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REVIEW

The crucial role of nucleoprotein in designing a protective vaccine and precision diagnosis for Crimean-Congo hemorrhagic fever virus

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Abstract: Crimean-Congo hemorrhagic fever virus (CCHFV) is a highly pathogenic agent that causes severe hemorrhagic disease with high mortality. Outbreak control requires rapid and accurate diagnosis together with effective vaccination strategies. The nucleoprotein (NP) is a central component of viral replication and of innate and adaptive immune responses, making it a promising target for diagnostic and vaccine development. NP is highly conserved across CCHFV isolates, which supports broad molecular and serological detection. Several studies have demonstrated that NP-based assays provide sensitive and specific results, a critical requirement for outbreak management. In vaccine research, NP has been shown to induce strong cellular immune responses that contribute to long-term protection. Multiple platforms, including recombinant proteins, DNA constructs, and viral vectors, have successfully incorporated NP with promising preclinical immunogenicity. Despite this progress, challenges remain in ensuring safety and achieving consistent immune responses across populations. Advances in nanotechnology, structural vaccinology, and bioinformatics may further improve the effectiveness of NP-based products. In conclusion, NP represents a key element for both reliable diagnostics and the induction of protective immunity. Future research should focus on optimizing NP-based platforms for translation into clinical applications and improving global preparedness against CCHFV.

Keywords: Crimean Congo, biological threat, nucleoprotein, vaccine development

INTRODUCTION

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne virus that can cause severe disease in humans and livestock [1]. CCHFV possesses a tri-segmented negative-sense RNA genome and is classified within the Orthonairovirus genus of the Nairoviridae family. Clinical symptoms include fever, diarrhea, fatigue, and drowsiness, with severe cases presenting with kidney lesions, liver failure, and lung damage, and a mortality rate of approximately 30% [1,2]. CCHFV poses a widespread threat to global public health due to its potential prevalence, high mortality, nosocomial infections, and difficulties in treatment and prevention [3]. Currently, there is no licensed vaccine against Crimean-Congo hemorrhagic fever. In 2019, the World Health Organization estimated that 3 billion people worldwide are at risk of CCHFV infection [4]. CCHFV causes acute illness in humans, primarily transmitted through tick bites. In animals, it is asymptomatic but poses a major threat to humans in close contact, especially in farms, clinics, and abattoirs. Previous studies have highlighted the immunogenic potential of the CCHFV nucleoprotein (NP) [3,5,6]. NP is abundantly expressed during infection and plays a critical role in the viral lifecycle by packaging the viral genome and antigenome [7]. The significant immune responses elicited by NP, including neutralizing antibodies and CD4+/CD8+ T cells, indicate its suitability as a vaccine antigen [8, 9]. The current study aims to enhance vaccine design against CCHF by incorporating NP alongside glycoproteins [10]. Utilizing bioinformatics approaches, the identification of conserved, immunogenic epitopes within NP could optimize vaccine formulations and diagnostic assays [11]. This integrated strategy may improve protective efficacy and advance prevention/control efforts for this important pathogen. In summary, NP represents a promising antigen for combating CCHFV through vaccination and diagnosis. The

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findings from ongoing research provide insights to guide the rational development of effective countermeasures against this global health threat.

Geographic Distribution

CCHF is one of the most important emerging tick-borne viral diseases, with sporadic human cases regularly reported across various regions globally. While CCHFV circulates naturally among several animal hosts, humans represent a dead end for the virus. The virus is maintained through a silent transmission cycle involving ticks, vertebrates, and subsequent tick reinfection. People in close contact with infected animals or their products face a heightened risk of acquiring the disease. Delayed diagnosis of CCHF can also lead to outbreaks through secondary nosocomial infections [2,6]. CCHFV has an extensive geographic presence as an emerging/re-emerging pathogen. Reported regions include Africa, the Balkans, the Middle East, and Asia. Within the Middle East, cases have been documented in Iran, Iraq, Saudi Arabia, Oman, and the United Arab Emirates. Eastern European nations with local transmission include Albania, Bulgaria, Greece, Kosovo, Turkey, Georgia, and Russia. Its potential for aerosol spreads, high fatality rates, and lack of approved countermeasures qualify CCHFV as a priority pathogen of biosecurity concern [2,8,12].

