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REVIEW ARTICLE

Overview of West Nile Virus and Sandfly-borne Phlebovirus Infections in Anatolia

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ABSTRACT

Arthropod-borne (arbo) viruses are trafinsmitted to the susceptible hosts by blood-feeding arthropods such as mosquitoes, sandflies and ticks. Arboviral infections have had a significant global public health impact during the last decades due to their resurgence and dynamic epidemiologic features. In Turkey, cases and outbreaks due to previously underestimated arboviral infections have emerged since 2009. In this manuscript, previous and current data on two of the major arboviral infections, West Nile virus and Sandfly-borne Phleboviruses have been overviewed with a special emphasis on clinical presentation and laboratory evaluation. *J Microbiol Infect Dis 2014; Special Issue 1: S22-S31*

Key words: Arboviruses, West Nile Virus, WNV, Phlebotomus fever, Phlebovirus, Turkey

Türkiye'de Batı Nil Virusu ve Flebovirus Enfeksiyonlarına Genel Bakış

ÖZET

Artropod kaynaklı (arbo) viruslar, duyarlı konaklara sivrisinek, kum sineği ve kene gibi artropodların kan emmesi yoluyla bulaşır. Son yıllarda yeniden ortaya çıkan ve epidemiyolojik özelliklerinde değişiklikler izlenen çeşitli arboviral enfeksiyonlar, tüm dünyada önemli halk sağlığı sorunları oluşturmaktadır. Türkiye'de 2009 yılından günümüze, daha önce dikkat çekmemiş bazı arboviruslara bağlı olgular ve salgınlar ortaya çıkmıştır. Bu derlemede, iki önemli arbovirus olan Batı Nil virusu ve kum sineği (tatarcık) kaynaklı fleboviruslarla ilgili ülkemize ait veriler, klinik ve laboratuvar bulguları özellikle vurgulanarak ele alınmaktadır.

Anahtar kelimeler: Arboviruslar, Batı Nil Virusu, BNV, Filebotomus ateşi, Filebovirus, Türkiye

INTRODUCTION

Arthropod-borne (arbo) viruses are transmitted to the susceptible hosts by blood-feeding arthropods such as mosquitoes, sandflies and ticks. There are over 530 viruses registered in the International Catalogue of Arboviruses, where 134 have been documented to cause illness in humans. Arboviruses are taxonomically diverse, belonging to eight viral families and 14 genera. During the last three decades, there was a dramatic global emergence/resurgence of arboviral diseases. Although some arboviruses that cause human disease have been newly recognized, the greatest problem has been the resurgence of diseases that were once thought to be controlled or confined to distinct geographic regions. The geographic distribution of both vectors and viruses has exhibited a global expansion, accompanied by more frequent and intense epidemics, influenced by the interaction of many factors such as global warming, demographic changes and modern transportation. Many arboviruses have become established in new geographic locations where susceptible vectors and hosts provide permissive conditions.[1,2]

Turkey is located in the northeastern part of the Mediterranean region and is included in the endemic zone for various arboviruses. Arboviral diseases in Turkey have gained considerable attention following the emergence of Crimean Congo hemorhagic fever. However, evidence for the circulation of other mosquito and sandfly-borne viral infections with sig-

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nificant public health impact are also present.[3] In this review, we aim to discuss current data on two of the major arboviral infections that have triggered outbreaks as well as case clusters since 2009 in Turkey, West Nile virus and Sandfly-borne Phleboviruses with a special emphasis on clinical presentation and laboratory evaluation.

WEST NILE VIRUS

West Nile virus (WNV) is an enveloped positive-sense RNA virus, classified in the Japanese encephalitis serocomplex of the Flavivirus genus within the family Flaviviridae. In nature, WNV is maintained within an enzootic cycle between various bird species as amplifying hosts and ornithophilic mosquito vectors. Humans, horses, and other mammals mostly become infected by blood sucking of infected mosquitoes. However, they are considered as incidental or dead-end hosts for WNV that do not contribute to the virus life cycle, due to insufficient levels of viremia observed in these species. Blood transfusion and organ transplantation from viremic donors have also been identified as potential transmission routes as well as documented laboratory-acquired infections. Moreover, cases due to intrauterine virus transmission and breast-feeding have been reported.[4,5]

