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Multicentre evaluation of central nervous system infections due to Flavi and Phleboviruses in Turkey

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Summary *Objectives:* Flavi- and Phleboviruses associated with central nervous system (CNS) infections including West Nile Virus (WNV), Tick-borne Encephalitis Virus (TBEV) and Toscana Virus (TOSV) cause significant morbidity and mortality in humans. In this study, the impact of these agents have been investigated in CNS infections at referral hospitals in two provinces in Turkey, where circulation of these viruses have previously been recognized.
Methods: In the study, 258 samples from 126 individuals from Ankara and 113 samples from 108 individuals from Izmir provinces collected in 2010 were included. Viral RNAs were investigated by multiple genus and strain specific primers. Commercial serological assays were employed in screening and reactive results were evaluated with additional assays and by plaque reduction neutralization assay.
Results: Two cases of WNV CNS infections, 14 cases of TOSV infections and one TBEV-exposed individual were identified via serological testing. WNV infections in 61 and 56-year old individuals from Ankara presented with fever and encephalitis without skin rash and residual neurologic damage. TOSV-associated cases from both provinces mainly displayed signs of meningitis. TOSV exposure was documented for the first time from Izmir.
Conclusions: WNV, TBEV and TOSV infections must be considered in cases of meningoencephalitis of unknown etiology in Turkey.

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Introduction

Human infections with vector-borne viruses (also called arboviruses) are among the most important emerging infectious diseases, due to their impact on public health and changing epidemiological features.[1] The arboviruses that manifest as central nervous system (CNS) infections are serious and may produce a fatal outcome or permanent neurological sequelae in the affected individuals.[2] The Flavivirus genus of the family Flaviviridae consists of a group of highly important human pathogens, many of which possess the capacity to induce a spectrum of CNS diseases in infected hosts, including Japanese encephalitis virus (JEV), Tick-borne encephalitis virus (TBEV), and West Nile virus (WNV).[3,4] All flaviviruses circulate in transmission cycles consisting of vertebrate hosts and insect vectors, in which humans mostly act as dead-end hosts.[5] Natural cases of human infection usually follow the bite of an infected tick or mosquito, although incidental cases related to other transmission mechanisms, including the use of infected blood products and organ transplants or, in case of TBEV, oral transfer through consumption of unpasteurized milk products have been reported as well.[5,6]

The family Bunyaviridae consists of more than 150 viruses and 16 serogroups, classified in five genera: Bunyavirus, Hantavirus, Nairovirus, Phlebovirus and Tospovirus. The Phlebovirus genus includes 37 recognized virus species, mostly transmitted to vertebrates by phlebotomine sandflies, which are geographically distributed in Europe, Africa, Central Asia and the Americas.[7] In the phlebovirus genus, sandfly fever Naples virus (SFNV), sandfly fever Sicilian virus (SFSV) and antigenically-related strains circulating in Europe have been associated with an acute, influenza-like febrile disease.[8] However, Toscana virus (TOSV), a variant of SFNV, have been observed to be among the most frequent viral pathogens involved in aseptic meningitis occurring during summer in France, Italy, Spain and other countries around the Mediterranean, accounting for as high as 81% of the viruses detected.[8–10]

Although located in an endemic region, data available on Flavi- and Phlebovirus-related CNS infections in Turkey is relatively limited.[11] Serologic evidence for WNV and TBEV exposure is well-documented, however, reports of acute infections are rare.[11] Recently, cases of TOSV meningoencephalitis and exposure to major SFNV and SFSV serotypes as well as TOSV have been identified.[12] The aim of this study was to investigate the presence and impact of WNV, TBEV and TOSV-associated CNS infections in two regions in Turkey.

Materials and methods

Setting and samples

The study was performed in two university hospitals, Hacettepe University Hospital in Ankara (Central Anatolia, 39°56′N – 32°52′E) and Dokuz Eylul University Hospital in Izmir (Aegean region, Western Anatolia, 38°26′N–27°09′E) (Fig. 1). Both centres are tertiary care and major referral hospitals of their regions. Ankara is the capital and second most densely-populated city in Turkey (approximate population: 4.8 million) and Izmir is the third most densely-populated city (approximate population: 3.9 million) (http://www.turkstat.gov.tr/Start.do). The clinical samples comprise 125 serum and cerebrospinal fluid (CSF) pairs and 8 single CSF samples from Ankara (collected April–October 2010) and 113 CSFs from Izmir (collected January–December 2010) from patients with the preliminary diagnosis of aseptic meningoencephalitis of presumed viral aetiology. Two separate samples were evaluated from 7 and 5 individuals from Ankara and Izmir, respectively. All samples were interpreted as negative for bacterial, mycobacterial and fungal cultures as well as rapid antigen assays. In CSF samples, Polymerase Chain Reaction (PCR) assays for Herpes simplex virus (HSV) type 1/2, Human Herpesvirus 6, Enteroviruses and *M. tuberculosis* were non-reactive. The samples, obtained within 1–5 days after the onset of symptoms, were stored in aliquots in −20 °C and −80 °C for future analysis. Clinical history and laboratory data of the patients were retrieved from hospital medical records. The study protocol was approved by local authorities.

WNV, TBEV and phlebovirus molecular testing

All samples were subjected to nucleic acid purification and reverse transcription using random hexamers via commercial assays (High Pure Viral Nucleic Acid Kit, Roche Diagnostics, Germany; RevertAid First Strand cDNA Synthesis Kit, Fermentas, Lithuania; Access RT-PCR kit, Promega, Madison, WI, USA) as directed by the manufacturers. All samples were screened for flaviviruses using a real-time reverse transcription (RT) PCR demonstrated to amplify all recognized members of the genus Flavivirus including WNV and TBEV, in addition to in house WNV-specific real-time PCR, targeting the NS5 region, as described.[13,14] For the detection of phleboviruses, a variety of primers targeting different genes were employed in independent reactions including a consensus primer set targeting the viral polymerase gene in the L segment of the viral genome and targeting the nucleoprotein gene in S segment of the SFNV complex including TOSV.[15,16] All assays were performed in nested or real-time RT-PCR formats where appropriate as previously described.[13–16] For nucleic acid testing, cell culture supernatants of Vero cells inoculated with WNV strain NY99-4132 and TOSV isolate ISS.Phl.3 and PS cells inoculated with TBEV strain K23 were processed as described above, as positive controls.

WNV, TBEV and phlebovirus serological testing

Screening for TBEV and WNV IgM and IgG class immunoglobulins in all samples were performed via commercial enzyme-linked immunosorbent assays (ELISA) and immunofluorescence assays (IFA) (anti-WNV ELISA and IFA, anti-TBEV ELISA and IFA; Euroimmun, Germany). Samples reactive in initial testing were further evaluated via a commercial IFA incorporating antigens of major Flaviviruses including WFV, TBEV, JEV, Yellow Fever Virus (YFV) and Dengue virus serotypes 1–4 (Flavivirus Profile 2 IFA IgM and IgG; Euroimmun, Germany). Specific antibody synthesis in CSF was evaluated for TBEV via a commercial assay (anti-TBEV ELISA IgG in CSF, Euroimmun, Germany) in serum-CSF pairs. Intrathecal synthesis of WNV-specific IgG antibodies were