International Journal of Infectious Diseases 14S (2010) e213–e216

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Case Report

A newly identified Crimean-Congo hemorrhagic fever virus strain in Turkey

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ARTICLE INFO

Article history:
Received 20 January 2009
Received in revised form 13 July 2009
Accepted 16 July 2009
Corresponding Editor: Jane Zuckerman, London, UK

Keywords:
Crimean-Congo hemorrhagic fever
Turkey
Phylogenetic analysis

ABSTRACT

Crimean-Congo hemorrhagic fever (CCHF) is a fatal viral disease that occurs in approximately 30 countries. It has the most extensive geographic range among the tick-borne viruses that affect human health. Recently, a 6-year-old boy presented with complaints of fever, fatigue, and loss of appetite. He revealed a history of tick bite in rural Istanbul three days prior to presentation. A hyperemia was detected at the site of the tick bite. Laboratory tests showed that alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, and creatine phosphokinase levels were elevated and that the prothrombin time and activated partial thromboplastin time were prolonged. Anti-CCHF virus IgM ELISA and a reverse transcriptase-PCR assay for CCHF RNA were both positive. Phylogenetic studies revealed that the virus was a new AP92-like CCHF strain, which was named KMAG-Hu-07-01 (accession number **EU057975**). This patient could provide important information on the transmission dynamics of CCHF infection.

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1. Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a fatal disease caused by a *Nairovirus* of the family *Bunyaviridae*.[1] The virus is transmitted to humans through the bites of infected ticks or from direct contact with viremic animals or humans.[2] Isolation of CCHF virus and/or CCHF disease have been reported in more than 30 countries in Africa, Asia, southeastern Europe, and the Middle East.[3,4] This disease is also an important public health issue in Turkey. The first cases in Turkey were detected in the town of Tokat in the Kelkit Valley region in the northern part of the country (Figure 1) in 2002.[5,6]

Between 2002 and 2008, a total of 2508 confirmed cases, including 133 deaths, were reported to the Ministry of Health of Turkey, with the number of cases increasing over the years.[7] The majority of cases (95%) were reported from middle and eastern Anatolia, particularly from the cities of Tokat, Sivas, Yozgat, Çorum, and Erzurum.[8] Sporadic cases have been reported from the southeastern and western parts of Turkey as well.

To date, all of the CCHF cases detected in Istanbul have been imported cases in which the patients had become infected outside of Istanbul. Herein, we report a mild infection with a newly emerged CCHF strain in Turkey. The strain was named KMAG-Hu-07-01. This new strain is closely related to the AP92 strain, which has only been detected in ticks in Greece.[9,10]

2. Case report

A previously healthy 6-year-old boy, living in the Küçükçekmece neighborhood in Istanbul, was admitted to Haseki Education and Research Hospital with complaints of fever, fatigue, and loss of appetite. The patient had been bitten by a tick on his head three days earlier, when he had attended a picnic in the rural Balkan area (Arnavutköy-Taşoluk region) of Istanbul. The boy's father had removed the tick on the day of the bite.

On admission, the patient appeared well. His body temperature was 39 °C. His blood pressure was 95/60 mmHg and his pulse was 92 beats/min. His weight and height were at the 25th and 50th percentiles, respectively. A hyperemia was detected on his scalp. There was no hepatosplenomegaly, lymphadenopathy, petechiae, or ecchymoses. The results of the respiratory, cardiovascular, gastrointestinal, and neurological examinations were completely normal. Laboratory test results were as follows: white blood cell (WBC) count 6.1×10^9/l, red blood cell (RBC) count 4.84×10^{12}/l, platelet count 175×10^9/l, hemoglobin level 11.3 g/dl, erythrocyte sedimentation rate 10 mm/h, and C-reactive protein 3 mg/dl. Enzyme levels were elevated: alanine aminotransferase (ALT) 89 U/l (normal <40 U/l), aspartate aminotransferase (AST) 263 U/l (normal <40 U/l), lactate dehydrogenase (LDH) 547 U/l (normal 125–243 U/l), and creatine phosphokinase (CK) 7690 U/l (normal

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doi:10.1016/j.ijid.2009.07.017



Figure 1. Location where new the CCHF strain was acquired in Istanbul relative to the previously identified endemic zone (Kelkit Valley region of Turkey).

34–174 U/l). The prothrombin time (PT) and activated partial thromboplastin time (aPTT) were prolonged at 20.1 s and 43.7 s, respectively.

