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REVIEW ARTICLE

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Crimean–Congo haemorrhagic fever virus: Past, present and future insights for animal modelling and medical countermeasures

E. J. Mendoza[1] | B. Warner[1,2] | D. Safronetz[1,2] | C. Ranadheera[1]

[1]Zoonotic Diseases and Special Pathogens, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada

[2]Department of Medical Microbiology, University of Manitoba, Winnipeg, MB, Canada

Correspondence
Charlene Ranadheera, Zoonotic Diseases and Special Pathogens, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada.
Email: charlene.ranadheera@phac-aspc.gc.ca

Summary

Crimean–Congo haemorrhagic fever (CCHF) is a widespread tick-borne viral zoonosis with a case-fatality rate ranging from 9% to 50% in humans. Although a licensed vaccine to prevent infection by the CCHF virus (CCHFV) exists, its ability to induce neutralizing antibodies is limited and its efficacy against CCHFV remains undetermined. In addition, controlling CCHF infections by eradication of the tick reservoir has been ineffective, both economically and logistically, and the treatment options for CCHF remain limited. In this review, we first critically discuss the existing animal models to evaluate therapeutics for CCHF. We then review the therapeutic options for CCHF that have been investigated in human cases, followed by investigational drugs that have been evaluated in pre-clinical studies. We highlight the importance of understanding human prognostic factors in developing an animal model for CCHF that recapitulates hallmarks of human disease and its implication for selecting therapeutic candidates.

KEYWORDS

animal models, Crimean–Congo haemorrhagic fever, prognostic markers, therapeutics

1 | CRIMEAN-CONGO HAEMORRHAGIC FEVER

Crimean–Congo haemorrhagic fever (CCHF) is a tick-borne disease caused by the CCHF virus (CCHFV). CCHFV was characterized during the Crimean outbreak in 1944 and later that the same agent was the cause of disease in the Congo in 1956 (Casals, 1969). A member of the Orthonairovirus genus within the Nairoviridae family, CCHFV is primarily transmitted through direct contact of skin or mucous membranes with infectious blood or tissues, by the bite of infected Hyalomma species ticks or contact with viremic livestock (Vorou, Pierroutsakos, & Maltezou, 2007). Furthermore, fatal nosocomial spread of CCHFV in the hospital setting is very common (Oestereich et al., 2014). Upon infection, CCHF progresses through four stages of disease: incubation, pre-haemorrhagic, haemorrhagic and convalescence. The duration of the incubation stage is usually 1–3 days when CCHFV is transmitted via tick bite and 5–13 days when transmitted via contact with infected blood or tissues. The pre-haemorrhagic stage follows, with non-specific viral symptoms such as fever, muscle soreness, chills, photophobia, headache and nausea. In non-severe cases, individuals clear the infection and the pre-haemorrhagic stage associated symptoms resolve. In severe cases, the disease progresses to the haemorrhagic stage occurs 3–6 days after infection, with symptoms such as petechiae and haemorrhaging of internal organs, gastrointestinal system, gums and nose (Shayan, Bokaean, Shahrivar, & Chinikar, 2015). Fatal cases are usually a result of multiple organ failure. The fatality rate of CCHF has ranged from 9% to 50% in past outbreaks (Dilber et al., 2010).

Those who survive the haemorrhagic stage experience the convalescent phase, which is characterized by memory loss, headache, dizziness, weak pulse, hair loss, anorexia and vision abnormalities (Shayan et al., 2015). Long-term sequelae, such as neurological problems and impaired vision, have been documented; however, these are rarely permanent (Hoogstraal, 1979). To date, it is still unclear whether survivors develop immunity to subsequent CCHFV infections, but IgG responses lacking in fatal cases are induced in non-fatal cases in humans (Ergonul, Tuncbilek, Baykam, Celikbas, & Dokuzoguz, 2006).

Crimean–Congo haemorrhagic fever is a geographically widespread zoonotic disease, ranging from the Middle East and Asia to South Africa and Eastern Europe. However, most documented CCHF cases have been reported in Turkey. CCHFV remains a risk group-4 pathogen for its ability to cause severe to fatal disease in humans and the absence of effective options for pre- or post-exposure prophylaxis. Under these circumstances, CCHFV is still deemed a possible weapon for bioterrorism (Bronze, Huycke, Machado, Voskuhl, & Greenfield, 2002). In this review, we explore the past, summarizing historical measures of CCHFV control and prevention. We then review the present state of existing animal models to evaluate therapeutics and interventions tested in human cases of CCHFV infection. Finally, we discuss investigational drugs in pre-clinical studies. This review aimed to highlight the importance of developing an animal model for CCHF that recapitulates hallmarks of human disease in order to optimize pre-clinical drug testing and thereby improve our chances in developing an effective medical countermeasure for humans.

Impacts

- Crimean–Congo haemorrhagic fever (CCHF) is a public health concern, ranging from the Middle East and Asia to South Africa and Eastern Europe. Standard of care remains the most widely used treatment for CCHF, but other pharmaceutical options are being considered in both clinical and pre-clinical settings
- While small immunodeficient animals are currently used for the study of CCHF, the development of models that recapitulate hallmarks of human disease is crucial for predicting drug efficacy in humans
- Increased understanding of the prognostic factors associated with CCHF fatality may allow for the optimization of animal models and thus, provide guidance for therapeutic selection for CCHF

2 | PAST: HISTORY OF CCHFV CONTROL MEASURES

In multiple instances attempts to control CCHF, the eradication of the CCHFV tick vector has been inefficient, both economically and logistically (Keshtkar-Jahromi et al., 2011). It remains difficult to control CCHFV by culling domestics animal hosts because reservoirs, such as cattle, goats and sheep, often remain asymptomatic even when highly viremic (Whitehouse, 2004). As many nosocomial outbreaks of CCHFV have occurred in endemic areas (Burney, Ghafoor, Saleen, Webb, & Casals, 1980; Conger et al., 2015; Naderi, Sheybani, Bojdi, Khosravi, & Mostafavi, 2013; Pshenichnaya & Nenadskaya, 2015; Van de Wal, Joubert, van Eeden, & King, 1985), isolation rooms for CCHF have been established in treatment units throughout Turkey, Iran, Pakistan, Russia, Georgia, Bulgaria, India, Kazakhstan and Kosovo. In addition, adequate training for the use of personal protective equipment and effective waste management and disinfection in the hospital setting have been implemented (Fletcher et al., 2017). However, as CCHF cases often enter healthcare facilities with non-specific flu-like symptoms, late recognition and diagnosis of CCHF cases may hinder the effectiveness of these measures in reducing the occurrence of hospital-acquired CCHFV infection (Fletcher et al., 2017).

