

Investigation of the presence of Zika, Dengue, Chikungunya, and West Nile virus in *Aedes* type mosquitoes in the Eastern Black Sea area of Turkey

Türkiye'nin Doğu Karadeniz Bölgesindeki *Aedes* tipi sivrisineklerde Zika, Dengue, Chikungunya ve Batı Nil virüsü varlığının araştırılması

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ABSTRACT

Objective: Arboviruses are a group of viruses transmitted by arthropods, and are characterized by a wide geographic distribution that causes various infections in humans, both in the enzootic and urban cycles, in environments associated with the presence of vectors. Dengue (DENV), Chikungunya (CHIKV), Zika (ZIKV), yellow fever, and West Nile virus (WNV) infections are increasingly public health problems that spread throughout the world. The best known vectors of these viruses are the invasive vectors of *Aedes aegypti* and *Aedes albopictus*. The aim of this study is located in the Black Sea in northeastern Turkey located in established populations of *A. aegypti* and *A. albopictus* was to investigate the presence of viruses transmitted by mosquito species.

Methods: From April to October 2016, it was studied on mosquitoes in the provinces from the western border

ÖZET

Amacı: Arbovirüsler, eklembacaklılar tarafından bulaşan bir virüs grubudur ve vektörlerin varlığı ile ilişkili ortamlarda hem enzootik hem de kentsel döngüler içerisinde insanlarda çeşitli enfeksiyonlara neden olan geniş bir coğrafi dağılım ile karakterizedirler. Dengue virüsü (DENV), Zika virüsü (ZIKV), Chikungunya virüsü (CHIKV) ve Batı Nil virüsü (BNV) enfeksiyonları, dünyaya yayılan ve giderek artan halk sağlığı problemleridir. Bu virüslerin en bilinen vektörleri; invaziv vektörler olan *Aedes aegypti* ve *Aedes albopictus*'tur. Bu çalışmanın amacı, Türkiye'nin kuzeydoğusunda yer alan Karadeniz bölgesinde kurulmuş populasyonlarda bulunan *A. aegypti* ve *A. albopictus* sivrisinek türleri tarafından bulaştırılan virüslerin varlığını araştırmaktır.

Yöntem: 2016 yılının Nisan-Ekim ayları arasında batı sınır kapısından Ordu iline kadar olan illerde ve iç bölgelere giriş noktaları olan bölgelerde sivrisinekler

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gate to the province of Ordu and areas with entry points to the inner regions. A total of 267 mosquitoes were collected from 51 different regions of the Black Sea region. All samples were tested for the presence of Dengue, Chikungunya, Zika, and West Nile virus.

Results: A total of 267 mosquito samples, 38 *A. aegypti* and 229 *A. albopictus*, were obtained, of which eight were male and 259 were female. No positivity was detected in terms of DENV, CHIKV, ZIKV and WNV in *A. aegypti* and *A. albopictus* mosquitoes included in the study. No positivity was found in panflavivirus.

Conclusion: The fact that none of the investigated viruses were detected in *A. aegypti* and *A. albopictus* mosquitoes is an important data that vectors in this region have not yet encountered DENV, CHIKV, ZIKV and WNV. In our country, where cases of foreign travel related to these viruses are seen, there may be a possibility of autochthonous transmission in case of encountering viruses that will come from outside and existing vectors. For this reason, similar studies should be carried out in other regions and healthy and up-to-date maps should be created in terms of vector and virus tracking. Thus, when the presence of these viruses entering the country is detected, it will be possible to take quick and effective measures.

Key Words: Zika virus, Dengue virus, Chikungunya virus, West Nile Virus, Flaviviruses, *Aedes*

üzerinde çalışılmıştır. Karadeniz bölgesinin 51 farklı bölgesinden toplam 267 sivrisinek toplanmıştır. Tüm örnekler Dengue, Chikungunya, Zika ve West Nile virüsünün varlığı açısından test edilmiştir.

Bulgular: 38 *Aedes aegypti* ve 229 *Aedes albopictus* olmak üzere toplam 267 sivrisinek örneği elde edilmiş, örneklerin 8'i erkek, 259'u dişi olarak belirlenmiştir. İncelemeye alınan *Aedes aegypti* ve *Aedes albopictus* sivrisineklerinde DENV, CHIKV, ZIKV ve BNV açısından pozitiflik saptanmamıştır. Panflavivirus açısından pozitiflik bulunmamıştır.

