Antiviral Research 146 (2017) 146–148

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

A neonatal death associated with Crimean-Congo hemorrhagic fever (Republic of Kalmykia, Russia, June 2016)

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ARTICLE INFO

Article history:
Received 7 June 2017
Received in revised form
25 August 2017
Accepted 29 August 2017
Available online 4 September 2017

Crimean-Congo hemorrhagic fever virus (CCHFV) is a negative-sense single-stranded RNA virus that belongs to the genus Nairovirus in the *Bunyaviridae* family (Plyusnin et al., 2011; Lvov et al., 2015). CCHFV is the etiological agent of Crimean-Congo hemorrhagic fever (CCHF), a severe disease in humans that can be transmitted via *Ixodidae* tick bites, contact with the biological fluids of infected patients and vertical transmission (Plyusnin et al., 2011; Lvov et al., 2015; Pshenichnaya et al., 2017). Ticks from the genus *Hyalomma* (mainly *H. marginatum*) are both the reservoir and vector for CCHFV in dry steppes in southern European Russia (Lvov et al., 2015). CCHF fatality rates can reach 40% (Lvov et al., 2015; Aslam et al., 2016). CCHFV is endemic in Africa, the Middle East, southern Europe, and Asia, including the southern Russian Plain (the Republic of Kalmykia, Stavropol Krai, Rostov and Astrakhan regions of Russia). From 2000 to 2015, there were 668 registered cases of CCHF in this area of Russia (Lvov et al., 2015; State report). Thus, CCHF is becoming a serious problem for Russian health care.

Here, we describe the neonatal death of a newborn child who was infected with CCHFV during the antenatal period in the Republic of Kalmykia, Russia, in 2016.

On June 2, 2016, a previously healthy 26-year-old pregnant woman (at 34–35 weeks of gestation) was bitten by a tick in the yard of a rural house located in the suburbs of Elista (the regional center of the Republic of Kalmykia; 46°18′28″ N, 44°15′20″ E). The disease manifested acutely on the following day with symptoms that included increases in body temperature to up to 39.0 °C, chills and a sore throat. The woman treated her fever symptomatically with paracetamol. However, her symptoms persisted, and her temperature increased to up to 39.9 °C. On June 5, the woman was admitted to a regional hospital at 34–35 weeks of gestation with a preliminary diagnosis of CCHF. This diagnosis was confirmed via RT-PCR performed using a CCHFV-FL kit (R-V22-50, AmpliSens, Moscow, Russia). Subsequently, specific IgM antibodies against CCHFV were detected using a Vector-Crimea-CHF-IgM ELISA kit (D5054, Vector Best, Koltsovo, Russia). All diagnostic tests were performed in accordance with the manufacturers' instructions.

On June 5, the patient began exhibiting labor activity, and a living male fetus was born 16 h after the start of labor. The early postpartum period was characterized by 2100 mL of uterine bleeding. The patient's condition was severe, and signs of brain edema and secondary encephalopathy were observed. Beginning on June 11, hemorrhagic syndrome developed, including gastric, uterine, nasal and gingival bleeding. The woman was treated with Viferon, ribavirin, ceftriaxone, dexamethasone, fluconazole, Canephron, Cytoflavin, and quamatel. Treatments added beginning on June 15 included Metrogyl, Asparcam, Fenibut, Diacarb, Relanium, oxetacaine, ascorutin, and ciprofloxacin as well as the following types of infusions: 5% glucose; 0.9% NaCl; vitamins B1, B6 and C; 1% CaCl; 25% $MgSO_4$; 4% KCl; and 10% albumin. Transfusions of packed red blood cells and platelets were also administered.

With intensive therapy, the patient's condition began to

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http://dx.doi.org/10.1016/j.antiviral.2017.08.018

Fig. 1. Location of the Republic of Kalmykia in Russia.

improve. On June 27, the convalescent patient was discharged to her home.