Another attempt to control CCHFV was the development of an inactivated CCHFV vaccine isolated from the brains of infected rats and mice in the 1970s. The vaccine gained approval from the Soviet Ministry of Health as a preventative measure against CCHF after demonstrating safety and immunogenicity in humans. Although this vaccine elicited robust T-cell responses against CCHFV and high antibody levels upon initial vaccination, four doses were required to induce even low levels of CCHFV-neutralizing antibodies (Mousavi-Jazi, Karlberg, Papa, Christova, & Mirazimi, 2012). To date, the protective efficacy of this vaccine in humans has not been established in controlled clinical trials and the crude nature of vaccine preparation makes it a less likely candidate for widespread use (Buttigieg et al., 2014). In addition, as the correlates of protection against CCHFV remain unclear, the development of an efficacious vaccine to prevent the disease remains an obstacle in the field. Thus, in the absence of adequate preventative options, therapeutic intervention is the primary mode of preventing mortality from CCHF, although their efficacy remains elusive.

3 | PRESENT: CURRENT ANIMAL PLATFORMS AND TREATMENTS FOR CCHF

3.1 | Animal models for studying CCHF

The evaluation of therapeutic candidates for CCHF has been hindered due to the historical lack of an animal model that recapitulates hallmarks of CCHF in humans. Many animal models that have been evaluated thus far develop viremia upon infection, but do not develop any clinical features of disease (Table 1). Other than humans, the only mammals that develop disease upon CCHFV infection are those lacking a fully functional immune system, including neo-natal mice (Smirnova, 1979), signal transducer and activator of transcription 1 (STAT-1) knockout mice (Bente et al., 2010) and interferon α/β receptor (IFNAR−/−) knockout mice (Oestereich et al., 2014). Thus, these are currently the most extensively used animal models for the pre-clinical evaluation of interventions against CCHFV.

TABLE 1 Animal models for Crimean–Congo haemorrhagic fever

Animal	CCHFV strain and inoculation route	Time to death	Signs of disease	Advantages and disadvantages
Adult animals: White mice White rats Cotton rats Young white mice Guinea pigs Rabbits Syrian hamsters Rhesus macaques Sheep Calves Donkeys	Various CCHFV isolates and doses (i.c or i.p.) (Smirnova, 1979)	Non-lethal (Smirnova, 1979)	• Increased viremia • Anti-CCHFV antibodies (Smirnova, 1979)	Disadvantages: • Non-lethal • No clinical signs of disease
Newborn animals: White rats Cotton rats	Various CCHFV isolates and doses (i.c or i.p.)	Varied time to death (Smirnova, 1979)	• Increased viremia (Smirnova, 1979)	Advantages: • Lethal models • Cost-effective Disadvantages: • Immature immune system prevents evaluation of vaccines
Neonatal mice	$10^{3.5}$ PFU CCHFV IbAr 10200 (i.c or i.p.) (Logan et al., 1989)	4–6 days post-infection (i.p.) (Logan et al., 1989)	• Increased viremia • Lesions (Logan et al., 1989)	Advantages: • Lethal model • Cost-effective Disadvantages: • Immature immune system prevents evaluation of vaccines
Adult STAT-1 knockout mice	10^{2} PFU CCHFV IbAr 10200 (i.p.) (Bente et al., 2010)	2–5 days post-infection (Bente et al., 2010)	• Increased viremia • Fever • Leukopenia • Thrombocytopenia • Elevated liver enzymes (Bente et al., 2010)	Advantages: • Increased clinical hallmarks of infection compared to neonatal mice Disadvantages: • Cost • Immunodeficiency may not mirror human immune parameters during infection

(Continues)

TABLE 1 (Continued)

Animal	CCHFV strain and inoculation route	Time to death	Signs of disease	Advantages and disadvantages
IFNAR−/− mice	10^1–10^6 FFU (i.p.) IbAr 2000 (Bereczky et al., 2010)	2–5 days post-infection (Bereczky et al., 2010)	• Increased viremia • Virus detected in spleen, liver, kidney and brain • Enlarged liver (Bereczky et al., 2010)	Advantages: • Increased clinical hallmarks of infection compared to neonatal mice Disadvantages: • Cost • Immunodeficiency may not mirror human immune parameters during infection
Humanized mice (Hu-NSG™-SGM3)	10^4 TCID50 (i.p.) Turkey-200406546 10^4 TCID50 (i.p.) Oman- 199809166 (Spengler et al., 2017)	Turkey: 13–23 days post-infection Oman: Non-lethal (Spengler et al., 2017)	• Increased viremia • Mentation • Ataxia • Dehydration • Dyspnoea • Weight loss • High levels of viral antigen in the liver, spleen and brain (Spengler et al., 2017)	Advantages: • Increased clinical hallmarks of infection, neurological signs • Immune competency may better mirror human immune parameters during infection Disadvantages: • Cost

i.c., intracranial; i.p., intraperitoneal; FFU, focus-forming units; PFU, plaque-forming units; TCID50, median tissue culture infective dose.

3.1.1 | Neo-natal mice

The earliest experimental systems in the 1970s that were able to induce lethality utilized either newborn mice, rats, or young mice infected intracranially or intraperitoneally (Salehi, Salehi, Adibi, & Salehi, 2013). Newborn mice were found to be the most sensitive of this group and were the commonly used animal model for the study of CCHF disease until only recently. The newborn mouse model of CCHFV has been used to study disease pathogenesis, efficacy of treatments and transmission dynamics of the virus via ticks (Logan, Linthicum, Bailey, Watts, & Moulton, 1989; Smirnova, Zubri, Savinov, & Chumakov, 1973; Tignor & Hanham, 1993). The early use of newborn mice for studying CCHFV was necessary due to the lack of disease seen in infection of adult animals of several species, in which viremia could be detected, but show no apparent signs of disease before clearing the virus (Smirnova, 1979). However widely used early on, newborn animal models are typically not representative of disease progression in adult animals or in humans and newborns are often susceptible to a wide range of pathogens that do not cause overt disease in mature animals. Interestingly, several larger animals have been experimentally infected with CCHFV such as sheep, cattle, donkeys and ostriches to study the persistence of the virus in these species, rather than to model human disease (Smirnova, 1979; Swanepoel et al., 1998). Since the advent of genetic engineering techniques, the use of knockout mice has proven to be a more suitable model for the study of CCHFV pathogenesis and pre-clinical testing of CCHFV vaccines and therapeutics.