1. BACKGROUND, GENOMIC AND PROTEOMIC ANALYSIS OF CCHFV

1.1. History

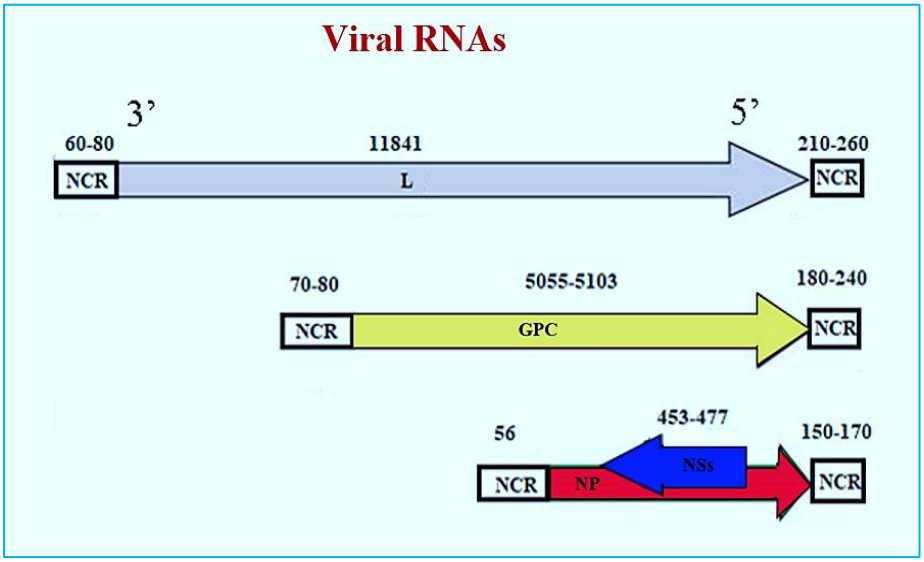
CCHFV was first recognized in 1944 during an outbreak among military personnel in Crimea, Russia, and later isolated in the Congo region in 1956 [13,14]. Subsequent studies confirmed its identity as a highly pathogenic tick-borne virus, leading to the current designation as CCHFV [6,13,15]. Since then, CCHFV has been established as a major public health threat, particularly in Africa, Asia, and Eastern Europe, where its geographic range and incidence are expanding [3]. Mortality is usually 30–50% and can reach 80% in some outbreaks [4]. Still, no approved vaccine or effective antiviral exists [15], and the risk of hospital transmission shows the urgent need for better tools.

1.2. Genome and Proteins

CCHFV possesses a tripartite, linear, single-stranded RNA genome of negative polarity that is encapsidated by viral NP to form distinctive spherical ribonucleoprotein complexes (RNPs) (shown in Figure 1) [3,5]. The small (S) segment is approximately 1,700 nucleotides in length and encodes NP in the viral sense, as well as a non-structural protein (NSs) in the opposite, complementary sense [7]. NP binds to viral RNA and is critical for genome packaging, while NSs act as interferon antagonists to suppress host immune responses [8]. The medium (M) segment measures approximately 5,400 nucleotides [10]. It directs the production of a 160 kDa GPC (glycoprotein precursor) that is cleaved by cellular proteases into the mature glycoproteins glycoprotein N (Gn) and glycoprotein C (Gc), which are found on the virion envelope. The M segment additionally encodes a non-structural protein (NSm) involved in glycoprotein maturation [15]. The largest (L) segment is approximately 12,100 nucleotides in length [1]. It produces an approximately 200 kDa RNA-dependent RNA polymerase with cap-snatching endonuclease and methyltransferase activities essential for transcription and replication of the viral genome [16-18]. Also, the M segment of the virus contains a large GPC, which is processed into seven proteins, including the structural glycoproteins Gn and Gc. These surface glycoproteins are potential vaccine candidates as they have been shown to be targets of neutralizing antibodies. [11,16,19]. GPC also yields a 38 kDa glycoprotein (GP38) and a heavily glycosylated mucin-like domain through additional processing steps [20,21].

CCHFV is an enveloped virus containing a tri-segmented, negative-sense RNA genome [3]. The virion possesses surface glycoproteins Gn and Gc, which facilitate receptor binding and host cell entry. Some evidence suggests an additional protein, GP38, may associate with the viral envelope, though its localization and function remain unclear [7]. The three genomic segments - small (S), medium (M), and large (L) - encode distinct viral proteins. The S segment expresses the NP in one reading frame and an NSs in the opposite frame [8]. Segment M is more complex, directing the production of GPC that host proteases process into glycoproteins Gn and Gc, a mucin-like domain (MLD), GP38, and the NSm involved in glycoprotein maturation [10]. Notably, the L segment of CCHFV is unusually large for bunyaviruses and produces an RNA-dependent RNA polymerase (RdRp) containing an ovarian tumor (OTU) protease domain [1]. The RdRp facilitates transcription and replication of the viral genome [11]. Following receptor-mediated endocytosis, CCHFV undergoes pH-dependent membrane fusion and releases its ribonucleoprotein complexes into the host cytosol [19]. Viral mRNAs initiate translation while the RdRp synthesizes antigens and NP and L proteins package new genomes [3]. Mature virions bud from the Golgi apparatus before exiting via exocytosis [22]. CCHFV proteins also subvert host cell apoptosis and immunity, representing important targets for therapeutic intervention [19].

Figure 1: . Schematic representation of the Crimean–Congo hemorrhagic fever virus (CCHFV) genome.



The CCHFV genome has three segments of negative-sense RNA: small (~1.6 kb), medium (~5.4 kb), and large (~12.1 kb). They code for NP (nucleoprotein), GPC (glycoprotein precursor), and L (large) proteins, respectively, and are flanked by non-coding regions (NCRs). Figure created by the authors for illustrative purposes.

2. INVESTIGATION ON NP AS A KEY GENE IN CCHFV TRI-SEGMENTED GENOME

2.1. NP

NP of CCHFV binds genomic and antigenomic RNAs and interacts with the viral RNA-dependent RNA polymerase (L protein) to form RNPs [4], which are enveloped by a lipid bilayer containing surface glycoproteins [6]. NP can self-assemble into nucleocapsid-like structures and plays essential roles in viral packaging, transcription, and replication, as well as in immune evasion through modulation of apoptosis and suppression of interferon induction [4,6]. Due to its high abundance during infection and strong sequence conservation, NP is highly immunogenic and represents a key viral structural protein for studying CCHFV biology [4,7].

