Title

New circulation of genotype V of Crimean-Congo haemorrhagic fever virus in humans from Spain

Running title

New clade of Crimean-Congo haemorrhagic fever virus in Spain.

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Keywords

Crimean-Congo haemorrhagic fever virus; emerging disease; haemorrhagic fever; tick-borne disease; zoonosis; Spain.

Abstract

Crimean-Congo haemorrhagic fever (CCHF) is a widespread tick-borne viral disease caused by the Crimean-Congo haemorrhagic fever virus (CCHFV). CCHFV has been implicated in severe viral haemorrhagic fever outbreaks. During the summer of 2016, the first two cases with genotype III (Africa III) were reported in Spain. The first aim of our study was to determine the presence of CCHFV among patients with febrile illness during the spring and summer periods in 2017 and 2018. Finally, we perform a phylogenetic analysis to determine the genotype of the virus.

Methods

We prospectively evaluated patients older than 18 years who came to the emergency with fever. Specific IgM and IgG antibodies against CCHFV by ELISA and one immunofluorescence assay against two different proteins (nucleoprotein and glycoprotein C) was done. Moreover, a Real Time PCR was performed. A phylogenetic analysis was carried out to characterize the complete CCHFV genome.

Results

A total of 133 patients were selected. The mean age was 67.63 years and 60.9% were male. One-third of the patients presented an acute undifferentiated febrile illness. Three patients had anti-CCHFV IgG antibodies, suggesting a previous infection. One patient was found confirmed anti-CCHFV IgM antibodies and positive RT-PCR. Phylogenetic analysis indicated that the virus corresponds to the European genotype V. This patient came to the emergency in August 2018 presenting an acute febrile syndrome with thrombopenia and liver impairment.

Conclusions

We describe a new circulation of European genotype V CCHFV in Spain. Moreover, this study suggests that CCHFV is an identifiable cause of febrile illness of unknown aetiology in Spain. Thus, CCHF could be suspected in patients with fever, liver damage, and/or haemorrhagic disorders, particularly in people with risk activities who present in the spring or summer.

Introduction

Crimean-Congo haemorrhagic fever (CCHF) is a viral infectious disease caused by the homonymous microorganism, the Crimean-Congo haemorrhagic fever virus (CCHFV), a tick-borne virus of the genus *Nairovirus* and the *Nairoviridae* family. This virus is transmitted to humans by infected tick bites, by direct contact with humans or animals with a high viral load, and by direct contact with blood or other bodily fluids or tissues of viraemic humans or livestock [1,2]. To date, six viral genotypes have been identified. Genotypes I, II and III are mainly distributed in Africa, genotype IV in Asia, genotype V in East Europe, and genotype VI in Greece [3]. Genotype VI is the only one that seems to be non-pathogenic [4]. This is the reason why in Greece, where the AP92 strain circulates, it exists an elevated seroprevalence [5] with only 1 known case of mortality [6]; which differs with its neighbour country Turkey where the local viral strain is more aggressive in terms of morbidity and mortality [7].

In Europe, this virus has caused major outbreaks in the eastern region [8] (principally in Balkan countries, Turkey, and Russia). However, in recent years, its epidemiology has been changing, a fact that has been associated with climate change [9]. Due to this epidemiological modification, CCHFV is currently considered endemic in areas of Southwest Europe since human cases have been identified in western Spain since the summer of 2016 [10,11]. These cases were produced by the Africa III genotype, and it seems that it could be introduced from Morocco through migratory birds carrying premature forms of infected ticks. Currently, its enzootic cycle has been established in some areas of Spain, with infected ticks identified in wild and domestic animals [12], which makes epidemiological control more difficult [10,13]. No other CCHFV genotype has been described in humans in Spain.

From a clinical point of view, this infection can present with a wide clinical spectrum, from an asymptomatic or oligosymptomatic disease to a life-threatening infectious condition with fever, vomiting and diverse haemorrhagic manifestations that may lead to multi-organ failure and death [14]. Laboratory analyses are frequently altered, with leukopenia, thrombocytopenia and elevated transaminases [15]. A strong clinical suspicion is needed in order to obtain a clear and fast diagnosis, initiate supportive treatment if needed, and activate biosafety measures to prevent transmission.