3.1.2 | STAT-1 and IFNAR−/− knockout mice

STAT-1 and IFNAR−/− knockout mice have both recently been used as lethal models of CCHF disease (Bente et al., 2010; Bereczky et al., 2010; Zivcec et al., 2013). These animal models are defective in the innate immune response, which is believed to be critical in protecting mice from productive infection with CCHFV. STAT-1 deficiency prevents the upregulation of genes due to a signal by either type I or type II interferons. These knockout mice succumb to infection within 3–5 days and suffer from fever, leukopenia, thrombocytopenia and elevated liver enzymes (Bente et al., 2010). STAT-1 knockout mice also have high levels of viremia in the liver and spleen along with elevated pro-inflammatory cytokine production. In contrast, the genetic defect of IFNAR−/− mice specifically affects the interferon type I signalling. IFNAR−/− mice infected with CCHFV develop acute disease and succumb to infection within 5 days (Bereczky et al., 2010; Zivcec et al., 2013). High viral loads are seen in the spleen, liver, kidneys, heart, lungs, lymph nodes and blood of the mice (Bereczky et al., 2010; Zivcec et al., 2013). Additionally, there is significant enlargement of the liver in infected IFNAR−/− mice. An advantage to using these mouse models is that they show characteristics of human disease such as early spikes in viremia in the blood, elevated liver enzymes, histopathological lesions in the liver and lymphocyte depletion in the spleen (Bente

et al., 2010; Bereczky et al., 2010; Swanepoel et al., 1989; Zivcec et al., 2013). Both models are useful in studying the pathogenesis of disease as well as testing treatment options. They can also provide insights into immunological mechanisms of disease and viral clearance. Although using immunocompromised mice for the testing of vaccines is not ideal, these models are also a suitable model to test vaccines, providing an option that was not available with the use of newborn animals.

3.1.3 | Humanized mice

An interesting advance in animal models that could potentially serve as a model for CCHF disease is humanized mice. In contrast to previous mouse models of CCHFV infection, these mice develop functional human immune systems, including production of T and B cells following the engraftment of hematopoietic stem cells, PBMCs or foetal liver and thymus (Brehm, Shultz, & Greiner, 2010). The functional human immune systems present are capable of mounting cell-mediated and humoral immune responses against viral challenge. Different types of humanized mice have been used for the study of various human diseases such as cancer, autoimmunity, graft rejection, as well as infectious diseases like human immunodeficiency virus and dengue fever (Koboziev et al., 2015; Li & Wood, 2014; Mathew & Akkina, 2014; Pearson et al., 2008; Simpson-Abelson et al., 2008). Due to the difficulty in finding animals models that accurately recapitulate human CCHF disease, humanized mice are an interesting option for future research. Their use may provide researchers and clinicians with a suitable option for pre-clinical studies in the light of the lack of CCHF models in higher animals such as NHPs. An immunocompetent animal model that might better reflect the course of human disease could go a long way for the development of vaccines and therapeutic options.

Recently, immunodeficient NOD/SCID mice depleted of mouse hematopoietic stem cells, engrafted with human CD34 + hematopoietic stem cells and engineered to express human stem cell factor (SCF), human granulocyte/macrophage-colony-stimulating factor 2 (GM-CSF) and human interleukin-3 (IL-3) have been developed. These humanized mice, termed Hu-NSG™-SGM3, developed progressive disease with significant weight loss and showed high levels of viral antigen in the liver, spleen and brain when infected with CCHFV Turkey-200406546 isolated from humans. Hu-NSG™-SGM3 infected with this isolate demonstrated neurological disease and liver histopathological features such as vacuolar degeneration and increased single-cell necrosis, comparable to human cases of CCHF. These animals succumbed to CCHFV by 23 days post-challenge, demonstrating promise as a lethal model for CCHFV. Compared to immunodeficient mouse models of CCHF, the time course of infection in Hu-NSG™-SGM3 is more consistent with that observed in human fatalities of CCHFV, with 1–13 days for incubation and another 5–14 days to death. In contrast, Hu-NSG™-SGM3 mice infected with CCHFV Oman-199809166 demonstrated milder disease with minimal weight loss and complete recovery from illness (Spengler et al., 2017).

3.2 | Therapeutics used to treat CCHF patients

To date, pharmaceutical options for the treatment of CCHF remain limited, with supportive care as the primary mode of treatment for patients who experience the disease (Jabbari, Besharat, Abbasi, Moradi, & Kalavi, 2006; Soares-Weiser, Thomas, Thomson, & Garner, 2010). However, some therapeutic candidates such as ribavirin, methylprednisolone and convalescent serum have also been administered to human cases of CCHF both inside and outside of clinical trial settings (Arda, Aciduman, & Johnston, 2012; Sharifi-Mood et al., 2013; Van Eeden et al., 1985). While some of these treatments demonstrate some therapeutic benefit in humans, no statistical significance has been established thus far.

3.2.1 | Current standard of care for CCHF

Supportive care for CCHF patients includes maintenance of fluid and electrolyte balance, ventilation support to maintain adequate levels of oxygen and mild sedation. Depending on the disease severity at the time of clinical presentation, patients may also require hemodynamic support, including transfusions of erythrocytes, platelets and fresh-frozen plasma transfusion. Obtaining a complete blood count and accordingly providing blood replacement therapy is an indispensable component of the current standard of care for severe CCHF cases (Jabbari, Tabasi, Abbasi, & Alijanpour, 2012). Secondary bacteraemia may also arise in CCHF patients and are usually treated with the appropriate antibiotic regimens, such as ceftriaxone and levofloxacin (Sunbul et al., 2015). Co-infections with malaria have also been documented and should be treated with anti-malarials, such chloroquine, doxycycline or primaquine (Christova et al., 2015; Sharifi-Mood, Metanat, Rakhshani, & Shakeri, 2011). However, treatments which may exacerbate bleeding are strongly avoided when disseminated intravascular coagulation is present. Administration of high doses of methylprednisolone demonstrated promise in CCHF patients presenting with virus-associated hemophagocytic syndrome (VAHPS) and has also been used in the current treatment regimen (Dilber et al., 2010). Delayed diagnosis and initiation of supportive care negatively impact the efficacy of treatment and disease prognosis (Jabbari et al., 2012).

3.2.2 | Ribavirin: A highly debated antiviral treatment for CCHF

Ribavirin (1-(β-D-Ribofuranosyl)-1H-1,2,4-triazole-3-carboxamide) is a small-molecule drug that has been implicated for the treatment of infections caused by a variety of DNA and RNA virus infections, such as respiratory syncytial virus and hepatitis C virus (Dusheiko et al., 1996; Hall et al., 1983). It has also demonstrated efficacy in the treatment of haemorrhagic fever caused by Lassa virus infection (McCormick et al., 1986). Synthesized in the 1970s, ribavirin is a purine nucleoside analogue, which can directly inhibit viral transcription by binding viral polymerases or by incorporation into nascent RNA genomes, causing viral RNA mutagenesis.

