Autochthonous Crimean–Congo Hemorrhagic Fever in Spain


SUMMARY

Crimean–Congo hemorrhagic fever (CCHF) is a widely distributed, viral, tickborne disease. In Europe, cases have been reported only in the southeastern part of the continent. We report two autochthonous cases in Spain. The index patient acquired the disease through a tick bite in the province of Ávila — 300 km away from the province of Cáceres, where viral RNA from ticks was amplified in 2010. The second patient was a nurse who became infected while caring for the index patient. Both were infected with the African 3 lineage of this virus. (Funded by Red de Investigación Cooperativa en Enfermedades Tropicales [RICET] and Efficient Response to Highly Dangerous and Emerging Pathogens at EU [European Union] Level [EMERGE].)

CHF IS A SEVERE VIRAL DISEASE CAUSED BY A NAIROVIRUS OF THE BUNYAVIRIDAE family. Humans become infected through tick bites or contact with viremic patients or animals. Clinically, CCHF is characterized by fever, coagulopathy, and hepatitis.1 The disease is widespread geographically and has been identified in more than 30 countries in Africa, Asia, the Middle East, and Europe. In Europe, CCHF has been reported only in countries located in the southeastern part of the continent: Russia, Georgia, Ukraine, Bulgaria, Albania, Kosovo, Greece, and Turkey.2 Here we report the epidemiologic and clinical course of two patients who contracted the disease autochthonously in Spain.

INDEX PATIENT

A 62-year-old man who had a history of hypertension and obstructive sleep apnea and had been living in Madrid presented to the Infanta Leonor University Hospital with a 2-day history of high fever, abdominal pain, malaise, nausea, and diarrhea. During the ensuing hours, purpuric lesions and hematomas developed at venipuncture sites. The next day, the patient was transferred to the intensive care unit (ICU) because of severe coagulopathy, with macroscopic hematuria, purpuric skin...
lesions and hematomas, a low platelet count, and prolonged prothrombin and partial-thromboplastin times. The patient's family reported that four days before admission, while visiting relatives, the patient had walked through the fields in San Juan del Molinillo, a small village located in Ávila, a province of central-western Spain (Fig. 1). When he returned to his relatives' home, he noticed a tick on his left knee. The patient lived and worked in Madrid and had not traveled abroad. After learning that the patient may have had a tick bite, clinicians initiated treatment with doxycycline.

On the seventh day of illness, the patient's clinical condition deteriorated rapidly. He had macroscopic hematuria, worsening of purpuric skin lesions and hematomas, fulminant hepatic failure, severe respiratory insufficiency, encephalopathy, hypoglycemia, and severe metabolic acidosis. Later that day, he was transferred to the ICU at Gregorio Marañón University General Hospital to be evaluated for liver transplantation. During the next 24 hours, the patient had distributive shock, oliguric renal failure, very high liver-enzyme levels, and persistent metabolic acidosis. All tests for routine infections were negative. The patient died on the ninth day of illness. The family consented to necropsy. Data regarding treatment, laboratory analyses, and clinical variables are provided in Figure S3 and Tables S1, S2, and S3 in the Supplementary Appendix, available with the full text of this article at NEJM.org.

Analysis of serum samples obtained on the sixth and eighth days of the patient's illness revealed 1.0×10⁸ and 1.2×10⁹ viral copies per milliliter, respectively. The CCHF virus was isolated on the sixth day of illness, the dose of ribavirin was reduced to 500 mg every 8 hours, in keeping with doxycycline.

We did not identify cytopathic cellular inclusions or inflammatory infiltrates in any of the organs examined, and endothelial swelling was not a prominent feature. There was massive hepatocyte necrosis, with sparing of narrow periporal and pericentral rings, and mild sinusoidal congestion. No Kupffer-cell hyperplasia or inflammatory infiltrates were observed. The hepatocytes had a swollen appearance and widespread necrosis. In general, the hepatocytes contained cytoplasmic macro- and microvesiculation (Fig. 2D and 2E, respectively). Although most mucosae were preserved, the appearance of the colon was striking owing to its complete epithelial denudation. The crypts were filled with basophilic mucoid material and walled by sloughed apoptotic cells, again without inflammatory infiltrates (Fig. 2A and 2B). Occasional microthrombi were observed. The bone marrow showed hemorrhages and a preserved megakaryocyte population with a normal morphologic appearance. The spleen showed slight lymphoid depletion and hemorrhage but no necrotic areas.

SECOND PATIENT
A 50-year-old female nurse was the second patient. On August 23, 2016 (Fig. 1), she had assisted with the endotracheal intubation of the index patient and with the insertion of femoral venous and arterial catheters. Profuse bleeding complicated placement of the catheters, and the nurse's hands were in direct contact with the patient's blood, although the skin was not punctured. The nurse lived and worked in Madrid and reported no recent travel abroad or to the countryside. She had had no recent tick bites.

