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CCHF virus variants in Pakistan and Afghanistan: Emerging diversity and epidemiology

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ABSTRACT

Background: Crimean Congo hemorrhagic fever (CCHF) has been reported from more than 30 countries in Africa, Asia, Eastern Europe and Middle East. The disease is considered endemic in Pakistan and neighboring countries like Iran and Afghanistan.
Objectives: This study aimed to explore the genetic diversity of CCHF virus (CCHFV) detected in Pakistan and Afghanistan based on analysis of partial S-segment sequences.
Study design: During 2011, one hundred samples satisfying the CCHF case definition were tested by (ELISA) and RT-PCR for detection of IgM antibodies and viral RNA, respectively. Phylogenetic analysis was carried out on partial S-segment nucleotide sequences using MEGA 5.0.
Results: Out of one hundred collected during 2011, 49 (49%) were positive for CCHF either by ELISA/RT-PCR or both. The mean age of the CCHFV positive cases for was 29.7 years (range 18–56 years) and overall mortality rate was 20.4%. All CCHF virus isolates from this study clustered with strains previously reported from Pakistan, Iran and Afghanistan within the Asia-1 genogroup. Four distinct sub-clades were found circulating within Asia-1 genogroup. Six CCHFV strains found in Pakistan and Afghanistan grouped into a new sub-clade-D.
Conclusions: Data from this study shows that endemic foci of CCHFV span the international border between Pakistan and Afghanistan with genetically diverse variants circulating in this region. Our findings emphasize to establish a laboratory based surveillance program and devise health policy measures to control CCHF infection especially in Baluchistan.

1. Background

Crimean Congo hemorrhagic fever virus (CCHFV) belongs to the genus Nairovirus within the family Bunyaviridae [1]. It contains single stranded, three negative sense RNA segments known as small (S), medium (M) and large (L) which encode nucleocapsid protein, glycoproteins (Gn and Gc) and viral RNA polymerase, respectively [2–4]. Based on geographical origin and phylogenetic analyses of S gene segment, CCHFVs has been classified into seven distinct genetic groups; African group 1 comprises viruses from Senegal, African group 2 includes strains from Uganda and South Africa, African group 3 contains isolates from South and West Africa. Viruses from Europe include isolated detected in Russia, Turkey and Balkan region (Bulgaria, Kosovo) forming European group 1, and a single isolate of Greek AP92 comprising European group 2. Viruses from Pakistan, Middle East, Iran and Afghanistan cluster within Asia group-1 while Asia group-2 comprises isolates from Pakistan, China, Uzbekistan and Kazakhstan [5–10]. The existence of genogroups reflects the circulation of CCHFV, in multiple areas separated by topographic barriers, rather than across single geographic region. Diversity of climate, and vegetation in various regions has steered the evolution of distinct species of ticks and vertebrates, and progressive adaptation of CCHFV to these regionally exclusive hosts has led to the emergence of local virus variants [11]. Phylogenetic analysis has shown evidence of genome reassortment and recombination during co-infection of a single host, indicating the potential for emergence of novel variants in future [7,8].

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CCHFV circulates in an enzootic tick-vertebrate-tick cycle and humans get infected through tick bites or direct contact with CCHF patients and viremic tissues or blood [12]. Other substantial transmission risks include uncontrolled population movement, infected migratory birds and livestock trade that may facilitate the introduction of new CCHF viruses in previously unaffected regions [5,13,14]. CCHF has been reported from over 30 countries in Africa, Asia, Eastern Europe and Middle East with mortality rates ranging between 15–60% [15]. Characteristic clinical features include sudden onset of fever, headache, dizziness, nausea and myalgia with development of complications including hemorrhagic manifestations with multi organ failure [1,16] due to hepatic impairment and coagulopathy [17].

In Pakistan, the first case of Crimean Congo hemorrhagic fever was reported in Rawalpindi in 1976 [18]. Since then sporadic cases and more than 14 outbreaks have been reported with significant number of fatalities particularly from the western and northwestern regions of country as well as bordering areas of Afghanistan [19]. During these outbreaks, health care workers including attending physicians were infected and died of CCHFV due to insufficient biosafety and control measures [20–22]. Similar findings with nosocomial mortalities have been reported from many other countries like Bulgaria, Dubai, Turkey and Iraq [23].

2. Objectives

The purpose of the current study was to understand the genetic diversity of CCHF viruses from Pakistan and Afghanistan at a molecular level through analyses of partial S-segment sequence.

3. Study design

3.1. Sample selection and collection

The study was carried out from January to December 2011 at the National Institute of Health in Islamabad, Pakistan. One hundred blood samples were collected from suspected patients of CCHF. The case definition used for identification of CCHF patients included fever, malaise, myalgia, bleeding from various orifices and low platelet count. CCHF was confirmed by reaction of IgM antibody using ELISA kit (Biological Diagnostic Supplies Limited, BDSL®) in accordance with the manufacturer's instructions.