2.2. Conservation of the CCHFV tri-segmented genome

CCHFV shows extensive genetic diversity, categorized into seven clades based on the genetic relatedness of the S segment. These clades are geographically clustered, with three in Africa, two in Asia, and two in Europe. The nucleotide variation between strains can be as high as 20% for the S segment, 31% for the M segment, and 23% for the L segment [8,9]. Genetic analysis of partial M sequences reveals a close relationship between Iranian isolates at less than 1.4% nucleotide diversity [8]. However, the M segment and GPC demonstrate up to 19.3% nucleotide and 14.8% amino acid differences between virus genotypes. Distinct geographic genotypes correlate with observations of S segment comparisons, though greater diversity exists in the M segment and proteins. This likely reflects different tick vectors and vertebrate hosts across virus life cycles [8]. Previous work mapped the main antigenic region of NP between amino acids 201–306, which reacted strongly with convalescent human sera. Comparisons among CCHFV isolates in this region reveal a high level of conservation, with homology ranging from 92–100% [11].

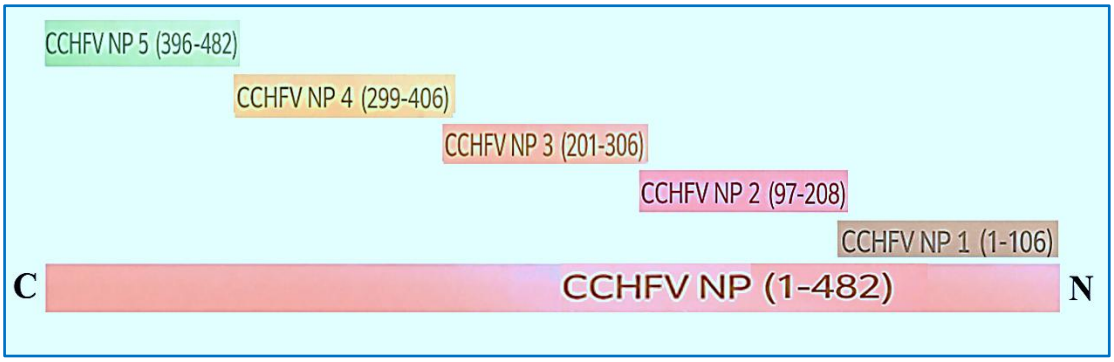
2.2.1. NP-Based Serological Diagnostics

NP of CCHFV is a highly conserved and immunogenic antigen, making it ideal for serological diagnostic assays. One study developed an optimized ELISA for detecting IgG antibodies against CCHFV NP. Most conventional serological methods require manipulation of live virus under BSL-4 conditions, but recombinant NP (rNP) expressed in a baculovirus system enables safe detection of NP-specific antibodies [11]. In this study, rNP derived from the Chinese CCHFV strain 8402 (91.9–92.5% amino acid homology with strain AP929) demonstrated high sensitivity and specificity when compared with the indirect immunofluorescence assay (IFA). The His-tagged rNP allows production without BSL-4 facilities, enhancing accessibility and safety [11]. Sequence analysis identified the NP 201–306 amino acid region as highly conserved and strongly antigenic across global CCHFV strains, supporting its utility in detecting antibodies from diverse lineages. Overall, the validated rNP-based IgG ELISA provides a sensitive, specific, and practical tool for CCHFV serodiagnosis and epidemiological surveillance, addressing key limitations of previous diagnostic methods [10,11].

3. NP CONSERVATION AND SEROLOGICAL APPROACHES FOR CCHFV DETECTION

NP of CCHFV is highly conserved and immunogenic, making it an ideal target for serological diagnostic assays. Conserved hyper-antigenic regions within NP allow precise identification of linear and conformational epitopes, facilitating the development of epitope-based diagnostics. NP-based assays include ELISA, IFA, PRNT, HI, and Western blot, while PCR remains essential for viral detection and strain differentiation [9]. Accurate diagnosis is challenging due to non-specific symptoms, assay limitations, and biosafety requirements. In Kenya, NP-based ELISA demonstrated high seropositivity in livestock and lower rates in rodents and febrile patients, with isolates from the Africa 3 lineage indicating multi-species circulation and the need for ongoing surveillance [5,9,23]. Recombinant NP (rNP) spanning amino acids 201–306, derived from Chinese strains, has been shown to detect antibodies against diverse global CCHFV strains, highlighting its cross-reactivity and broad applicability in serodiagnostic platforms, as illustrated in Figure 2 [10,11].

Figure 2: Schematic overview of truncated nucleoprotein (NP) fragments used for epitope mapping.



The amino acid positions and designations of the truncated NP fragments, which were expressed as fusion proteins with GST on the N-terminal side, are indicated. Figure adapted from Wei et al., 2010 and Saijo et al., 2002 [10,11].

