Abstract: Crimean Congo hemorrhagic fever (CCHF) has the most extensive geographic distribution of the medically significant tick-borne viruses. Its causative agent is a negative-sense, single-stranded RNA virus belonging to the family Bunyaviridae, genus Nairovirus. The virus can be transmitted mainly through direct contact with blood or tissues from infected livestock or through bites of Hyalomma ticks. CCHF is a public health problem in many regions of the world, including Africa, Middle East, southern and Eastern Europe, and Western Asia. Crimean-Congo hemorrhagic fever (CCHF) virus causes a hemorrhagic and toxic syndrome disease in humans and high mortality rates of up to 50%. The levels of liver enzymes, creatinine phosphokinase, and lactate dehydrogenase are raised, and bleeding markers are prolonged. Infection of the endothelium has a major pathogenic role. Besides direct infection of the endothelium, indirect damage by viral factors or virus-mediated host-derived soluble factors that cause endothelial activations and dysfunction are thought to occur. In diagnosis, enzyme-linked immunosorbent and real-time reverse transcriptase polymerase chain reaction are used.

Key Words: Crimean haemorrhagic fever (CCHF), Nairovirus, Ribavirin.

Introduction:

The disease was first observed in the Crimea by Russian scientists in 1944 and 1945 and given the name Crimean haemorrhagic fever. In 1969 it was recognized that the pathogen causing Crimean haemorrhagic fever was the same as that responsible for an illness identified in 1956 in the Congo and linkage of the two place names resulted in the current name for the disease and the virus. Congo hemorrhagic fever (CCHF) virus. It contains RNA and is inactivated by lipid solvents and detergents. CCHFV is a member of the Nairovirus genus of the family Bunyaviridae. Other genera within the family include Orthobunyavirus, Hantavirus, Phlebovirus, and Tospovirus. According to the most recent report from the International Committee on the Taxonomy of Viruses, there are seven recognized species in the genus Nairovirus containing 34 viral strains. The most important serogroups are the CCHF group, which includes CCHFV, and Hazara virus, which has not been demonstrated to be pathogenic to humans, and the Nairobi sheep disease group, which includes Nairobi sheep disease and Dugbe viruses. Only three members are known to be pathogens of humans, namely, CCHFV, Dugbe and Nairobi sheep disease viruses, although the latter is primarily a pathogen of sheep and goats. Crimean-Congo hemorrhagic fever (CCHF) virus is transmitted to humans by Hyalomma ticks or by direct contact with the blood of infected humans or domestic animals. Human infections occur through tick bites, direct contact with blood or tissue of infected livestock, or nosocomial infections and can result in severe hemorrhagic fever with case fatalities of (ca. 30%)[1-3].

Geographical distribution:

Congo hemorrhagic fever (CCHF) virus occurs from Sub-Saharan Africa to western China, reflecting the broad distribution of Hyalomma ticks, the predominant vector [2]. [4-7]. The disease is endemic in many countries in Africa, Europe and Asia, and during 2001, cases or outbreaks have been recorded in Kosovo, Albania, Iran, Pakistan, and South Africa [9]. CCHF virus has been reported from the Near, Middle, and Far East (countries such as Iraq, Pakistan, United Arab Emirates, Kuwait, Oman, and China [7,9-12] and from several African countries [13,14]). Besides, there are several reports on CCHF virus in the former Yugoslavia [15,16], but CCHF virus strains from this area have not been characterized up to now. The first case of Crimean-Congo haemorrhagic fever (CCHF) in South Africa was diagnosed in 1981 when the virus was isolated from the blood of a schoolboy who died after being bitten by a tick in the North West Province [17]. The geographic range of CCHF virus is the most extensive one among the medically important tickborne viruses. The serious building of Gujarat Congo virus attack is soon taking the heavier toll in villages in Gujarat near Ahmedabad known as Chagodhar where maximum cases of fever were recorded.

Structure:

Like other nairoviruses, CCHF virus is an enveloped single stranded negative-sense RNA virus and its tripartite
genome consists of a small (S), a medium (M) and large (L) segment which encode for the nucleocapsid protein (NP), the envelope glycoproteins G1 and G2 and an RNA-dependent polymerase, respectively. The three RNA genome segments (S, M, and L) are complexed with nucleocapsid protein to form ribonucleocapsid structures. The nucleocapsids and RNA-dependent RNA polymerase are packaged within a lipid envelope that contains the viral glycoproteins, G1 and G2 (also referred to as Gn and Gc, respectively). S encodes the nucleocapsid protein (N) and a nonstructural protein (NSs). The G1 and G2 glycoproteins are present as spikes in the viral envelope as shown in Figure 1

**Figure 1: Structure of CCHF virus**

![Structure of CCHF virus](image)

