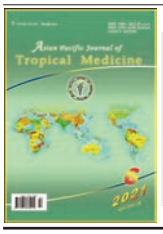

Review Article

Asian Pacific Journal of Tropical Medicine

apjtm.org

doi: 10.4103/1995-7645.315899

Impact Factor: 1.94

Crimean-Congo hemorrhagic fever from the immunopathogenesis, clinical, diagnostic, and therapeutic perspective: A scoping review

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ABSTRACT

Crimean-Congo hemorrhagic fever virus (CCHFV) is responsible for widespread tick-borne zoonotic viral disease CCHF in African, Middle Eastern, Asian, and European countries. CCHFV can be spread to humans through tick bites or contact with infected animals or humans, and it often progresses from asymptomatic to severe/lethal illness, with fatality rates ranging from 10% to 40% in humans. Today, CCHF is growing into a significant public health concern due to its very high prevalence, severity of the condition, and lack of available vaccines and specific treatments. Recent research has been drawn towards a more accurate study of CCHFV characteristics, including the structure, genetic diversity, mechanisms involved in pathogenesis and immunopathogenesis, and clinical features. In addition, the use of animal models (mouse and non-human primates) and advanced diagnostic tools in recent years has resulted in a significant advance in CCHF related studies. In this context, we summarized the latest findings about CCHF research, its health complications, animal models, current diagnosis, vaccination, and CCHF treatments, and therapeutic strategies. Furthermore, we discussed existing deficiencies and problems in CCHFV analysis, as well as areas that still need to yield conclusive answers.

KEYWORDS: Crimean-Congo hemorrhagic fever virus (CCHFV); CCHF disease; Immunopathogenesis; Animal models; Diagnosis; Vaccines; Therapeutic approaches

1. Introduction

Crimean-Congo hemorrhagic fever (CCHF) caused by Crimean-Congo hemorrhagic fever virus (CCHFV) is one of the most severe viral illnesses in humans[1]. In 1944, an unfamiliar pathogen outbreak was first confirmed during the Crimean conflict, later referred to as a hemorrhagic fever[2]. In 1969, it was discovered that the pathogen causing Crimean hemorrhagic fever was similar to the pathogen causing a distinct disease identified in the Congo in 1956[3]. Therefore, these two locations lead to the name of the disease's discovery. The most efficient vector of CCHFV is ticks of the genus *Hyalomma marginatum*, which are widely distributed across many countries of Africa, Europe (south Russia, Turkey, Balkan countries, and Spain), and Asia (from China and India to the Middle East)[3,4]. Furthermore, *Hyalomma* vectors have the ability to adapt to a wide variety of environments, which has resulted in their global distribution through migratory birds. Global distributions of CCHF are among numerous areas[5,6]. At the moment, Middle Eastern countries are the principal areas of CCHF activity. Today, *Hyalomma* ticks are far more prevalent or even epidemic in the world. A new study reveals that their geographical range is growing rapidly. Moreover, migratory birds possess a significant role in disseminating *Hyalomma* ticks into farther areas and potentially exposing human populations to CCHFV, which would result in approximately 10 000 to 15 000 cases of human infections annually[4]. Often, CCHF can cause a wide spectrum of asymptomatic to lethal disease in humans, and there is no cure in animals or a vaccine that should be approved[7]. Thus, in endemic areas, CCHF will create a slew of complications in diagnosis, care, and prevention. Currently, several research trials are being done on animal models that rely on the identification of viral proteins and pathology associated with the infection, as well as on virus pathology in animals that have been exposed to the virus[3,8,9]. Also, with the contribution of novel animal models in recent years, the development of vaccines and therapeutic approaches for CCHF is rapidly expanding[10,11]. This review discussed the recently published data regarding general characteristics, clinical features, laboratory findings, immunopathogenesis systems, and animal

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How to cite this article: Hamidinejad MA, Esmaeili Gouvarchin Ghaleh H, Farzanehpour M, Bolandian M, Dorostkar R. Crimean-Congo hemorrhagic fever from the immunopathogenesis, clinical, diagnostic, and therapeutic perspective: A scoping review. Asian Pac J Trop Med 2021; 14(6): 254-265.

Article history: Received 9 November 2020 Revision 2 May 2021
Accepted 8 May 2021 Available online 25 June 2021

models of CCHFV. Additionally, we showed current progress in the production of CCHF vaccines and clinical approaches.

This study was executed under supervision of ethical committee of Baqiyatallah University of Medical Sciences, Tehran, Iran (ethical code IR.BMSU.REC.1398.256).

2. Characteristics of CCHFV

2.1. Taxonomy and phylogenetics

CCHFV is a member of the Bunyaviridae family's Nairovirus class. This family comprises five groups and over 350 virus types, only three of which cause hemorrhagic fever in humans: *Nairovirus*, *Phlebovirus*, and *Hantavirus*. *Nairoviruses* are the tick-borne viruses comprising 34 different viruses that are classified into seven distinct serological serogroups[2,12]. CCHFV is a member of the CCHF serogroup, as is another virus known as the Hazara virus, which has not been proven to cause disease in humans[12,13].