It can also directly inhibit translation by inhibiting viral capping enzymes (Tam, Lau, & Hong, 2001). Ribavirin can also indirectly mediate antiviral activities via immunomodulatory effects, by inducing antiviral type 1 cytokines (IFN-γ, TNF-α, IL-2) and suppressing pro-viral cytokines (IL-4, IL-10) (Tam et al., 2001). Lastly, it inhibits host inosine monophosphate dehydrogenase, disrupting intracellular GTP concentration needed for viral replication (Tam et al., 2001). CCHFV is sensitive to ribavirin in a strain-dependent manner in vitro (Watts, Ussery, Nash, & Peters, 1989). CCHFV strains IbAr 10200 (Nigeria), SPU 128/81(South Africa) and HY-13(China) were highly sensitive to ribavirin, with 50% effective inhibitory doses (ED50) between 4–6 μg/ml; CCHFV strains UG 3011 (Uganda), AP-92 (Greece) and SPU 41/84 (South Africa) were less sensitive to ribavirin, with ED50s between 9 and 16 μg/ml (Watts et al., 1989).

While the World Health Organization and Centers for Disease Control and Prevention promote the use of ribavirin for the treatment of CCHF, efficacy of ribavirin in human CCHF cases remains controversial. In favour of ribavirin as a CCHF therapeutic, the efficacy of oral ribavirin in suspected and confirmed CCHF patients was 34% and 80% in a study conducted in Iran, respectively (Mardani, Jahromi, Naieni, & Zeinali, 2003). Eight CCHF patients all survived severe disease when administered oral ribavirin within a mean of 5.5 days (Ergönül et al., 2004). A multivariate analysis of confirmed CCHF patients at a Turkish hospital demonstrated that those treated with oral ribavirin and admitted within 2 days of symptom onset were at decreased risk of experiencing severe disease (Ozbey, Kader, Erbay, & Ergönül, 2014). In a similar multivariate analysis of CCHF patients stratified by disease severity at another site in Turkey, ribavirin was found to be effective in reducing the case-fatality rate among moderately ill patients (Dokuzoguz et al., 2013).

In contrast, most current studies support the conclusion that ribavirin is not effective in the treatment of CCHF. A meta-analysis conducted on one randomized controlled trial and seven observational studies demonstrated that ribavirin did not improve survival in CCHF patients and was unable to provide significant clinical benefit (Ascioglu, Leblebicioglu, Vahaboglu, & Chan, 2011). In other studies, oral ribavirin treatment of CCHF patients did not confer significant benefit regarding viral load or disease progression compared to patients who did not receive ribavirin, nor did it improve survival rates (Bodur et al., 2011; Ceylan, Calıca, Ak, Akkoyunlu, & Turhan, 2013; Elaldi et al., 2009). Additionally, patients given ribavirin experienced delayed return to normal leucocyte counts and longer hospitalization times. However, these findings were not statistically significant (Koksal et al., 2010). The discrepancies surrounding the efficacy of ribavirin for the treatment of CCHF may be a result of multiple factors, including the administration of additional supportive therapies that may increase the likelihood of survival and variation in the initiation of treatment relative to symptom onset. Randomized controlled trials with larger sample sizes may be necessary to confirm the benefit of ribavirin for the treatment of CCHF (Akinci, Bodur, Sunbul, & Leblebicioglu, 2016).

3.2.3 | Methylprednisolone

Methylprednisolone is a synthetic corticosteroid that has been licensed for the treatment of inflammatory ailments such as arthritis and bronchitis, as well as autoimmune diseases, such as systemic lupus erythematosus and multiple sclerosis (Sloka & Stefanelli, 2005). Methylprednisolone binds intracellular glucocorticoid receptors, which activates the expression of genes that modulate the immune system and aid in the restoration of blood barrier disruption. The anti-inflammatory effects of methylprednisolone are believed to be of benefit in cases where disease pathogenesis is mediated by deleterious host immune responses (Tam et al., 2012). Methylprednisolone has been evaluated in human trials for viral ailments, such as coronavirus-associated severe acute respiratory syndrome (SARS) and dengue virus. However, these studies failed to establish statistically significant efficacy of corticosteroid treatment in patients (Auyeung et al., 2005; Ho et al., 2003; Tam et al., 2012). To date, the efficacy of methylprednisolone against CCHFV challenge has not been evaluated in animal studies, however, it has been used in conjunction with other supportive care measures in humans.

High-dose methylprednisolone (HDMP) demonstrated efficacy in reducing fever and increasing platelet counts when administered to children infected with CCHFV in the haemorrhagic stage of disease presenting with VAHPS. HDMP appeared to reduce the amount of blood products required to compensate for haematological effects of infection. However, efficacy of HDMP remains inconclusive due to the additional administration of intravenous immunoglobulin (IVIG) and/or granulocyte colony-stimulating factor (G-CSF) prior to treatment (Dilber et al., 2010). Combination therapy comprising HDMP, fresh-frozen plasma (FFP) and IVIG demonstrated efficacy in treating patients presenting with hemophagocytic lymphohistiocytosis (HLH) caused by macrophage activation (Erduran, Bahadir, Palanci, & Gedik, 2013).

3.2.4 | Convalescent blood products

Convalescent blood products harvested from recovered patients aims to provide pathogen-specific antibodies generated during disease progression to newly infected patients and has been successful for the treatment of diseases such as measles, whooping cough, typhoid fever, scarlet fever and mumps (Berger, 2002; Dewar, 1946; McGuinness, Stokes, & Mudd, 1937; Stoll, 1919). Although the efficacy of convalescent blood products against CCHFV challenge has not yet been evaluated in animal studies, the use of convalescent serum harvested from recovered CCHF patients to treat newly infected patients was proposed as early as 1945 (Keshtkar-Jahromi et al., 2011). Intramuscular administration of convalescent serum was first used to treat CCHF patients in 1967, but demonstrated lack of efficacy. Subsequently, in 1970, 61 patients intramuscularly administered 80 mL of convalescent serum either once or twice daily for up to 4 days following symptom onset did not exhibit statistically improved outcomes over non-treated patients (Leshchinskaya &

Martinenko, 1970). A patient administered 300 mL of convalescent serum during a nosocomial outbreak of CCHF in Dubai survived illness and experienced a shorter convalescent period compared to other survivors (Suleiman et al., 1980). In the South African CCHF outbreak of 1985, five CCHF patients treated with 2 transfusions of hyperimmune serum survived infection; four of five demonstrated improvement of symptoms after administration of the first dose compared to two untreated patients who succumbed to infection. Although the results were not statistically significant, this study suggested that continuous transfusions may better impact disease outcome (Van Eeden et al., 1985). Furthermore, these findings would suggest administration of protective antibodies may be a successful direction to explore.

