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Recent advances in research on Crimean-Congo hemorrhagic fever

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Abstract

Crimean-Congo hemorrhagic fever (CCHF) is an expanding tick-borne hemorrhagic disease with increasing human and animal health impact. Immense knowledge was gained over the past 10 years mainly due to advances in molecular biology, but also driven by an increased global interest in CCHFV as an emerging/re-emerging zoonotic pathogen. In the present article we discuss the advances in research with focus on CCHF ecology, epidemiology, pathogenesis, diagnostics, prophylaxis and treatment. Despite tremendous achievements, future activities have to concentrate on the development of vaccines and antivirals/therapeutics to combat CCHF. Vector studies need to continue for better public and animal health preparedness and response. We conclude with a roadmap for future research priorities.

Keywords

Crimean-Congo hemorrhagic fever; ecology; epidemiology

1. Introduction

Crimean-Congo hemorrhagic fever (CCHF) is characterized by fever and hemorrhagic manifestations with fatality up to 30%¹. It is endemic in focal areas in Asia, Europe and

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Africa, with geographic distribution following that of *Hyalomma* ticks, the main vectors of CCHF virus (CCHFV). Apart from the bite of an infected tick (Figure 1), the virus can be transmitted to humans by direct contact with blood or tissues of viremic patients or animals. Nosocomial and intra-family transmission have been reported^{2, 3}.

The disease typically presents an incubation phase (1–9 days), prehemorrhagic and hemorrhagic phases (in severe cases), and convalescence⁵. The hemorrhagic manifestations range from petechiae and epistaxis to extended ecchymosis and bleeding from various systems (Figure 2).

CCHFV (genus *Nairovirus*, family *Bunyaviridae*) is an enveloped single-stranded negative sense RNA virus with a tri-segmented genome consisting of a small, medium and large RNA segments, encoding for the nucleocapsid protein (N), the glycoproteins Gn and Gc and the RNA-dependent RNA polymerase, respectively. CCHFV is characterized by a great genetic variability with complex evolutionary patterns⁶. Due to its high pathogenicity and the lack of approved vaccines and specific intervention strategies, CCHFV must be handled under biosafety level 4 (BSL-4) containment. The recent emergence of CCHFV causing either sporadic human infections^{7, 8} or epidemics in previously unaffected areas^{9, 10}, has raised animal and public health concerns. As a result, great progress has been made in CCHF pathogenesis, diagnostics and epidemiology/ecology, while efforts are underway to design effective vaccines and treatment strategies including antiviral and immunotherapeutic compounds. This article summarizes and discusses the progress in the field over the past decade and identifies knowledge gaps and future research perspectives.

2. Advances in eco-epidemiology

CCHFV is maintained in nature by ixodid ticks mainly of the genus *Hyalomma*^{11, 12}. Additional tick species from the genera *Dermacentor*, *Boophilus*, *Amblyomma*, *Rhipicephalus*, and *Haemaphysalis*, have been implicated in harboring CCHFV in the field or were shown to be experimentally infected, but there is little evidence for a role of these species in natural transmission or maintenance of CCHFV¹³. Thus, it appears that *Hyalomma* ticks are necessary for the maintenance of active CCHFV foci even during periods of enzootic or silent activity. Studies on the vectorial abilities of soft ticks (family *Argasidae*) confirmed that these ticks cannot transmit the virus despite getting infected while feeding on viremic hosts and detectable virus in blood remnants¹⁴. Thus, reports on the vectorial capacity of other ticks are unreliable and less convincing^{15–17}. Detection of CCHFV RNA in those tick species is likely the result of virus uptake while feeding on viremic hosts.