This patient's child was a newborn male born preterm who weighed 2500 g. At one minute after birth, his Apgar score was 6–8 points (Ehrenstein, 2009). Infection with CCHFV was confirmed using a specific real-time RT-PCR kit as described above. A few hours after birth, the newborn's condition deteriorated dramatically. He developed respiratory failure that was managed using mechanical ventilation and treated with maximum inotropic support and with intravenous ribavirin, amikacin, and cefotaxime. On June 10, he developed febrile syndrome with a temperature of up to 38.2 °C. Despite all efforts, including intensive therapy and transfusions of packed red blood cells and platelets, hemorrhagic syndrome developed on June 15, and the newborn patient died due to severe disseminated intravascular coagulation and circulatory failure. A summary of the laboratory findings for the woman and the newborn is presented in Table S1.

Serum samples were collected from the woman and the newborn, and viral RNA was extracted using a QIAamp Viral RNA Mini Kit (52904, Qiagen, Germany). The complete genomes of two CCHFV isolates from the woman and the newborn (excluding the 5′ and 3′ ends) were obtained using 27 primer pairs (Table S2). Primers were designed based on the reference sequences for CCHFV isolate K323_27 (KX013471-KX013473) (Lukashev et al., 2016). Overlapping fragments of viral genomes were amplified using the Super Script III One-Step RT-PCR System with Platinum Taq DNA Polymerase (12574-035, Invitrogen, USA) and sequenced using the Sanger method on an ABI-Prism 3500XL device (Applied Biosystems, USA). Genomes were assembled using Lasergene 7.0 software (DNASTAR, USA). The complete genomes of the two CCHFV isolates, which were designated CCHF/Russia/Kalmykia/Shch_1/2016 (for the woman) and CCHF/Russia/Kalmykia/Shch_2/2016 (for the newborn), were submitted to GenBank under accession numbers KY982864, KY982866, and KY982868 and KY982865, KY982867, and KY982869, respectively.

For phylogenetic analysis, both sequences were compared with sequences available in GenBank for CCHFV strains from Russia, Central Asia and Africa; supporting information and accession numbers for these sequences are provided in Table S2. Sequence alignments were performed using the CLUSTAL W software program (Thompson et al., 1994). Phylogenetic analysis was performed using the Jukes-Cantor substitution model. Trees for all segments were reconstructed using the neighbor-joining (NJ) tree algorithm in MEGA 6.0 software (Pollock and Goldstein, 1995). The statistical significance (robustness) of the tree topology was evaluated using 1000 bootstrap replicates.

Phylogenetic analysis showed that both of the isolated strains belonged to lineage V of CCHFV, which is found in southern European Russia (Deyde et al., 2006). These strains were closely related to CCHFV isolates from Stavropol Krai, which borders the Republic of Kalmykia (Fig. 1). They were most closely related to the strain 3758-ST-2007 (KR814848, KR814867, and KR814886) (see Fig. 2), with 99% identity for each segment. No single nucleotide variations (SNVs) were observed in the sequences of the two isolated strains. Therefore, it can be stated that these strains are endemic to the southern Russian Plain. The lack of SNVs in the sequences of isolates from the woman and the newborn allows for the assumption that the transplacental barrier had no significant impact on the genetic structure of the virus and was unlikely to act as a bottleneck for CCHFV transmission.

Thus, we have reported the first case of antenatal CCHFV infection and neonatal death that has been well documented using molecular genetic techniques. The obtained data revealed that CCHFV easily penetrated the placental barrier without the significant selection of viral populations. Therefore, for pregnant women with CCHF, the risk of CCHFV-induced death of the fetus is extremely high; this risk must be taken into account in anti-epidemic planning to minimize the incidence of this serious and potentially lethal illness.