In the majority of the individuals exposed to WNV, asymptomatic seroconversion or subclinical infections occur, whereas a febrile illness called "West Nile Fever" develops in 20% and WNV neuroinvasive disease (WND) in less than 1% of the affected individuals. West Nile fever is characterized by abrupt onset of fever, headache, backache, malaise, anorexia, myalgia, chills, vomiting, rash, fatigue, and eye pain. While a wide range of clinical presentations can be observed in WND with signs and symptoms of meningitis, encephalitis and myelitis, more than half of the persons display signs, symptoms, or laboratory evidence of brain parenchymal involvement and are classified as encephalitis. WNV-associated encephalitis is reported to occur more frequently in persons over 55 years and in those with underlying immunosuppression, history of hypertension and/or cardiovascular disease. WNV-related diseases are frequently diagnosed via detection of specific immunoglobulins. Detection of viral RNA via polymerase chain reaction (PCR) in clinical specimens is possible within a few days after onset, due to the rapid clearance of the virus. However, prolonged viremia without detectable immunoglobulins has been noted in rare cases and in patients with immune suppression, under chemotherapy and in transplant recipients.[4-6]

WNV infections have been documented in northern Africa, Israel, India, Australia, and Europe. WNV has emerged in the United States in 1999 and has progressively spread in the American continent thereafter.[6] Since 1994, the virus has caused outbreaks of severe neuroinvasive disease in humans and horses in Europe and the Mediterranean Basin.[7] Various genetic lineages of WNV have been identified, the prominent being lineages 1 and 2. The majority of the strains responsible of the European and the Mediterranean Basin outbreaks belonged in Lineage 17 Turkey is located in the endemic zone for WNV in the Old World.

Seroepidemiology and Vector Surveillance in Anatolia

Serosurveillance efforts in blood donors and various mammalian species have provided evidence for widespread WNV exposure in Anatolia.[3] The inital evidence for WNV circulation in Anatolia has been provided over three decades ago, in 1971, after an investigation of antibodies against various arthropod-borne viruses performed via hemagglutination inhibiton (HI) method followed by confirmation by neutralization.[8] This study revealed antibodies against WNV in 20% and 0.9% of sheep from Ankara (Central Anatolia) and Hatay (Southern Anatolia) provinces, respectively. Animal and human WNV exposure in Aegean and Southeast Anatolia were suggested in the following studies.[9,10] However, high seroprevalence rates (42.8% in various provinces of Southeastern Anatolia) reported in these studies are probably due to serological cross-reactions commonly observed in assays that detect group-specific antibodies, such as HI.[11] Human WNV exposure in the Aegean border of Anatolia was verified in 1980, by the demonstration of WNV specific antibodies in 21.5% of the sera collected from residents of the region.[12] Moreover, virus activity was also confirmed in Southeast Anatolia in 2007, where the presence of WNV neutralizing antibodies was revealed in.[9] 4% of the individuals from Sanliurfa and Siverek provinces.[13] In a recent survey performed in Kızıltepe region of Mardin province, the neutralizing antibody seroprevalence was reported as 17%, age over 50 and certain occupational risk groups were identified as risk factors for virus exposure.[14] These cross-sectional data from residents obtained in various dates and research groups clearly demonstrate the circulation of WNV in South-Southeast Anatolia where clinicians and public health officials must

consider WNV as suspected agent among cases with febrile and/or neuroinvasive diseases of undetermined etiology and appropriate testing should be performed in probable cases.

A serosurvey undertaken in 10 representative provinces, including Hatay, Adana, Antalya (Mediterranean), Mugla, Izmir (Aegean), Sanliurfa (Southeast Anatolia), Bursa (Northwest Anatolia) and Ankara (Central Anatolia), the presence of WNV neutralizing antibodies were investigated in a variety of mammalian species.[15] Overall, 2.5% assmules, 4% of cattle, 37.7% of dogs, 13.5% of horses, 20.4% of humans and 1% of sheep tested positive and virus exposure was observed in all provinces studied. These results indicate WNV infections in a wide range of mammals that can contribute to the long-term survival of WNV in the absence of overt disease. Provinces located in Central Anatolia have also been studied extensively for human WNV exposure. In a seroprevalence study with 2516 asymptomatic blood donors from Ankara, Konya, Yozgat and Sivas provinces, WNV neutralizing antibodies of high avidity were detected in 0.56%.[16] A following report demonstrated a 0.8% prevalence in a different cohort from Ankara province.[17] An equine serosurveillance performed in a location where symptomatic infections were observed in Eskişehir province, WNV-specific antibodies were detected in 31.6% of the samples.[18] In two studies utilizing ELISA assays, WNV seroreactivity was observed as 2.4% in blood donors and 2.85% in goats from Ankara and Samsun provinces, respectively.[19,20] WNV IgG was also detected via ELISA in 5.4% of a cohort of schizophrenic patients and 3.1% of controls.[21] However, since these findings lack specificity confirmation via neutralization, possible antigenic cross-reactions or false positive results could not be ruled out in these studies. Attempts of detecting viral RNA in various groups without WNV-related clinical disease have been unsuccessful so far.[21,22] In a recent study in Tekirdağ province, a local seroprevalence of 1.7% was reported where an emergence of WNV-associated cases were also noted.[23]

