Suspected Crimean-Congo Haemorrhagic Fever Re-Infection: A Case Report with Atypical Serological Profile

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Case Report

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ABSTRACT

A 53-year-old male farmer presented with clinical manifestations consistent with Crimean-Congo Hemorrhagic Fever (CCHF) virus infection, reporting a recent history of tick bite. To confirm the diagnosis, molecular and serological assays were performed on four specimens collected following the onset of illness. Reverse transcription-polymerase chain reaction (RT-PCR) detected CCHF virus RNA in the initial sample, while serum immunoglobulin M (IgM) antibodies were undetectable in all specimens. Notably, a robust immunoglobulin G (IgG) antibody response was observed across all samples. The concurrence of detectable viral RNA with the presence of IgG antibodies, but the absence of IgM, in the acute phase sample suggests a potential case of CCHF virus re-infection. These findings hold significant implications for public health, particularly regarding our understanding of CCHF virus infection dynamics, diagnostics, and vaccine development strategies.

INTRODUCTION

Crimean-Congo Hemorrhagic fever (CCHF) is a paramount tick-borne viral disease [1]. The disease is characterized by a significant case fatality rate, ranging from 5% to 50%, and poses a considerable public health threat due to its potential for human-to-human transmission and the inherent complexities in managing infected individuals [1, 2]. The etiological agent is a negative-sense RNA virus classified within the genus *Orthonairovirus* (family *Nairoviridae*, order *Bunyavirales*) [3]. This virus is maintained in nature through a complex enzootic cycle involving ticks and vertebrate hosts, the intricacies of which remain under investigation. Human infection typically occurs via the bite of infected ixodid ticks or through direct, unprotected contact with infectious biological fluids or tissues from viremic livestock or infected individuals [4].

The advancement of scientific knowledge regarding the pathophysiology and various other aspects of CCHF virus biology has been inherently challenging due to the virus's classification as a biosafety level 4 (BSL-4) agent in numerous countries, necessitating high-containment laboratories for its manipulation. Furthermore, the largely

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asymptomatic nature of CCHF virus infection in most animal models has historically hindered the development of *in vivo* models that faithfully recapitulate the disease observed in humans. However, recent investigations have demonstrated that a specific subgroup of cynomolgus macaques infected with an early-passage strain of CCHF virus can develop clinical sequelae that, to varying degrees, mirror the manifestations of human CCHF disease [5]. This represents a significant advancement in our ability to study the pathogenesis of CCHF. Nevertheless, to circumvent the inherent limitations in direct virus manipulation, researchers have effectively utilized data derived from prospective studies, clinical laboratory analyses, necropsy examinations, and *in vitro* culture in cell lines to elucidate the pathogenesis of CCHF virus infection [6]. A robust CCHF virus-specific antibody response is generally associated with a favorable clinical outcome. Conversely, minimal or absent antibody responses have been frequently reported in fatal cases [7, 8]. Characteristically, serum immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies become detectable during the first week of illness, with IgM typically persisting for approximately five months and IgG potentially lasting for several years [9, 10].

Herein, we present a case report detailing an atypical antibody profile against CCHF virus, strongly suggestive of a re-infection, although the possibility of a relapse cannot be entirely excluded.

CASE PRESENTATION

On July 14, 2017, a 53-year-old male patient was admitted to Vali-e-Asr Hospital, located in Shahrebabak (Kerman province, Iran). The patient, a farmer by occupation, reported a history of an ixodid tick bite three days prior to the onset of illness. Upon admission, clinical examination revealed a fever (oral temperature >38 °C), malaise, rigors, tachycardia, a respiratory rate of 18 breaths per minute, and a blood pressure of 110/70 mmHg. Notably, there was no clinical evidence of hepatosplenomegaly or lymphadenopathy.

Upon admission, initial laboratory investigations revealed white blood cell (WBC) count of 4.5×10^9/L, with a lymphocyte proportion of 57.7%. The platelet count was 190×10^9/L. Coagulation parameters were within the normal range, with a prothrombin time (PT) of 12.4 seconds, an activated partial thromboplastin time (aPTT) of 26 seconds, and an international normalized ratio (INR) of 1.0. Liver function tests were also unremarkable, showing an aspartate aminotransferase (AST) level of 21 IU/L and an alanine aminotransferase (ALT) level of 20 IU/L. However, microscopic examination of the urine demonstrated haematuria (+1). Beyond the microscopic haematuria, there were no other clinical signs of haemorrhage. The patient had no known history of immunodeficiency or autoimmune disorders. Furthermore, the patient denied any prior episodes of similar clinical symptoms or tick bites, with the exception

of a tick bite three days prior to the onset of illness.This history makes interpretation of serological findings difficult and suggests reasons such as asymptomatic initial infection, relapse, or persistent infection.