2.2. Structure and genetic diversity

CCHFV-RNA has a negative, one-stranded genome found in the thick capsid and has a diameter of 90 to 120 nm of the spheroidal particle. The CCHFV genome is divided into three segments: Large (L), Medium (M), and Small (S) based on their respective size (Figure 1)[1,6]. The L segment encodes RNA-dependent RNA polymerase (RdRp). M segment encodes non-structural proteins and glycoprotein, which dissociate into two structural glycoproteins (Gn, Gc), which are essential factors in the cell junction process prior to endocytosis, hemagglutination, triggering host immune response, and virus entry into host cells[1,8]. Besides, this gene is the main factor in immunity and pathogenicity. The S segment with viral polymerase has been involved in virus replication and transcription. Moreover, it encodes the nucleocapsid that responsible for the encapsidation of the virus[8,14].

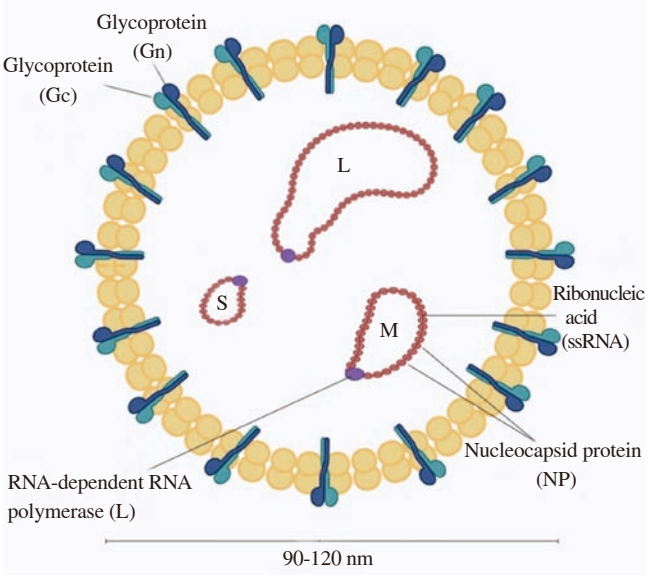

Figure 1. Schematic representation of CCHFV structure.

Due to its wide regional range, CCHFV has the most genetic variation of tick-borne viruses. Several studies have shown that geographically distinct isolates of CCHFV strains can differ by up to 5% in the amino acid composition of the NP and L proteins and up to 25% in the glycoprotein precursor[8]. Nowadays, based on genetic diversity among RNA sequences (in particular S segment) of CCHFV strains, they are divided into seven to eight lineages (three from Africa, two from Asia, and two from Europe)[15,16]. The general impact of genetic variation on viral immunopathogenesis is still poorly understood. However, it should be noted that various factors including host condition, quantity of medical treatment, and individual vulnerability, contribute to the fatality rate that varies worldwide from 2% to 80%[2,17,18]. Also, for instance, observed differences in mortality rates caused by the same genetic lineage of CCHF, named AP92, across countries suggest that it is virulent in some (Turkey and Greece) and benign in others (China)[19-21].

2.3. Tick–host–virus interactions

Ixodes ticks, specially the *Hyalomma* genus, are important for preserving and transmitting CCHF. Additionally, some ticks may transmit the disease through contact with an infected animal or through cohabitation with a tick. Moreover, some species of mammals serve as a key reservoir host for CCHF, which usually do not present any symptoms[22,23].

In the first step for the virus entrance to the tick body, it needs to be able to prevail the midgut and salivary gland barriers and the immune system of the tick body[24]. Virus infection may influence the behavior, gene expression, and survival of the tick. The tick bite and the extended process of its feeding on the host is the first contact between the host and the tick, and Then, pathogens and other toxins transmit to the host through saliva secretions[25]. Various factors determine the transmission efficiency of the virus to the host, such as the volume of salivary glands secreted inside the host, since it is one of the main ways of the pathogen transmission to the host by the arthropod vectors; the attachment time, owing to its influence on the level of tick-host interaction; and abiotic agents (climatic and environmental), because of their impact in the affluence and aggressiveness of ticks that increase the chances of the host bite by the tick[26].