In addition to the fundamental role played by the presence and abundance of the most prominent tick vectors, an adequate density of reservoir hosts seems to be necessary in order to reach a critical transmission rate of CCHFV^{12, 18}. For other tick-borne diseases, such as tick-borne encephalitis or Lyme disease, it has been speculated that changes in host abundance, social habitats, economic fluctuations, environmental conditions, and to a lesser extent climate, have increased the disease incidence rate^{19–22}. Climate change, however, has been frequently linked to CCHF outbreaks. The development of a process-driven model for

H. marginatum, the main vector in the Mediterranean region²³, has provided an adequate framework to study the impact of climate features on virus spread by the tick vector in an endemic area²⁴ and to evaluate the effect of host abundance on viral transmission²⁵. It has been proposed that such study should precede the active surveillance of the tick vectors²⁶. Results from modeling approaches indicated that the Balkans are the area in the Mediterranean basin where climate trends might have a larger impact on the spread of *H. marginatum*²⁴. The modeling study provided evidence that the most important factor for increased transmission of CCHFV might be the increased abundance of large hosts (e.g. wild and domestic ungulates), on which adult ticks feed, thus, allowing further amplification through transovarial transmission²⁴. Further field surveys investigating the infestation and infection rates of different wild animals (mammals and birds) would enable a better understanding of the virus transmission cycle.

In the past years, the importance of habitat fragmentation and its consequences in the maintenance of active CCHFV foci have been discussed. There is evidence that a fragmented landscape with multiple smaller patches of vegetation within a matrix of unsuitable tick habitat, may lead to isolated populations of both ticks and hosts producing an amplification cycle with ticks feeding on infected hosts²⁷. Due to the isolation of these host populations, the local movements of hosts are limited, and, therefore, new naive animals carrying uninfected ticks do not dilute the prevalence rate in the isolated patch²⁷. For CCHFV eco-epidemiology, the degree of habitat patchiness contributes to the increased contact rate among reservoir hosts, humans, and ticks.

3. Advances in basic virology

Humans are the only known host that develops disease after CCHFV infection. The major pathological abnormalities of CCHF are related to vascular dysfunction resulting in hemorrhagic manifestations largely driven by erythrocyte and plasma leakage into the tissues²⁸. Endothelial damage can contribute to coagulopathy by deregulated stimulation of platelet aggregation, which in turn activates the intrinsic coagulation cascade, ultimately leading to clotting factor deficiency and hemorrhages. Vascular leakage may be caused either by destruction of endothelial cells or by a disruption of the endothelial cell junctions. It is still unknown whether vascular dysfunction is due to a direct effect of virus on the endothelial cells or a consequence of a cytokine storm. In fact, for other hemorrhagic fever viruses, such as Ebola and Dengue viruses, there is a correlation among the level of the pro-inflammatory response, vascular leakage, and disease severity^{29–33}. Key players in disease progression are interleukin (IL)-10, IL-1, IL-6 and tumour necrosis factor- α (TNF- α)³². In vitro studies showed that CCHFV replicates in human dendritic cells and macrophages resulting in the release of TNF- α , IL-6 and IL-8, which then can activate endothelial cells in vitro^{34, 35}. In contrast to milder CCHF cases, elevated levels of pro-inflammatory mediators (such as TNF- α , IL-6, IL-10) and serum markers of vascular activation (sICAM-1 and sVCAM-1) have been detected in fatal CCHF cases^{36–38}. These clinical observations are mirrored in an in vitro experiment, in which it was demonstrated that CCHFV infected endothelial cells can up-regulate ICAM-1 and VCAM-1³⁹. It was shown that CCHFV induces apoptosis in vitro late post infection in human target cell lines^{40, 41}. This suggests that CCHFV may induce plasma leakage from the vessels and cause loss of lymphocytes by

apoptosis. However, it remains unclear whether induction of apoptosis is due to direct effects of virus replication to indirect effects induced by certain pro-inflammatory mediators known to induce apoptosis.