**Replication:**

The viral glycoproteins are responsible for the recognition of receptor sites on susceptible cells. They attach to the host receptors by their Gn-Gc glycoprotein dimer. The virus is then endocytosed into the host cell via a vesicle [18]. Replication occurs in the cytoplasm, Bunyaviruses are known to bud from Golgi membranes and the budding site seems to be defined by retention of the glycoproteins GN and GC at that particular site. GN is localised to the Golgi compartment, whereas GC is found in the endoplasmic reticulum. These viruses are not dependent on a helper virus. The ribonucleocapsid segments are released into the cytoplasm commencing transcription [18]. Transcription and replication occur in the cell and the newly synthesized virions are released by budding. Replication cycle of viruses in the family Bunyaviridae. Steps in the replication cycle are numbered as follows:

- Attachment of virions to cell-surface receptors;
- Entry via endocytosis followed by membrane fusion, allowing viral ribonucleocapsids and RNA-dependent RNA polymerase access to the cytoplasm;
- Primary transcription;
- Translation of viral proteins;
- Replication of vRNA via a cRNA intermediate;
- Assembly of virions at the Golgi or plasma membrane;
- Egress by budding into the Golgi followed by exocytosis, or budding through the plasma membrane [19].

**Figure 2: Replication cycle of viruses in the family Bunyaviridae**

![Replication cycle of viruses in the family Bunyaviridae](image)
Life cycle:
The natural cycle of CCHFV includes transovarial (i.e., passed through the eggs) transstadial (i.e., passed directly from immature ticks to subsequent life stages) transmission among ticks in a tick-vertebrate-tick cycle involving a variety of wild and domestic animals. A substantial number of cases of CCHF have been acquired by contagion, particularly through contact with the blood of patients with hemorrhages, either in the home or in hospitals. The most important transmitters of the infection to man are species of the genus *Hyalomma*, the life history of which is shown in the Figure 3 below [20].

Figure 3: Life history of transmitters of Crimean-Congo fever to man

Pathogenesis:
The pathogenesis of CCHF is not well described. A common pathogenic feature of haemorrhagic fever viruses is their ability to disable the host immune response by attacking and manipulating the cells that initiate the antiviral response [21]. This damage is characterised by marked replication of the virus together with dysregulation of the vascular system and lymphoid organs [22]. Infection of the endothelium has an important role in CCHF pathogenesis [23,24]. The endothelium can be targeted in two ways—indirectly by viral factors or virus-mediated host-derived soluble factors that cause endothelial activations and dysfunction, and/or directly by virus infection and replication in endothelial cells [23]. Endothelial damage contributes to haemostatic failure by stimulating platelet aggregation and degranulation, with consequent activation of the intrinsic coagulation cascade. Capillary fragility is a common feature of CCHF, suggesting infection of the endothelium. In one study of CCHF patients, the levels of interleukin 1, interleukin 6, and tumour necrosis factor alpha were higher among those patients that subsequently died compared with those that survived [23]. The disseminated intravascular coagulation score was higher among fatal cases, correlating positively with interleukin 6 and tumour necrosis factor alpha levels, and negatively with interleukin 10 levels [25].

Clinical manifestations:
Human beings are the only known host of CCHF virus in which disease is manifested [19,26]. The incubation period is 2-9 days [3]. The typical course of CCHF infection has four distinct phases: [27]
- Incubation,
- Prehaemorrhagic,
- Haemorrhagic,
- Convalescence periods

The incubation period could differ depending on several factors including viral dose and route of exposure e.g., it could be shorter with bloodborne transmissions. The prehaemorrhagic period is characterised by the sudden onset of fever (39°C - 41°C), headache, myalgia, and dizziness [8, 19]. On average, fever persists for 4-5 days. Additionally, symptoms of diarrhoea, nausea, and vomiting are also seen in some cases [28,29]. Hyperaemia of the face, neck, and chest, congested sclera, and conjunctivitis are commonly noted. The prehaemorrhagic period lasts an average of 3 days (range: 1-7 days) [27]. The haemorrhagic period is short (usually 2-3 days), develops rapidly, and usually begins between the third to fifth day of disease. There is no relation between the temperature of the feverish patient and onset of haemorrhage [27]. Bleeding from other sites, including the vagina, gingival bleeding, and cerebral haemorrhage have been reported [30]. The most common bleeding sites are the nose, gastrointestinal system (haematemesis, melena, and intra-abdominal), uterus (menometrorrhagia) and urinary tract (haematuria), and the respiratory tract (haemoptysis) [31]. Hepatomegaly and splenomegaly have been reported to be occurring in one-third of patients. In Turkey, hepatomegaly was detected in 20-40% of cases [28,32-34]. And two studies reported splenomegaly, with frequencies of 14% and 23% [33,34]. The convalescence period begins in survivors about 10-20 days after the onset of illness. In the convalescent period, labile pulse, tachycardia, temporary complete loss of hair, polyneuritis, difficulty in breathing, xerostomia, poor vision, loss of hearing, and loss of memory have been reported, although none of these findings were noted in the recent outbreak in Turkey.
**CCHF reservoirs and vectors:**