Based on the patient's clinical presentation and history of a recent tick bite, clinicians suspected CCHF on July 15th. Consequently, serum samples were submitted to the Department of Arboviruses and Viral Haemorrhagic Fevers, National Reference Laboratory, Pasteur Institute of Iran, for laboratory confirmation. Concurrently, antiviral therapy with oral ribavirin was initiated, according to the CCHF treatment protocol [8, 10]. The patient made a full recovery and was discharged on July 20th.

Molecular and serological assays were employed for laboratory analysis. Viral RNA detection was performed using four distinct reverse transcription-polymerase chain reaction (RT-PCR) assays: a) a commercial real-time RT-PCR assay (Fast Track Diagnostics, Luxembourg); b) specifically, a conventional RT-PCR targeting the S segment using F2 and R3 primers [11] ; c) a real-time RT-PCR assay developed by Atkinson *et al*. (2012) [12]; and d) an in-house one-step SYBR Green-based real-time RT-PCR targeting a 189-base pair (bp) region of the small viral RNA (S) segment. Subsequent sequencing and phylogenetic analysis were performed as previously described [7]. The nucleotide sequence of the amplified viral RNA was determined using Sanger sequencing. Subsequently, the query sequence was aligned with Iranian CCHF virus sequences and reference sequences representing various CCHF virus genotypes. Sequence alignments were performed using the Clustal W algorithm implemented within MEGA version 6 software [13]. The genetic distance between the query sequence and existing CCHF virus genotypes was estimated employing the Kimura-2 parameter model to account for nucleotide substitutions within the alignment. The standard error of the estimated distances was calculated using the bootstrap method with 1000 replicates. Furthermore, to evaluate the phylogenetic placement of the query sequence among existing CCHF virus genotypes, maximum likelihood (ML) and neighbor-joining (NJ) phylogenetic analyses were conducted. All distance and phylogenetic analyses were performed using MEGA version 6 software [13].

Detection of IgM and IgG antibodies was performed using commercially available VectoCrimean-CHF-IgM/IgG enzyme-linked immunosorbent assay (ELISA) kits (Vector-Best, Novosibirsk, Russia). Quantification of IgG antibodies was subsequently conducted using a human CCHF virus IgG ELISA Kit, Quantitative (Alpha Diagnostic Intl, Texas, USA). Four serum samples were obtained from the patient at the following time points post-symptom onset: day 2 (July 15th), day 13 (July 26th), day 29 (August 11th), and day 70 (September 21st).

The aforementioned first and second serum samples were analyzed using the four RT-PCR assays. Of these, only the in-house one-step SYBR Green-based real-time

RT-PCR assay demonstrated a positive result with the first sample, exhibiting a cycle threshold (Ct) value of 26.4 (Table 1). All other assays and the second serum sample yielded negative results. The viral strain identified in this patient was designated Iran-5811. The amplicon

generated by the SYBR Green-based real-time RT-PCR was subsequently sequenced, and the resulting sequence was deposited in GenBank under accession number MH557793.

Table 1. Results of different molecular and serological diagnostic assays for four specimens.

Sample (Collection Date)	RT-PCR Assay Results	IgM Antibody	IgG Antibody (Titer, U/mL)
1st (July 15th)	Homemade: Positive Fast Track Diagnostics Kit: Negative Atkinson *et al*. [13]: Negative F2,R3 [12]: Negative	Negative	422
2nd (July 26th)	Homemade: Negative Fast Track Diagnostics Kit: Negative Atkinson *et al*. [13]: Negative F2,R3 [12]: Negative	Negative	1230
3rd (August 11th)	Homemade: Not Done Fast Track Diagnostics Kit: Not Done Atkinson *et al*. [13]: Not Done F2,R3 [12]: Not Done	Negative	1553
4th (September 21st)	Homemade: Not Done Fast Track Diagnostics Kit: Not Done Atkinson *et al*. [12]: Not Done F2,R3 [12]: Not Done	Negative	1644

Note: Serum samples were collected on day 2 (July 15th), day 13 (July 26th), day 29 (August 11th), and day 70 (September 21st) post-symptom onset. (Tick bite: July 11th, Symptom Onset: July 14th, Recovery: July 20th).