An efficient and rapid host response against virus invasion is the production of type I interferons (IFN- α/β). Besides their role as antiviral messengers, IFNs possess a wide range of biological activities including inhibition of cell proliferation, regulation of apoptosis, and immune-modulation^{42, 43}. CCHFV is IFN-sensitive⁴⁴ and despite the strong inhibitory effect of type I IFNs on its replication in vitro, CCHFV still runs its devastating course in the human host, probably because virus-induced IFN production in the host is inefficient or not timely. It has been shown that IFN has no significant activity against an already established CCHFV infection⁴⁵. This indicates that CCHFV strongly counteracts IFN signalling. It has been demonstrated that CCHFV employs a range of strategies to evade and counteract the innate immune response. RIG-I recognition is circumvented by removal of the 5' triphosphate group from the viral genome⁴⁶, IRF-3 activation, mediated by a particle recognition pathway is delayed by a yet to be identified viral factor⁴⁵, and NF- κ B activation and the antiviral action of ISG15 are down regulated by the OTU domain on the L protein⁴⁷. The knowledge that IFN plays a key role in controlling CCHFV replication was utilized for the development of CCHF animal models in adult mice lacking IFN responses^{48–50}. Recently, these models have been used to understand the pro-inflammatory response during active CCHFV infections⁵⁰. Most probably, capillary fragility caused by CCHFV is due to multiple host-induced mechanisms in response to CCHFV infection.

4. Advances in clinical virology

A prompt and accurate laboratory diagnosis during the first days of the disease is critical for both patient's management and prevention of transmission. Virus isolation is seldom tried for CCHF diagnosis, due to the time-consuming procedure and the biocontainment needed for handling specimens. Molecular methods are increasingly used for the detection of CCHFV in acute clinical samples. Furthermore, in combination with genetic characterization an immediate insight into the molecular epidemiology of the disease causing CCHFV strain is achieved. The tremendous genetic variability of CCHFV as well as the potential for reassortment and recombination events has to be considered for the design of diagnostic assays and vaccines.

4.1. Molecular methods

Several real-time PCR-based diagnostic assays for rapid detection of CCHFV in clinical samples have been described; primers are designed either to detect the majority of CCHFV genetic lineages or they are lineage-specific^{51–58}. A commercial quantitative real time RT-PCR kit is currently available. Assays using simple equipment have been developed, including a low-density microarray⁵⁹, a high-density resequencing DNA microarray⁶⁰, a method based on padlock probes with colorimetric readout⁶¹, and a loop-mediated isothermal amplification assay⁶². Some of these assays seem more practical than the real-time PCR for rapid detection of CCHFV in the field and in rural hospitals with resource-poor settings, but assay efficiency under those conditions remains to be evaluated on site.

The padlock probes have been used for in situ rolling circle amplification detection of viral and complementary RNA molecules, enabling studies of CCHFV replication⁶³.

4.2. Serologic methods

Serological methods have greatest use after the 5th day of illness. Antibody production is delayed or even absent in CCHF patients with severe disease. Enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescent assay (IFA) kits are commercially available for the detection of human IgM and IgG CCHFV-specific antibodies. The CCHFV N protein induces an early, strong and long-lasting immune response in humans and therefore the native or recombinant N protein is often used as the antigen in serological assays. Capture ELISAs for detection of CCHFV-specific IgM (μ -capture) and IgG (rheumatoid factor capture) have been developed⁶⁴, while the recent development of an indirect ELISA for the parallel measurement of CCHFV IgG and IgM antibodies using recombinant N protein as antigen removes the reliance on polyclonal antibody preparations⁶⁵.

The characteristics, performance, and on-site applicability of serologic and molecular assays for diagnosis of CCHF have been evaluated⁶⁶. An international external quality assessment of CCHFV molecular diagnostics showed differences in performance depending on the method used, the type of CCHFV strain targeted, the virus load of the sample and the laboratory performing the test, indicating that there is a need for improved performance and standardized protocols⁶⁷. As with most viral diseases, for accurate laboratory diagnosis a combination of molecular and serologic methods should be applied taking always into consideration the day of sampling during illness, the course of the disease and the immunological status of the patient.

Neutralization assays are of great confirmatory value; however, CCHFV has to be handled in BSL-4 containment making application of this diagnostic assay difficult. A recently described pseudo-plaque reduction neutralization test was suggested as an alternative, fast, reproducible and sensitive method for the measurement of CCHFV neutralizing antibodies⁶⁸.

5. Advances in prophylaxis and therapy

A number of studies regarding treatment and prevention have been recently performed. No specific therapy has been confirmed as effective enough for the treatment of CCHF patients. Differential diagnosis is very important. The initial nonspecific symptoms of CCHF can mimic other infections, leading to misdiagnosis and delay of proper treatment. CCHF patients must be closely monitored for effective supportive treatment^{69, 70}.