4 | FUTURE: ADVANCES IN ANIMAL MODELS AND THERAPEUTICS FOR CCHFV

4.1 | Prognostic factors in human CCHFV cases

Prognostic factors that predict mortality in CCHFV cases continues to be of crucial importance in the field. In the medical setting, the ability to predict the clinical course of disease allows physicians to make crucial decisions about disease management, such as whether a patient could be managed at a local hospital or if they should be transported to medical centres with greater intensive care capacities (Akinci et al., 2016). Identifying the factors more strongly associated with fatality may allow for the optimization of animal models, which should ideally exhibit similar clinical signs and laboratory findings as humans. The selection of therapeutic agents that specifically address these signs and biomarkers may then show more benefit in preventing mortality in human cases.

4.1.1 | Clinical signs associated with mortality

Many studies have attempted to understand clinical signs associated with mortality in humans by comparing those seen in fatal and non-fatal CCHF cases (Akinci et al., 2016; Ergonul, Celikbas, Baykam, Eren, & Dokuzoguz, 2006; Ozturk et al., 2012). Early in the disease course, CCHF patients present with fever and loss of appetite, however, these are not significantly associated with mortality. Bleeding is a key feature of the subsequent haemorrhagic stage of CCHFV infection (Cevik et al., 2008). As depicted in Table 2., hematemesis (blood in vomit), epistaxis (bloody nose), gingival bleeding and melena (blood in stool), have been shown in multiple studies to be significantly associated with death (Cevik et al., 2008; Ergonul, Celikbas et al., 2006; Ergonul, Tuncbilek et al., 2006; Kaya et al., 2014; Ozturk et al., 2012). Bleeding from the skin was a significant prognostic factor in the study by Ozturk et al. (2012). In addition, an overall consensus between these studies shows that somnolence is another clinical sign significantly associated with mortality. While petechiae, maculopapular rash, hepatomegaly, splenomegaly, eccymosis and jaundice are often seen in CCHF patients, they are currently not significant sign of mortality.

To date, neonatal mice, IFNAR−/− mice, STAT-1 knockout mice and Hu-NSG™-SGM3 mice have been unable to demonstrate any of these clinical signs upon CCHFV infection. However, IFNAR−/− mice, STAT-1 knockout mice and Hu-NSG™-SGM3 mice demonstrate loss of appetite and show CCHFV antigen in the liver and spleen (Bente et al., 2010; Spengler et al., 2017; Zivcec et al., 2013).

4.1.2 | Coagulation parameters

Abnormal blood clotting as a result of thrombocytopenia has been established as a mortality factor in CCHF cases (Akinci et al., 2016). In a study comparing coagulopathy markers of fatal CCHF cases (n = 59) with non-fatal patients (n = 74), significant prognostic factors associated with mortality were identified. Decreased platelet count (p = .001), longer prothrombin time (PTT; p = .0001), longer activated partial thromboplastin time (aPTT; p = .003), higher international normalized ratio (INR; p = .008) and lower fibrinogen level (p = .041) were associated with mortality (Onguru et al., 2010). Independent predictors of mortality were platelet count <20 × 109 cells/L and PTT > 60 s (p < .05) (Onguru et al., 2010).

The IFNAR−/− mouse model has demonstrated statistically significant decreases in platelet counts and fibrinogen levels, as well as prolonged aPTT compared to wild-type mice during CCHFV infection, mimicking human CCHFV infection (Zivcec et al., 2013). Similarly, the STAT-1 knockout mouse model for CCHFV exhibit decreased platelet counts; however, the other coagulation markers have not yet been documented in this model (Bente et al., 2010). These factors have yet to be examined in the Hu-NSG™-SGM3 mouse model (Spengler et al., 2017).

4.1.3 | Other haematological findings

Many studies have demonstrated that abnormal leucocyte counts and elevated liver enzymes are hallmarks of CCHFV infection and can be used to predict fatal outcome in patients (Akinci et al., 2016). In a retrospective study comprising CCHFV patients confirmed by PCR or anti-CCHFV IgM antibodies, the leucocyte, lymphocyte, neutrophil and monocyte levels were compared between fatal (n = 36) and non-fatal (n = 184) cases. Using Receiving Operating Curve analysis, significant risk factors for mortality established in this study were increased neutrophil levels (p = .01) and decreased lymphocyte (p = .037) and monocyte (p = .001) levels. Independent predictors of mortality were leucocytes >2,950 per µl, lactate dehydrogenase >967.5 U/L and alanine aminotransferase >119.5 U/L, which increased mortality risk by 7- to 12-fold (Bastug et al., 2016). This study suggests the importance of the mononuclear immune response for patient survival and these markers may be of interest when evaluating the efficacy of therapeutics in animal models for CCHFV in the future. In addition, decreased CCHFV-specific IgG and IgM have been significantly associated with mortality in human cases, suggesting the importance of the humoral immune response in survival against

TABLE 2 Clinical signs and laboratory findings associated with mortality in human CCHF cases and their presence in animal models for CCHFV infection

	Humans					Animals		
	Ergonul, Celikbas et al. (2006); Ergonul, Tuncbilek et al. (2006)	Cevik et al. (2008)	Ozturk et al. (2012)	Kaya et al. (2014)	Ergönül et al. (2017)	IFNAR−/− mice	STAT-1 KO mice	Humanized mice
Clinical signs								
Hematemesis	Yes (.009)	Yes (.030)	Yes (<.001)	–	–	N	N	N
Epistaxis	ns	ns	Yes (.002)	–	–	N	N	N
Gingival bleeding	–	Yes (.044)	Yes (<.001)	–	–	N	N	N
Melena	Yes (.001)	Yes (<.001)	Yes (<.001)	–	Yes (.001)	N	N	N
Skin bleeding	–	–	Yes (<.001)	–	–	N	N	N
Somnolence	Yes (.022)	Yes (.004)	–	Yes (.003)	–	N	N	N
Anorexia	–	ns	–	–	–	Weight loss	Weight loss	Weight loss
Petechiae	–	ns	–	ns	Yes (.03)	N	N	N
Maculopapular rash	ns	ns	–	ns	–	N	N	N
Ecchymosis	ns	Yes (.007)	ns	ns	Yes (.04)	N	N	N
Hepatomegaly	–	No	–	ns	–	Virus +	Virus +	Virus +
Jaundice	–	–	–	Yes (.01)	–	N	N	N
Splenomegaly	–	ns	–	–	–	Virus +	Virus +	Virus +
Fever > 38°C	ns	ns	ns	–	–	–	–	–
Laboratory findings								
Increased viremia	–	–	–	$p < .0001$	–	Y	Y	Y
Elevated AST	Yes (.004)	Yes (<.001)	Yes $p = .009$	–	Yes (<.05)	Y	–	–
Elevated ALT	Yes (<.001)	Yes (<.001)	No $p = .107$	–	Yes (<.05)	Y	Y	–
Elevated CPK	ns	Yes (.004)	No $p = .061$	–	–	–	–	–
Elevated LDH	ns	Yes (<.001)	Yes (<.006)	–	Yes (<.05)	–	-	–
Decreased fibrinogen	Yes (.012)	ns	Yes (.027)	–	Yes (<.001)	Increased	–	–
PT elongation	Yes (.002)	Yes (<.001)	Yes (.003)	<.001	Yes (<.001)	N	–	–
aPTT elongation	Yes (<.001)	Yes (.002)	Yes (<.001)	.001	Yes (<.001)	Y	–	–
Elevated INR	–	Yes (<.001)	Yes (.005)	<.001	–	–	–	–
Thrombocytopenia	Yes (.038)	Yes (.036)	Yes (<.001)	–	–	Y	Y	Y
Leukopenia	–	ns	–	–	ns	–	Y	N
Leukocytosis	ns	ns	–	–	–	–	N	Y
Elevated C3	–	–	Yes (.048)	–	–	–	–	–

