



Investigation of The Prevalence of Tick-Borne Encephalitis Virus Antibodies in Patients with Preliminary Diagnosis of Crimean-Congo Hemorrhagic Fever

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ABSTRACT

Objective: The tick-borne encephalitis virus (TBEV), a *flavivirus* transmitted by *Ixodes* spp. ticks, can cause a clinical picture characterized by nonspecific symptoms, as well as more specific conditions such as encephalitis and myelitis. Most patients admitted and followed with a preliminary diagnosis of Crimean-Congo hemorrhagic fever (CCHF) are from regions with a risk of tick exposure. The aim of this study is to determine the epidemiology of TBEV.

Material and Method: A total of 272 adult patients admitted with a preliminary diagnosis of CCHF between April and September 2021 in the Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Sivas Cumhuriyet University, were included in the study. The diagnosis of CCHF was defined using the criteria established by the CCHF Scientific Committee of the Turkish Ministry of Health. To determine the seroprevalance of TBE, TBEV-IgG antibodies were investigated in patient serum samples using the ELISA method (Anti-TBE Virus ELISA (IgG), Euroimmun, Germany). The results were evaluated by calculating the ratio of the extinction value of the patient sample to the extinction value of calibrator 2. Ratios below 0.8 were considered negative, between 0.8 and 1.1 were considered borderline, and greater than 1.1 were considered positive. Patients with no detected antibodies against TBEV in their serum samples were considered seronegative.

Results: The mean age of the 272 patients included in the study was 49.46 ± 17.48 years (Range: 18-98 years), with 181 (66.5%) being male. All patients' TBEV antibody levels were evaluated as negative. The provinces of residence of the patients were Sivas, Giresun, Tokat, Yozgat, and Erzincan. A history of tick exposure was found in 204 (75%) of the patients, with 143 (79%) of male patients and 61 (67%) of female patients reporting tick exposure.

Conclusion: In this study, the absence of antibodies against TBEV indirectly demonstrated the absence of TBEV in the tick population. However, no study has been conducted to detect the presence of TBEV in the tick population in Sivas province, and our study is the first to address this issue. Nevertheless, further seroepidemiological studies are required.

Keywords: Tick-Borne Encephalitis Virus, Crimean Congo Hemorrhagic Virüs, ELISA

Kırım-Kongo Kanamalı Ateşi Ön Tanılı Hastalarda Kene Kaynaklı Ensefalit Virüsü Antikorlarının Yaygınlığının Araştırılması

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ÖZET

Amaç: *Ixodes* spp. keneleri tarafından bulaşan bir *flavivirus* olan kene kaynaklı ensefalit virüsü (TBEV), non-spesifik semptomlar ile karakterize bir klinik tabloya neden olabileceği gibi daha spesifik bir durum olan ensefalit ve miyelit tablosu oluşturabilmektedir. Kırım-Kongo kanamalı ateşi (KKKA) ön tanısı ile yatırılarak takip edilen hastaların çoğu kene ile temas riski olan bölgelerden gelmektedir. Bu çalışmanın amacı TBEV'nin epidemiyolojisinin belirlenmesidir.

Gereç ve Yöntem: Sivas Cumhuriyet Üniversitesi Tıp Fakültesi Enfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Anabilim Dalı servisinde KKKA ön tanısı ile Nisan-Eylül 2021 tarihleri arasında takip edilen 272 erişkin hasta çalışmaya dahil edildi. KKKA tanısı, Sağlık Bakanlığı Türkiye KKKA Bilimsel Kurulu tarafından oluşturulan kriterler kullanılarak tanımlandı. TBE seroprevalansını belirlemek amacıyla hasta serum örneklerinden ELISA yöntemi ile (Anti-TBE Virus ELISA (IgG) Euroimmun, Almanya) TBEV-IgG antikorları araştırıldı. Sonuçlar, hasta örneğinin ekstinksiyon değerinin kalibratör 2'nin ekstinksiyon değerine oranı hesaplanarak değerlendirilmiş ve bu oran 0.8'den küçük olarak saptandığında negatif, 0.8 ile 1.1 arasında ise ara değer, 1.1'den büyük saptandığında ise pozitif olarak kabul edilmiştir. Serum örneklerinde TBEV'ye karşı oluşmuş antikor saptanmamış hastalar seronegatif olarak değerlendirildi.

