starling (*Stumus vulgaris*)	1	Negative
Zibrit golden chicken (*Gallus gallus*)	1	Negative
Dead sea sparrow (*Passer moabiticus*)	3	Negative
Common buzzard (*Buteo buteo*)	5	Negative
Indian peafowl (*Pavo cristatus*)	5	Negative
Wild turkey (Meleagris gallopavo)	1	Negative
Green pheasant (*Phasianus versicolor*)	1	Negative
Mallard duck (*Anas platyrhynchos*)	1	Negative
TOTAL	**141**	

et al (2012) .'s research. In his investigation, Pir (2016) found that 4.3% of domestic birds had antibodies against WNV. According to him, high seropositivity zones are found around the Kızılırmak river and may be brought on by migrating birds passing through the delta. In their research, Ergünay et al. (2014) discovered a 9.9% seropositivity rate for WNV in ducks near the province of Kars. It was found to be 1.1% (4/378) in geese near Türkiye's northeast by Yildirim et al. (2018). WNV seropositivity rates were reported to be 3.1% for hens, 0.8% for ducks, 1.8% for geese, and 17.9% for turkeys by Pir and Albayrak (2017). 32 out of 736 serum samples were discovered to be positive (4.3%) for WNV antibodies. Out of 155 bird blood serum samples taken in Malaysia, WNV IgG ELISA was used to detect seropositivity in 30 samples (27 migratory birds and 2 indigenous water birds). They claimed that mosquitoes may be connected to WNV illnesses reported in local and migratory aquatic birds (Ain-Najwa et al. 2020).

According to estimates, migratory birds are one of the main vectors of WNV transmission into previously uninfected nations (Rappole et al., 2000). Additionally, the WNV viremia time in investigations on several bird species was no longer than a week (del Amo et al., 2014). Due to the fact that ELISA-positive birds had not recently been infected to WNV and were therefore unlikely to be at the viremia stage, neutralized antibody testing on sera was not conducted on them (Jourdain et al., 2011). For instance, it may take about 12 days for wild birds to migrate from Spain to Germany and Holland. Under these

circumstances, the migratory birds may contract a fly-borne illness cycle, and since the stress of the migration does not affect the duration of the viremia phase, the disease may not spread (Chevallier et al., 2010).

Numerous bird species are susceptible to WNV infection, which can develop and manifest as a variety of clinical signs. Although some animals are immune to the virus, others can have MSS flaws. Domestic goose fatalities and abnormalities have been documented in Canada and Israel. Infection with WNV is fatal to wild birds in Europe (Zeller & Schuffenecker, 2004). White storks and domestic geese showed signs of encephalitis and paralysis during the WNV outbreak in Israel (Malkinson et al., 2002). Many domestic and wild birds were found to have WNV infection in an American zoo (Austin et al., 2004; Steele et al., 2000). In this study, samples from the province of Isparta, Burdur and Antalya antibodies against WNV could not be found. Similarly, in more than 4000 blood serum samples taken from 3300 wild birds, Balança et al. (2009) found a seropositivity rate of fewer than 1%.

According to an OIE report from 1999, domestic geese in Israel had a 27% prevalence of WNV infection. Geese 60 to 70 days old may exhibit clinically detectable central nervous system abnormalities (Swayne & Spackman, 2013). However, because they do not frequently come into contact with flies, birds raised indoors are less prone to contract the disease. Chickens and turkeys are not clinically affected by WNV (OIE, 2000). Similar to this, Calle (2000) reported that out of 277 birds of 74 species kept indoors, WNV seropositivity was not found in 36 of them. Therefore, the fact that we were unable to identify seropositivity in samples obtained from the SDU Agricultural Faculty Research Farm may be related to the fact that these animals are cared for, fed, and housed indoors.

CONCLUSIONS

When WNV infection is discovered in wild bird species, people and domestic mammals may also be at risk. Our nation offers a suitable habitat for WNV hosts and vectors to survive, similar to arboviral infections, in terms of geography and climate. The illness can also be widely prevalent in the nations that are next to us. Based on the findings of this study conducted in the Western Mediterranean region, which is on the migratory routes of migrating birds and features lakes and wetlands, we advise conducting additional serosurvey investigations in densely populated animal groups.

DECLARATION

Ethics Approval

This study was approved by Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee at the meeting dated 20.06.2013 with the number of 36 decisions.

Conflict of Interest

The authors declared that there are no conflicts of interest.

Consent for Publication

For this study, a permit warrant has been taken by the article named as Research Permit from TR Ministry of Forestry and Water Affairs, Nature Reserve and General Directorate for National Parks dated 21st August, 2013 and numbered 72784983-488.04-156205.

Author Contribution

Idea, concept and design: MK, NM

Data collection and analysis: MK, NM, KA, AA

Drafting of the manuscript: YY, HSS, YSA

Critical review: MK, KA, SH, OB

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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