4. DRUG OPTIONS FOR TREATING CCHF

Severe CCHF is a hemorrhagic fever characterized by uncontrolled inflammation and cytokine storms, resulting in significant immunopathology. Ribavirin remains the only approved treatment, underscoring the urgent need for effective vaccines and novel therapies [13]. Limited clinical attempts have explored anti-inflammatory interventions to mitigate the hyperinflammatory response. In one comparative study, high-dose methylprednisolone combined with ribavirin improved outcomes compared with ribavirin alone, with corticosteroids appearing particularly beneficial in severely ill patients, although cohort sizes were small. Experimental data from type I interferon–blocked mice indicate that genetic deletion of the TNF receptor or antibody-mediated blockade of TNF signaling can protect against lethal disease, suggesting that clinically approved anti-TNF agents and other cytokine-targeted drugs warrant further evaluation, as shown in Table 1. Antibody-based therapies have also shown promise: plasma or antibodies from CCHF survivors, as well as mouse and human monoclonal antibodies, can benefit severely ill patients. However, some antibodies effective in neonatal models failed in lethally infected adults, and protection does not necessarily require neutralizing activity. Notably, the protective effect of a GP38-targeting monoclonal antibody was dependent on complement activity [1].

Table 1: The Drugs approved for CCHF treatment.

Therapeutic agent	Pharmacological class	Molecular target	Evidence from preclinical studies	Clinical-level evidence	Key remarks
Ribavirin	Nucleoside analogue antiviral	RNA-dependent RNA polymerase (RdRP)	Variable and controversial efficacy reported in rodent models	Clinical outcomes remain inconsistent across patient populations	Overall, limited benefit; early initiation may be critical; potential use only within combination therapies
Favipiravir	Nucleoside analogue antiviral	RNA-dependent RNA polymerase (RdRP)	Demonstrated antiviral activity in rodent and non-human primate models	Clinical benefit has not been conclusively established	Delayed administration is effective in animal studies; robust clinical trials are required

2′-Deoxy-2′-fluorocytidine	Nucleoside analogue antiviral	RNA-dependent RNA polymerase (RdRP)	Preclinical assessment has not yet been performed	No clinical evidence is currently available	Additional preclinical investigations are necessary
Molnupiravir	Nucleoside analogue antiviral	RNA-dependent RNA polymerase (RdRP)	No significant antiviral activity observed in rodent models	Clinical efficacy data are lacking	Further clinical development appears unlikely
Convalescent plasma or survivor-derived antibodies	Neutralizing and non-neutralizing antibody-based therapy	Viral structural proteins	Preclinical efficacy has not been systematically evaluated	Limited and inconclusive clinical findings were reported	Further preclinical and clinical studies are needed to clarify the therapeutic potential
Monoclonal antibodies	Neutralizing and non-neutralizing antibody-based therapy	Viral structural proteins	Limited supportive evidence from rodent studies	No validated clinical data available	Comprehensive preclinical and clinical validation is required
Corticosteroids	Anti-inflammatory agents	Host immune response modulation	Preclinical investigations have not been reported	Clinical benefit remains uncertain	Current evidence does not support routine use; controlled studies are warranted

5. CCHF VACCINATION

CCHF is a severe, often fatal disease endemic in parts of Africa, Asia, the Middle East, and Southern Europe. Its increasing incidence has led the World Health Organization (WHO) to designate it as a priority for the development of countermeasures [19,24]. While viral envelope glycoproteins are logical vaccine targets due to their role in host cell entry, their antigenic variability facilitates immune evasion. In contrast, the NP is highly conserved and abundantly expressed, making it an attractive vaccine antigen [19]. Multiple vaccine platforms, including subunit, viral vector, DNA, and mRNA vaccines, have been evaluated in preclinical studies, showing variable protective efficacy [1,16,17]. RNA-based vaccines expressing NP alone have achieved complete protection against clinical disease, while combining NP with the glycoprotein precursor further enhanced protection against both disease and viral replication, with even a single 100 ng RNA dose proving protection [25]. Similarly, a DNA vaccine encoding NP and glycoproteins from the CCHFV Hoti strain, administered intramuscularly with electroporation, was well tolerated in cynomolgus macaques and induced robust antibody and T-cell responses. These immune responses correlated with the absence of viremia, reduced tissue viral loads, and improved hematological parameters post challenge [25,26].

5.1 Inactivated Vaccine Platform

The only experimental inactivated CCHFV vaccine to date is the Bulgarian vaccine, developed in the 1970s by propagating a virulent strain in suckling mouse brains, followed by chloroform inactivation and heat treatment at 58 °C to denature viral proteins while preserving conformational epitopes [18,23]. This vaccine has been administered on a limited basis in Bulgaria but remains unapproved by major regulatory authorities. Wider development has been constrained by sporadic case occurrence outside endemic areas and the lack of small animal models that fully mimic human disease [22,27,28]. In a key study, healthy individuals immunized with a four-dose schedule displayed markedly higher frequencies of interferon-gamma (IFN-γ) secreting $CD4^+$ and $CD8^+$ T cells, as well as increased IgG titers, compared with those receiving a single dose [23]. Neutralizing antibody titers in the four-dose group were two- to four-fold higher than in the single-dose group, suggesting that booster doses are important for optimal protection. While the first dose induced detectable antibodies, subsequent doses significantly enhanced both humoral and cellular immunity. These findings indicate that the inactivated vaccine platform can elicit protective immune responses when optimally administered, and further refinement of production methods and immunization regimens could improve safety, availability, and immunogenicity for the Bulgarian vaccine or novel formulations [23,29].