Inter-genotype genetic distance analysis revealed that the Iran-5811 strain exhibited the greatest genetic distance to the Europe-2 genotype (0.255 substitutions per site) and the least genetic distance to the Africa-1 and Africa-3 genotypes (0.177 substitutions per site; Table 2).

Phylogenetic analysis, as depicted in Figure 1, indicated that the Iran-5811 strain clustered closely with the Iran-Kerman22 strain (GenBank accession number KM401454), which was reported in 2012 from the Kerman province of Iran.

Table 2. Inter-genotype genetic distances of the Iran-5811 strain with various CCHF virus genotypes

CCHF virus genotypes	Genetic distance (substitutions per site)	Standard error
Africa-1	0.177	0.034
Africa-3	0.177	0.031
Asia-2	0.180	0.033
Asia-1	0.180	0.034
Europe-1	0.187	0.035
Africa-2	0.197	0.032
Europe-2	0.255	0.041

Given the expected kinetics of a typical antibody response to CCHF virus infection, the absence of detectable anti-CCHF virus IgM antibodies in the second serum sample was unexpected. Specifically, ELISA analysis did not reveal the presence of anti-CCHF virus IgM in this sample.

This intriguing observation prompted the analysis of a third serum sample collected at day 29 post-symptom onset, which also yielded a negative result for anti-CCHF virus IgM antibodies. Consistent with these findings, a

fourth sample obtained at day 70 post-symptom onset similarly lacked detectable anti-CCHF virus IgM antibodies. To further investigate the humoral immune response, all four serum samples were tested for anti-CCHF virus IgG antibodies. Remarkably, strong positive IgG ELISA results were observed not only in the convalescent-phase samples (2nd, 3rd, and 4th serum samples) but also in the acute-phase serum (1st sample). Subsequent IgG titration demonstrated an increasing trend in IgG antibody levels over time (Table 1).
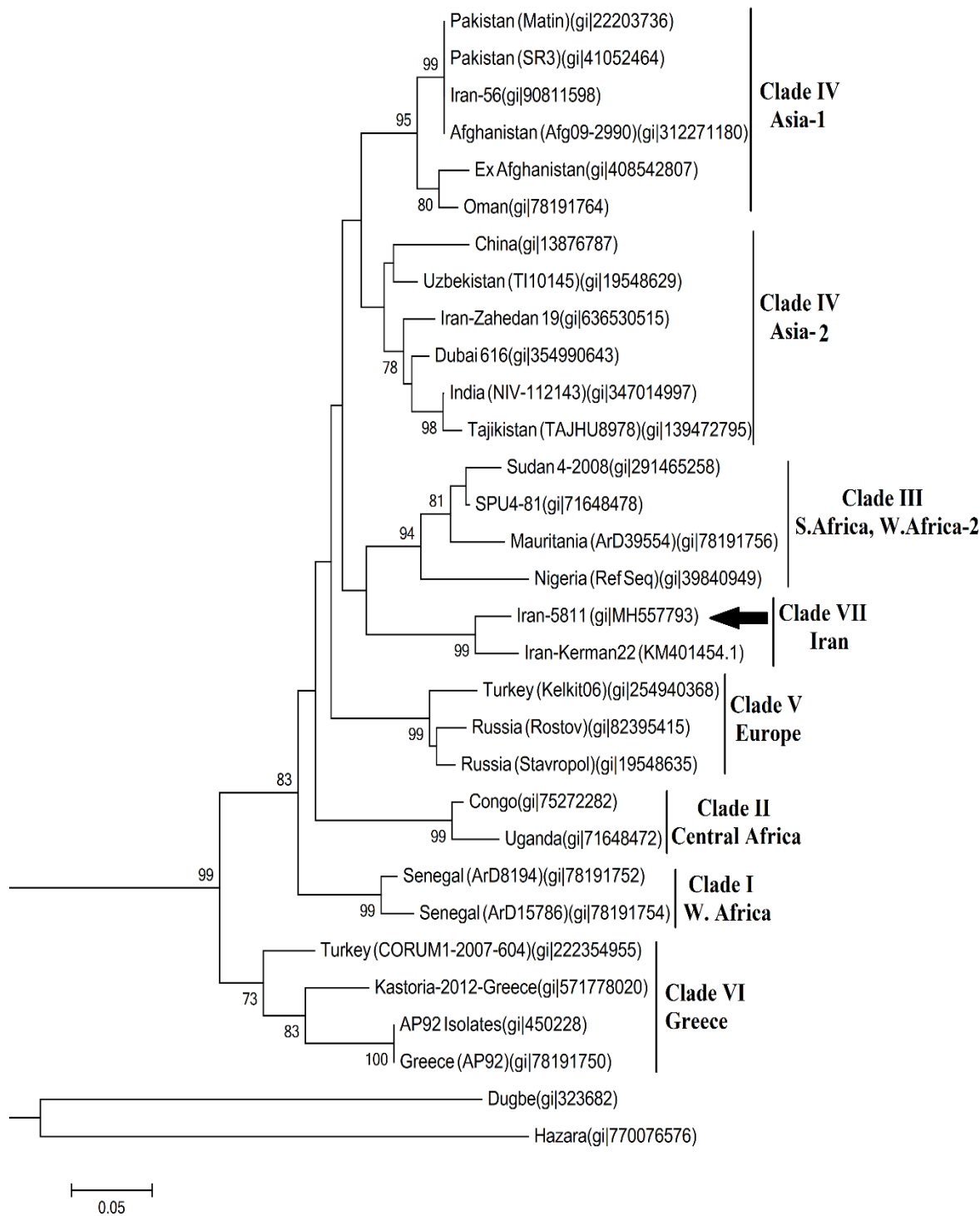
Fig. 1. Phylogenetic Analysis of the Iran-5811 Strain. (A) Neighbor-joining (NJ) phylogenetic tree constructed using bootstrap analysis with 1000 replicates and the Kimura-2 parameter substitution model. (B) Maximum likelihood (ML) phylogenetic tree constructed using bootstrap analysis with 1000 replicates and the Kimura-2 parameter substitution model. Both phylogenetic analyses demonstrate that the identified CCHF virus strain, Iran-5811, clusters within the Kerman-22 sub-clade, exhibiting strong statistical support (bootstrap values of 99% and 96%, respectively). However, this sub-clade did not form a robust higher-level clade with other existing CCHF virus genotypes or publicly available Iranian CCHF virus sequences. The majority of Iranian sequences were excluded from the analysis due to a lack of significant and consistent branching patterns with the query sequence.