Bulgular: Çalışmaya alınan 272 hastanın yaş ortalaması ± standart sapma (SD) 49,46 ± 17,48 (Yaş aralığı: 18 -98) yıl olup 181'i (%66,5) erkek idi. Tüm hastaların TBEV antikor düzeyleri negatif olarak değerlendirildi. Hastaların ikamet ettiği iller Sivas, Giresun, Tokat, Yozgat ve Erzincan idi. Hastaların 204'ünde (%75) kene temas öyküsü bulunurken, bu sayı erkek hastalarda 143 (%79), kadın hastalarda ise 61 (%67) olarak saptandı.

Sonuç: Çalışmamızda TBEV'ye karşı oluşmuş antikor saptanmamış olması kene popülasyonunda TBEV'nin yokluğunu dolaylı yoldan ortaya koymuştur. Ancak Sivas ilinde kene popülasyonu üzerinde TBEV'nin varlığını saptamaya yönelik bir çalışma bulunmamaktadır ve çalışmamız bu konuda yapılmış ilk çalışma olma özelliğini taşımaktadır. Bununla birlikte, daha fazla seroepidemiolojik çalışmanın yapılması gerekmektedir.

Anahtar Kelimeler: Kene Kaynaklı Ensefalit virüs, Kırım Kongo Kanamalı Ateşi, ELISA

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Introduction

Crimean-Congo Hemorrhagic Fever (CCHF) is a zoonotic infection caused by the CCHF virus, which belongs to the *Nairovirus* genus within the *Nairoviridae* family.¹ The clinical presentation and outcomes of CCHF, which manifests as a hemorrhagic fever syndrome, vary widely. While 88% of infected individuals remain asymptomatic, symptomatic cases exhibit a spectrum of clinical manifestations ranging from mild illness, characterized by nonspecific symptoms such as fever, headache, myalgia, arthralgia, nausea, and vomiting, to severe conditions including major hemorrhages, hemodynamic instability, liver damage, and neurological disorders.² The CCHF virus is transmitted to humans via infected ticks (through tick bites or crushing of ticks) or through direct contact with the infected blood or tissues of viremic animals.³

Tick-borne encephalitis virus (TBEV), a member of the *Flavivirus* genus within the *Flaviviridae* family, is an RNA virus.⁴ The virus can be transmitted via contact with ticks of the *Ixodes* species as well as through the consumption of unpasteurized dairy products from infected animals (e.g., goats). The disease was first described in Austria in 1931, and its causative agent was isolated in the eastern regions of Russia in 1937.⁵ Annually, over 10,000 cases of the disease are reported, making TBEV the second most prevalent neurotropic flavivirus worldwide.⁶ Furthermore, the number of cases has been steadily increasing in recent years.⁶ The disease typically progresses in two phases. The first phase involves nonspecific symptoms such as fever and headache, followed by an asymptomatic period. The second phase is characterized by neurological complications, including encephalitis and myelitis.⁷

The aim of this study is to investigate the seroprevalence of TBEV, another tick-borne infection, among patients hospitalized with a preliminary diagnosis of CCHF.

Materials and Methods

This study is a retrospective cohort study. The study included patients aged 18 years and older who were hospitalized in the Department of Infectious Diseases and Clinical Microbiology at Sivas Cumhuriyet University Faculty of Medicine between April 1, 2021, and September 31, 2021. Ethical approval for the study was granted by the Clinical Research Ethics Committee of Sivas Cumhuriyet University on April 19, 2023 (approval number: 2023-04/61). Cases suspected of Crimean-Congo hemorrhagic fever (CCHF) were defined using the CCHF case definition criteria developed by the Turkish Ministry of Health's Scientific Committee on CCHF. Patients presenting with at least two symptoms such as fever, headache, widespread body pain, arthralgia, fatigue, diarrhea, and bleeding, as well as having visited or resided in an endemic area for CCHF within the last two weeks, or having a history of tick exposure with thrombocytopenia ($<1.5 \times 10^5/\text{mm}^3$) and/or leukopenia ($<4 \times 10^3/\text{mm}^3$), were considered suspected cases of CCHF.⁸ The diagnosis

of CCHF was confirmed by the Turkey Republic Ministry of Health's General Directorate of Public Health through the detection of anti-immunoglobulin M antibodies and Crimean-Congo Hemorrhagic Fever Virus (CCHFV) Polymerase Chain Reaction (PCR) in patient serum samples.