5.1. Supportive treatment

The most important step in the management of CCHF patients is supportive treatment^{70, 71}. Figure 3 summarizes an algorithm of CCHF case management.

Patients with a confirmed CCHF diagnosis are usually hospitalized for monitoring. Laboratory tests, like complete while blood cells count, serum electrolytes and

transaminases, renal function tests, and coagulation parameters (such as prothrombin time, partial thromboplastin time and international normalized ratio) are very informative and critical. Patients with organ failure must be monitored in intensive care units, especially for respiratory and renal complications^{69, 72}.

Fluid substitution, blood transfusion, and other supportive therapies, such as fresh frozen plasma and platelet transfusions, should be initiated as soon as possible if indicated. Intravenous immunoglobulin (IVIG) should be given to severe CCHF patients with refractory thrombocytopenia^{69, 72}. There is also a study reporting the efficacy of methylprednisolone in CCHF cases⁷³.

5.2. Antiviral therapy

Ribavirin is the only antiviral drug that has been used for the treatment of CCHF cases. It is a broad spectrum synthetic purine nucleoside analogue that acts by several mechanisms which are not totally understood yet. Ribavirin inhibits CCHFV replication in vivo and in vitro in a concentration-dependent manner⁷⁴. Its effectiveness in humans is largely based on clinical observations⁶⁹. Overall, however, the effectiveness of ribavirin is controversial. Some studies have suggested that ribavirin is effective when given in the early stage of the disease⁷⁵. However, other recent studies do not support the efficacy of ribavirin. One such study reported that despite a decline in the use of ribavirin from 68% to 12% in Turkey during 2004–2007, there was no change in CCHF mortality rate, which remained stable at around 5%⁷⁶. A randomized prospective study performed in the Black Sea region of Turkey did not determine a positive effect on clinical and laboratory parameters in CCHF patients given ribavirin and hospitalization was even prolonged in ribavirin-treated patients; mortality rates were similar in patients receiving ribavirin or not⁷⁷. A systematic review and meta-analysis did not support the idea that ribavirin is effective in the CCHF treatment, since the results of patients treatment with ribavirin were compared with those of patients without ribavirin treatment and found that ribavirin did not increase the survival rate, did not shorten the length of hospitalization and did not reduce the need for blood and blood products⁷⁸. Similar results have been reported from another meta-analysis reporting drug-related adverse effects in patients receiving ribavirin⁷⁹. On the other hand, combination therapy might be beneficial as shown in a study involving six patients who were treated with corticosteroids and ribavirin early in infection⁸⁰.

Flusin et al. reported that the combination of ribavirin and small interfering RNAs (siRNAs) exhibited a synergistic antiviral effect against Hazara virus in an animal model suggesting that the treatment might be also beneficial for CCHFV⁸¹. Others investigated compounds/drugs such as recombinant and natural interferon-alpha, however no human studies have been conducted⁸².

5.3. Specific immunoglobulin therapy

In the past, some CCHF patients have been treated with horse immunoglobulin and human convalescent serum. However, data regarding the effectiveness of this approach is still limited^{69, 79}. Specific immunoglobulin derived from plasma of convalescent CCHF patients has been given intramuscularly for prophylactic and therapeutic purposes, particularly in

Bulgaria⁸³. In a study performed in Turkey, CCHFV hyperimmunoglobulin prepared from healthy donors was given to 15 patients, who had high CCHF viral load (10^8 copies/mL). The application of anti-CCHF hyperimmunoglobulin may represent a new therapeutic approach, particularly for high-risk patients⁸⁴.

5.4. Post exposure prophylaxis

The benefit of post exposure prophylaxis following contact with CCHFV is debatable. However, studies on health care workers with a high risk of transmission and percutaneous exposure suggest that ribavirin prophylaxis might be beneficial^{85–87}. It is recommended that ribavirin be started immediately after injury/exposure based on the knowledge that the benefit of ribavirin is best when administered early in infection⁷⁰. According to these studies, ribavirin prophylaxis can be recommended for health personnel with high risk exposure to CCHFV such as through percutaneous injury^{88, 89}. Ribavirin prophylaxis is administered orally as a 2g loading dose, followed by 4 g/day for 4 days and 2 g/day for 6 days⁹⁰. Future studies need to address the efficacy, dose and duration of ribavirin prophylaxis for CCHF exposure.