The prominent mosquito species responsible for WNV transmission are Culex mosquitoes although other genera have also been observed to support virus replication and may act as competent vectors.[5] In Europe, Cx. pipiens sensu stricto, Cx. theileri, Cx. modestus s.s., Cx. univittatus, Ochlerotatus caspius and Anopheles maculipennis s.l. are considered to act as WNV vectors and potentially involved in virus transmission.[7] In the Anatolian fauna although Cx.pipiens is known to be widespread, many other mosquito species with vector capacity for WNV are also exist.[24] The number of studies involving virus surveillance in probable vectors from Turkey are limited. In a region with serologically-proven human WNV exposure in Southeast Anatolia, 6457 mosquito specimens collected were classified as Cx. pipiens (56%), Oc. caspius (24%), and Aedes spp. (20%).[25] However, assays for viral antigen and RNA detection were negative and attempts of virus isolation were unsuccessful. WNV RNA could not be demonstrated in 402 samples obtained from 27 different wild avian species from Kizilirmak delta and the adjacent wetlands in northern Anatolia neither in hard ticks removed from a variety of mammalian species from Black Sea region.[26,27] A total of 4698 mosquitoes (99.6% of which were Cx. pipiens s.l.) captured across Western Anatolian provinces associated with the 2010 WNV outbreak were observed as negative for viral RNA.[28] Similarly, no evidence for WNV antigen or RNA could be demonstrated in samples collected during summer 2013 in Ankara province, where confirmed WND cases were identified through 2009 to 2013 (K. Ergunay, unpublished data). Morphological identification of these specimens revealed the activity of Cx. pipiens s.l. (74.4%), An. maculipennis (20.9%), An. claviger (2.1%), Culiseta annulata (1.6%) and Cx. theileri (1%) in the study area. The first detection of WNV infections in mosquitoes was accomplished in 2012, during a pan-arboviral surveillance in field-collected specimens around Edirne province, Eastern Thrace.[29] In this study, 9261 mosquitoes that comprise Oc. caspius (90.9%), Cx. pipiens s.l. (4.7%), An. pseudopictus (3%) and An. maculipennis s.l. (1.3%) species were evaluated and WNV Lineage 1 clade 1a sequences were identified in 15.6% and 36.3% of the Oc. caspius and Cx. pipiens s.l. pools, respectively. Specimens morphologically classified as Cx.pipiens s.l. were further characterized as Cx.pipiens pipiens via molecular barcoding. Comparison of partial WNV sequences detected in human and equine infections in Anatolia and sequences in mosquitoes revealed 94.00-96.34% similarity, which provided an epidemiological link between WNV activity in mosquitoes and vertebrate infections.

CLINICAL CASES

Before 2010, data from very few documented cases due to WNV was available from Anatolia. WNV infection was identified in a 40 year-old patient who underwent non-myeloablative bone marrow transplantation for acute myelogenous leukemia in