The CCHF virus must infiltrate and surmount the host's epithelial cells to proliferate and spread[27]. The virus then replicates to high titers at the injection site and in the epithelial cells, Dendritic cells, and macrophages, helping to move the virus to different infection sites. All that eventually leads to early infection of the local lymph nodes and peripheral blood-borne monocytes carrying the systematic dissemination of the virus[28]. Moreover, the major clinical features of CCHF, including bleeding and high vascular permeability and the attendance of viral antigens in endothelial cells (ECs), indicating that endothelium is the principal target of the virus. Also, Kupffer cells and hepatocytes have been demonstrated as significant targets in CCHF. The CCHFV receptor in the target cells is yet unknown, but according to some studies, the CCHFV glycoproteins (Gn, Gc) are involved in the primary binding of the virus to the plasma cell membrane of the host. Furthermore, Gc has mediated virus entry into the host[29].

2.4. Transmission

CCHF Human infection typically happens by a tick's bite, squashing an infected tick with ungloved hands, or through contact with the tissues or blood of infected humans or animals (wild or agricultural)[23]. Also, there is a possibility of human-to-human transmission by close contacts (family members) or hospital-acquired infections[30,31]. Furthermore, extreme infant-maternal trans-transmission of CCHF has been confirmed to result in infant and fetal deaths[3,32].

The latest reports indicate that the risk of human infection increases during the spring and summer when ticks eat and multiply[33,34]. Furthermore, studies have found a high correlation between the *Hyalomma* tick's capacity for transmitting the CCHFV to humans and the ecosystem in which they grow. For example, it has been demonstrated that CCHFV has a substantial capacity for spread in areas with both small mammals (hedgehogs and hares) and large mammals (cattle and sheep)[5,35]. Previous studies have found that individuals who are in contact with animal blood, as well as medical personnel and laboratory workers are at higher risk for acquiring this disease[36]. In Turkey, for instance, almost 90 percent of all human cases recorded were in farmers, abattoir employees, and butchers[37].

3. Clinical/laboratory findings

Studies have implicated that various vertebrate species, including birds, fish, amphibians, reptiles, and mammals, can be infected by CCHFV. However, it seems that severe or fatal CCHF can only occur in humans. Also, CCHF may appear as an asymptomatic, mild, or subclinical disease in humans[7,38]. However, it is still unknown why some human cases of CCHF develop severe or lethal illnesses, but others mild or asymptomatic.

In general, CCHF can be divided into four different stages, including incubation, pre-hemorrhagic, hemorrhagic, and convalescence, respectively (Figure 2). The incubation phase begins