(Continues)

TABLE 2 (Continued)

	Humans					Animals		
	Ergonul, Celikbas et al. (2006); Ergonul, Tuncbilek et al. (2006)	Cevik et al. (2008)	Ozturk et al. (2012)	Kaya et al. (2014)	Ergönül et al. (2017)	IFNAR−/− mice	STAT-1 KO mice	Humanized mice
Elevated C4	–	–	Yes (.025)	–	–	–	–	–
Decreased IgM	–	–	Yes (.003)	–	–	–	Not detected	–
Decreased IgG	–	–	Yes (.040)	–	–	–	–	–
Decreased IgA	–	–	ns	–	–	–	–	–
Increased IL-6	–	–	–	Yes (<.001)	.037	Y	Y	–
Decreased IL-10	–	–	–	ns	ns	Increased	Increased	–
Increased TNF-α	–	–	–	Yes (<.001)	ns	Y	Y	–
Increased IFN-γ	–	–	–	ns	ns	Y	Y	–
Increased IL-8	–	–	–	–	Yes (.037)	–	–	–

Y, sign exhibited in animal model; N, sign not exhibited in animal model; Yes, finding associated with mortality in human CCHF cases; ns, finding not significantly associated with mortality in human CCHF cases; *p*-values indicated in brackets where available. Expressions in IFNAR−/− and STAT-1 KO mice were compared with respective WT mice infected with CCHFV.

CCHFV (Ozturk et al., 2012). It is still unknown whether virus neutralization or other antibody-mediated mechanisms are correlated with protection.

Both the IFNAR−/− and STAT-1 knockout mouse models have demonstrated leukopenia when infected with CCHFV (Bente et al., 2010; Zivcec et al., 2013). These factors have yet to be examined in the Hu-NSG™-SGM3 mouse model (Spengler et al., 2017).

4.1.4 | Cytokines and chemokines

In another comparison between fatal (n = 11) and non-fatal (n = 20) cases of CCHFV, increased IL-6 (326.1 ± 74.9 vs. 70.7 ± 17.4 pg/mL; $p < .001$) and TNF-α (161.3 ± 26.3 vs. 77.3 ± 7.2 pg/mL; $p < .001$) were identified as significant markers of mortality. IL-10 levels were decreased in fatal cases compared to survivors, but not to a statistically significant level. IFN-γ was similar between both groups (Kaya et al., 2014). A more recent student study similarly showed increased IL-6 and TNF-α in fatal cases (n = 5) compared with non-fatal cases (n = 22), with IL-8 being an additional marker for fatality (Ergönül et al., 2017).

IFNAR−/− mice also develop strong pro-inflammatory immune responses following CCHFV infection, exhibiting significant increases in G-CSF, IFN-γ, CXCL10 and CCL2 concentrations. Similar to humans, at time of death, IFNAR−/− mice infected with CCHFV exhibit significant increases in IL-6 and TNF-α, as well as GM-CSF, IL-1α, IL-1β, IL-2, IL-12p70, IL-13, IL-17, CXCL1, CCL3 and CCL5 (Zivcec et al., 2013). STAT-1 knockout mice also exhibit increases in IL-6 and TNF-α; however, in contrast to human clinical findings, these animals demonstrate an increase in IL-10 when lethally infected with CCHFV (Bente et al., 2010). Cytokines and chemokines have yet to be evaluated in the Hu-NSG™-SGM3 mouse model (Spengler et al., 2017).

4.2 | CCHF therapeutics in pre-clinical development

As mentioned previously, the CCHF therapeutics which have been tested in human clinical trials have demonstrated some therapeutic benefit, although not statistically significant. New investigational drugs typically need to fulfil the "two-animal" rule of the US Food and Drug Administration (FDA) in order to be made available for human use. This rule requires that (i) the drug has demonstrated safety and efficacy in two different animal models of infection or one well-characterized animal model that recapitulates the major hallmarks of human disease; and (ii) does not cause adverse events in humans (FDA, 2014).

However, as ribavirin and methylprednisolone are clinically approved drugs that have undergone phase I safety testing in humans and in the absence of any therapeutic with proven benefit for CCHFV, ribavirin was repurposed and evaluated in phase II or III efficacy trials. However, the lack of significant benefit conferred by ribavirin for CCHF patients in these trials may have been a predictable outcome. For example, licensed drugs which were repurposed for the treatment of Ebola virus during the 2014–2016 West African outbreak

TABLE 3 Therapeutic options for the treatment of Crimean–Congo haemorrhagic fever