Ankara province. In this case, a second attack of fever with headache and sudden onset of severe weakness of limbs were observed after acute graft versus host disease had been relieved with immunosuppressive agents. Diagnosis was established by detecting viral RNA in blood. Symptoms of the patient subsided gradually with supportive measures without further complications.[30] In 2003, WNV RNA was identified in CSF of an individual, presenting with a febrile disease and meningoencephalitic symptoms during immunosuppression due to a renal transplantation in Izmir province (Prof. Ayşın Zeytinoğlu, unpublished data). WNV-associated encephalitis was also reported from Ankara province in a 62-year old woman in 2009, as well as in 56 and 61-year old men in 2010.[31,32] The symptoms observed in these patients were consistent with mild encephalitis following an febrile episode, including high fever, headache, malaise, confusion and alteration of consciousness without focal neurological manifestations. CSF examinations demonstrated lymphocytic pleocytosis and increased protein levels and the diagnosis was established via detection of specific IgM antibodies an sera and/or CSF. An outbreak of acute WNV infections emerged in 2010 in Turkey, mainly in 15 provinces located in the western Anatolia and involved 12 serologically-confirmed and 35 probable cases with a case fatality rate of 21%[33]. Forty of the 47 cases displayed WND with most common symptoms being fever (100%), headache (85%), nausea/vomiting (75%), alteration of consciousness (57.5%), while convulsions were observed in 15%. For WND and fatal outcome, advanced age and underlying conditions such as diabetes mellitus, hypertension, chronic obstructive pulmonary disease were identified. In the outbreak, specific diagnosis was made via demonstration of IgM and IgG antibodies in sera. However, CSF examination was performed in a limited number of cases and no data regarding neurological evaluation or nucleic acid detection were described. WNV infection was suggested in an 11-year old girl from Ankara province with complaints of abrupt-onset fever, abdominal pain, diarrhea, fatigue, difficulty in walking, dizziness, and eruption on the hands and feet, who also developed signs of meningeal irritation.[34] However, no data regarding the performance and interpretation of the diagnostic assays were provided. In 2011, concominant cases of WND in horses and a human from Eskişehir and Ankara provinces, respectively, were reported.[18] The human case, an 61-year old woman, presented with confusion, disorientation, myoclonic jerks and resting tremor of both upper extremities after a febrile episode. Laboratory evaluation revealed mild leukocytosis and elevated CSF protein without pleocytosis was observed. Partial WNV sequences were detected in CSF (as well as in equine infections) and characterized as lineage 1 clade 1a, providing the initial evidence for the presence of lineage 1 strains in Turkey. Surprisingly, these epidemiologically-related infections occurred during an unfavorable season for WNV transmission due to the diminished vector activity, probably due to overwintering viruses in hibernating mosquitoes. A fatal case of WNV encephalitis was also reported from Ankara province in a 76 year-old male with complaints of fever, impaired consciousness and generalized tremors with CSF pleocytosis and elevated protein levels.[35] A case of WND was also reported from Ankara province in 2012.[36] This case, an 87 year-old woman with a recent febrile episode was admitted to the hospital with altered consciousness, myoclonic jerks in facial muscles and left extremity. Positive serum and CSF PCRs established the diagnosis and cycle sequencing of the amplicons revealed lineage 1 clade 1a viruses, confirming the circulation of WNV lineage 1 strains in Central Anatolia. In this case, serum samples obtained within three weeks after the diagnosis were negative for specific antibodies via commercial assays and plaque reduction neutralization assay, indicating that WNV seroconversion may be delayed or absent in elderly individuals without overt diseases associated with immunosuppression. During August 2012, a cluster of cases were identified in Edirne and Tekirdağ provinces of Eastern Thrace, including 6 patients with West Nile fever and one with WND.[23] The diagnosis was based on the detection of viral RNA+IgM in four, only RNA in one and IgM+low avidity IgG in two cases. The most common symptom was fever (>38°C), followed by headache, malaise/fatigue, myalgia/arthralgia, muscle stiffness/lower back pain, anorexia, nausea-vomiting, diarrhea, supraorbital/retrobulbar and abdominal pain. Neuroinvasive disease was documented in a 65 year-old male, who demonstrated meningoencephalitic as well as focal neurologic symptoms characterized by mental status changes, confusion, neck stiffness without Kernig or Brudzinski's sign, paresis and myoclonic seizures of the right arm with increased protein and pleocytosis in CSF. WNV isolates in RNA-detectable patients were characterized as lineage 1, suggesting the widespread circulation of these strains in Turkey. In two cases, WNV and Toscana virus coinfections were identified. The clinical presentations in these patients were not significantly more severe than WNV cases[23] (Figure 1a).