The aim of this study was to evaluate whether CCHFV is a cause of acute febrile syndrome, to determine the risk factors and to describe the main clinical and laboratory characteristics of the acute cases identified. In this way, sequencing in samples with CCHFV were done.

Methods

Study type and sample collection

Descriptive, cross-sectional study that was carried out during the months of May to October in 2017 and 2018 at the emergency of University Salamanca Hospital (HUS) located in western Spain. It covers an area of 12,350 km^2 encompassing 362 municipalities with a population of 331,473 individuals. All patients with an age above 18 years who came to HUS for a febrile illness without an aetiologic diagnosis were eligible. Patients were evaluated by the emergency department and were

included if they presented with fever without an aetiological diagnosis. Serum and plasma samples were taken to determine the presence/absence of CCHFV. Details were collected from the medical records.

Immunological techniques

The antibodies for CCHFV were analysed according to methodology described previously [16] . Briefly, we used a commercial immunological kit (Vector Best, Novosibirsk, Russia) according to the manufacturer's instructions. The cut-off was calculated as the mean of the adjusted optical density (OD) of the negative control serum plus 0.2. All samples were tested in triplicate and the mean values were used for this study. Moreover, *in-house* ELISA and immunofluorescence assay (IFA) (Euroimmun, Germany) were used to confirm the positive results.

CCHFV detection by RT-PCR

Total RNA was extracted from the plasma samples using the QIAamp viral RNA Mini kit (QIAgen, Germany) according to the recommendations of the supplier. The RNA was eluted in 60 µL of RNase-free water and stored at -80ºC until needed. Furthermore, a real-time polymerase chain reaction (RT-PCR) described by Atkinson *et al.* [17] was used for the detection of the CCHFV genome in all the samples. This procedure consists in a nested PCR that reduces nonspecific amplification of RNA, it is performed by two successive PCR, including the conversion of the RNA to DNA at the first reaction, by the use of a inverse transcriptase.

Positive acute or previous cases of CCHFV

Acute cases were defined as the presence of confirmed IgM (by at least two different assays) and/or positive RT-PCR. Previous cases were characterized by the presence of IgG antibodies confirmed by two of the performed tests in the absence of markers of acute infection. Cases with a single IgG positive result, not confirmed by any other assay, were considered as probable.

Phylogenetic analysis

To characterize the complete CCHFV genome, we performed a phylogenetic analysis of the full S, M, and L segments

Ethics Statement

The study protocol was approved by the Clinical Research Ethics Committee of Investigation with Drugs of University Salamanca Hospital (HUS), Salamanca, Spain (CEIMC PI 91 09/2017). The procedures described here were carried out in accordance with the ethical standards described in the Revised Declaration of Helsinki in 2013. All clinical and epidemiological data were anonymized.

Statistical analysis

All the data were statistically analysed using the SPSS Statistics 23.0. software (*Statistical Package for the Social Sciences*). Proportions were calculated for the qualitative variables and standard deviation (SD) and interquartile range (IQR) was calculated for the mean and median respectively.

Results

One-hundred thirty-three patients were included in this study. The main epidemiological and clinical data are shown in **table 1**. The mean age (±SD) was 67.63 years (±18.8), and 81 (60.9%) were male. Most of these individuals presented with respiratory and genitourinary symptoms and were diagnosed with nonspecific febrile syndrome in the emergency department from HUS.

Table 1. Main epidemiological and clinical characteristics of patients

Characteristics	N= 133, n (%)
Age mean ± SD, years	67.6 (18.8)
Median age, years	73 (IQR, 54.5-82)
Male gender	81 (60.9)
Urban population	101 (68.7)
Emergency department initial diagnosis	
Respiratory syndrome	37 (27.8)
Genito-urinary syndrome	32 (24.0)
Febrile syndrome without focus	30 (22.5)
Fever after tick exposure	8 (6.0)
Neurological syndrome	6 (4.5)
Gastrointestinal syndrome	6 (4.5)
Biliary and hepatic infection	4 (3.0)
Cutaneous affectation	4 (3.0)
Mononucleosis syndrome	2 (1.5)
ENT infection	2 (1.5)
Fever associated to haemodialysis process	2 (1.5)

