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The First Fatal Case of Crimean-Congo Hemorrhagic Fever Caused by the AP92-Like Strain of the Crimean-Congo Hemorrhagic Fever Virus

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Crimean-Congo hemorrhagic fever (CCHF) is the most widespread tick-borne viral disease, which has been reported in more than 30 countries in Africa, Asia, southeastern Europe, and the Middle East (1). The disease is an important public health threat owning to a high fatality rate (up to 50%), lacks any specific antiviral therapy or FDA-approved vaccine, and the possible human-to-human transmission (1,2). The causative agent, CCHF virus (CCHFV), is a *Nairovirus* in the family *Bunyaviridae*, which is transmitted to humans by tick bites, as well as by direct contact with blood or tissue from viremic livestock or infected humans (3). Thus, farmers, animal herders, slaughterhouse workers, veterinarians, and healthcare workers are at risk of contracting CCHFV. In Iran, CCHFV is endemic and has been reported in most provinces (26 out of 31) (3). Evidently, the genetic diversity of CCHFV is not related to its pathogenicity in humans (4,5). However, strain AP92, unlike other CCHFV isolates, seems to be avirulent in humans (1,2). To date, the AP92 strain has not been documented in Iran.

In October 2015, a serum sample was collected from a 60-year-old man with suspected CCHFV infection, living in Mazandaran province (Fig. 1). The patient had been admitted to the department of Arboviruses and Viral Hemorrhagic Fevers (National Ref. Lab.) of the Pasteur Institute, Iran. He was a farmer and stockman, living in a village called Kelardasht, and he had experience slaughtering livestock. Interestingly, he had used to squish ticks by his hands. The patient had presented with high fever (>38°C), severe muscle pain, fulminant gastrointestinal bleeding, and thrombocytopenia (platelet count; 5,000/ml). He underwent ribavirin therapy, but died the next day after a hemorrhage developed.

The patient's serum sample was analyzed for CCHFV using serological and molecular assays. Specifically, an ELISA for anti-CCHFV IgM and IgG (6) revealed that neither was present. To detect the CCHFV genome, viral RNA was extracted using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. To amplify the genome, reverse transcriptase-PCR (RT-PCR) was carried out using the F2 (5′-TGGACACCTTCACAAACTC-3′) and R3 (5′-GACAATTCCCTACACC-3′) primers which target a 536-bp region in the S segment along with the Qiagen One-Step RT-PCR kit (Qiagen), according to the manufacturer's protocol. The RT-PCR mix consisted of 10 μl 5 × Qiagen OneStep RT-PCR Buffer, 2 μl dNTP Mix (containing 10 mM of each dNTP), 0.6 μM each primer, 2 μl Qiagen OneStep RT-PCR Enzyme Mix, and 500 ng extracted RNA in a 50 μl total reaction volume. The RT-PCR was strongly positive for CCHFV.

To perform phylogenetic analysis, the PCR product was sequenced by Macrogen (Seoul, South Korea) and verified using BLAST <http://blast.ncbi.nlm.nih.

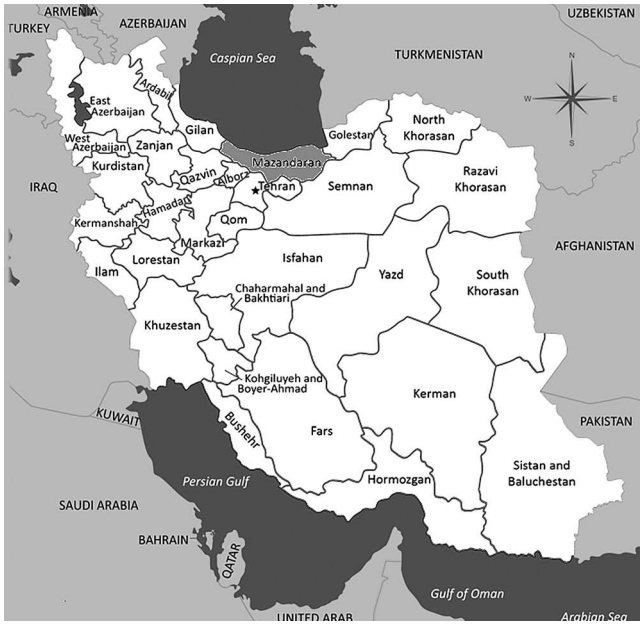

Fig. 1. Geographical location of Mazandaran province where the CCHFV strain Iran-4675 was isolated.