5.2. Adenoviral Vector Vaccine Platform

Adenoviruses are well-characterized viral vectors with high carrying capacity and broad cell tropism, enabling efficient antigen delivery for vaccine development [23]. For CCHFV, adenoviral vectors expressing the N gene induced strong IgG and T-cell responses in mice, providing partial protection against lethal challenge [30]. Despite challenges such as pre-existing immunity and limited transgene expression, continued optimization could enhance their potential for CCHFV vaccine design [23]. In this platform, NP served as the

primary antigen, demonstrating its capacity to induce both humoral and cellular immune responses when delivered via adenoviral vectors.

5.3 DNA Vaccine Platform

DNA vaccines are attractive for CCHFV due to their ability to induce protective immunity via in vivo antigen expression, straightforward design, and lack of interference from pre-existing vector immunity [23]. Although no recombinant DNA vaccines have been approved for bunyaviruses, two leading CCHFV candidates have shown strong immunogenicity in animal models [31]. One encodes ubiquitin-fused Gn, Gc, and NP, while another uses transcriptionally competent virus-like particles (tc-VLPs). In mice, DNA vaccination incorporating NP elicited a balanced Th1/Th2 response and conferred protection against lethal challenge despite modest neutralizing antibody titers. Boosting with tc-VLPs shifted immunity toward a Th2 profile post-challenge. These results highlight NP's role in driving cellular immunity, particularly a Th1 response, which may be critical for durable protection. Continued optimization of antigen selection, delivery systems, and immunization regimens could further improve the efficacy of DNA vaccines for CCHFV. This evidence supports including NP as a core antigen in DNA-based CCHFV vaccine strategies to achieve balanced and long-lasting immunity.

5.4. Subunit Vaccine Platform

The CCHFV envelope polyprotein (EPP) is cleaved into five components: mucin-like variable region, Gp38, Gn, NSm, and Gc, all of which can serve as targets for subunit vaccine design. In one in silico study, protein sequences of EPP and RdRP from the Nigeria/IbAr10200/1970 strain were analyzed for T- and B-cell epitope prediction, MHC binding affinity, and multi-epitope vaccine construct design [32,33]. Selected epitopes were linked with appropriate linkers, fused to a β-defensin adjuvant, and optimized for antigenicity, allergenicity, solubility, and physicochemical stability. Table 2 lists eight predicted cytotoxic T-lymphocyte (CTL) epitopes, and Table 3 summarizes four helper T-lymphocyte (HTL) epitopes with their cytokine-inducing profiles (IL-4, IL-10). Molecular docking and dynamics simulations confirmed strong and stable binding with human immune receptors (TLR2, TLR3, TLR4), and codon optimization suggested high expression potential in Escherichia coli K12. The final 427–amino acid construct incorporated CTL and HTL epitopes along with ten B-cell epitopes, designed to elicit balanced immune responses, including IL-4–mediated B-cell activation and IL-10–driven regulation of inflammation. Although this construct targeted glycoprotein-derived epitopes rather than NP, incorporating conserved NP epitopes into future subunit designs could broaden immune coverage and durability, since NP has demonstrated strong T-cell immunogenicity in other vaccine platforms [23,32,33].

Table 2: Selected CTL epitopes for multi-epitope-based vaccine construction and their antigenicity, immunogenicity, toxicity, allergenicity, conservancy, cross-reactivity with the human proteome, and interacting MHC Class-I allele prediction.

Source protein	CTL epitope sequence	Residue range	HLA supertype	Composite prediction score	Predicted antigenicity score	Predicted immunogenicity score	Toxicity prediction	Allergenicity assessment	Epitope conservation (%)	Associated MHC class I alleles	Cross-reactivity evaluation
EPP	KKIEISGLK	1438–1446	B27	0.9605	0.9491	0.13994	Predicted to be non-toxic	Not applicable	100.00	HLA-A*30:01 HLA-A*03:01 HLA-A*11:01	No cross-reactivity detected
EPP	HREVEINVL	411–419	B39	2.5097	0.7096	0.31548	Predicted to be non-toxic	Not applicable	100.00	HLA-B*08:01 HLA-B*40:01	No cross-reactivity detected
EPP	TEAIVCVEL	1231–1239	B44	1.4217	1.1768	0.22941	Predicted to be non-toxic	Not applicable	100.00	HLA-B*40:01 HLA-B*44:03 HLA-B*44:02	No cross-reactivity detected
RDRP	YLYIVITLY	1411–1419	A1	1.7622	0.9588	0.32448	Predicted to be non-toxic	Not applicable	100.00	HLA-B*15:01 HLA-A*30:02 HLA-A*03:01 HLA-A*26:01 HLA-A*01:01 HLA-B*35:01 HLA-A*32:01 HLA-B*57:01 HLA-A*68:01 HLA-B*58:01 HLA-A*11:01 HLA-B*53:01 HLA-A*33:01 HLA-A*02:01 HLA-A*23:01 HLA-B*44:02 HLA-A*24:02 HLA-B*44:03	No cross-reactivity detected
RDRP	VTDIVVGAI	902–910	A1	1.4576	1.0628	0.27164	Predicted to be non-toxic	Not applicable	100.00	HLA-A*01:01 HLA-B*51:01 HLA-A*68:02 HLA-A*02:06 HLA-A*32:01	No cross-reactivity detected
RDRP	LMSFLNWRV	3263–3271	A2	1.3555	1.1247	0.26439	Predicted to be non-toxic	Not applicable	100.00	HLA-A*02:01 HLA-A*02:03 HLA-A*02:06 HLA-A*68:02 HLA-A*32:01	No cross-reactivity detected
RDRP	YHSIAELTM	42–50	B39	2.0951	0.6095	0.22589	Predicted to be non-toxic	Not applicable	100.00	HLA-B*35:01 HLA-B*08:01 HLA-B*51:01 HLA-A*24:02 HLA-B*53:01	No cross-reactivity detected