ENT: Ear, nose, and throat disorder

Serological and molecular diagnosis detecting CCHFV showed that seven patients had positive results in some test. One patient (Case 1) had anti-CCHFV IgM antibodies by two serologic assays. This patient also had positive results by Nested RT-PCR (**table 2**). Case 2 also presented IgM positive results by VectorBest EIA, but not confirmed by the other performed analysis and result by Nested RT-PCR was negative. However, case 2 and case 3 and 4 had anti-CCHFV IgG confirmed by 2 or more of the performed assays, indicating previous infection. Finally, other patient (case 5) presented a positive result for IgG in one assay while an undetermined result was obtained in another assay, being classified as indeterminate.

The nucleotide sequence of the different CCHFV segments from the confirmed acute infection (case 1) was done. Phylogenetic analyses were shown in **Figure 1.** The samples from Salamanca Spain 2018 (case 1) belong to genotype V, Europe. Is the first time that this genotype is described in human infected with CCHFV from Spain.

The main clinical data of patients with acute and previous CCHF are described in **table 3.** The patient with confirmed acute CCHF was a 53-years-old man, involved in cattle husbandry in Béjar, Salamanca province (coordinates: 40.38641 latitude -5.76341 longitude), a small city with 12,961 inhabitants, near to the Portuguese border. He presented in the emergency department at the beginning of August 2018 with a history of fever of 5 days, chills, mouth ulcerations (but not any haemorrhagic oral bullae) and acute leg myalgias with no bleeding symptomatology. Laboratory analysis revealed leukopenia, thrombopenia, increase of transaminases with an anicteric cholestasis, and prolongation of activated partial thromboplastin time. Also, an hemophagocytic syndrome was raised in order of the presence of hyperferritinemia (>10,000 ng/mL), hypertriglyceridemia and increase of lactate dehydrogenase (LDH) Also, it is important to asseverate that no nosocomial cases were reported.