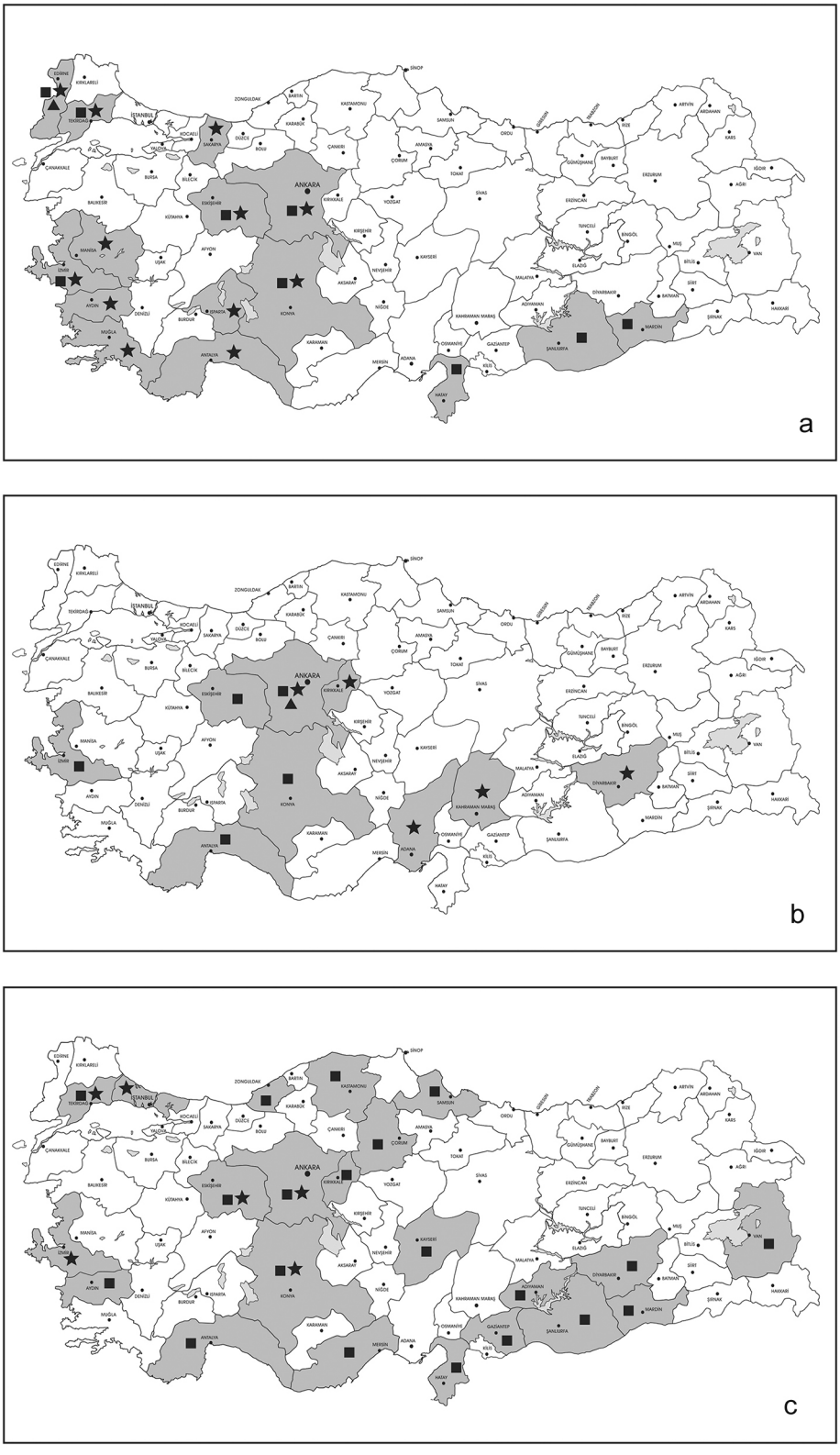

Figure 1 a,b,c. Distribution of West Nile virus (a), Phleboviruses associated with sandfly fever (Sicilian, Naples and Turkey viruses) (b), and Toscana virus in Anatolia (★: human case, ■ : human seroprevalence, ▲: virus in vectors)