										HLA-A*23:01 HLA-B*40:01	
RDRP	IQNRITGLY	2775–2783	B62	1.4651	0.6585	0.23824	Predicted to be non-toxic	Not applicable	100.00	HLA-B*15:01 HLA-A*30:02 HLA-A*01:01 HLA-A*32:01 HLA-A*03:01 HLA-B*57:01 HLA-A*26:01 HLA-B*58:01 HLA-A*30:01 HLA-A*11:01 HLA-B*35:01 HLA-B*44:03 HLA-B*44:02 HLA-B*53:01	No cross-reactivity detected

Table 3: Predicted HTL epitopes and their antigenicity, toxicity, cytokine-inducing properties, conservancy, cross-reactivity, and interacting MHC class-II alleles.

Protein	Epitopes	Position	Antigenicity	Toxicity	IC50 (nM)	IL4 pred	IL10 pred	IFN-epitope	Conservancy (%)	Interacting MHC class II alleles	Cross-reactivity
RDRP	EVLINRNSLKARSE	1240–1254	1.1352	Non-toxic	13.3	Inducer	Inducer	Positive	100.00	HLA-DRB1*08:02 HLA-DQA1*02:01/DQB1*03:01 HLA-DRB5*01:01 HLA-DRB1*13:02 HLA-DRB1*15:01	Zero
RDRP	VHEKRNOPPSVENVO	700–714	1.2275	Non-toxic	5	Inducer	Inducer	Positive	80.00	HLA-DQB1*02:02 HLA-DRB1*02:02 HLA-DRB3*02:02 HLA-DRB1*09:01	Zero
EPP	KAFSAMPKTSLCFYI	1508–1522	0.5604	Non-toxic	10	Inducer	Inducer	Positive	100.00	HLA-DRB1*09:01 HLA-DRB1*11:01	Zero
EPP	ILFFMFGWRILFCFK	1610–1624	1.1537	Non-toxic	6.3	Inducer	Inducer	Positive	86.67	HLA-DQA1*01:01/DQB1*05:01 HLA-DRB3*01:01 HLA-DPA1*01:03/DPB1*02:01 HLA-DPA1*03:01/DPB1*04:02	Zero

5.5. Virus-Like Particle Vaccine Platform

NP can self-assemble into VLPs when overexpressed in insect cells, although the assembly mechanisms remain incompletely understood [4]. VLPs mimic the native virion structure while lacking infectious material, combining high immunogenicity with an excellent safety profile [2,23]. For some viruses, nucleocapsids or glycoproteins autonomously form VLPs during replication, and VLP-based vaccines have demonstrated protective efficacy for other pathogens. The baculovirus expression system supports high-level protein production, facilitating CCHFV VLP formation. The conserved 235–305 amino acid region of NP contains strong antigenic domains, making it a suitable immunogen for VLP design [23]. Preclinical candidates include DNA constructs and modified vaccinia Ankara (MVA) vectors encoding the full GPC or individual NP subunits, which conferred full or partial protection in murine models [19]. MVA, a replication-deficient vaccinia strain, supports high-level transgene expression and has shown promise for CCHFV vaccines, though immune responses require optimization [23–25]. Inactivated virus preparations and combined DNA/VLP regimens achieved partial protection, while subunit or MVA-N vaccines failed to protect [19]. Glycoprotein-focused strategies induced antibodies without protection, underscoring the need to incorporate conserved antigens such as NP [19,27]. Table 4 summarizes all vaccine platforms evaluated for CCHFV to date, and Table 5 provides a comparative overview of these platforms along with the specific role of NP in CCHFV vaccine development. Overall, VLP approaches merit continued development through optimized antigen selection, production processes, and identification of correlates of protection [22,23,29].

5.6 NP in Vaccine Design

NP of CCHFV is highly conserved and abundantly expressed, making it a prime candidate for inclusion in vaccine platforms. It has been evaluated in multiple formats, including recombinant NP subunits, DNA vaccines encoding NP, viral-vectored vaccines (e.g., adenovirus, MVA) expressing NP, and VLPs generated by NP overexpression in baculovirus–insect-cell systems [4,6,7,19,23,25,26]. Preclinical studies demonstrate that NP can induce strong $CD4^+$ and $CD8^+$ T cell responses and contribute to protection. However, NP alone may not completely block viral replication; combining NP with glycoprotein antigens frequently improves protection against both disease symptoms and viremia [19,23,25,26].