Sonuç: *A. aegypti* ve *A. albopictus* türü sivrisineklerde, araştırılan virüslerden hiçbirinin saptanmaması henüz bu bölgedeki vektörlerin DENV, CHIKV, ZIKV ve BNV ile karşılaşmadıkları yönünde önemli bir veri elde edilmiştir. Bu virüslere bağlı yurt dışı seyahati kaynaklı vakaların görüldüğü ülkemizde, dışardan gelecek olan virüslerle mevcut vektörlerin karşılaşması durumunda otoktanöz bulaş ihtimali söz konusu olabilecektir. Bu nedenle diğer bölgelerde de benzer çalışmalar yapılarak vektör ve virus takibi açısından sağlıklı ve güncel haritalar oluşturulmalıdır. Böylece ülkeye giriş yapan bu virüslerin varlığı saptandığında hızlı ve etkin tedbirler almak mümkün olacaktır.

Anahtar Kelimeler: Zika virüs, Dengue virüs, Chikungunya virüs, Batı Nil Virüsü, Flavivirüsler, *Aedes*

INTRODUCTION

Dengue, Chikungunya, Zika, Yellow Fever, and West Nile Virus infections are increasingly public health problems that spread throughout the world. The best-known vectors of these viruses are the invasive vectors of *A. aegypti* and *A. albopictus*. The formation of arboviral diseases depends on

complex interactions between the vector, the pathogen, and the environment. The sensitivity of mosquitoes to viral pathogens varies depending on temperature, relative humidity, location, and many other variables (1,2). There is a strong relationship between the distribution of infectious diseases and their vectors. Numerous studies have developed risk maps of arboviral illnesses according to this vector

distribution (3-5). Dengue fever is the most common disease among arboviral diseases and is known to have pandemic potential. Dengue virus causes severe influenza-like disease and can sometimes be severe or even fatal (6). The incidence of the disease has increased by 30 times in the last 50 years. Severe dengue infections were first seen in the 1950s during outbreaks in the Philippines and Thailand. Today it affects Asian and Latin American countries, and it has become a significant cause of hospitalization and death in children and adults (7). Chikungunya fever is the second most common arboviral disease. It is usually characterized by sudden fever elevation seen with joint pain. Other common symptoms are muscle pain, headache, nausea, weakness, and rash. The virus may cause acute, subacute, or chronic disease. It is endemic in Africa but is increasingly spreading across the Indian Ocean, Asia, the South Pacific, Southern Europe, the Caribbean, and Central America (6). The rapid emergence of the virus has been linked to the geographic expansion of the vectors *A. aegypti* and *A. albopictus* (9). Outbreaks were observed in the Americas and some European countries after 2005 (1,2,10). ZIKV was first detected in Uganda in 1947, and then epidemics were recorded in Africa, the Americas, Asia, and the Pacific (11). The symptoms of the disease caused by ZIKV include mild fever, skin rash, conjunctivitis, muscle and joint pain, weakness, headache, and mild symptoms, which last for 2-7 days. However, in 2015, with the association of microcephaly and Guillain-Barré syndrome in an outbreak in Brazil, it suddenly attracted the attention of the whole world (12-14). The World Health Organization (WHO) declared that ZIKV was a global public health emergency in February 2016 (11). The geographical distribution of ZIKV is closely related to the distribution of *Aedes* mosquitoes, the primary vector of ZIKV transmission (15). *A. aegypti* and *A. albopictus* are the primary mosquito vectors for ZIKV (16-17). The presence of *A. aegypti* and *A. albopictus* mosquitoes in Turkey has been shown in previous studies (18,19). It is known that the West

Nile virus is transmitted by mosquitoes of the species *Culex pipiens*, but this virus may also be transmitted by mosquitoes of the genus *Aedes* (20,21). Infection is often asymptomatic. Fever, polyarthropathy, rash, lymphadenopathy, headache, and myalgia are the main findings in symptomatic patients. In its neuroinvasive form, CNS involvement is present and may be fatal. West Nile virus is maintained in nature in a cycle involving transmission between birds and mosquitoes. People, horses, and other mammals can be infected. It is widespread in Africa, Europe, Asia, the Middle East, and Australia (22). In the past, Southern Europe, Israel, the Middle East, Romania, and Russia have had outbreaks. Recent epidemiological changes have occurred, and infections leading to fatal meningitis or encephalitis occurred during the 1999-2010 West Nile virus epidemic seen in the Americas (23). In Turkey, in 2010, autochthonous cases started to be detected (24). The aim of study is located in the Black Sea in northeastern Turkey located in established populations of *A. aegypti* and *A. albopictus* was to investigate the presence of viruses transmitted by mosquito species. Thus, the possibility of autochthonous transmission of these viruses in our country can be evaluated.