- The CCHF virus may infect a wide range of domestic and wild animals. Many birds are resistant to infection, but ostriches are susceptible and may show a high prevalence of infection in endemic areas. Animals become infected with CCHF from the bite of infected ticks.
- A number of tick genera are capable of becoming infected with CCHF virus, but the most efficient and common vectors for CCHF appear to be members of the *Hyalomma* genus. Trans-ovarial (transmission of the virus from infected female ticks to offspring via eggs) and venereal transmission have been demonstrated amongst some vector species, indicating one mechanism which may contribute to maintaining the circulation of the virus in nature.
- However, the most important source for acquisition of the virus by ticks is believed to be infected small vertebrates on which immature *Hyalomma* ticks feed. Once infected, the tick remains infected through its developmental stages, and the mature tick may transmit the infection to large vertebrates, such as livestock. Domestic ruminant animals, such as cattle, sheep and goats, are viraemic (virus circulating in the bloodstream) for around one week after becoming infected.
- Humans who become infected with CCHF acquire the virus from direct contact with blood or other infected tissues from livestock during this time, or they may become infected from a tick bite. The majority of cases have occurred in those involved with the livestock industry, such as agricultural workers, slaughterhouse workers and veterinarians [35].

**Diagnosis:**
The diagnosis is suggested on clinical grounds when the patient has a history of a tick bite or of exposure to ticks in the environment, and after an incubation period of 2 - 7 days develops an illness of sudden onset of muscle pains, headache fever and a rapidly evolving severe illness with the development of a haemorrhagic state with bleeding from the mucous membranes and petechiae in the skin, associated with thrombocytopenia and leucopenia.

**Isolation of virus:**
The diagnosis may be confirmed in the laboratory by intracerebral inoculation of baby mice with blood of a patient; the mice sicken about 1 week after inoculation. The virus is identified by using known specific Congo virus antiserum in an immunofluorescent test. The development of antibodies in patients’ serum as the illness progresses may be demonstrated in immunofluorescent tests using chamber slides with tissue culture cells infected with Congo virus.

**Serology:**
IgM and IgG antibodies are detectable by ELISA and immunofluorescence assays from about 7 days after the onset of disease. Specific IgM declines to undetectable levels by 4 months post-infection, but IgG remains detectable for at least 5 years. Recent or current infection is confirmed by demonstrating seroconversion, or a fourfold or greater increase in antibody titre in paired serum samples, or IgM antibodies with IgM antibody capture (MAC)-ELISA in a single sample [25]. ELISA methods are quite specific and much more sensitive than immunofluorescence assays and neutralisation tests. Recently, a recombinant nucleoprotein-based IgG ELISA for serological diagnosis of CCHF virus infections was developed [25].

**Molecular method:**
Molecular-based diagnostic assays, such as the reverse transcription-polymerase chain reaction (RT-PCR), provide a useful complement to serodiagnosis and now often serve as the front-line tool in the diagnosis of CCHF, as well as other viral hemorrhagic fevers. Reverse transcription-polymerase chain reaction detects the genetic material of the virus, and due to the high sensitivity of reverse transcription-polymerase chain reaction, positive results can often be obtained from samples which are culture negative. The assay can be applied retrospectively to stored serum samples.

**Treatment:**
**Supportive care:** Treatment is supportive and may require intensive care. Supportive therapy is the most essential part of case management, and includes the administration of thrombocytes, fresh frozen plasma, and erythrocyte preparations. Replacement therapy with these blood products should be done after checking the patient’s complete blood count, which should be done once or twice a day. The intravenous preparation of ribavirin is recommended for treatment of viral hemorrhagic fevers, and the oral form for postexposure prophylaxis [36]. Intravenous ribavirin should be administered within 6 days of illness onset as follows: 30 mg/kg loading dose, followed by 16 mg/kg 4 times a day for 4 days, then 8 mg/kg 3 times a day for 6 days [37]. Mild cases do not need to be treated with ribavirin. In case management, severe cases should be defined and treated.

**Contraindication:** It should be noted that men and women who take ribavirin for prophylaxis should avoid conception for six months after taking it because of ribavirin’s teratogenic effects. Ribavirin is teratogenic in experimental animals. Its use may be contraindicated in pregnant women; however, given the seriousness of the disease, ribavirin must be considered [37]. Although ribavirin should not be used when renal impairment is present, it may be necessary for severe disease in which the potential benefit may outweigh the risks.

**Immunotherapy:**
A new specific immunoglobulin CCHF-Venin has been prepared the plasma pool of boosted donors, by a combined ethanol-polyethyleneglycol fractionation method with an ion-exchange purification step. The final product is free from immunoglobulin aggregates, vasoactive substances, and polyethyleneglycol, and meets national
and international requirements for intravenous immunoglobulin [30].

Conclusion:
Prevalence needs to be measured in animals and in at-risk humans in endemic areas; and a useful animal model needs to be developed. Further research is needed to determine the efficacy of specific treatment with ribavirin and other antiviral drugs, and develop a safe and effective vaccine for human use.

References:
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