	Mechanism of action	In vitro data	Animal data	Human data
Standard of care	Compensates for fluid and electrolyte loss, support ventilation and treat secondary infections (Jabbari et al., 2012)	Not available	No animal data available	In a meta-analysis compiling data of patients only provided with supportive care, 215 of 365 (68%) survived CCHF(Soares-Weiser et al., 2010)
Ribavirin	Inhibits viral replication and indirectly modulates host immune response (Tam et al., 2001)	Inhibits CCHFV replication in Vero E6 cell line (Oestereich et al., 2014)	IFNAR−/− mice (100 FFU CCHFV): Protected 1/7 (14%) when administered on 0 dpi (Oestereich et al., 2014) STAT-1 knockout mice (10 PFU CCHFV): Protected 6/6 (100%) when daily regimen was initiated 1 hr post-infection or 1 dpi (Bente et al., 2010). STAT-1 knockout mice (1,000 PFU CCHFV): Protected 60% when given 1 hr post-infection and 0% at 1 dpi (Bente et al., 2010)	Pro-ribavirin: Improvement in survival and disease severity (Dokuzoguz et al., 2013; Mardani et al., 2003; Ozbey et al., 2014). Anti-ribavirin: No statistically significant improvement in outcome for CCHF patients treated with ribavirin (Ascioglu et al., 2011; Bodur et al., 2011; Ceylan et al., 2013; Elaldi et al., 2009; Koksal et al., 2010)
Methylprednisolone	Modulates host immune response (Tam et al., 2012)	Not available	No animal data available	5/5 (100%) CCHF patients survived when administered doses 20–30 mg per kg/day intravenously for 5 days, after administration of IVIG and/or G-CSF (Dilber et al., 2010)
Convalescent blood products	Antibody-mediated effects (neutralization, antibody-dependent cell cytotoxicity)	Not available	No animal data available	1 patient treated with convalescent serum survived disease (Suleiman et al., 1980). 5/9 (56%) patients treated with hyperimmune serum survived disease (Van Eeden et al., 1985)
Monoclonal antibodies	Antibody-mediated effects (neutralization, antibody-dependent cell cytotoxicity)	Anti-Gc mAbs neutralized CCHFV infection of SW-13 cells Anti-Gn mAbs demonstrated less neutralizing activity against CCHFV in SW-13 cells (Bertolotti-Ciarlet et al., 2005)	Neonatal mice: 8A1 (Anti-Gc): 100% protection when administered 1 day before infection, 20% when administered 1 dpi 11E7 (Anti-Gc): ~75% protection when administered 1 day before or after infection 6B12 (Anti-Gn): 100% protection when administered 1 day before infection, ~95% protection when administered 1 dpi 10E11 (Anti-Gn): 95% protection when administered 1 day before infection, 90% protection when administered 1 dpi (Bertolotti-Ciarlet et al., 2005)	Not tested in human CCHF cases
Favipiravir (T-705)	Inhibits viral RNA polymerase (Janeba, 2015; Li, Chan, & Lee, 2015; Salata et al., 2015)	Inhibits CCHFV replication in Vero E6 cell line (Oestereich et al., 2014)	IFNAR−/− mice (100 FFU CCHFV): Protected 5/5 (100%) when treatment initiated up to 2 dpi (Oestereich et al., 2014)	Not tested in human CCHF cases

(Continues)

TABLE 3 (Continued)

	Mechanism of action	In vitro data	Animal data	Human data
Arbidol	Inhibits viral entry (Blaising et al., 2013)	Inhibits CCHFV replication in Vero E6 cell line (Oestereich et al., 2014)	IFNAR−/− mice (1,000 FFU CCHFV): Protected 0/5 (0%) when administered 1 day before infection (Oestereich et al., 2014) IFNAR−/− mice (10 FFU CCHFV): Protected 1/5 (20%) when administered 1 day before infection (Oestereich et al., 2014)	Not tested in human CCHF cases
Chloroquine	Inhibits uncoating and post-translational modifications, modulates host immune response (Savarino et al., 2003)	Inhibits CCHFV replication in Vero and Huh7 cell lines (Ferraris et al., 2015)	No animal data available	Not tested in human CCHF cases
Chlorpromazine	Inhibits formation of clathrin-coated pits, preventing clathrin-mediated endocytosis and viral uncoating (Wang et al., 1993)	Inhibits CCHFV in Vero and Huh7 cell lines (Ferraris et al., 2015)	No animal data available	Not tested in human CCHF cases

demonstrated a lack of effectiveness in patients (Dunning et al., 2016; Gupta-Wright, Lavers, & Irvine, 2015). From these observations, it has been suggested that a lack of prior efficacy testing in current animal models for infection may contribute a drug's lack of benefit in clinical trials (Mendoza, Qiu, & Kobinger, 2016). Thus, future CCHFV drug candidates are more commonly undergoing pre-clinical testing in the available animal models for CCHFV, which may allow for better prediction of effectiveness when tested in human patients (Table 3).

4.2.1 | Ribavirin, revisited

Although ribavirin has been evaluated in human cases of CCHFV, it has not been evaluated in any animal model for CCHF until recently. In a study using the IFNAR−/− mouse model for CCHF, ribavirin did not demonstrate statistically significant efficacy against the Afg09-2990 strain of CCHFV in infected IFNAR−/− mice when administered on 0 dpi (1/7; 14%) (Oestereich et al., 2014). In contrast, ribavirin was able to protect STAT-1 knockout mice from 10 PFU CCHFV challenge when a daily treatment was initiated 1 hr post-infection (6/6; 100%) and 24 hr post-infection (6/6; 100%) (Bente et al., 2010). However, when challenged with 1,000 PFU CCHFV, only 60% of animals survived when treatment was initiated 1 hr post-infection and no animals survived when treatment was initiated 24 hr post-infection (Bente et al., 2010).

4.2.2 | Monoclonal antibody treatment

Monoclonal antibodies (mAbs) targeted to the Gn and/or Gc surface glycoproteins of CCHFV originally developed to identify CCHFV in 1987 are currently being investigated as a treatment for CCHF (Blackburn, Besselaar, Shepherd, & Swanepoel, 1987). In vitro, Gc-specific, but not Gn-specific mAbs were able to neutralize CCHFV infection of SW-13 cells (Bertolotti-Ciarlet et al., 2005). Up to 100% protection was achieved when suckling mice were administered anti-Gc mAbs 24 hr prior to lethal CCHFV challenge; however, only up to 70% protection was achieved when administered 24 hr post-CCHFV infection. In contrast, up to 90% protection was achieved when anti-Gn mAbs were administered 24 hr post-infection, suggesting that other mechanisms, such as antibody-dependent cell cytotoxicity or complement-mediated cell lysis, may play a role in protection against CCHFV (Bertolotti-Ciarlet et al., 2005). It would be of interest to assess the efficacy of these and other antibody-based therapeutics in a model that better recapitulates the human disease such as IFNAR−/− and STAT 1 KO mice.

4.2.3 | Arbidol and Favipiravir (T-705)

Arbidol is a broad-spectrum antiviral, which interrupts clathrin-dependent trafficking, thereby inhibiting viral entry (Blaising et al., 2013). Arbidol has been used to treat influenza virus in humans and elicits inhibition of virus entry and replication for viruses, such as hepatitis C virus in vitro (Pécheur et al., 2016). Favipiravir (T-705; 6-fluoro-3-hydroxy-2-pyrazinecarboxamide) is a broad-spectrum selective inhibitor of viral RNA polymerase, which has demonstrated efficacy against influenza virus infections in humans (Furuta et al., 2013). In a study by Oestereich et al. (2014), both arbidol and T-705 suppressed virus replication by ≥3 log units in vitro. However, arbidol did not provide protection or prolonged the time to death even when administered to IFNAR−/− mice at a dose of 150 mg/(kg day) 1 hr prior to lethal CCHFV challenge. In contrast, T-705 administered at a dose of 300 mg/(kg day) 2 days post-infection survived a lethal CCHFV challenge and did not develop clinical disease or virus replication in blood or organs (Oestereich et al., 2014).