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gov/Blast.cgi>. The strain was designated as ''Iran-4675'', and the sequence was submitted to GenBank with the accession number of KT899991.

A data set was made that included the newly submitted sequence shown above as well as 39 other sequences from GenBank in both nucleotide and amino acid levels. A multiple sequence alignment was performed using the Clustal W method. To validate the analysis, the maximum likelihood method, combined with 10,000 Bootstrap replicates, was used to construct a phylogram (Fig. 2). The number of base substitutions per site was estimated, using Bootstrap (10,000 replicates) as variance estimation method and the Kimura 2-parameter substitution model. Bootstrap values above 70% were considered significant. Phylogenetic analysis was conducted using MEGA6 (7). Phylogenetic analysis of the sequence showed that a CCHFV strain in question was closely related to the AP92 strain, which was first reported in Greece (8) (Fig. 2). Therefore, this report constitutes the first evidence of CCHFV closely related to the AP92 strain in Iran. As shown in the phylogenetic trees, the Iran-4675 isolate clustered within clade VI (Greece), demonstrating high similarity to the AP92 and KMAG-Hu-07-01 isolates. Furthermore, its high similarity to AP92 manifested within nucleotide identities (91%; 470/515 nt), amino acid identities (98%; 168/171 aa), and pairwise distance (0.084; SE, 0.015). The overall mean distance of this data set was estimated as 0.207 (SE, 0.013). Regarding Iran-4675, the highest distance was 0.255 in pairwise with Zahedan-19, and the lowest distance was in pairwise with AP92 and AP92-like strains reported from Turkey and Greece (9–11).

Among the *Arboviruses*, CCHFV is the most genetically diverse, and all CCHFV strains have been classified into 7 lineages. Namely, Asia 1 and 2, Africa 1–3, and Europe 1 and 2 (1), of these 4 lineages have been found in Iran (3), indicating Iran as the broadest CCHFV genetic diversity among endemic countries (12). In 1975, the AP92 strain of CCHFV was first isolated from ticks of the species *Rhipicephalus bursa* in Greece (8). This strain is significantly different in sequence from other CCHFV isolates (more than 20% nucleotide variation), and it has been proposed that the AP92 strain does not cause disease in humans. However, 2 studies from Turkey have described a mild clinical disease caused by an AP92-like CCHFV (11,13).

We have herein reported the first fatal case of fulminant CCHF caused by an AP92-like strain. Moreover, this is the first evidence of an AP92-like CCHFV strain in Iran; the infection was ultimately fatal, perhaps because the adaptation of CCHFV to region-specific hosts, leading to differences in pathogenicity in humans. Several lines of evidence have indicated that in fatal cases of CCHFV, no anti-CCHFV IgM or IgG antibodies are detectable (1). The present study corroborated those results detecting no IgM or IgG antibodies to CCHFV in the patient's serum sample. Therefore, in cases of suspected CCHFV with severe hemorrhage, diagnostic detection must target viral RNA as well as antibodies.