The patient was defined as a suspected CCHF case based on the history of a tick bite, the clinical symptoms, and the laboratory findings. He was isolated in a single room and contact isolation procedures were implemented. Supportive therapy consisted of hydration and delivery of fresh frozen plasma. To definitively diagnose the patient with CCHF, whole blood samples were drawn into EDTA-containing vacutainer tubes on the third day after admission. The blood samples were sent to the local and national reference laboratories: the Microbiology and Clinical Microbiology

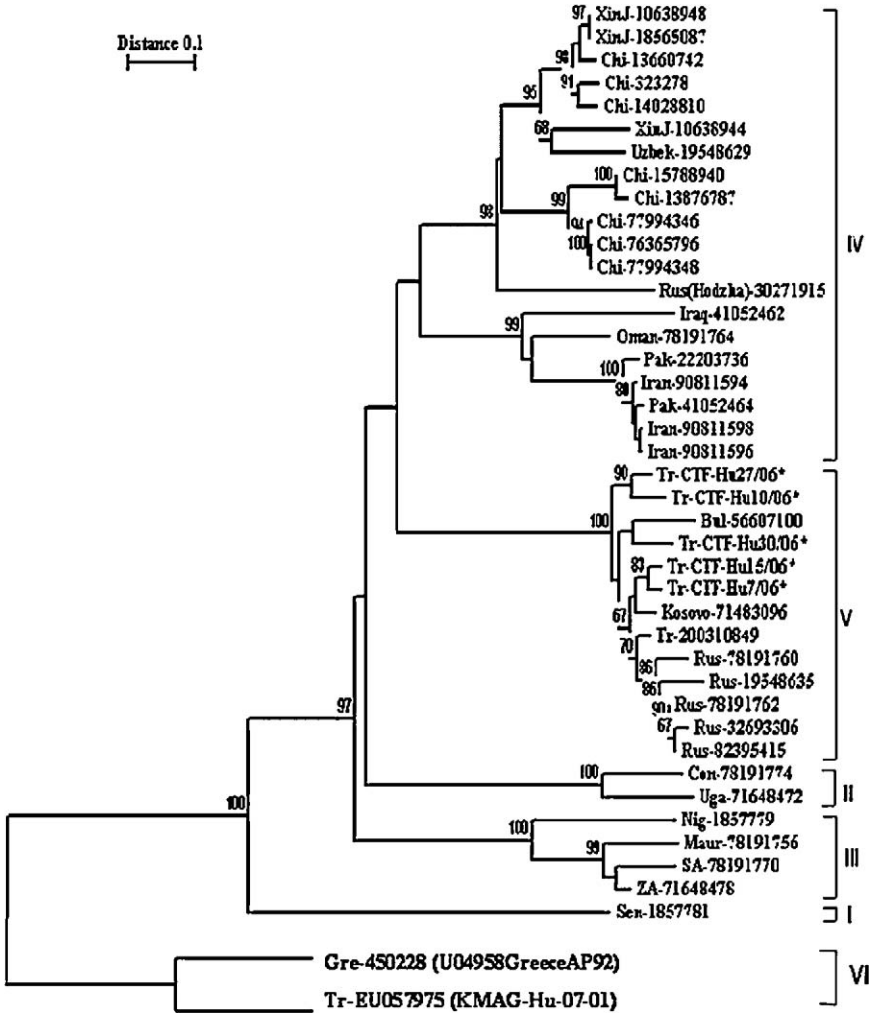


Figure 2. Phylogenetic trees for CCHF strains. I: West Africa; II: Democratic Republic of Congo; III: Southern/West Africa; IV: Asia/Middle East; V: Europe/Turkey; VI: Greece (AP92). Previous isolates from Turkey and the new KMAG-Hu-07-01 virus are highlighted on the tree.

Department of the Istanbul University Cerrahpasa Medical Faculty (local) and the Virology Laboratory of the Refik Saydam Hygiene Center in Ankara (national). Serologic testing for anti-CCHF virus IgM and IgG was done by ELISA. IgM antibodies specific for CCHF virus were found. Anti-CCHF virus IgG was absent. Molecular data were also obtained by real-time reverse transcriptase-PCR (RT-PCR). RNA was extracted from 200 μl of whole blood using a commercial RNA extraction kit (High Pure Viral Nucleic Acid Kit, Roche Diagnostics, Germany) and cDNA synthesized using the Omniscript reverse transcription kit (Qiagen, Germany), according to the manufacturer's instructions. RT-PCR for CCHF virus RNA was positive. In the design of the primers used during the diagnostic screening for CCHF virus RNA, all S segment sequences from Turkey and from several eastern European countries were downloaded from GenBank and were aligned using the Clustal Wallis software program. Primers for the S segment of the virus were selected for the first and second rounds of amplification. The sites of the possible primers were selected visually and were evaluated using the Primer test option of the Primer Express software (Applied Biosystems, USA). After the initial diagnostic PCR, the amplicon was sequenced. The obtained sequence closely matched the sequence of the AP92 strain. For exclusive amplification of AP92-strain RNA, we chose primers from the S segment of this strain. For molecular analyses and comparisons, a 439-bp segment (between nucleotides 218 and 657, numbered according the AP92 strain (U04958)) of the S segment was used. Phylogenetic analysis of the obtained sequence of the new CCHF virus revealed that the strain is closely related to the AP92 strain, which has been found in Greece. The nucleotide sequence divergence between the new strain and AP92 was 17.43% (200/218 nt). However, at the amino acid sequence level (146 amino acids), the two strains differed only at two positions (1.36%). The sequence of the new Turkish strain was deposited in GenBank under the accession number **EU057975**. The strain was named KMAG-Hu-07-01 (Figure 2).