On the first day of her illness, August 27, fever, asthenia, and arthromyalgias developed (Fig. 1). On the second day, the patient was admitted to the ICU at Infanta Leonor University Hospital owing to the presence of petechiae, thrombocytopenia, and a mild increase in aminotransferase levels. On the third day of illness, vaginal bleeding started, coinciding with expected time of her normal menstruation period. On the fourth day of illness, CCHF was suspected. Empiric treatment with ribavirin was started, with an oral dose of 1000 mg administered every 6 hours and continued for the next 24 hours. The drug was administered intravenously thereafter. On the sixth day of illness, the dose of ribavirin was reduced to 500 mg every 8 hours, in keeping
Figure 1. Clinical Events and Locations.
Panel A shows a timeline of events related to the two patients and their contacts. The color blue denotes the day of infection for each patient. Panel B shows the geographic location of Crimean–Congo hemorrhagic fever (CCHF) worldwide and in Spain, according to the World Health Organization. Circle 1 marks the geographic area (39.63° north and 7.33° west), where hyalomma ticks infected with the CCHF virus have previously been identified. Circle 2 marks San Juan del Molinillo (Ávila), where the index patient became infected. Circle 3 marks Madrid, where the first and second cases were detected and where the patients received the diagnosis and were treated. DOI denotes day of illness, GMUGH Gregorio Marañón University General Hospital, HLIU high-level isolation unit, ICU intensive care unit, ILUH Infanta Leonor University Hospital, and RT-PCR reverse-transcriptase–polymerase chain reaction.
with the protocol for the treatment of CCHF from the World Health Organization. The patient was subsequently transferred to the high-level isolation unit at La Paz University Hospital.

On admission to this unit, the patient was awake, alert, and fully oriented to time and place. Physical examination revealed subconjunctival hemorrhage in the right eye, cutaneous petechiae on pressure areas, and vaginal bleeding. Hypoxemic respiratory failure associated with a moderately sized pleural effusion in the right lung led to treatment with oxygen by nasal cannula until the 15th day of illness. On the 9th day of illness, vaginal bleeding stopped. Levels of aminotransferase and lactate dehydrogenase began to decrease on the 9th day of illness, and platelet levels began to increase on the 11th day. Mild renal impairment persisted until the 20th day. Complete data regarding the administered treatment, laboratory results, and clinical variables are available in Tables S4 through S7 in the Supplementary Appendix.

On the 9th day of illness, we discontinued treatment with ribavirin because severe hemolytic anemia was suspected. The patient received transfusions of a total of 5 units of platelets before we performed any invasive procedures and when the platelet count fell below 10,000 per cubic millimeter. The transfusions took place on the 6th and 8th days of illness.

Levels of CCHF virus in the blood were highest in a stored sample obtained on the 2nd day of illness, at 3.6×10^7 copies per milliliter. We also cultured the virus from a plasma sample on the 7th day of illness. The first negative result for viremia, obtained by means of real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay, was observed on the 20th day of illness (Fig. 3). Anti-CCHF virus antibodies were not detectable on the 2nd day of illness. IgM titers increased to 1:640 on the 6th day of illness and started to decrease after the 15th day. The titer for IgG antibodies remained constant (1:640), with the exception of an isolated decrease that was probably the result of a technical issue. RT-PCR assays of several fluid samples were performed;
vaginal fluid was positive on the 4th day of illness, saliva on the 8th day, and conjunctival, nasal, and rectal swabs were sporadically positive, with very low viral titers. After the 14th day of illness, RT-PCR assays of all body fluids were negative. On the 22nd day of illness, the measures taken in the high-level isolation unit were discontinued when two consecutive RT-PCR assays of the blood were negative.

**Identification of Virus and Contact Tracing**

**Identification of Virus**

We used two PCR methods designed to amplify two different targets of the CCHF viral genome (Fig. S1 in the Supplementary Appendix). The sequences in the plasma samples (592 bp of the S segment) obtained from both patients were 100% identical. Nucleotide sequences were compared with those available in the database of the National Center for Biotechnology Information with the use of the Basic Local Alignment Search Tool (BLAST), and the sequences showed a 99% identity with those from the African 3 lineage, such as the Mauritania ArD39554 (GenBank accession number, DQ211641) strain of the CCHF virus. Phylogenetic analysis also revealed 99% identity with African 3 lineage sequences (GenBank accession numbers, KY492289 and KY492290) (Fig. S2 in the Supplementary Appendix).

**Contact Tracing**

The Public Health Service from Comunidad de Madrid and the occupational health services at each hospital performed contact tracing to identify all persons exposed to either patient. Contacts took their body temperature twice daily for 14 days after exposure. Among the 437 people who were exposed, 386 were classified as having a high risk of acquiring the infection and 51 as having a low risk (definitions are available in the
Supplementary Appendix). In addition, 59 health care workers in the high-level isolation unit had protected exposures while caring for the second patient. None contracted symptomatic CCHF. Serologic testing was not performed in contacts to rule out asymptomatic disease.