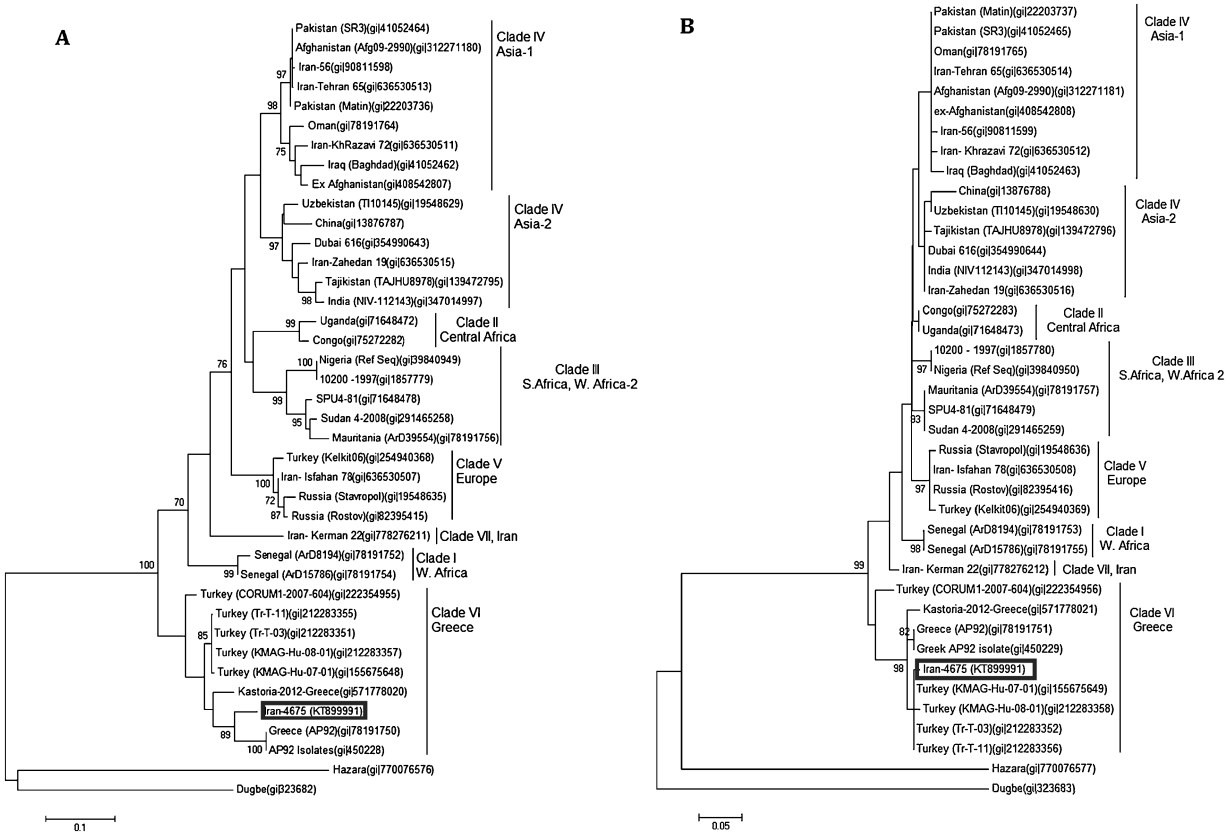

Fig. 2. (A) Maximum-likelihood phylogenetic tree (nucleotide sequence) with Bootstrap 10,000 and Kimura 2-parameter substitution model. (B) Maximum-likelihood phylogenetic tree (amino acid sequence) with Bootstrap 10,000 and p-distance substitution model.

The AP92 strain of CCHFV is assumed to be avirulent in humans; in fact, on this basis, it has been suggested as a candidate for vaccine studies (2). However, based on the present report, as well as 2 studies from Turkey that described clinical cases of CCHFV infection by an AP92-like strain, further investigation should be carried out regarding the virulence of AP92 in genetically different populations. This suggestion is corroborated by the data from Akıncı et al. (14), indicating that host genetics (which differ among populations) are associated with the clinical course of CCHFV. In rural areas of Iran, some traditional habits among villagers, especially herdsmen may put them at risk of CCHFV.

In conclusion, this study reports a case of fatal CCHFV infection by a strain closely related to AP92. The latter strain is genetically quite different from other CCHFV strains that have been reported in Iran, and far from any other strain of CCHFV in the world.

Conflicts of interest None to declare.

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