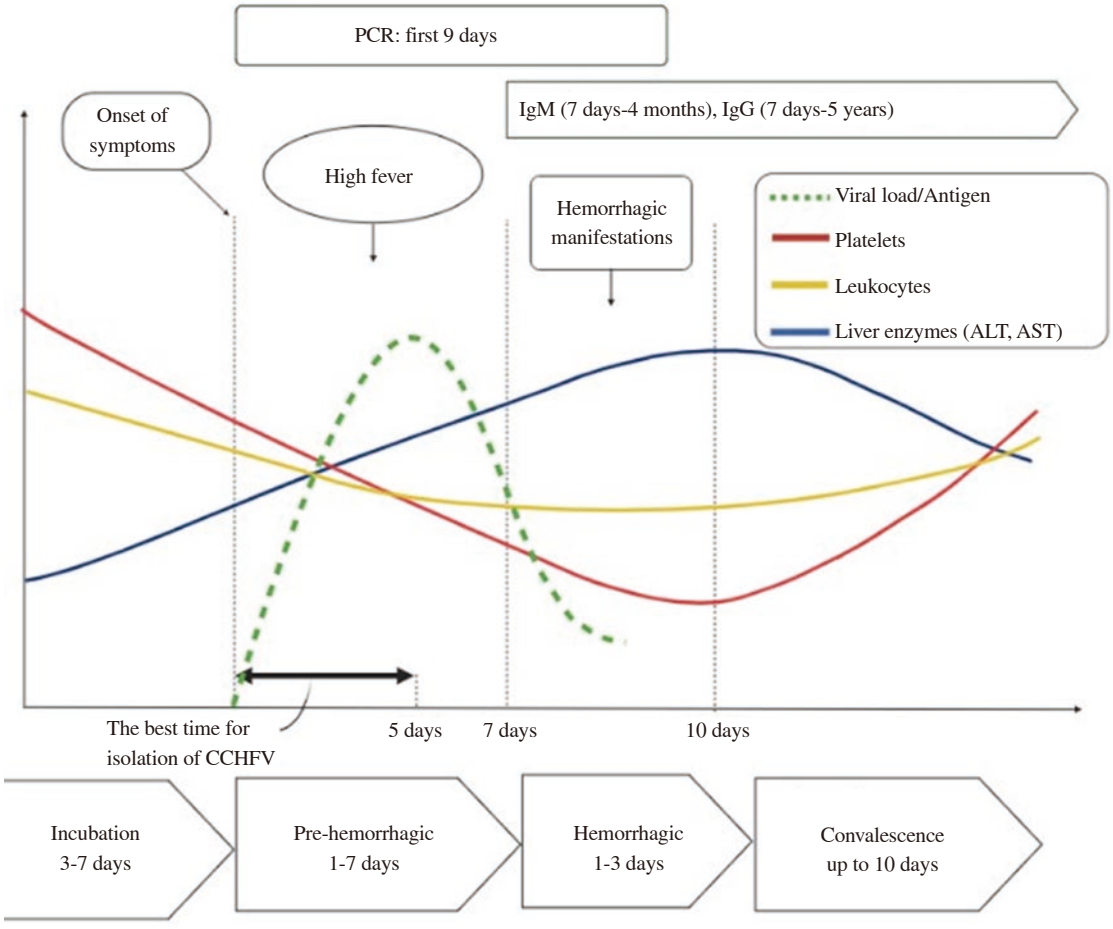

Figure 2. Phases of Crimean-Congo hemorrhagic fever. Incubation phase: after infection with CCHFV, the incubation period starts and lasts up to 7 days. This process is symptom-free[39]. Pre-hemorrhagic phase: this phase usually occurs shortly after the incubation phase and is characterized by generalized symptoms. High fever is the most common feature in this process. In addition, the first five days of the pre-hemorrhagic phase are the safest time to isolate CCHFV. Furthermore, the RT-PCR test will diagnose this disease in early stage. Additionally, serological diagnostic approaches based on CCHFV-specific IgM and IgG (ELISA, IFA) are only applicable after the first five days of disease[1]. Hemorrhagic phase: If detection erupted, the disease would progress to the hemorrhagic process at an alarmingly fast rate. This CCHF phase is characterized by hemorrhagic manifestations such as decreased platelet and/leukocyte counts, elevated liver enzymes, and pro-inflammatory cytokines[30,40,41]. Generally, the patient can start feeling better 10-20 days after the symptoms has finished.

following the infection with CCHFV. Regarding virus exposure way (*i.e.*, needle sticks, tick-bites, or respiratory exposure) and also viral load, this phase would continue for approximately 3-7 days[23,39]. The pre-hemorrhagic phase can occur soon after the incubation phase and continue for 1-7 days. Through this phase, symptoms are commonly unspecific. So, CCHF during the pre-hemorrhagic phase remains indistinguishable from viral fevers. Also, high fever is the main symptom of the pre-hemorrhagic stage, which can reach 39 ℃-41 ℃[1]. Furthermore, other signs are headache, myalgia, diarrhea, retro-orbital pain, muscle aches, photophobia, a stiff neck, and some non-specific symptoms. This phase is usually short but can remain for one week. The entrance of CCHFV into the host's epithelial cells and establishment of the infection causes endothelial dysfunction and leakage of red blood cells (RBCs) and plasma from capillaries into the tissues. The initiation of the coagulation cascade and increased bleeding takes place as a result of endothelial dysfunction. Additionally, activating coagulation is likely to lead to the development of disseminated intravascular coagulation (DIC) and resulting in multi-organ loss and shock. Additionally, the leaking of the vasculature seen in affected patients occurs as a consequence of their overt infection with the CCHFV or as a result of cytokine discharge. The phase described above will occur in certain people and lasts between one and three days, with a fatality risk of 10% to 40%. During this time, the heavy bleeding and lower blood cell count will identify CCHF. This stage is characterized primarily by bleeding from mucous tissues (hematemesis, epistaxis, hemoptysis, melena, and hematuria), as well as gastrointestinal, gingival, and cerebral hemorrhages. Furthermore, the most common symptom at this stage is bleeding through the skin. In addition, the greatest probability of viral propagation is during this stage[30,40,41]. The recovery period starts ten to twenty days after the first symptoms of CCHF appear. This process typically involves ten days of pronounced fatigue, dizziness, changes in libido, and compromised memory.

Thrombocytopenia, hepatocellular cytolysis, and leukopenia have been shown as the main laboratory complications observed in almost all CCHFV infected cases[42,43]. Thrombocytopenia features include increased activated partial thromboplastin time (aPTT), decreased prothrombin time (PT), and hypofibrinogenemia. Furthermore, common laboratory findings show raised levels of creatine kinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) in the patients[1]. CCHF often causes liver damage manifested as changes in liver enzymes ratio (AST, ALT)[43,44]. Moreover, some studies have reported neuropsychiatric disorders as the primitive symptoms of CCHF, which include confusion, disorientation, mood alteration, aggression, cerebral/cerebellar edema, and encephalopathy[1,45,46]. Besides, both cardiac and pulmonary disorders have also been demonstrated by other studies in CCHF infected cases[47,48]. Generally, progression to advanced (severe) CCHF is identified by thrombocytopenia, hemorrhagic manifestation, prolonged clotting times, elevated levels of circulating liver enzymes (ALT, AST), and high levels of inflammatory cytokines[43,44,49]. Fatal outcomes commonly occur following DIC, shock, and multi-organ failure (liver, spleen, heart, lung, and intestinal tissues) at the end of hemorrhagic phase (Table 1)[42,43].