Sandfly-borne Phleboviruses

Sandfly-borne phleboviruses (SBPs) are enveloped RNA viruses, classified in the Phlebovirus genus of Bunyaviridae family. Phlebovirus genus consists of over 50 distinct viruses grouped within 9 viral species or serotypes and several tentative species.[37] Certain phlebovirus serotypes are the causative agents of Sandfly fever, also known as Phlebotomus, Papatacci or three-day fever, a self-limited febrile disease characterized by high fever headache, retroorbital pain, photophobia, myalgia/arthralgia, malaise, chills and occasionally, gastrointestinal symptoms.[38] Sandfly fever Sicilian virus (SFSV) and Sandfly fever Naples virus (SFNV) are well-known agents of sandfly fever whereas Sandfly fever Cyprus virus (SFCV) and Sandfly fever Turkey virus (SFTV), the latter initially characterized during an outbreak in Anatolia, also cause a similar disease.[38,39] Toscana virus (TOSV) differs from other phleboviruses due to its neurotropism, causing febrile disease and aseptic meningitis or meningoencephalitis in the affected individuals.[39] Although novel phleboviruses have been characterized in sandflies and human exposure have been demostrated for some strains, their association with human clinical disease is not clear.[37] Virus transmission to humans and animals occurs during blood meal of female phlebotomine sandflies of the Psychodidae family. So far, no reservoirs for SBPs have been defined and the viruses appear to circulate between susceptible species and vectors.[37] The main vector for SFSV and SFNV is Phlebotomus papatasi while P. perfiliewi and P. perniciosus have been established as vectors of TOSV.[38,39] SBPs are widely distributed in the Mediterranean region, in Africa, the Indian subcontinent, the Middle East and central Asia, following the distribution of vector sandflies.[37] The diagnosis of SBP-associated diseases can be achieved via the detection viral nucleic acids via PCR and demonstration of virally-induced IgM. Similar to WNV, nucleic acids can only be detected during the early stages of the clinical disease due to low and transient viremia.[37,38]

Seroepidemiology and Vector Surveillance in Anatolia

Evidence for SBP circulation in Turkey dates back to 1976, when PRNT-confirmed seroprevalence rates of 22% and 62% for SFSV and SFNV were reported in 50 adults residing in Antalya province. Interestingly, the evaluated sera were collected n 1955, indicating even an earlier activity of these viruses in the Mediterranean parts of Anatolia.[40] Seroprevalence rates of 0.84% for SFSV and 13.9% for SFNV were reported in 1980 from the Aegean region, as well.12 Exposure to SFNV and TOSV was revealed in residents of Akbük and Olukbaşı towns (Aydın province) in 2002, where febrile diseases suggesting sandfly fever previously emerged. However, acute cases or virus in vectors could not be identified in this study.[41] In a large serosurvey involving 1533 blood donors from central Anatolian provinces of Ankara, Konya, Eskişehir and Black Sea province of Zonguldak, widespread exposure to SFSV, SFNV, SFTV and TOSV as well as multiple infections with different virus serotypes were recognized.[42] This study demonstrated TOSV neutralizing antibodies in residents of all provinces investigated. In a following seroepidemiology study, VNT-confirmed TOSV seroreactivity of 5.2% was observed, verifying virus circulation in Central Anatolian provinces of Ankara and Eskişehir and further demonstrated TOSV circulation in residents from other provinces (Kırıkkale, Çorum and Kayseri) of this region.[43] Similarly, previous evidence for TOSV circulation in Black Sea region is confirmed by the detection of neutralizing antibodies in residents of the neighboring Kastamonu and Samsun provinces. An interesting finding is the detection of virus exposure in residents of Mediterranean region of Anatolia (Hatay, Mersin and Antalya provinces) and eastern-southeastern Anatolia (Mardin, Van, Gaziantep, Urfa, Adıyaman and Diyarbakır provinces) as well. Risk factors associated with TOSV IgG reactivity were described as male gender, residing in rural areas, frequent sighting of mosquitoes/sandflies and working outdoors. TOSV-specific antibody prevalence was also observed to increase with age. These epidemiological features were consistent with previous observations reported from other TOSV endemic countries.[39] Recently, a seroprevalence of 14.4% was observed in residents of Tekirdağ province, providing initial data from the Eastern Thrace region.[23]

Many species of Phlebotomine sandflies, some of which are known to transmit SBPs, including P. papatasi and P. perfiliewi, are present in Anatolia.[3,44] Despite their activity and serological evidence of human exposure, virus detection in potential vectors have been rarely performed. In 2002, no evidence of SBP infection could be demonstrated in 2000 sandflies captured around Akbük town (Aydın province).[41] In a study undertaken in Ankara province, including neighbourhoods associated with sandfly fever emergence, SFTV sequences were characterized in P. major s.l. sandflies, indicating this species as vectors involved in virus transmis-

sion.[45] Moreover, human and bovine blood meals were identified in infected sandflies, demonstrating virus exposure in non-human mammals. SBP RNA could not be detected during an extensive field survey in Ankara province in 2013. Nevertheless, presence of sandfly species established as SFSV, SFTV and TOSV vectors were observed (K. Ergunay, unpublished data).