On the day of admission, ribavirin was not administered because it was not available. The patient's symptoms, PT and aPTT had already improved on the second day of hospitalization. No hemorrhagic signs were observed during the course of the disease, and all abnormal laboratory test results (ALT, AST, LDH, and CK) returned to normal by day 10. The child was defined as a mild CCHF case and was discharged with complete recovery.

3. Discussion

Crimean hemorrhagic fever was first described in 1944, when approximately 200 Soviet military personnel were infected in Crimea in the wake of World War 2. The virus was identified in 1967 and was found to be similar to a virus isolated in 1956 from a febrile patient in Congo. The virus was then named Crimean-Congo hemorrhagic fever virus.[11] Over the last decade, climate, environmental, and anthropogenic factors have driven the expansion of CCHF-endemic areas and have triggered the onset of community outbreaks.[12] Such outbreaks were recorded in Turkey in 2002–2008.[4–8] In April 2008, a cluster of suspected CCHF cases occurred in southern Bulgaria, a few kilometers from the border with Greece.[13] Turkey and all Balkan countries with the exception of Greece have recorded the circulation of CCHF strains among animal hosts, ticks, and humans, and CCHF is considered to be endemic in these countries.[14] The wide outbreaks that occurred in Turkey were preceded by several decades of serologic evidence of zoonotic transmission of CCHF.[12] In previous studies,[4–8] all of the CCHF patients identified in Istanbul were imported cases; that is, they had been infected outside of Istanbul. The present patient was the first CCHF case to be infected within the city.

The typical course of CCHF infection has four distinct phases: incubation (3–7 days), pre-hemorrhagic (4–5 days), hemorrhagic (2–3 days), and convalescence (10–20 days). The pre-hemorrhagic period is characterized by the sudden onset of fever (39–41 °C), headache, myalgia, and dizziness. The hemorrhagic period is short and usually begins between the third and fifth days of the disease. Hemorrhagic manifestations range from patchy to large hematomas appearing on the mucous membranes and the skin. Our patient was within the pre-hemorrhagic period when admitted and presented with complaints of fever, fatigue, and loss of appetite. The clinical and laboratory findings showed that the case was mild according to the severity criteria defined by Ergonul et al.[15]

In 1967, when the virus was first named CCHF virus,[11] the antigenic structures of the viruses from various geographic regions were thought to be indistinguishable. However, nucleic acid sequence analysis has since revealed extensive genetic diversity. All previously identified Turkish CCHF virus isolates from the recent outbreak have clustered closely with CCHF viral strains from southwest Russia and the Balkan countries (lineage V in Figure 2). The strains from southeast Russia are closely related to the European strains. Because of their low genetic divergence, the European strains are grouped together, except for the Greek AP92 strain.[9,10] The AP92 strain, isolated from *Rhipicephalus bursa* ticks in Greece, differs from European strains and forms a very distinct clade, quite separate from the other CCHF virus lineages. This genetic difference may be attributable to the different species of tick vector and/or to genetic isolation due to the intervening mountain ranges.[9]

In this report, we have described a newly identified CCHF strain from Turkey. This strain of CCHF was found in a different area of Turkey than previously identified Turkish strains, and the new strain is genetically quite different from those found previously in Turkey. It is closely related to the AP92 virus found previously only in *Rhipicephalus bursa* ticks in Greece.

The current treatment approach for CCHF is based on general supportive measures, monitoring the patient's hematologic and coagulation status, with replacement of cells and factors as needed, and the use of ribavirin.[16] Ribavirin is the recommended antiviral agent for infected patients, although its mechanism of action is not clear. It should be noted that there is no evidence from randomized clinical trials to support the use of ribavirin to treat human CCHF, and ribavirin's effectiveness has only been described in observational studies.[4] A recent study suggested that mild cases do not need to be treated with ribavirin.[17] On admission, ribavirin could not be given to our patient because it was not available. Thereafter, it was not administered to the patient, since his case was subsequently defined as mild.

In conclusion, this study describes a case of CCHF infection in a child, caused by a virus strain closely related to the AP92 strain. The newly identified strain of CCHF is genetically quite different from strains previously found in Turkey. It is closely related to the AP92 virus previously found only in Greece. The fact that the infection was most likely acquired in the rural Balkan part of Istanbul is even more interesting. This new focus of infection could present a good opportunity to describe the transmission dynamics of CCHF infection.

Conflict of interest: No conflict of interest to declare.

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