Currently, case fatality rates of CCHF in the world is ranging from 10% to 40%. This broad range may be due to variations in the virus, the way of exposure, and the dose of the infective virus[50,51]. In regard to the special condition of pregnant mothers (*i.e.*, immunocompromised status), CCHF can be dangerous for both mother and fetal/neonatal[52]. Moreover, infected pregnant mothers have a higher mortality rate (up to 33%) compared to the overall lethality in humans. The CCHF-related mortality rate in fetal and neonatal is 58.5%[53]. Moreover, the high risk of nosocomial diseases is another concern of CCHFV infections during pregnancy[54,55].

4. Diagnosis

In brief, the diagnosis process of CCHF is comprised of 2 central stages: virus isolation and virus detection. Based on the studies, the best time for virus isolation is the initial five days of disease (in the pre-hemorrhagic phase) when the viremia is high (Figure 2). There are various cell lines for virus isolation, including LLC-MK2, CER, SW-13, and BHK-21. In addition to cell culture, intracerebral inoculation of newborn mice can also be used for virus isolation; this method has higher sensitivity than the cell culture[56].

Detection methods of CCHF are based on molecular and serological techniques (Figure 2). The molecular techniques are

Table 1. Clinical/laboratory abnormalities of CCHF disease.

Associated with severe disease	Associated with severe/fatal disease	Associated with death	References
Decreased platelet numbers Decreased leukocytes numbers Prolonged clotting times Hemorrhagic manifestation High viral loads Elevated levels of liver enzymes (ALT, AST) Absence antibody responses	Elevated levels of inflammatory cytokines	Disseminated intravascular coagulopathy (DIC); Multi-organ failure (liver, spleen, lung, heart, and intestinal tissues); Shock	[42–44,49]

mainly used in the early stage laboratory diagnosis of CCHF during the first days after infection. For example, reverse transcriptase-polymerase chain reaction (RT-PCR) can be employed in CCHF diagnosis by detection of the viral RNA in infected samples during the initial days of the disease[57]. Moreover, RT-PCR can detect both local CCHFV strains and several genetic lineages of the virus[58,59]. Enzyme-linked immunosorbent assays (ELISA) and IFA (in-house or commercial) can be used as the serological methods in the CCHF diagnosis by detection of the CCHFV-specific IgM and IgG antibodies[60]. Unlike biochemical approaches, which can be used to diagnose disease at an early stage, serological procedures can be used to diagnose disease only within the first five days of infection (Figure 2); IgM and IgG antibodies would be detected only after five days of illness. Furthermore, most serological assays use N protein (NP) as the CCHFV antigen[61].

Study analysis using in situ hybridization (ISH) and immunohistochemistry (IHC) can help in the diagnosis of CCHF and its pathogenesis[62]. Plaque reduction neutralization tests (PRNT) can be used to quantify the titer of CCHFV neutralizing antibody[63]. Recent advances in diagnostic and research laboratory instruments, including next-generation sequencing (NGS), provide a beneficial tool for better investigation on the CCHFV. Moreover, the whole genome sequence (WGS) of the CCHFV has been recently obtained by the use of de novo NGS[64,65]; analysis of WGSs would result in developing effective diagnostic tools and more studies on recombination, reassortment events, and virus evolution[66,67]. Furthermore, a CCHFV μ-capture ELISA with increased sensitivity/specificity and a CCHFV IgG immune complex (IC) ELISA have recently been developed for detection of CCHFV-specific IgM and IgG antibodies, respectively[68].

It is worthy of mentioning that CCHF is a highly contagious disease and generates serious complications. So, the sampling and diagnosis process of this disease suspected cases must only be conducted in high-containment laboratories (BSL-4) by expertized staff[69].

5. Immunopathogenesis system

Infection with CCHFV can result in inducing a series of immune responses, including the inflammatory immune responses, innate immune responses, and adaptive immune responses, which are crucial for the host defense against the pathogens. However, CCHFV may either disrupt or cause a delay in these antiviral responses[37]. Levels of inflammatory cytokines and chemokines may be increased as a result of inflammatory immune responses to CCHFV, which would result in an intensifying effect in the immune-pathogenesis of the virus[70,71].

5.1. Inflammatory and innate immune responses

The innate immune response is the body's first line of defense against the CCHFV. Studies on the effects of CCHFV in mice lacking of type Ⅰ interferon system revealed the critical role of host innate immune responses in limiting CCHFV pathogenesis, which led to developing severe or fatal illness in mice[47,48].