DISCUSSION

This case report elucidates an atypical pattern of antibody response to CCHF virus, characterized by a robust IgG antibody response during the viremic phase, notably unaccompanied by a discernible IgM antibody response. Typically, anti-CCHF virus IgM antibodies become detectable approximately 5 to 7 days post-symptom onset, subsequently followed by the appearance of anti-CCHF virus IgG antibodies, typically detectable within a few days thereafter. Anti-CCHF virus IgM and IgG antibodies are generally reported to persist at detectable levels for approximately five months and up to five years, respectively (9).

The observed pattern of immune response in this case is analogous to that observed during secondary dengue virus infection, a flavivirus characterized by four distinct serotypes (14). In primary dengue virus infection, IgM and IgG antibodies typically become detectable approximately five and seven days post-symptom onset, respectively. In contrast, during secondary dengue virus infection, a rapid and robust IgG antibody response is detectable as early as one to two days post-symptom onset, whereas the IgM antibody response is typically weaker and may be undetectable (15, 16). Consequently, the aforementioned atypical pattern observed in the Iran-5811 case raises the possibility of a secondary infection with a genetically distinct Crimean-Congo Hemorrhagic Fever (CCHF) virus variant. However, the possibility of viral relapse, albeit undocumented for CCHF virus, cannot be definitively excluded. Notably, viral relapse has been documented in cases of Ebola virus infection, another viral hemorrhagic fever, as late as 10 months following the initial diagnosis [17, 18]. Furthermore, the patient in this report is presented with a recent history of a tick bite, which corroborates the hypothesis of a recent viral exposure. The detection of a positive IgG antibody response with a titer of 442 U/mL on day 2 post-symptom onset is unprecedented. This, coupled with the subsequent rise in titers, strongly suggests a secondary exposure that provided a rapid boost to the immune system, thereby conferring protection against severe disease and resulting in the observed minimal clinical consequences. Alternatively, the possibility of antigenic cross-reactivity cannot be entirely dismissed. While antigenic cross-reactivity is a recognized phenomenon among arboviruses, the observed increasing IgG titers, in conjunction with the positive RT-PCR result, strongly support the diagnosis of an acute CCHF virus infection.