Figure 1: Phylogenetic analysis

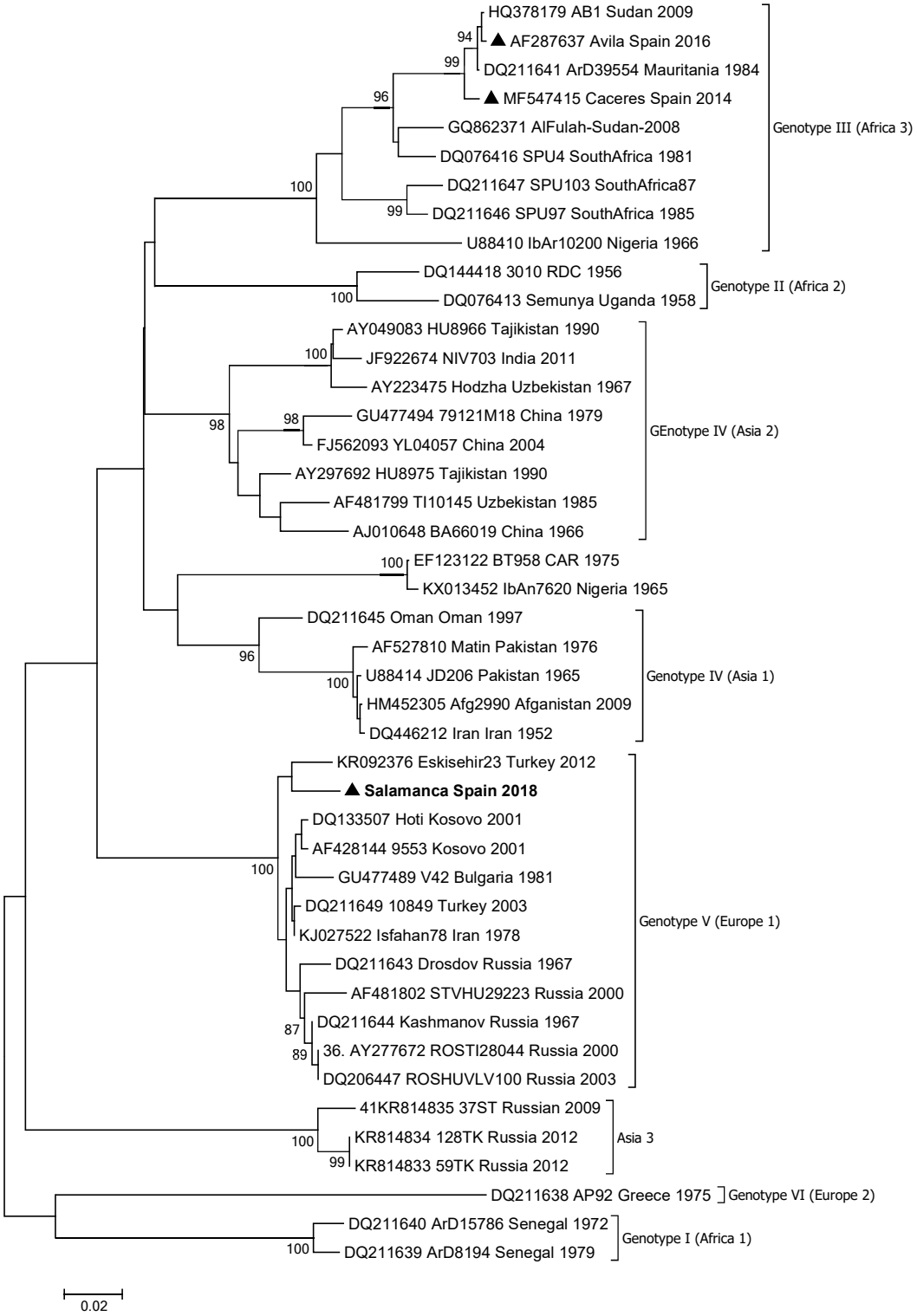

Table 2. Serological and nested RT-PCR results

Case	ELISA IgG		IFA (IgG)		ELISA IgM		IFA IgM		Nested RT - PCR	Final classification
	Vector Best	NCM*	GP**	NP***	Vector Best	NCM	GP	NP		
1	0.2 (-)	2 (+)	Negative	Negative	5.5 (+)	0.7 (-)	Negative	Positive	Positive	Confirmed acute infection
2	10 (+)	2.5 (+)	Negative	Negative	10.2 (+)	0.8 (-)	Negative	Negative	Negative	Confirmed previous infection (Undetermined acute infection)
3	10 (+)	9 (+)	Negative	Negative	0.02				Negative	Confirmed previous infection
4	1 (+)	1.3 (+)	Negative	Negative	0.01				Negative	Confirmed previous infection
5	3.3 (+)	0.9 (+/-)	Negative	Negative	0.01				Negative	Indeterminate previous infection
6	1.7 (+)	0.6 (-)	Negative	Negative	0.01				Negative	Negative
7	1.9 (+)	0.1 (-)	Negative	Negative	0.03				Negative	Negative

* NCM: National Centre of Microbiology

** GP: Glycoprotein

*** NP: Nucleoprotein

Table 3. Main clinical and laboratory data of patients with CCHFV

	Acute infection by CCHFV, N=1	Previous infection by CCHFV, N=3
Age mean ± SD, years	53	68 ± 28.2
Sex	Male	2 males / 1 female
Urban population	Yes	2/3
Comorbidity	Non	2/3 arterial hypertension 1/3 chronic renal failure, hypothyroidism, depressive syndrome, dyslipidemia and stroke 1/3 essential thrombocytosis
First clinical diagnosis	Acute viral hepatitis	2/3 genitourinary tract infection 1/3 acute pancreatitis
Range fever duration, days	5-10	3-7
Chills	Yes	2/3
Abdominal pain	Non	1/3
Cutaneous signs (suggesting tick bite)	Leg sore	0
Muscles soreness	Leg myalgias	1/3
Any bleeding symptomatology	Non	Non
Risk factors	Cattle husbandry	Non
Laboratory data		
Hemogram, ±SD		
Haemoglobin, g/dL	14.1	9.6 ± 2.4
White blood cells, x $10^3/mm^3$	3.1	7.0 ± 1.9
Polymorphonuclear leukocytes x $10^3/mm^3$	8.1	5.4 ± 1.5
Lymphocytes x $10^3/mm^3$	3.6	1.1 ± 5.6
Platelets, x $10^3/mm^3$	41	207.6 ± 158.5
Liver function tests, ±SD		
C-Reactive Protein (CRP), mg/L	15.16	20.2 ± 15.5
Activated Partial Thromboplastin Time, sec	43.8	34.2 ± 5.1
Aspartate Aminotransferase (AST), U/L	347	151.3 ± 110.2
Alanine Aminotransferase (ALT), U/L	161	74.7 ± 50.1