CLINICAL CASES

In Anatolia, the clinical presentation of sandfly fever is surprisingly well-known (called as "Tatarcık humması", "Yakarca ateşi") in regions with known or probable virus activity among residents as well as physicians. However, since the infection is self-limited and common during the sandfly season, the majority of the affected individuals do not seek medical assistance unless there is an underlying disease that worsens the patients' condition, travel-associated infection or an outbreak. In 1997, a SBP infection presenting as meningitis in a 15-year-old girl, after a vacation in Turkey was reported.[46] In this case, a febrile disease of three-day duration accompanied by frontal headache, nausea and arthralgia was followed by signs of severe meningitis and a lymphocytic infiltrate in the CSF. ELISA and immunoblot assays in serum revealed an infection with a SFSV-like virus and the patient recovered without sequelae. During 2007-2008, cases of febrile diseases associated with sandfly bites were investigated in Izmir (Aegean coast), Ankara (Central Anatolia) and Adana (Mediterranean Anatolia) provinces.[47] Medical records also suggested similar cases since 2004 from the districts of Ödemiş, Kiraz and Beydağı in İzmir province. One hundred and sixty six patients from Kozan district (Adana province), [39] patients from Çömlekçi, Karaburç and Çayağzı villages of Kiraz district (Izmir province) and 55 patients from Mamak district (Ankara province) were included in the investigation. Recorded symptoms in the patients included high fever (39-40 °C) fever, headache, fatigue, muscle and joint pain, nausea,vomiting and less commonly, watery diarrhea. Laboratory findings of leucopenia, thrombocytopenia, elevated AST, ALT and creatinine phosphokinase (CPK) were also noted. Most of the affected individuals required hospitalization. During the evaluation of patient samples, SFTV was isolated and identified as the responsible agent for some of the cases.[47] Virus activity continued during 2008-2010 in Ankara province, as new SFTV cases were documented, via PCR or serology.[48,49] In addition to the symptoms of sandfly fever, aggravated gastrointestinal symptoms were observed in these cases, with marked elevation of hepatic enzymes, creatine kinase, as well as alkaline phosphatase (AP) and gamma-glutamyl transpeptidase (GGT) in some cases. Leucopenia and thrombocytopenia were also noted.[48] While the febrile period fever lasted for 3-6 days, complete recovery required up to 30 days, with a well-characterized post-infectious asthenia syndrome. It is evident that SFTV triggers a more pronounced clinical symptomatology. Moreover, interleukin 6, 10 and interferon gamma levels were found to be higher in patients infected with SFTV.[50] During summer 2009, an outbreak of febrile disease, clinically compatible with sandfly fever occurred in various different districts of Kırıkkale province.[51] A preliminary diagnosis of sandfly fever was considered in 5 cases due to IgM reactivity and in 3 cases due to IgM seroconversion 6 days later via a commercial IIFT. Clinically, fever and myalgia-arthralgia were detected in all of the cases, followed by diarrhea and nausea-vomiting, headache and conjunctival hyperemia. Since multiple IIFT seroreactivities observed in the cases were not confirmed by virus-specific assays, the infecting serotype could not be precisely characterized. Nevertheless, the laboratory findings were suggestive of SFTV, with leukopenia noted in all cases and thrombocytopenia, elevated ALT, CPK and C-reactive protein (CRP). During July-August 2010, a total of 40 patients from Kahramanmaras province (Southeastern Anatolia), with a history of sandfly bites and clinical presentation suggesting sandfly fever were admitted to the hospital.[52] Serological evidence for SFSV-SFCV or an antigenically-related virus was detected in 9 cases whereas in 2 IgM positive individuals, SFSV real-time PCR also yielded positive results. Sandfly fever was suggested in a 14 year-old male patient with fever and bicytopenia but information on diagnostic assay or the virus serotype were not available.[53]

Although very few sporadic cases of SFSV-like isolates involved in encephalitis or meningoencephalitis have been recognized, a case of central nervous system infection due to SFTV was reported, indicating that the strain might be neuroinvasive as well.[54] The patient was a 63-year-old female from Diyarbakir province (southeastern Anatolia) with high fever, alteration of consciousness, elevated liver enzymes, blood urea nitrogen and creatinine. Lumbar puncture demonstrated increased protein and glucose levels, without significant microscopic findings in CSF, where SFTV nucleic acids were detected and characterized. It is also suggested that

the meningitis case defined by Becker et al. could be due to SFTV, mistakenly identified as SFSV due to cross reactions in the ELISA/immunoblot assays[54] (Figure 1b).