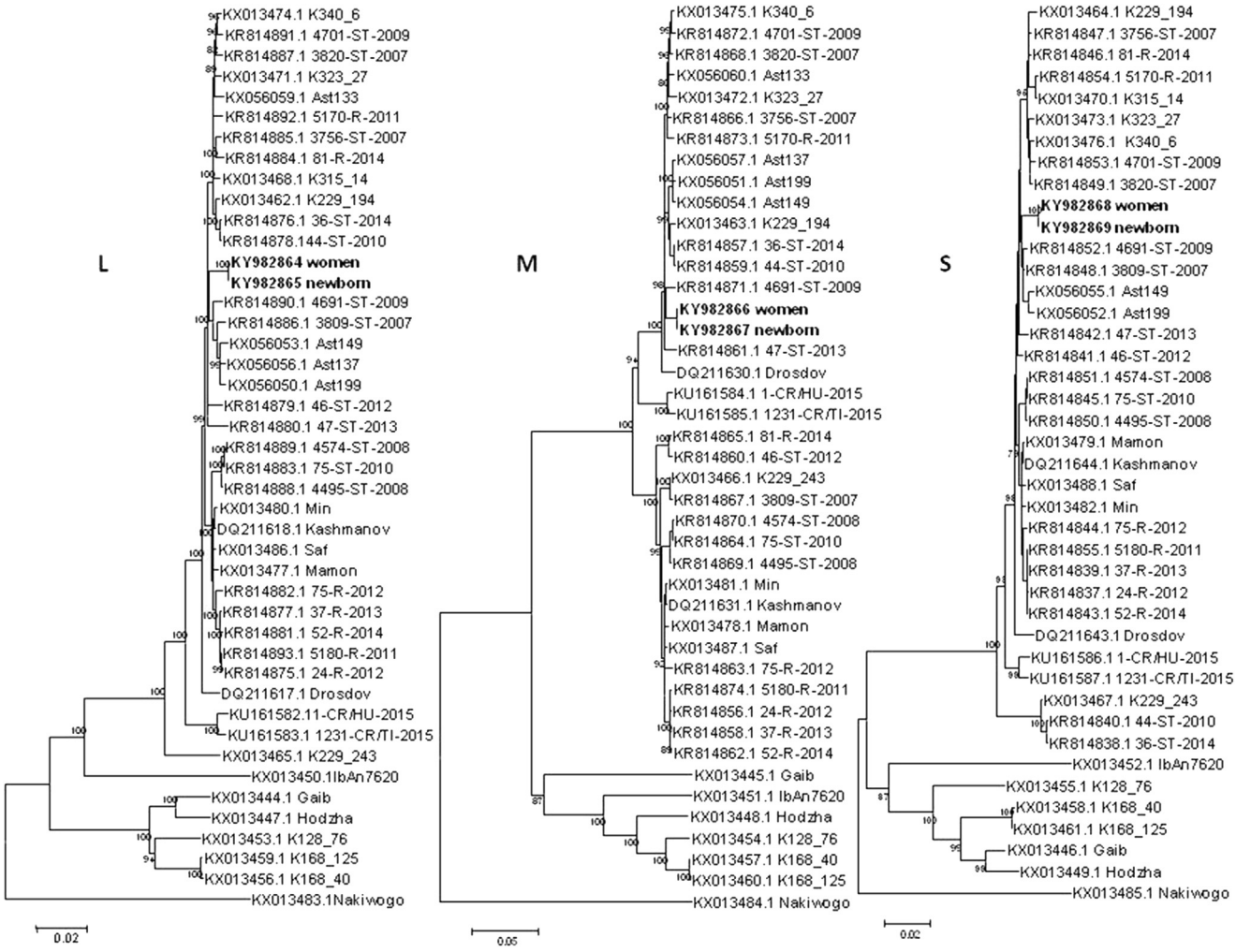

Fig. 2. Phylogenetic trees for complete L, M and S sequences of CCHFV (at the nucleotide level). Phylogenetic analysis was performed using the Jukes-Cantor substitution model, and trees were reconstructed using the neighbor-joining (NJ) tree algorithm in MEGA 6.0 software. The robustness of the trees was tested using 1000 bootstrap replicates. The locations on the tree of the CCHF-Russia_Kalmykia-Shch_1-2016 and CCHF-Russia_Kalmykia-Shch_2-2016 strains are indicated in gray. Accession numbers for nucleotide sequences of the L, M, and S segments of CCHFV are provided in Supplementary Table S2.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.antiviral.2017.08.018.

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