5.5. Modes of prevention

As with all tick-borne diseases, persons have to pay attention to avoid tick bites. Since CCHFV is highly infectious after direct contact with secretions or blood of viremic patients or animals, in particular livestock, individuals occupied in animal husbandry and slaughterhouses, as well as healthcare workers, must follow infection control procedures, such as use of gloves, masks, gowns and face shields, which proved to be sufficient for protection^{87, 91}. Barrier nursing and patient isolation are needed while managing CCHF patients.

5.6. Vaccines

Initial attempts to develop CCHF vaccines began in the 1960s⁶⁹. The first inactivated vaccine was based on brain tissue derived from CCHFV infected suckling mice and rats. This vaccine was approved in 1970 by the Soviet Ministry of Health. In the same year, serum samples were collected from approximately 2,000 healthy individuals before vaccination and after 2nd and 3rd vaccination, and tested for the presence of neutralizing CCHFV antibodies. Neutralizing CCHFV antibodies developed within 1–4 weeks after the 3rd dose, but titers decreased 3–6 months thereafter^{69, 82}. In 1974, the Soviet vaccine was licensed in Bulgaria and was administered to military, medical personnel and agricultural workers in places where CCHF is endemic. The Bulgarian Ministry of Health reported that over a 22-year period the number of CCHF cases decreased four-fold after the introduction of vaccination⁸⁵. Modern vaccine approaches need to be urgently developed, like virus-like particle-based or DNA vaccines. Discovery and evaluation of new CCHFV vaccine candidates are difficult in the absence of proper animal model and a lack of interest by industry. The recently developed knockout mouse models may be temporary solutions to drive urgently needed research efforts for vaccine development^{69, 91}.

6. Ten-year research roadmap

CCHFV is the most widely distributed tick-borne hemorrhagic fever virus with endemic areas in more than 30 countries. Recent emergence and re-emergence of CCHFV underline the importance of this pathogen for global human and animal health. The tick vector of CCHFV is widely distributed in the Old World; its movement and expansion will define known and new endemic regions for this pathogen and CCHF disease. Despite improving knowledge on the biology of the virus, a better understanding of the clinical aspects of the disease and the establishment of rapid and sensitive diagnostics over the past 10 years, there are still many gaps to be filled. Future research needs to particularly focus on the vector-virus interaction, the impact of infection on livestock and other animals, and the development of vaccines and treatment strategies for humans and animals.

A priority future goal should be studies on CCHFV dynamics in reservoirs and vectors. The role of birds, climate change and reservoir/host abundance for the distribution of ticks and the establishment of new CCHFV endemic areas should be investigated. The creation of an epidemiological predictive risk map for CCHF would be of tremendous impact for 'One Health' preparedness.

The development of countermeasures such as vaccines and treatment options are urgent priorities. Vaccine strategies should be based on new recombinant technologies as they are generally safer, better suited for production purposes, and, probably easier approved by authorities. Gain in knowledge and better understanding of the virus biology have already produced targets for intervention, but new targets need to be identified and current strategies need to be moved forward towards efficacy testing and approval. The lack of proper animal models is a huge drawback and the development of immunocompetent CCHF disease models is an urgent short-term need. The lack of success in this area of research most likely has multiple reasons, but CCHFV adaptation through tissue culture passaging could be a simple explanation. Therefore, there is a need to establish tick colonies for virus seed stock propagation; those colonies would also be a tremendous tool to study the interaction of the virus with its tick vector.

Overall, the path forward for the next 5–10 years is laid out by the needs for public and animal health preparedness and response. Given the importance and urgency of CCHF for the health systems of many nations, short- and long-term deliverables are being expected over the next decade.

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Figure 1.
Tick on the back of a patient.

Figure 2.
A patient with Crimean-Congo hemorrhagic fever presenting ecchymosis on the right lower extremity and the pelvic area.

Figure 3.
Algorithm of CCHF case management.