MATERIAL and METHOD

Study area, specimen collection and identification

From April to October 2016, mosquitoes were collected in the provinces from the western border gate to the province of Ordu and in areas with entry points to the inner areas. Within this scope, samples were collected from Artvin, Rize, Trabzon, and Ordu provinces for ten days each month. Mosquito larvae and pupae samples were collected with standard larvae scoops, larval pipettes, and light traps. Systematic evaluations of the samples were performed in the laboratory with stereomicroscopes. Thus, the samples obtained from the field studies were grouped according to their types, put into separate sampling containers, and sent to the laboratory with labels. Mosquitoes were grouped according to type and

according to the field in which they were collected, and a total of 46 pools were formed. A total of 267 mosquitoes were collected from 51 different areas in the Eastern Black Sea region. The generated mosquito pools were tested in the laboratory.

Specimen processing and viral RNA extraction

Mosquitoes were placed in Eppendorf tubes with ten mosquitoes per pool and homogenized using sterile sand in Dulbecco's PBS supplemented with 0.2% BSA and antibiotics (100 IU of penicillin/mL and 100 g of streptomycin/mL). The homogenates were clarified by low-speed centrifugation at 1500 rpm for 15 min at 4 °C, and supernatants were aliquoted and stored at -80 °C. Viral RNA was extracted from 400 µL of supernatant using the QIAGEN EZ1 Virus Mini Kit v2.0 and EZ1 advanced extraction robot (QIAGEN, Hilden, Germany) according to the manufacturer's instructions with an internal control.

Real time RT-PCR

Each sample was tested for the presence of West Nile virus (25), Zika virus (26), Chikungunya virus (27), Dengue virus (28), and also panflavivirus (29), using an ABI 7500 real-time PCR (polymerase chain reaction) platform (Applied Biosystems, Foster City, CA). Real-time RT-PCR was performed using the SuperScript III Platinum One Step qRT-PCR System kit. Primers and probes used in this study was given in Table 1.

Ethical Statement

Ethics committee approval was not required as the study was a study conducted in vector mosquitoes and was not conducted in humans or animals. The study was carried out with the approval of the Ministry of Health, Public Health Agency of Turkey, dated 13.06.2016, number 10712557-136.99-E.397 and barcode 00025198221.

Table 1. Primers and probes

| Virus | Primer name | 5-----3 |
|-------------------|-------------------------|---|
| West Nile virus | WN/ProC/10/F | CCTGTGTGAGCTGACAACTTAGT |
| | WN/ProC/153/R | GCGTTTTAGCATATTGACAGCC |
| | WN/ProC/89-113/FT Probe | (FAM)-CTGGTTTCTTAGACATCGAGATCT-(Tamra) |
| Zika virus | Zik1/835/F | AAGCTYCGCGTCCTTTACCAAG |
| | Zik1/911/R | CCAAATTGTCCYGGTCTTCCT |
| | Zik1/860/FT Probe | (FAM)-CCAATGTCYTCMGCCTGGACACCTTT-(Tamra) |
| Chikungunya virus | Chik/E1/10366/F | AAGCTYCGCGTCCTTTACCAAG |
| | Chik/E1/10554/R | CCAAATTGTCCYGGTCTTCCT |
| | Chik/E1/10486/FT Probe | (FAM)-CAATGTCYTCMGCCTGGACACCTTT-(Tamra) |
| Dengue virus | DENV-F | 5'-UTR region (Trioplex, Real-time RT-PCR Assay (CDC)) |
| | DENV-R1 | |
| | DENV-R2 | |
| | DENV-P | |
| Panflavivirus | Flavi all S | TACAACATgATggggAARAgAgARAA |
| | Flavi all AS 2 | gTgTCCCAgCCNgCKgTgTCATCWgC |
| | DEN4 F | TACAACATgATgggRAAACgTgAGAA |