In VLP-based approaches, the conserved NP region (aa 235–305) functions as an immunodominant core that supports particle assembly and enhances antigenicity [4,23]. Thanks to its strong immunogenicity and cross-strain conservation, NP is considered a key component in multi-antigen vaccine designs aimed at eliciting balanced humoral and cellular immune responses. As summarized in Table 5, NP's specific role across different vaccine platforms underscores its significance in CCHFV vaccine development.

Table 4: CCHF vaccination platforms and their functions.

Type of vaccine	Antigen	Function
DNA vaccine	M segment	Equally Th1 and Th2 response
mRNA vaccine	S segment	Induce both humoral and cellular immune responses
Exosome-based vaccine	Whole virus	T-cell activities
Inactive vaccine	Whole virus	T-cell activities
Plant-expressed vaccine	GN-GC	Antibody response
Subunit vaccine	GN-GC	Neutralizing antibody response
VSV-based vaccine	GN-GC	Induce both humoral and cellular immune responses
MVA vaccine	M, S segment	Induce both humoral and cellular immune responses
VLP vaccine	NP	Induce both humoral and cellular immune responses
Adenoviral vector vaccine	NP	(a) Upregulated IgG response (b) Protection via CD4+ and CD8+ T cells and non-neutralizing antibody responses

GN-GC, glycoprotein N-glycoprotein C; MVA, modified vaccinia virus Ankara; NP, nucleoprotein; VLP, virus-like particle; VSV, vesicular stomatitis virus. Information summarized and adapted from the published literature cited in the text.

Table 5: Comparative Summary of Vaccine Platforms and the Role of NP in CCHFV Development

Vaccine Platform	Description	Role of NP	Advantages	Limitations/Challenges
DNA Vaccine	Plasmid-based delivery of viral antigen genes for in vivo expression.	The NP gene is expressed to induce cellular immunity; it is often combined with glycoproteins for broad immunity.	Induces balanced Th1/Th2 response; easy to produce; no pre-existing vector immunity interference.	Moderate neutralizing antibody response alone; optimization needed for durability and potency.
Subunit Vaccine	Purified recombinant viral proteins (e.g., glycoproteins Gn/Gc) are used as vaccine antigen.	NP may be included as a conserved antigen to enhance T cell responses, though most subunits focus on glycoproteins.	Safe, well-characterized, potentially strong antibody responses.	Subunit of Gn/Gc alone is sometimes insufficient; NP addition is needed to improve protection.
Virus-Like Particle (VLP) Vaccine	Non-infectious particles mimicking virion structure, assembled from expressed viral proteins including NP.	NP self-assembles into VLP cores, enhancing immunogenicity by mimicking natural virus morphology and promoting strong cellular immunity.	High immunogenicity without live virus; mimics authentic virion structure.	Complex manufacturing; need to confirm proper assembly and stability.
Adenoviral Vector Vaccine	Recombinant adenovirus delivering NP and/or glycoprotein antigens in vivo to stimulate an immune response.	NP expressed to elicit CD4+, CD8+ T cell and antibody responses; partial protection was reported.	Robust induction of cellular immunity; well-studied vector.	Pre-existing anti-vector immunity may reduce efficacy; partial protection from NP alone.
Inactivated Vaccine	Chemically or physically inactivated whole virus vaccines.	NP is present in the whole virion; it contributes to cellular immunity with other viral components.	Comprehensive antigen presentation.	Safety concerns, complex production, and lack of widespread approval.

mRNA Vaccine	Synthetic mRNA encoding NP and other antigens delivered for in vivo expression.	NP included to enhance cellular immunity alongside glycoproteins (not detailed extensively here but relevant given platform trend).	Rapid design, scalable manufacture, potent immune responses.	Stability and delivery challenges; emerging technology for CCHFV.

NP, nucleoprotein; VLP, virus-like particle. Information summarized and adapted from the published literature cited in the text.

6. IMMUNITY

6.1. NP-Mediated Immune Mechanisms

The cellular immune response to CCHFV is driven by the activation of both natural killer (NK) cells and cytotoxic T lymphocytes (Tc cells), with NK cells often emphasized due to their prominent role in early infection. Antibodies contribute by recognizing antigens on infected cell surfaces, which may occur through two pathways: (i) intracellular processing of viral proteins, including NP, and presentation via MHC class I; or (ii) antigen presentation during viral budding. NP elicits both humoral and cellular immune responses through multiple mechanisms, including NK cell activation via antibody-dependent cellular cytotoxicity (ADCC), Tc cell stimulation, and balanced Th1/Th2 polarization. When used as a vaccine antigen, NP can also be presented via MHC class II on antigen-presenting cells (APCs), promoting cytokine production (e.g., IL-2, IL-4, IL-6, IL-10, IL-12, TNF-α, IFN-γ, IL-17), memory Tc formation, and cross-presentation for enhanced cytotoxic responses. High NP expression and accessibility on the cell surface reinforce its value as a target for immune monitoring and vaccine development [7,34].