3.2. Extraction of RNA

Total RNA was extracted from serum samples using QIAamp Viral RNA mini kit (Qiagen, Germany) according to manufacturer's instructions and stored at −70 °C until further use.

3.3. Nested-RT PCR and nucleotide sequencing of amplicon

Nested Reverse transcriptase polymerase chain reaction (RT-PCR) was performed for detection of CCHFV RNA in serum samples. During first round, 530 nucleotides region of S gene was amplified Q3 using primer set CCHF-F2 (TGGACACCTTCACAAACTC) and CCHF-R3 (GACAAATTC CCTGCACCA) [24]. Briefly, 100 μl PCR reaction volume containing 5 μl of template RNA, 10 μl of 10X reaction buffer(ABI Buffer II), 3.5 μl of 2.5 mM dNTPs, 5 μl of (10 mM) of each primer, 0.5 μl of reverse transcriptase (25 U/μl), 1 μl of *Taq* DNA polymerase (5 U/μl) and 70 μl of PCR grade water was subjected to amplification at the following conditions: 42 °C for 45 min followed by 40 cycles at 94 °C for 40 s, 38 °C for 40 s and 72 °C for 90 min. For second round of amplification, 2 μl of first round product was added to PCR reaction mix prepared as mentioned above but primers CCHF-F3 (GAATGTGCATGGGTTAGCTC) and CCHF-R2 (GACATCACAATTTCACCAGG), and annealing temperature was adjusted to 41 °C [24]. PCR amplicons from 2nd round were purified by Qiaquick PCR purification Kit (Qiagen, Germany) and directly sequenced on ABI 3100 genetic analyzer using Big Dye Terminator kit V.3.0.cycle sequencing kit (ABI Foster City Canada, USA). The nucleotide sequences were assembled, edited and

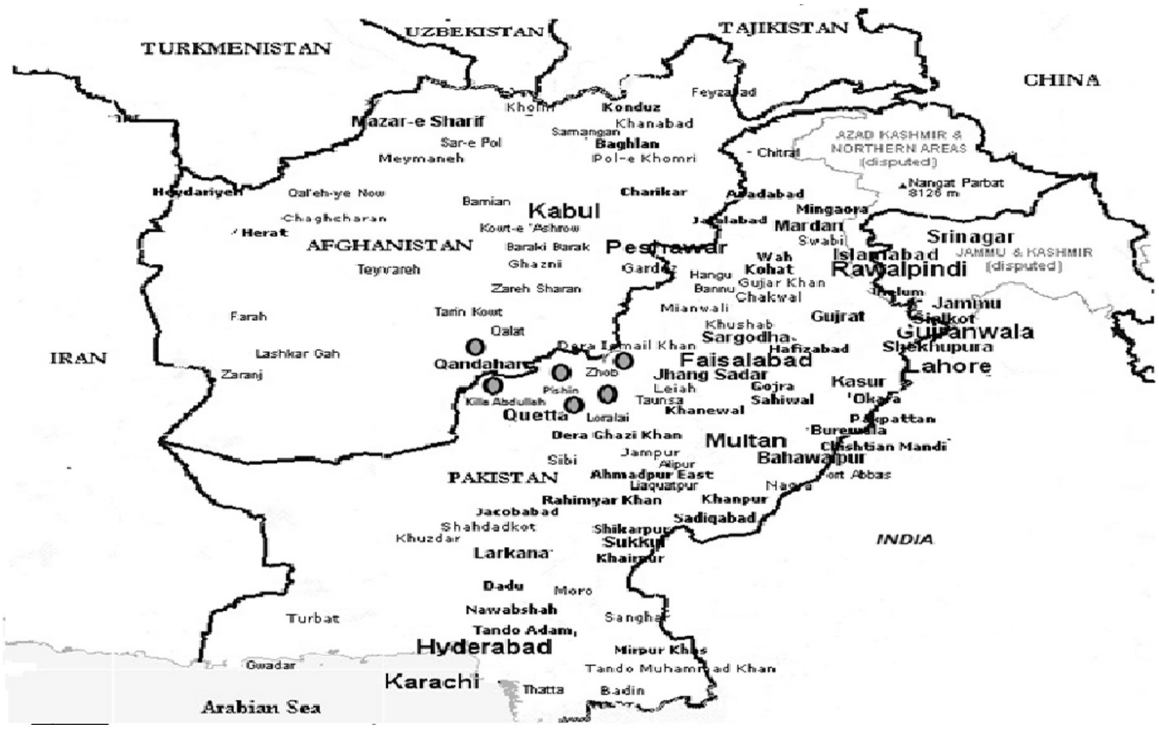

Fig. 1. Geographical location of confirmed cases of CCHF reported during 2011 in Baluchistan province of Pakistan and Kandahar province of Afghanistan. Each dot represents the disease affected area.

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