RESULTS

A total of 267 mosquito samples were examined. Female mosquitoes constitute 95.8% (254/267) of the study group. *A. albopictus* accounts for 86.2% (230/267) of mosquitoes. 62.5% (167/267) of the samples were collected from Artvin, 18.4% (49/267) from Trabzon, 14.6% (39/267) from Rize and 4.5% (12/267) from Ordu provinces. All of the samples collected in Trabzon consisted of *albopictus*, while

albopictus species were seen as dominant in Artvin and Ordu, and in Rize the type of *aegypti* was seen at a higher rate (Table 2). All samples were tested for the presence of Dengue, Chikungunya, Zika, and West Nile virus and none of the samples were positive. In addition, no positive results were found in the panflavivirus PCR assay performed for all samples. It was determined that these arboviruses were not found in the *Aedes* mosquitoes collected from the Eastern Black Sea region of Turkey (Figure 1).

Table 2. Properties of the studied mosquitoes

| | | Species | | | | Total |
|-----------|---------|-------------------|--------|----------------------|----------|-------------|
| | | <i>A. aegypti</i> | | <i>A. albopictus</i> | | |
| | | ♀ | ♂ | ♀ | ♂ | |
| Provinces | Artvin | 7 | 1 | 156 | 3 | 167 (62.5%) |
| | Ordu | 1 | 0 | 11 | 0 | 12 (4.5%) |
| | Rize | 21 | 7 | 11 | 0 | 39 (14.6%) |
| | Trabzon | 0 | 0 | 49 | 0 | 49 (18.4%) |
| | | 29 (10.8%) | 8 (3%) | 227 (85%) | 3 (1.2%) | 267 |



Figure 1. Provinces where mosquitoes are collected and viruses are investigated (<https://earth.google.com/web>)

DISCUSSION

The presence of *A. aegypti* and *A. albopictus* mosquitoes in the Black Sea region where mosquitoes were sampled was shown previously (18). However, the investigation of arboviruses in *Aedes* mosquitoes in this region is necessary to determine whether the people are at risk of these diseases. The presence of these species of mosquitoes, which are the primary vectors of those viral agents, poses a threat. Therefore, the existence of viruses in these vectors should be monitored at regular intervals. Although the sensitivity of vectors to viruses varies according to the conditions of the region in question, it is possible that viruses will be able to enter a country as international travels and trade are commonly practiced. In Turkey, there are some cases of foreign-origin infections with the Dengue, Zika, and Chikungunya viruses from time to time. These cases are generally seen in citizens who return after a trip abroad or in guests coming to visit Turkey from abroad (30,31). In our country, autochthonous cases have not been reported yet. However, the situation for West Nile virus is different. Confirmed cases of West Nile virus have been reported in Turkey since 2010 (24,31). West Nile cases in Turkey are diagnosed more often in the southern provinces. In our study, panflavivirus PCR tests were performed after Dengue,

Zika, Chikungunya, and West Nile virus PCR tests were found to be negative. As a result of those tests, no positivity was found. It was shown that other flaviviruses (yellow fever, Japanese encephalitis virus) were not present in *Aedes* mosquitoes in this region. However, a limitation of our study is that these virus were not investigated in non-*Aedes* mosquitoes. Especially for the West Nile virus, *Culex pipiens* mosquitoes play the primary vector role, and such mosquitoes need to be studied. In particular, this virus has been previously researched due to West Nile cases seen in the south of Turkey in 2010 (24,31). Some studies also investigated the presence and transmission of the West Nile virus in *Aedes* mosquitoes (20,21).

In conclusion, as a result of our research, none of the viruses investigated in *A. aegypti* and *A. albopictus* mosquitoes were detected; it is an important finding that the vectors in this region are not carrying the Dengue, Zika, Chikungunya, or West Nile viruses. Similar studies in other parts of the country are needed to create a vector and arboviral infection map of Turkey. New studies should be conducted in which the number of samples is higher and more diverse mosquitoes are investigated. Thanks to vector and virus monitoring, it will be possible to take fast and effective measures when the presence of these viruses is detected.

ETHICS COMMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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