Phylogenetic analysis revealed that the Iran-5811 strain formed a statistically significant cluster with the Kerman-22 strain (bootstrap value = 99%; Figure 1) (19). Both strains were identified within the same geographic region, specifically the Kerman province of Iran. However, these sequences did not exhibit significant genetic similarity to the Asia-1 and Europe-1 genotypes, which are the predominant CCHF virus genotypes circulating in the Kerman province (19). The Kerman province represents a region with a significant burden of CCHF in Iran. Consequently, high-risk populations, such as agricultural workers, are likely to experience repeated exposure to the virus. Based on this epidemiological context, a plausible scenario is that the patient had experienced a previous infection with a genetically distinct CCHF virus genotype (most likely Asia-1, given its prevalence in Iran) prior to this subsequent exposure to a Kerman-22 related strain. Notably, the possibility of CCHF virus re-infection has been previously reported by Ayatollahi *et al.* (2015) (20), who described a laboratory-confirmed case of CCHF in a 21-year-old butcher with a reported history of CCHF virus infection three years prior. However, the authors did not present definitive laboratory confirmation of the primary infection, suggesting that the diagnosis was likely based on clinical presentation, which can be less conclusive.

The findings presented in this case report have significant implications for both the laboratory diagnosis of CCHF virus infection and the development of effective CCHF virus vaccines. Specifically, within this study, only one of the four RT-PCR assays employed successfully detected CCHF virus RNA in the acute phase. This observation underscores the critical need for continued efforts in developing both universal and clade-specific CCHF virus detection and diagnostic assays, particularly considering the high genetic diversity of the virus and the potential for re-infection with genetically divergent strains. Therefore, it is imperative that global public health entities involved in the detection and diagnosis of CCHF virus incorporate the full spectrum of known CCHF virus genotypes, including newly emerging lineages such as Kerman-22, into their RT-PCR assay algorithms. This is crucial, as this case may have remained undetected in numerous settings globally due to limitations in current diagnostic assays. Notwithstanding the prevailing understanding that distinct CCHF virus genotypes are geographically restricted, instances of novel genotype detection in regions lacking prior reports of their presence underscore the dynamic nature of viral distribution. Consequently, the design of molecular diagnostic assays should not be solely predicated on the predominant strains circulating within a specific geographic area.

This case demonstrates an unusual immune response, which can be explained by reinfection or even relapse or persistence of infection. The application of current genotype-specific anti-CCHF virus monoclonal antibodies hold the potential to definitively determine whether this patient experienced sequential infections with distinct CCHF virus genotypes. Specifically, these tools could help us determine if a secondary exposure caused the mild disease seen in this case. We also want to know if this secondary exposure explains the anamnestic antibody response, which showed rapid increases in IgG antibodies but not much IgM. A limitation of the present study is that it was conducted as a single case and raises the possible scenario of reinfection, although final-

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confirmation requires multicenter research. These findings hold substantial significance for the advancement of detection methods, diagnostics, and vaccine development targeting this high-consequence pathogen, whose ongoing global expansion is a matter of increasing concern.

CONFLICTS OF INTEREST

The authors declare that they have no conflic of interests associated with this manuscript.

DATA AVAILABILITY

The datasets generated (RT-PCR raw data, IgG titers) are available from the corresponding author upon reasonable request. The viral sequence is publicly accessible in GenBank (accession: MH557793).

AI DISCLOSURE

No AI tools were used in the writing or preparation of this manuscript.

ETHICAL CONSIDERATIONS

This study was conducted in accordance with the national guidelines for CCHF surveillance. Written informed consent to participate in this study was obtained from the patient prior to any study-related procedures.

AUTHORS' CONTRIBUTIONS

MT, SA-M, TM, MH, and ZF conducted the molecular and serological assays. MHP, MSV, MF, VB, EM, and AAB contributed to the study design and were involved in data analysis and interpretation. TJ and SE performed the phylogenetic analyses. MSV, MHP, MF, VB, GKP, MMG, and TJ contributed to the writing of the manuscript. All authors critically reviewed and approved the final version of the manuscript.

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