While inflammatory cytokines and chemokines are useful for the antiviral responses in innate immunity response, higher levels of these molecules can cause pathological damage and subsequently progress the infection and death[72]. For example, recent studies have demonstrated elevated levels of the interleukin-8 (IL-8), IL-6, tumor necrosis factor (TNF)-α, and monocyte chemokine MCP-1 (CCL2) in the fatal/severe CCHF cases compared to non-fatal patients[49,73]. The presence of secreted expression of the trigger receptor on myeloid cells-1 (sTREM-1) has been identified by other researchers as a reinforcement of inflammatory responses in CCHF viruses. However, it remains to be empirically demonstrated how an abnormal amount of these inflammatory agents would result in driving pathogenic processes[74].

Interferons (IFNs) are critical compounds of the innate immune system in the antiviral response by inducing the expression of the antiviral proteins and also limiting the spread of the infection[75]. CCHFV replication causes activating innate immune system that would result in the induction and discharge of IFNs and subsequently upregulation of interferon-stimulated genes (ISGs) that involved in innate immune responses of the host to pathogens. However, some studies indicated that CCHFV caused a delay in IFNs production in infected cells, which resulted in the prevention of IFNs antiviral effect. Overall, IFNs could not alone suppress the CCHFV replication during progressing infection[76,77].

One of the well-known sensors of CCHFV is retinoic acid-inducible gene I (RIG-I) that may be disrupted by viral RNA to avoid sensing. However, CCHFV sensing is improved by more innate immune sensors[17,78]. Other studies in CCHF patients have shown a correlation between polymorphisms in toll-like receptors (TLRs) and the severity of the illness, which has suggested TLRs as a likely remarkable immune-sensing pathway in the control of virus. For example, polymorphisms in TLR7, 8, 9, and 10 have been found to correlate with illness severity in infected cases in Turkey[17,79]. In addition to TLRs, it has been found a correlation between polymorphisms in nuclear factor-kappa B and a higher risk of CCHF. However, more investigation is required to identify the association of related polymorphisms with CCHF in various populations of different geographic regions[80]. It has been found that host apoptotic pathways could disrupt CCHFV replication; CCHFV replication can induce apoptosis that would result in activation of caspase 3 cleaving the CCHFV nucleoprotein and subsequently suppressing

viral replication[81,82]. However, recent studies state that CCHFV can disrupt innate immune signaling through a domain named ovarian tumor-related deubiquitinase (OTU) encoded by L segment. OTU domain can disrupt innate immune responses by deubiquitinating proteins involved in innate immune signaling pathways[83]. Furthermore, ISG15 modifications that have been involved in direct antiviral responses can be cleaved by the CCHFV OTU domain. ISG15 acts as a regulator of host innate immune response to CCHFV infection[84–86]. In addition to deubiquitinase activity, the de-ISGylation has also been found in the OTU domain that may be essential for the domain's activity and also viral pathogenesis[86]. More researches have found the significant role of the OTU domain in overcoming interferon responses[83].

5.2. Adaptive immune responses

Unlike the innate immune response, the function of adaptive immune responses in the immunopathogenesis of CCHF is less well known, which is mainly due to the lack of proper animal models. However, recent studies have provided promising results in correlation to the function of adaptive immune responses in CCHF immunopathogenesis. Studies have found a correlation between low-to-absent anti-CCHFV antibody response with severe disease and death[43,44,87]. Moreover, antibody levels, host IgM and IgG antibody responses against both the glycoproteins and nucleocapsid protein of CCHFV could be taken as a predictor of illness outcome[87]. Nevertheless, it is yet to know whether antibody responses contribute to the prevention of initial CCHFV infection or not. CCHF survivors are usually seen with low neutralizing antibody responses, and even in the fatal cases of CCHF, these responses are undetectable. In this regard, some studies on CCHFV survivors in Turkey and South Africa recognized antibody responses against epitopes in the mucin-like domains (GP38, Gn protein) that are not likely to lead to neutralizing antibodies[88,89]. Nevertheless, conservation of non-neutralizing antibodies against fatal CCHF challenge has shown that other mechanisms (except neutralization) can also protect antibodies.

Recent CCHF related research, Spengler *et al*, has developed a humanized mouse model of CCHFV, and they found that CCHFV infection would result in activation of T cells. Also, they identified high levels of perforin as a marker of cytolytic activity in $CD8^+$ T cells[90]. Other studies have found a positive correlation between the numbers of circulating $CD3^+CD8^+$ T cells in CCHF patients and lethal outcomes[91]. Moreover, it has been shown a long-lived $CD8^+$ T cell response to the virus in some survivors of CCHF[92]. Other studies using STAT1-deficient mice have demonstrated early activation of $CD4^+$ and $CD8^+$ T cells against CCHFV infection. Some researchers identified a correlation between human leukocyte antigen alleles and the protection and sensitivity of CCHFV[93]. Thus, using updated viral vaccine studies for CCHF indicating that T-cell activity could be important in providing the bulk of the CCHF immunity[94,95]. A recent study on mice treated with an interferon blockade antibody by Lindquist *et al.* showed that adaptive immune responses could control CCHFV in mice. Moreover, they found out that adaptive immune responses, including cytolytic T-cell activity, not necessarily resulted in liver damage following CCHF infection. Thus, CCHFV could directly cause liver damage[96].