Epidemiological data for the included volunteers, such as age, gender, occupation, clinical and routine laboratory data, and history of tick attachment, were recorded individually for each volunteer. Serum samples collected on the day of hospitalization for diagnostic purposes (0.5 ml each) were stored at -80°C . The serum samples to be used in the study have been maintained under proper conditions up to the present, with no thawing or power interruptions during storage. On the day of testing, serum samples were removed 2 hours prior to application and allowed to thaw at room temperature. All reagents were brought to room temperature ($+18^\circ\text{C}$ to $+25^\circ\text{C}$) 30 minutes before use. The washing solution was diluted with water (1 part washing solution to 9 parts distilled water). Patient samples were diluted 1:101 with sample buffer. TBEV-IgG antibodies were investigated using the ELISA method (Anti-TBE Virus ELISA (IgG) (EI 2661-9601 G), Euroimmun, Germany (Lübeck)). The results were evaluated semi-quantitatively by calculating the ratio of the extinction value of the control or patient sample to the extinction value of calibrator 2. A ratio less than 0.8 was considered negative, a ratio between 0.8 and 1.1 was considered an intermediate value, and a ratio greater than 1.1 was considered positive.

Epidemiological data including age (years), gender, clinical symptoms and signs at hospital admission, routine laboratory test results, history of tick exposure, and occupation of the patients were collected from electronic patient records and entered into Microsoft Excel (Microsoft Office Version 365, USA; URL: <https://www.office.com/>). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 23.0 (IBM® SPSS®, Armonk, New York, USA; URL: <https://www.ibm.com/products/spss-statistics>). Age (years) data were reported as mean and standard deviation (SD), while gender, history of tick exposure, and serological results were reported as n (%). The normality of continuous variables was tested using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Anti-TBE IgG antibody levels determined by ELISA were recorded as optical density (OD) values for each patient.

Results

A total of 272 patients were included in the study, with 181 (66.5%) male and 91 (33.5%) female, with a mean age of 49.46 ± 17.48 years. A history of tick exposure was present in 204 (75%) of the patients. Among the male patients, 143 (79%) had a history of tick exposure, while 61 (67%) of the female patients had a similar history. Of the 272 patients included in the study, 250 were from Sivas, 15 from Giresun, 4 from Yozgat, 2 from Tokat, and 1 from Erzincan (Figure 1). Of the 272 patients (82.7%) admitted with a preliminary diagnosis of CCHF, 225 tested

positive for CCHF PCR, while 47 (17.3%) had negative results. The majority of the patients, 223 (82%), were engaged in farming and animal husbandry, and 97% had a history of living in or visiting rural areas. The history of tick

exposure and CCHF PCR results of the patients are detailed in Table 1. All patients' TBEV antibody levels were found to be negative.



Figure 1: Distribution of Patients with Preliminary CCHF Diagnosis by Provinces

Table 1: Gender distribution, tick exposure, CCHF-PCR results, and TBEV serology data of Patients by Provinces

	Number of patients by provinces n (%)	Gender distribution n (%)	History of tick exposure n (%)	CCHF-PCR results n (%)	TBEV serology n (%)
Sivas	250 (%92)	Male:163 (%65.2)	Yes: 184 (%73.6)	Positive: 207 (%82.8)	Negative: 250 (%100)
Giresun	15 (%5,6)	Male: 12 (%80)	Yes: 14 (%93.3)	Positive: 14 (%93.3)	Negative: 15 (%100)
Yozgat	4 (%1,4)	Male: 4 (%100)	Yes: 4 (%100)	Positive: 3 (%75)	Negative: 4 (%100)
Tokat	2 (%0.7)	Male: 1 (%50)	Yes: 1 (%50)	Positive: 1 (%50)	Negative: 2 (%100)
Erzincan	1 (%0.3)	Male: 1 (%100)	Yes: 1 (%100)	Positive: 0 (%0)	Negative: 1 (%100)

