Phylogenetic Characterization of Crimean-Congo Hemorrhagic Fever Virus, Spain

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Two cases of Crimean-Congo hemorrhagic fever were reported in Spain during 2016. We obtained the virus from a patient sample and characterized its full genomic sequence. Phylogenetic analysis indicated that the virus corresponds to the African genotype III, which includes viruses previously found in West and South Africa.

Crimean-Congo hemorrhagic fever (CCHF) is a severe disease transmitted to humans mainly by ticks, primarily of Hyalomma spp. This zoonotic disease is caused by CCHF virus (CCHFV), a nairovirus in the family Bunyaviridae, which was detected first in Crimea in 1944 and isolated 12 years later in the Democratic Republic of the Congo. Since the beginning of the 21st century, CCHFV has been spreading from disease-endemic areas to new regions previously considered free of the disease, particularly in areas where Hyalomma spp. ticks are present. In nature, CCHFV usually circulates between asymptomatic animals and ticks in an enzootic cycle.

CCHFV has a negative-sense and tripartite RNA genome (small [S], medium [M], and large [L] segments) with high genetic diversity. The sequences of the S segment, which is the most conserved at the nucleotide level, could be distributed in 6 lineages (1–6). Each genetic lineage has been linked to geographic regions in Africa, the Middle East, Asia, and Europe, where Hyalomma spp. ticks are present (7). Genotypes I, II, and III have been described in Africa; genotype IV in Asia; and genotypes V and VI in Europe. However, strains have moved between geographic regions; migrating birds, unregulated wildlife trade, livestock import and export, and a global movement of humans could have dispersed the virus or CCHFV-infectected ticks (8).

In western Europe, the presence of the virus had only been detected indirectly by means of serologic methods in the serum of 2 people from southern Portugal (9). However, genotype III CCHFV was detected in ticks from deer captured in western Spain in 2010 and ticks from birds migrating from Morocco in 2013 (10,11). No cases among humans in Spain had been reported until 2016, when 2 autochthonous cases were diagnosed (12). Here, we report the complete genomic sequence of the virus from 1 of these case-patients and show the phylogenetic relationships among the 3 segments.

The Study
We previously detected CCHFV in serum samples from the 2 patients in Spain with autochthonous CCHV (12); the index case-patient died. For this study, we obtained viral RNA (3.6 × 10⁷ copies/mL) from the secondary case-patient in a sample taken 4 days after onset of symptoms by using the QIAamp viral RNA Mini kit (QIAGEN, Hilden, Germany). We then amplified the virus in a single-step reverse-transcription PCR by using the SuperScript III One-Step RT-PCR system with the Platinum Taq High Fidelity DNA Polymerase kit (Invitrogen Life Technologies, Barcelona, Spain) with overlapping primers throughout the complete genome (Table). PCR conditions were amplification at 52.5°C for 30 min, 94°C for 2 min, and then 40 cycles at 94°C for 15 s, 48°C–55°C for 30 s, and 68°C for 1 min/kb, with a final extension cycle at 68°C for 5 min. We designed primers for each segment in most conserved regions after aligning available CCHFV sequences from all genotypes retrieved from GenBank (Table). We directly sequenced purified amplicons by using additional internal primers (data not shown). We assembled and analyzed the consensus sequence of each segment by using SeqMan Pro from the Lasergene Suite 12 (DNASTAR Inc., Madison, WI, USA). To get complete finished genomes, we used a hybrid-capture method as described by Blackley et al. (13) by using probes designed against the CCHFV sequences obtained from amplicon sequencing.

To characterize the complete CCHFV genome, we performed a phylogenetic analysis of the full S, M, and L segments (Figure, https://wwwnc.cdc.gov/EID/article/23/12/17-1002-F1.htm). The 3 segments were aligned by using ClustalW in MEGA 5.2 (http://www.megasoftware.net) and representative available CCHFV sequences from GenBank of all genotypes. We generated

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Because the virus is circulating in Spain, additional studies will be required to establish the distribution of the virus in this country.

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