Generally, despite all findings mentioned above about the role of the adaptive immune response in the immunopathogenesis of CCHFV, more studies are still needed to better knowledge of the interactions among virus and host adaptive immune response. While adaptive immune response and disease responses in cynomolgus macaques have been recently achieved, this is just a small step forward on the research and will pave the way for a better understanding of these two topics in the future[97].

6. Animal models

Until 2010, intracerebrally inoculated neonatal mice were the only available model used in CCHFV related studies. Like other developed animal models in laboratory research, they represented no illness following inoculation[23]. Currently, CCHFV studies commonly use mice lacking type Ⅰ or both type Ⅰ and type Ⅱ IFN responses[47,48,98]. These interferon deficient mice are typically susceptible to CCHFV and show an abrupt onset of severe illness and subsequently, death in approximately four days after the appearance of the disease. Moreover, these mice exhibited some of the behavior similar to humans after CCHFV infection, including elevated levels of inflammatory cytokines and also high amounts of liver enzymes (ALT, AST)[97,98].

In recent years, novel animal models of CCHFV have been developed in order to study interactions among hosts and viruses. For example, Hawman *et al*. has recently developed a novel model of the type Ⅰ interferon-deficient mice, in which mice lacking type Ⅰ interferon infected with a human clinical isolate named strain *Hoti* developed progressive illness with several days of obvious clinical symptoms and subsequent death about day 7 or 8 (Table 2)[99,100]. However, it has been demonstrated that other clinical isolates, including the Afg-09 strain, can cause quickly fatal illness in these mice[97]. These different clinical results of various CCHFV strains in mice suggested that there are probably major virulence determinants within CCHFV that have yet to be identified. Other research developed a mouse model in which MAR1-5A3 monoclonal antibody was used to block IFN receptor signaling, allowing for transient interferon signaling blockade in a variety of mouse genetic backgrounds[96,101]. In addition to the aforementioned mouse models

Table 2. Animal models of Crimean-Congo hemorrhagic fever virus (CCHFV).

Animal model	Features (advantages and limitations)	References
Type Ⅰ interferon- deficient mice	Either genetic knockout or antibody blockade. Develop viremia, liver failure, rapid-onset terminal disease, and inflammatory immune responses. Multiple CCHFV strains can be used. Useful for examining therapeutic interventions against CCHFV. Limited for examining host immune responses to CCHFV due to innate immune deficiencies and death prior to adaptive immune responses.	[99,100]
Humanized mice	Mouse engrafted with human $CD34^+$ hematopoietic stem cells. Strain-specific virulence observed. Exhibit a neurological-type disease.	[90,99]
Cynomolgus macaques	Immunocompetent macaques infected with a human clinical isolate of CCHFV called strain *Hoti*. Develop viremia, inflammatory immune responses, elevated levels of liver enzymes (ALT, AST), and prolonged clotting times. Represent a spectrum of disease outcomes from asymptomatic to lethal disease. Main sites of viral replication are liver and spleen. Useful preclinical model for therapeutic interventions against CCHFV. Valuable for studying host and viral determinants of disease outcome.	[99,102]

of CCHFV, a novel humanized mouse model engrafted with human $CD34^+$ hematopoietic stem cells (HSC) has been developed by Spengler *et al.* (Table 2)[90].

Until recently, there was no immunocompetent animal model for CCHFV. Elaine Haddock *et al*. have recently described a cynomolgus macaque model of CCHF infected with a human clinical isolate of CCHFV (strain *Hoti*) that exhibited many features of human cases of CCHF (Table 2). Moreover, these immunocompetent macaques developed a broad-ranging of clinical outcomes similar to human CCHF cases, as well as pathological complications, including high amounts of inflammatory responses (*i.e.*, cytokines and chemokines), primary viremia, low levels of platelets, and high amounts of ALT and AST[102].

