

Emergence and co-infections of West Nile virus and Toscana virus in Eastern Thrace, Turkey

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Abstract

The objective of this study was to identify the impact of West Nile virus (WNV) and Toscana virus (TOSV) in febrile diseases of unknown aetiology in Eastern Thrace, Turkey; this study was conducted during August–October 2012, and included 18 clinical cases and 296 blood donors for local serosurveillance. Antibodies were determined via commercial assays and further tested for specificity via neutralization assays (NA). Viral RNAs were sought via specific and/or generic primers. WNV infections were diagnosed in seven patients (38.8%), detected via RNA+IgM in four, RNA in one and IgM and 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, diarrhoea, supraorbital/retrorbular pain and abdominal pain. Neurological symptoms were noted in one individual. WNV strains in RNA-detectable patients were characterized as lineage I. TOSV RNA or IgM were identified in two individuals with confirmed WNV infections and in one patient without evidence of WNV exposure. The clinical and laboratory findings in individuals with WNV/TOSV co-infection were comparable to those in WNV-induced disease. The TOSV strain in the patient with detectable viral RNA was characterized as genotype A. In local blood donors, seroreactivity for specific WNV and TOSV immunoglobulins was observed in 1.7% (5/296) and 14.4% (26/180), respectively. These findings indicate the emergence of WNV and TOSV-associated diseases in Eastern Thrace. WNV/TOSV co-infections were documented for the first time.

Keywords: Thrace, Toscana virus, TOSV, Turkey, West Nile virus, WNV

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Introduction

West Nile virus (WNV) and Toscana virus (TOSV) are causative agents of major vector-borne viral infections that induce central nervous system manifestations as well as febrile

diseases in affected individuals [1,2]. WNV is a mosquito-borne Flavivirus (family *Flaviviridae*) maintained in an enzootic cycle between birds as amplifying hosts and mosquito vectors [1]. WNV infections in humans usually remain subclinical; a febrile illness called West Nile fever develops in 20% of exposed persons and neuroinvasive disease in <1% [3]. Since 1994, the virus has caused outbreaks of severe disease in humans and horses in Europe and the Mediterranean Basin [4]. TOSV, included in the *Phlebotomus* fever group in the *Phlebovirus* genus of the *Bunyaviridae* family along with Sandfly fever and Sicilian and Naples viruses, is transmitted to humans by the bite of phlebotomine sandflies [5]. While all three viruses may induce

a self-limited febrile disease known as phlebotomus, papatacci or sandfly fever, TOSV, having a distinct neurotropism, is considered to be one of the leading causes of human aseptic meningitis in endemic countries around the Mediterranean basin [5,6]. In this study, the activity and impact of WNV and TOSV were investigated in Eastern Thrace, Turkey, where no previous data were available.

Materials and Methods

Study area, setting and clinical cases

The study was carried out in the northwestern part of the Turkish Republic, also called the Eastern Thrace region, which includes territories of five provinces (Edirne, Tekirdag, Kırklareli, Canakkale and Istanbul) with a population of 1 569 388 (www.kalkinma.gov.tr/DocObjects/Download/10211/TR21_Trakya_Bolge_Plani.pdf). The region is bordered on the west-northwest by Greece and Bulgaria and separated from Anatolia or Asia minor by the Sea of Marmara (Fig. 1).

During early August 2012 two cases of febrile disease of unidentified aetiology were referred to the infectious diseases clinic of the Haydarpaşa Training and Research Hospital of Gulhane Military Medical Academy. The cases were military recruits, located in the Corlu district of Tekirdag province (Fig. 1). As the preliminary evaluations indicated WNV infections, this study was undertaken to identify further cases

and asymptomatic exposure in the region. The study was approved by the Turkish Ministry of Health Haydarpaşa Research and Training Hospital ethical board (HNEAH-KAEK 2012/197).

Adult patients with the clinical diagnosis of febrile disease of presumed viral aetiology observed during late July to early October 2012 at state hospitals in the Corlu and Cerkezkoy districts of Tekirdag province, infectious diseases clinics of Trakya University Hospital in Edirne province and Haydarpaşa State and Military Research and Training Hospitals in Istanbul province were included in the study (Fig. 1). After informed consent, physical examination and laboratory tests, which included haemoglobin, leucocyte and platelet counts, sedimentation rate, C-reactive protein (CRP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine phosphokinase (CPK), lactate dehydrogenase (LDH), total protein, albumin and creatinine, were performed. The participants filled out a questionnaire to identify risk factors for vector-borne infections. Sera and/or cerebrospinal fluid (CSF) were obtained from patients within 1–10 days after the onset of symptoms, and subjected to nucleic acid purification using the High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Mannheim, Germany) and reverse transcription using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Tokyo, Japan). For the detection of WNV RNA, nested and real-time reverse transcription PCRs, targeting distinct regions of the viral genome, were employed [7,8]. TOSV and other



FIG. 1. Map of the Eastern Thrace region, showing the study area. Provincial boundaries and district borders in Tekirdag province are demonstrated. See text for details (△, institution for patient evaluation; ▲, institution where confirmed WNV cases were identified; ⊥, mosquito sampling location).

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