Discussion

CCHFV was found in Spain for the first time in 2010, when it was detected in ticks feeding on wild animals at the margins of the Tajo River in the province of Cáceres [13] (that borders on the west with Portugal and on the north with the province of Salamanca, where this study was performed). CCHFV was probably introduced in this territory by migratory birds from North Africa [18]. Six years later, the first two human autochthonous cases, one of them with a fatal outcome, were reported [10]. More recently, infected ticks were reported in other territories in Spain, including ticks feeding on both wild animals and domestic animals, thus increasing the outbreak risk [12].

This study was performed to determine whether CCHFV could be a cause of acute febrile syndrome in Spanish emergency departments. To our knowledge, this study is the first conducted in Spain with this purpose. In terms of the impact of the disease, we found an elevated incidence and prevalence, with 0,75 % (1/133) of acute patients who were IgM-positive and with positive (RT-PCR) and an additional 2.2% (3/133) of patients who were IgG positive, suggesting previous infections. According to these results, testing for CCHFV in Spain should be performed before other tick-borne diseases such as Lyme disease, tularaemia, babesiosis or anaplasmosis, that have a lower incidence [19,20].

Likewise, in our hospital two new cases of CCHFV were diagnosed during May-July 2020. The first of them also presented a CCHVF compatible with genotype V. We do not have a clear explanation about the spreading of CCHVF in our area, some theories could be assessed: i) transport/import of animals for livestock use, ii) migratory birds from eastern Europe and iii) immigrants from these countries. Nevertheless, it has recently been detected the presence of CCHFV genotype Europe V in *Hyalomma lusitanicum* and *Dermacentor marginatus* ticks collected from red deer, fallow deer and Eurasian wild board, suggesting that genotype V introduction in Spain was probably from Eastern Europe [21]

Our positive acute case was a middle-aged man, involved in livestock husbandry. Clinical findings were characterised by fever, chills, myalgias, with thrombopenia and prolongated coagulation times, with no evidence of bleeding or hepatomegaly. These features are similar to those found in previous studies conducted in other endemic countries such as Georgia and Bulgaria [22–24]. The main

differences found between our patient with acute CCHFV and those reported in other endemic countries were the lack of any bleeding history or bleeding stigma and the lack of hepatomegaly or splenomegaly at the clinical examination.

According to our data, patients with CCHFV could present to the emergency department with a febrile syndrome, with thrombopenia, prolongated coagulation times, elevated transaminases levels, even without any haemorrhagic signs or symptoms. Spanish physicians should have this clinical suspicion when they face a patient with a similar clinical picture (especially in the period between May and October). All of this in order to rapidly start the supportive and biosafety measures, to avoid complications linked to the patient morbidity, and the possible nosocomial outbreaks [14,25].

Conclusion

A new genotype virus circulation was described in human from Spain. More studies will be required to establish the mechanism of dissemination and distribution of these virus. Moreover, this study suggests that CCHF is an identifiable cause of febrile illness in Spain; therefore, it is mandatory to suspect this disease when a patient comes to the emergency department with fever, thrombocytopenia and transaminase elevation, especially in spring and summer, and when patients have an occupational risk, following the protocols already established for this purpose. All of this is intended to initiate supportive treatment and isolation measures as soon as possible, thus reducing the mortality risk and avoiding the risk of a nosocomial outbreak.

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Conflict of interest

All authors declare no potential conflicts of interest and no sources of supporting.

Ethical considerations

The procedures described here were carried out in accordance with the ethical standards described in the Helsinki Declaration revised in 2013. Additionally, this study was approved September 7th, 2017 by the Bioethics Committee of CAUSA with the code *Cómite Ético CEIC: PI9109/2017*. At all times, we maintained the confidentiality of the patients' personal data.