7. Antiviral measures

Owing to the high prevalence of CCHFV over various geographical areas, proper preventive measures have been required to prevent infection against CCHFV. For this purpose, so far, several diverse vaccination and therapeutic approaches have been examined for CCHF. Nevertheless, Globally, no appropriate medication is available for this disease. However, a whole inactivated virus produced in the neonatal mouse brain is the only human CCHFV vaccine[103,104]; due to concerns about vaccine safety and efficacy, they are just used in Bulgaria and have not been licensed for general use in other countries. Studies linked to CCHFV have started in this regard to assist the creation of new individual CCHFV platforms. In preclinical studies, some of these vaccines have shown positive outcomes so far. At the moment, complete defense against lethal CCHFV infection in mice has been achieved through the development of several vaccination approaches, including improved vaccinia virus expressing CCHFV glycoproteins, DNA-based vaccination, and virus-like particle vaccination[94,101,105]. In addition to glycoproteins, the nucleoprotein of CCHFV encoded by S segment can also be used in developing modified vaccines[106,107]. Besides, based on some studies, nucleoprotein vaccines can induce immune responses directly, causing protection without the interference of glycoproteins. In this regard, the development of a vaccinia adenovirus expressing the nucleoprotein of CCHFV led to significant protection in mice against fatal CCHFV challenge. However, some studies on nucleoprotein-based vaccines have implicated that induced protection by these vaccines may be insufficient. It is worthy of mentioning that recent advances in the development of animal models, such as humanized mice and cynomolgus macaques, can contribute to the production of effective CCHFV vaccines[108].

Currently, both synthetic nucleoside analog ribavirin and immunotherapeutic approaches are used for the treatment of CCHF in humans. Hyperimmune human plasmas/serum that derived from either vaccinated individuals or CCHF survivors would be used in immunotherapeutic methods. Currently, ribavirin possesses the highest usage among other therapeutic approaches in CCHFV patients. Nevertheless, there are inconsistent clinical data for ribavirin efficiency and also for the results of treatment with ribavirin in CCHF cases[108-110]. For example, a recent ribavirin therapy on a CCHF patient in Spain led to decrease viral titers and also mutagenic impacts on CCHFV at the beginning of treatment[111]. Thus, this

treatment was eventually stopped due to a potential complication in ribavirin therapy termed hemolytic anemia[57,108,112]. According to the results of various human and animal studies, it is suggested that ribavirin may potentially improve clinical outcomes in the early stages of CCHF disease, particularly within the first four days where there are no serious symptoms, while also decreasing mortality[113]. Another therapeutic option for CCHF is favipiravir, which was initially used as a treatment approach for influenza virus infections in Japan[114]. Subsequent research revealed that favipiravir therapy inhibited CCHFV replication. Researchers observed that favipiravir could be given later in the disease and may lead to substantial results for advanced (severe) CCHF patients and also avoid death in those cases. These data say favipiravir can be used to treat CCHF. Oestereich *et al.* found that favipiravir and ribavirin could be combined with the same therapeutic effectiveness as a single medication in CCHF cases with a lower dosage of both medications. Additionally, they proposed that combined therapies could be beneficial in the treatment of CCHF in humans through reducing adverse side effects[97]. Other clinical trials, including employing a monoclonal antibody called mAb-13G8 that resulted in protecting neonatal mice from lethal infection, have recently been conducted either *in vitro* or *in vivo* and showed promising results[89,115].

Overall, the main agents of the current lack in required antiviral measures include lack of *in vivo* animal studies, the limitation in the abundance of infected humans with CCHFV, and the economic limitations in this field.

8. Conclusions

CCHF is a widespread tick-borne zoonotic infectious disease that is found in a variety of geographical areas. CCHF has great potential to be an endemic or even epidemic disease in different regions worldwide. The transmission of the virus to humans mainly happens by tick's bite or contact with infected animals or humans. CCHF appears in humans from asymptomatic to severe/lethal with a case fatality rate of up to 40%. Nowadays, CCHFV has gradually become a serious threat across some areas, owing to the high capability of transmission to new geographic regions, high severity of the disease, and the possibility of human-to-human transmission. Moreover, there is yet no FDA-approved vaccination and therapeutic approaches for CCHF. Also, most of the antiviral strategies to prevent and treat human CCHF remain controversial or experimental. However, such drugs are currently used, and their clinical results are inconsistent in CCHF patients. One of the main limits of CCHF vaccine development is the absence of specialized animal models; in terms of structural, immune, and therapeutic dimensions, mouse and primate models have been significantly improved. This advance in study tools of CCHFV would contribute to the development and evaluation of novel vaccines and therapeutic approaches in order to decrease or prevent CCHFV-induced fatality cases in humans. Generally, many aspects of the CCHFV remain poorly defined and need more consideration. Also, more attention should be paid toward the role of the ticks and wild and domestic animals involved in maintaining, transmitting, and pathogenesis of virus; this could lead to substantial measures regarding control of CCHF expansion. However, the recent significant advances in recognizing CCHFV structure, genetic diversity, life cycle, clinical/laboratory complications, and immunopathogenesis interactions among host and virus could be promising to overcome challenges ahead.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

We would like to thank the "Clinical Research Development Center of Baqiyatallah Hospital" for their kindly cooperation.

Authors' contributions

The conceptualization was done by HEG. The formal analysis and interpretation were done by HEG, MAH and MF. The resource and writing-original draft preparation were carried out by HEG, MAH, MF, RD and MB. The writing review and editing were performed by HEG, MAH. The supervision was done by HEG. The whole manuscript was read and approved by all authors.

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