TOSV infections from Turkey were initially reported in 2010, in 16 patients residing in Ankara, Konya and Eskişehir provinces of Central Anatolia via nucleic acid detection in serum.[42] Neck rigidity and/or Kernig's signs were frequent symptoms of the cases whereas muscle paresis, alterations of consciousness, tremor and/or cranial nerve involvement were relatively rare. Normal findings to pleocytosis with increased glucose and/or protein were observed in CSF evaluation. Partial sequence analyses performed in selected samples demonstrated the infecting isolate to be phylogenetically grouped with TOSV genotype A strains.[42] Subsequently, probable TOSV infections were identified in Izmir and Ankara provinces during the sandfly season in the following year, with similar clinical presentation and laboratory evaluation.[32] In a 43 year-old HIV-infected resident of Istanbul province, admitted to the hospital in Adana with high fever, headache, malaise, leucopenia, anemia and trombocytopenia, TOSV RNA was detected, suggesting virus circulating in Northwestern Anatolia.[55] During an investigation of a cluster of cases with febrile diseases of unknown etiology in Istanbul, Tekirdağ and Edirne provinces, TOSV-specific IgM was detected in a 35 year-old housewife with headache, malaise/fatigue, myalgia/arthralgia and laboratory findings of thrombocytopenia, leukopenia and increased AST, ALT and lactate dehydrogenase levels.[23] As discussed above, coinfections of WNV and TOSV were demonstrated in two individuals with febrile diseases via PCR and serology. Here, characterization of partial sequences also revealed genotype A strains, indicating a more disseminated zone of activity for this genotype in Anatolia.[23] TOSV RNA and IgM seroconversion was also detected in a 23 year-old female presenting with high fever (39-40°C), malaise, arthralgia, without neurologic symptoms in Ankara province in 2012.[56] Sequence analysis confirmed the previous reports of the circulation of genotype A strains in Central Anatolia (Figure 1c).

CONCLUDING REMARKS

Convincing evidence is now present to suggest that the activity of WNV is widespread in Anatolia. Current data also maintain that infections with SBPs, including major as well as local strains are endemic and may trigger outbreaks in various regions in the country. Serosurveillance studies demonstrated that for both agents, asymptomatic seroconversion after exposure is also common. It should be noted that significant portion of the presented data, especially case and outbreak reports, was generated during the last 5 years, although serologic evidence for virus activity has been provided much earlier. This clearly demonstrates the importance of serological and vector surveillance efforts prior to the emergence of clinical cases, to reveal the presence and to predict the zones of potential spread. It is known that the geographic and climatic conditions in Turkey generally favor the introduction and establishment of many arboviral agents as well as potential vector species.[3] Preliminary data have already suggested the activity of other arboviruses, including tick-borne encephalitis virus and Dengue or antigenically-similar agents.[3] Moreover, the recent detection of Aedes albopictus, the Asian tiger mosquito, a well-known vector for Chikungunya and a minor vector for Dengue in Eastern Thrace, as well as imported cases emphasize that the potential conditions for emergence of other arboviral infections are present.[57-59]

Increased awareness in clinicians and diagnostic microbiologists is also needed to identify imported and/or indigenous cases of less-frequent arboviral infections. Since the clinical picture in arboviral infections presenting with febrile disease or CNS symptoms are not specific and can easily be attributed to other causes, a thorough evaluation of patient history to identify potential risks and epidemiological factors is crucial for a correct diagnosis. Moreover, appropriate sampling, the choice of diagnostic assays in conjunction with the disease stage and interpretation of the results is required for precise virus characterization, given the serological cross-reactions observed in viruses belonging in the same family or genus. Since no specific treatment is currently available for the majority of the arboviral infections, timely identification of the etiology to initiate optimal curative or supportive therapy is also important for patient management. In conclusion, arboviruses must be considered as etiologic agents in cases with febrile diseases and neurological symptoms.

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