CCHFV-PCR: Crimean-Congo hemorrhagic fever virus polymerase chain reaction
 TBEV: Tick-borne encephalitis virus

Discussion

TBEV (Tick-borne encephalitis virus) is a neurotropic virus belonging to the *Flavivirus* genus of the *Flaviviridae* family.⁴ First discovered in 1931, this virus is endemic in Central, Eastern, and Northern Europe, as well as in Northeastern Asia.⁹ The existence of TBEV in nature is primarily maintained through transmission between wild mammalian hosts and migratory birds via ixodid ticks.¹⁰ The clinical presentation of the virus varies depending on its subtype, but it typically starts with nonspecific symptoms such as fatigue, headache, neck pain, high fever, and vomiting, progressing into a two-phase clinical course.⁷ In 20-30% of patients, a second phase follows an asymptomatic period of 2-10 days, leading to neurological involvement such as meningitis, encephalitis, myelitis, or radiculitis.¹¹

CCHFV, belonging to the *Orthonairovirus* genus of the *Nairoviridae* family under the *Bunyvirales* order, was first identified in 1944 in Crimea and has since been reported

in more than 35 countries.¹² Similar to TBEV, the primary route of transmission for CCHF virus is through tick attachment, which serves as the vector for the virus.¹¹ In addition to tick transmission, CCHF can also spread through contact with tissues, feces, or secretions from infected animals or humans.³ The initial symptoms of CCHF, which leads to viral hemorrhagic fever, can overlap with those of TBEV infection, which is transmitted through tick bites. Therefore, in this study, the presence of TBEV was investigated in patients who were initially diagnosed with CCHF.

TBEV is endemic in many regions of Europe and Asia; however, there is limited data on TBEV activity in Turkey.¹³⁻¹⁵ The first evidence of TBEV infection in Turkey showed an IgG positivity rate of 10.5%.¹³ A study evaluating TBEV exposure in 181 healthy blood donors and the impact of TBEV on central nervous system infections in Central/North Anatolia analyzed 2454 serum samples, finding a seropositivity rate of 1.9%.¹⁴ Another

study investigating TBEV seropositivity in 278 individuals with tick contact identified IgG positivity in 4 patients (1.4%).¹⁵ Except for one patient, all participants in this study were from the Central Anatolia region. In a study similar to ours, where TBEV seropositivity was assessed in patients with suspected CCHF, one patient had IgM positivity, and seven others had IgG positivity.¹⁶ In our study, TBEV seropositivity was investigated in patients admitted with a preliminary diagnosis of CCHF, but all results were negative. This supports the notion that TBEV is not a significant threat in our region.

TBEV spans a wide area, including Northern Asia and Europe, and has three subtypes. The European subtype is transmitted by *Ixodes ricinus*, and the Far Eastern and Siberian subtypes are transmitted by *Ixodes persulcatus*.^{6,17} The mortality rate for the European and Siberian subtypes is approximately 1–3%, while the Far Eastern subtype's mortality rate can rise to 20–40%.^{17,18} Only the European subtype, transmitted by *Ixodes ricinus*, is found in Turkey. However, no outbreaks have been reported thus far. The presence of the *Ixodes ricinus* tick responsible for transmitting the European subtype was reported in wild animals in our region in 2011 and 2012.¹⁹ However, there have been no reported TBE cases in our region to date. In our study, 204 out of 272 patients (75%) with a suspected CCHF diagnosis had tick contact. However, since the blood samples from these patients were collected during the acute phase before the formation of antibodies, no positivity was detected, which may explain the negative results.

In conclusion, the presence of the tick vector for TBEV in our region and the absence of antibodies to TBEV in our study support the idea that TBEV is not a significant threat in our region. However, further studies are needed to explore the presence and epidemiology of the virus in Turkey.

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Authorship Contribution

Concept/Hypothesis: MÖ, NE

Design: MÖ, YÇK, NE

Data Collection/Data Processing: MÖ, ANP, BB, TT

Data Analysis: NE, TT

Manuscript Preparation: MÖ, NE, YÇK, SAB, CÖ

Conflicts of interest

There are no conflicts of interest in this work.

Ethical Approval Statement

Ethical approval for the study was granted by the Clinical Research Ethics Committee of Sivas Cumhuriyet University on April 19, 2023 (approval number: 2023-04/61).

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