Informed consent

Non relevant.

References

[1] Bente DA, Fkorrester NL, Watts DM, McAuley AJ, Whitehouse CA, Bray M. Crimean-Congo hemorrhagic fever: history, epidemiology, pathogenesis, clinical syndrome and genetic diversity. Antiviral Res 2013;100:159–89. https://doi.org/10.1016/j.antiviral.2013.07.006.

[2] Whitehouse CA. Crimean Congo hemorrhagic fever. Antiviral Res 2004;64:145–60. https://doi.org/10.1016/j.antiviral.2004.08.001.

[3] Deyde VM, Khristova ML, Rollin PE, Ksiazek TG, Nichol ST. Crimean-Congo hemorrhagic fever virus genomics and global diversity. J Virol 2006;80:8834–42. https://doi.org/10.1128/JVI.00752-06.

[4] Papa A, Chaligiannis I, Kontana N, Sourba T, Tsioka K, Tsatsaris A, et al. A novel AP92-like Crimean-Congo hemorrhagic fever virus strain, Greece. Ticks Tick Borne Dis 2014;5:590–3. https://doi.org/10.1016/j.ttbdis.2014.04.008.

[5] Sidira P, Maltezou HCC, Haidich A-BA-B, Papa A. Seroepidemiological study of Crimean-Congo haemorrhagic fever in Greece, 2009–2010. Clin Microbiol Infect 2012;18:E16–9. https://doi.org/10.1111/j.1469-0691.2011.03718.x.

[6] Salehi-Vaziri M, Baniasadi V, Jalali T, Mirghiasi SM, Azad-Manjiri S, Zarandi R, et al. The first fatal case of Crimean-Congo hemorrhagic fever caused by the AP92-like strain of the Crimean-Congo hemorrhagic fever virus. Jpn J Infect Dis 2016;69:344–6. https://doi.org/10.7883/yoken.JJID.2015.533.

[7] Karakecili F, Cikman A, Aydin M, Binay U, Kesik O, Ozcicek F. Evaluation of

epidemiological, clinical, and laboratory characteristics and mortality rate of patients with Crimean-Congo hemorrhagic fever in the northeast region of Turkey. J Vector Borne Dis 2018;55:215. https://doi.org/10.4103/0972-9062.249479.

[8] Leblebicioglu H. Crimean–Congo haemorrhagic fever in Eurasia. Int J Antimicrob Agents 2010;36:S43–6. https://doi.org/10.1016/j.ijantimicag.2010.06.020.

[9] López R, Molina R. Cambio climático en España y riesgo de enfermedades infecciosas y parasitarias transmitidas por artrópodos y roedores. Rev Esp Salud Publica 2005;79:177–90. https://doi.org/10.1590/S1135-57272005000200006.

[10] Negredo A, de la Calle-Prieto F, Palencia-Herrejón E, Mora-Rillo M, Astray-Mochales J, Sánchez-Seco MP, et al. Autochthonous Crimean–Congo Hemorrhagic Fever in Spain. N Engl J Med 2017;377:154–61. https://doi.org/10.1056/NEJMoa1615162.

[11] Ministerio de Sanidad Consumo y Bienestar Social. Informe de situación y evaluación del riesgo de transmisión de Fiebre Hemorrágica de Crimea-Congo en España. Spain: Dirección General de Salud Pública, Calidad e Innovación; 2019.

[12] Negredo A, Habela MÁ, Ramírez de Arellano E, Diez F, Lasala F, López P, et al. Survey of Crimean-Congo Hemorrhagic Fever Enzootic Focus, Spain, 2011–2015. Emerg Infect Dis 2019;25:1177–84. https://doi.org/10.3201/eid2506.180877.

[13] Estrada-Peña A, Palomar AM, Santibáñez P, Sánchez N, Habela MA, Portillo A, et al. Crimean-Congo Hemorrhagic Fever Virus in Ticks, Southwestern Europe, 2010. Emerg Infect Dis 2012;18:179–80. https://doi.org/10.3201/eid1801.111040.

[14] Ergönül O. Crimean-Congo haemorrhagic fever. Lancet Infect Dis 2006;6:203–14. https://doi.org/10.1016/S1473-3099(06)70435-2.