4.2.4 | Chloroquine and chlorpromazine

Chloroquine is a broad-spectrum antiviral, which inhibits pH-dependent steps of viral replication, including uncoating and post-translational modifications, likely by increasing pH of the endosome, lysosome and Golgi vesicle. In addition, chloroquine elicits immunomodulatory effects, such as inhibiting the production and/or release of interleukin-6 and tumour necrosis factor α (Savarino, Boelaert, Cassone, Majori, & Cauda, 2003). Chloroquine has been used to treat malaria and has been shown to inhibit the replication of influenza virus, chikungunya virus and dengue virus in cell culture (Farias, Machado, de Almeida Junior, de Aquino, & da Fonseca, 2014; Khan, Santhosh, Tiwari, Lakshmana Rao, & Parida, 2010; Ooi, Chew, Loh, & Chua, 2006).

Chlorpromazine is a licensed anti-psychotic drug used for the treatment of bipolar disorder and schizophrenia (Gajwani et al., 2006; Leucht, Kitzmantel, Chua, Kane, & Leucht, 2008). Chlorpromazine has also demonstrated antiviral activity against adenovirus infection in Syrian hamsters (Diaconu et al., 2010). It has also inhibited Middle East respiratory syndrome coronavirus (MERS-CoV) and Ebola virus in cell culture (Bhattacharyya et al., 2010; De Wilde et al., 2014). Antiviral activity of chlorpromazine is likely mediated by interference of clathrin-coated pits, which prevents clathrin-mediated endocytosis and viral uncoating (Wang, Rothberg, & Anderson, 1993). Both chloroquine and chlorpromazine were able to inhibit CCHFV infection of Vero and Huh7 cell lines when added up to 6 hr post-infection and up to 24 hr (Ferraris et al., 2015). Next steps, would involve evaluating these drugs in animal models for CCHFV infection.

4.3 | CCHFV vaccines in pre-clinical development

The development of lethal animal models of CCHFV infection has allowed for the evaluation of newly developed vaccine candidates to protect against CCHFV. While some vaccines platforms have demonstrated protective efficacy in small animal models, our understanding of correlates of protection against CCHFV infection in humans remains incomplete (Buttigieg et al., 2014; Dowall, Carroll, & Hewson, 2017). Characterizing the specific immune responses optimal for protection against CCHFV infection in humans will be crucial for future vaccine development.

4.3.1 | Subunit vaccine

A Drosophila insect cell-based expression system was used to develop a subunit vaccine comprising the ectodomains of the structural CCHFV glycoproteins Gn and Gc (Kortekaas et al., 2015). The vaccine induced CCHFV-neutralizing antibodies in STAT-1 knockout mice when administered in a prime-boost regimen. However, the vaccine was unable to offer protection in the STAT-1 knockout mouse model for CCHFV infection when lethally challenged intraperitoneally with the IbAr10200 strain 42 days post-vaccination (Kortekaas et al., 2015). While the results of this study suggest that CCHFV-neutralizing antibodies may not correlate with protection, future studies should evaluate the role of virus neutralization in other animal models for CCHFV infection to determine whether this is a model-specific phenomenon or a feature that mimics the human response to infection.

4.3.2 | Modified Vaccinia virus Ankara-vectored vaccine

A recombinant vaccine using the attenuated poxvirus vector, Modified Vaccinia virus Ankara (MVA), to express the CCHFV glycoproteins was recently developed (Buttigieg et al., 2014). The vaccine, designated MVA-GP, induced CCHFV GP-specific IFN-γ cellular responses and anti-CCHFV GP antibodies in both IFNAR−/− mice and wild-type control mice when administered in a prime-boost regimen. The vaccine provided 100% protection (6/6) in the CCHFV IFNAR−/− mouse model when lethally challenged intradermally with the IbAr10200 strain 28 days post-prime vaccination (Buttigieg et al., 2014). Further studies showed that protection conferred by the MVA-GP vaccine in IFNAR−/− mice required both cellular and humoral responses (Dowall et al., 2016). Future research should evaluate if either or both of these responses are required for protection against CCHFV in human cases.

4.3.3 | DNA vaccine

A recombinant DNA vaccine encoding the open reading frame of the CCHFV M-segment was developed using the pWRG7077 mammalian expression vector (Garrison et al., 2017). The vaccine induced CCHFV-specific antibodies and CCHFV-neutralizing antibodies in IFNAR−/− mice when administered three times at 3 weeks intervals. The vaccine conferred 71% protection (5/7) in the IFNAR−/− mouse model for CCHFV infection when lethally challenged intraperitoneally with the IbAr10200 strain 4 weeks after receiving the final boost vaccination (Garrison et al., 2017). The study found no direct correlation between the anti-CCHFV glycoprotein antibody response and survival, suggesting that antibodies targeted to other CCHFV proteins and/or the cellular response may be involved in vaccine-mediated protection (Garrison et al., 2017). While DNA vaccines are advantageous for their cost-effectiveness, ability to induce robust cytotoxic T-cell responses and enhanced stability at different temperatures, they are limited in terms of immunogenicity, requiring adjuvants and electroporation to induce adequate antibody responses (Clem, 2011; Khan, 2013).

5 | CONCLUSIONS AND FUTURE DIRECTIONS

The potential for widespread geographic distribution and highly lethal outbreaks of disease make CCHF an important emerging infectious disease, and a renewed interest in developing vaccines and therapeutic approaches exists. The biocontainment requirements and the lack of suitable animal models have limited research advances in these areas. Due to the zoonotic nature of the disease and the lack of approved therapies, raising awareness among those at risk is critical for prevention of CCHF. While several therapeutic approaches have been examined,

their efficacy has been variable, and there remains a need for reliable therapeutics. The development and testing of reliable therapeutic options will rely upon advances in an understanding of disease pathogenesis in animal models, which to date have been lacking. Suitable animal models would open doors for potential vaccine and therapeutic research that has not been achieved so far. Neonatal and immunodeficient animals have limitations when assessing the course of disease and in testing potential vaccine platforms, but there is optimism that advances in animal models such as humanized mice will advance our understanding of disease progression, immunological correlates of protection against CCHF, and what approaches might best prevent disease. When evaluating pathogenesis of human viruses in animals, the ideal model should be able to host virus replication, exhibit similar prognostic markers as those seen in human cases and recapitulate hallmarks of human disease.

Advances in transgenic and knockout animal models with these characteristics would optimize pre-clinical development of therapeutic candidates which yield more benefit in human CCHF patients. Immune correlates of protection will also provide valuable information for development of specific therapies such as antibody development or anti-inflammatory mediators such as steroids. While our current understanding of CCHF has been established using limited animal models and clinical data, future directives should be aimed at developing more ideal models of infection for pre-clinical testing of vaccines and therapeutics for CCHF. Future advances in these areas would pave the way for informative research that works towards effective vaccines and medical countermeasures against CCHF.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The authors do not have any relevant affiliations or financial involvement with any organization or entity with a financial interest in the subject matter of the manuscript.

ORCID

C. Ranadheera http://orcid.org/0000-0002-5674-7061

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