**DISCUSSION**

These autochthonous cases of CCHF represent a change in the geographic distribution of the disease. Given the expanding distribution of the main vector,3,6-8 the appearance of these two cases in a previously unaffected region of Europe reinforces the notion that CCHF is a reemerging infectious disease.

The index patient acquired CCHF through a tick bite in the province of Ávila. The southern region of this province shares a border with the province of Cáceres, where the nucleic acid of the CCHF virus was detected in *Hyalomma lusitanicum* ticks obtained from deer in 2010.3 The viral nucleic acids amplified in the blood of both patients were identical and shared a genetic footprint with viruses of the African 3 lineage but not with sequences from Eastern Europe (Fig. S2 in the Supplementary Appendix). The region of the CCHF virus sequenced from infected ticks in Cáceres1 (positions 115 through 326 in the S fragment) is typically amplified by means of an in-house RT-nested PCR analysis (positions 123 through 764). The amplification of sequences in samples from ticks and from the case patients showed that the sequences were nearly identical to the ArtD39554 strain — 98% for the ticks and 99% for the patients. We therefore conclude that our index patient was probably infected by the same CCHF virus that was detected in ticks in 2010. This particular strain could have arrived in Spain through infected ticks carried by northward migrating birds from Morocco, the live-stock trade, the movement of infected animals, or other means.8,9

We did not suspect CCHF until the second patient presented with a clinical picture similar to that of the index patient. It is very likely that in the absence of a second case, this outbreak of CCHF would not have been discovered. Thus, it is possible that other cases of CCHF may have occurred in Spain in recent years. In our circumstance, more than 400 people — primarily health care workers — may have been exposed to the CCHF virus while not wearing the appropriate personal protective equipment. Although the nosocomial transmission of CCHF has been previously described,10 we have not detected any additional cases beyond that of the second patient, whose exposure to the virus was substantial.

Both patients fulfilled the criteria for severe CCHF.11,12 Although in the second patient we observed a rapid decline of viremia with the concomitant use of intravenous ribavirin, its efficacy for the treatment of CCHF remains controversial — a meta-analysis did not reveal evidence in favor of its use for this disease.13

The second case allowed us to study various body fluids for the presence of the CCHF virus, an aspect of infection for which there are very few data. Unlike Ebola virus disease (EVD),24 in CCHF the genetic material in all fluids cleared before the viremia did. Although the viral load in the second patient was low during the last week of infection, she did have viremia for 20 days, a fact that delayed her discharge from our high-level isolation unit. At this time, it is unclear whether patients who are clinically well but still have positive results on RT-PCR for the CCHF virus in their blood can be safely treated without the use of the precautions taken in the high-level isolation unit. In Turkey, patients are routinely discharged after improvement in the clinical picture and in laboratory results without confirmation of clearance of the viremia.15 However, there has been no evidence of subsequent spread of the CCHF virus in Turkey, where these discharge criteria are observed.

Our investigation of these cases provided us with the rare opportunity to perform a human necropsy — albeit inadvertently — in a case of CCHF.16-18 Massive liver necrosis in the absence of inflammatory infiltrates is consistent with the findings reported in other hemorrhagic fevers.19-21 No cytopathic inclusions have been reported in cases of CCHF,17 a finding that is in contrast with the Cowdry type A intranuclear inclusions seen in cases of Rift Valley fever29 and the cytoplasmic eosinophilic inclusions reported in cases of EVD21 and Marburg virus disease.22 In CCHF, the appearance of hepatocytes and the absence of inflammatory infiltrates provide support for the view that there is a primary cytopathogenic effect.

An unexpected finding in the necropsy was the selective and complete apoptosis of colonic...
enterocytes in the absence of inflammatory infiltrates. This feature of the disease is unusual because it is not accompanied by multigland failure and suggests massive, viral-induced cell damage. The in vitro activation of apoptotic pathways by the virus or by host cells has been reported.23 Similar findings have been described in cases of early infection with the simian immunodeficiency virus.24 It is possible that specific colonic targeting is typical of this particular strain. This characteristic would partly explain the ionic imbalances and diarrhea. The colonic epithelium could constitute a viral replication site. Findings from bone marrow provide support for a peripheral pathogenic mechanism for thrombocytopenia, as has been described in association with other viral infections.25

In conclusion, we report two autochthonous cases of CCHF occurring in Spain. Our observations highlight the importance of routine surveillance of vectors capable of spreading CCHF. When CCHF nucleic acid is amplified from infected ticks in geographic areas that have previously been unaffected by CCHF, clinicians should remain alert to the possibility of human cases.

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APPENDIX

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