[15] Mostafavi E, Pourhossein B, Chinikar S. Clinical symptoms and laboratory findings supporting early diagnosis of Crimean-Congo hemorrhagic fever in Iran. J Med Virol 2014;86:1188–92. https://doi.org/10.1002/jmv.23922.

[16] Monsalve Arteaga L, Muñoz Bellido J, Vieira Lista C, Vicente Santiago B, Fernández Soto P, Bas, I et al. Crimean-Congo haemorrhagic fever (CCHF) virus-specific antibody detection in blood donors, Castile-León, Spain, summer 2017 and 2018. Eurosurveillance. 2020 Mar 12;25(10):1900507. do JLM, et al. Crimean-Congo haemorrhagic fever (CCHF) virus-specific antibody detection in blood donors, Castile-León, Spain, summer 2017 and 2018. Eurosurveillance 2020;25:1900507. https://doi.org/10.2807/1560-7917.es.2020.25.10.1900507.

[17] Atkinson B, Chamberlain J, Logue CH, Cook N, Bruce C, Dowall SD, et al. Development of a Real-Time RT-PCR Assay for the Detection of Crimean-Congo Hemorrhagic Fever Virus. Vector-Borne Zoonotic Dis 2012;12:786–93. https://doi.org/10.1089/vbz.2011.0770.

[18] Leblebicioglu H, Eroglu C, Erciyas-Yavuz K, Hokelek M, Acici M, Yilmaz H. Role of Migratory Birds in Spreading Crimean-Congo Hemorrhagic Fever, Turkey. Emerg Infect Dis 2014;20:1331–4. https://doi.org/10.3201/eid2008.131547.

[19] Ministerio de Sanidad Consumo e Igualdad. Sociedad Española de Medicina Tropical y Salud Internacional. Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica. Guía de actuación ante picadura de garrapata. Spain: 2016.

[20] Vázquez-López ME, Pego-Reigosa R, Díez-Morrondo C, Castro-Gago M, Díaz P, Fernández G, et al. Epidemiología de la enfermedad de Lyme en un área sanitaria del noroeste de España. Gac Sanit 2015;29:213–6. https://doi.org/10.1016/j.gaceta.2015.01.008.

[21] Moraga-Fernandez A, Royo-Hernandez L, Habela MA, Ruiz Fons F, Calero-Bernal R, Gortazar C, et al. Detection of New Crimean-Congo Hemorrhagic Fever Virus Genotypes in Ticks Feeding on Deer and Wild Boar, Spain. Transbound Emerg Dis 2020;Preprint. https://doi.org/10.2139/ssrn.3314445.

[22] Kuchuloria T, Imnadze P, Chokheli M, Tsertsvadze T, Endeladze M, Mshvidobadze K, et al. Viral hemorrhagic fever cases in the country of Georgia: Acute Febrile Illness Surveillance Study results. Am J Trop Med Hyg 2014;91:246–8. https://doi.org/10.4269/ajtmh.13-0460.

[23] Christova I, Younan R, Taseva E, Gladnishka T, Trifonova I, Ivanova V, et al. Hemorrhagic fever with renal syndrome and Crimean-Congo hemorrhagic fever as causes of acute undifferentiated febrile illness in Bulgaria. Vector Borne Zoonotic Dis 2013;13:188–92. https://doi.org/10.1089/vbz.2011.0938.

[24] Bower H, El Karsany M, Alzain M, Gannon B, Mohamed R, Mahmoud I, et al. Detection of Crimean-Congo Haemorrhagic Fever cases in a severe undifferentiated febrile illness outbreak in the Federal Republic of Sudan: a retrospective epidemiological and diagnostic cohort study. PLoS Negl Trop Dis 2019;13:e0007571. https://doi.org/10.1371/journal.pntd.0007571.

[25] Fletcher TE, Gulzhan A, Ahmeti S, Al-Abri SS, Asik Z, Atilla A, et al. Infection prevention and control practice for Crimean-Congo hemorrhagic fever—A multi-center cross-sectional survey in Eurasia. PLoS One 2017;12:e0182315. https://doi.org/10.1371/journal.pone.0182315