6.2. Antibody-Mediated Effector Mechanisms (ADCC, ADCP, CDC)

Antibody-mediated mechanisms bridge innate and adaptive immunity in CCHFV infection. ADCC involves NK cell–mediated killing of infected cells via Fc gamma receptor (FcγR) engagement with antigen-specific antibodies, functioning similarly to Tc cells. Antibody-dependent cellular phagocytosis (ADCP) is mediated by macrophages and dendritic cells through Fc receptor–dependent uptake of antibody-coated targets, enhancing antigen presentation and activating both cellular and humoral responses. Complement-dependent cytotoxicity (CDC) relies on the complement cascade, initially triggered by natural antibodies, to eliminate infected cells prior to full activation of adaptive immunity [7,34].

6.3. T Cell Epitope Mapping in CCHFV Survivors

A study of eleven male survivors of CCHFV infection in South Africa examined peripheral blood mononuclear cells (PBMCs) collected 10 months to 13 years post-infection. Cells were stimulated with 36 peptide pools covering NP, glycoprotein N (GN), and GC. Enzyme-linked immunospot (ELISpot) assays detected IFN-γ responses to 16 peptides, mapping to 10 distinct epitopic regions, most within NP.

Two peptides, N262–280 and N298–316, elicited strong responses in multiple survivors, predominantly mediated by $CD8^+$ T cells, as confirmed by CD8 depletion assays. No single immunodominant epitope was identified, and response breadth/magnitude did not clearly correlate with time since infection; however, participants tested more than a decade post-infection showed weaker responses. These epitopic regions are illustrated in Figure 3 and summarized in Table 6 [27,20].

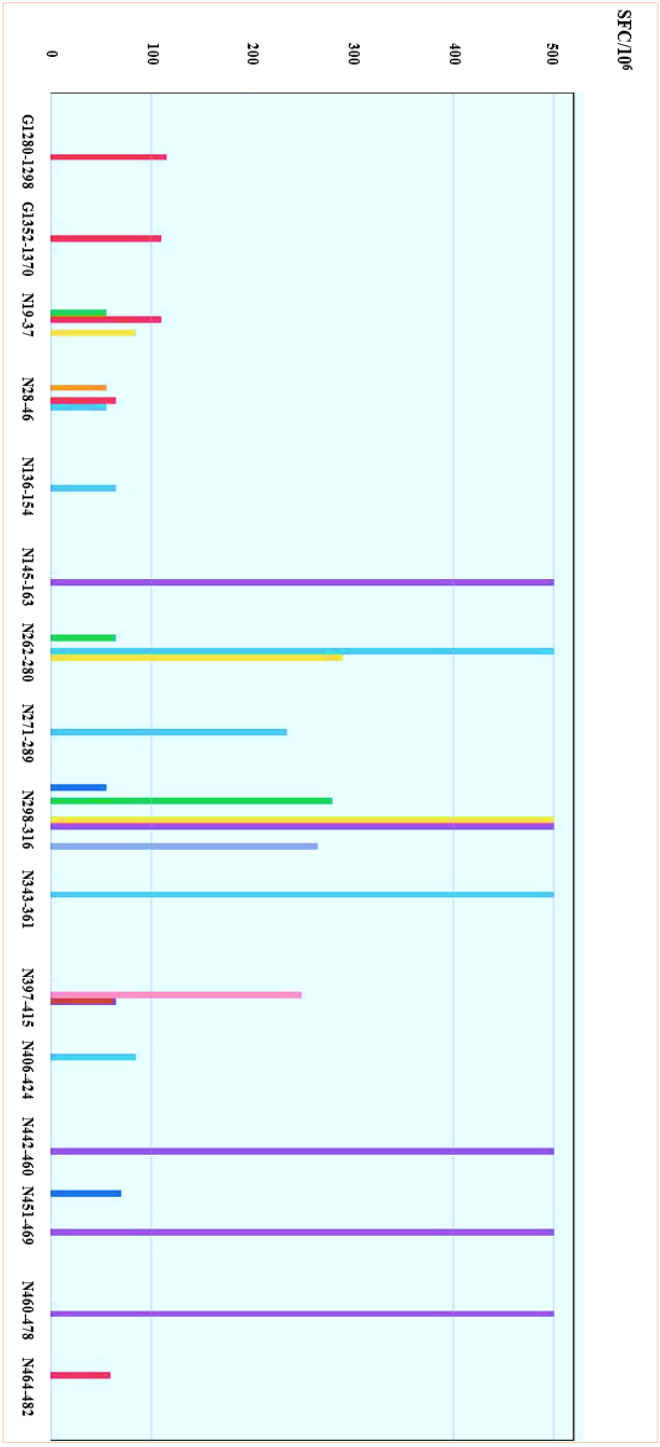
In summary, ten distinct T cell epitopes of CCHFV were identified in survivors, predominantly in the NP, with two in GC. Peptides N262-280 and N298-316 elicited the strongest $CD8^+$ T cell responses, as confirmed by depletion assays, which are shown in Figure 4 [27].

Blocking IFN-γ signaling in infected mice significantly increased mortality, underscoring its critical role in viral control [34]. The predominance of NP epitopes, similar to findings in Hantaan virus, may relate to NP abundance during replication and supports its potential as a multi-epitope vaccine target. In contrast, limited GN/GC responses and the failure of previous subunit vaccines suggest glycoproteins alone may be insufficient for T cell–mediated protection. The study identified long-lived T cell responses to CCHFV, focusing on epitope mapping rather than precise quantification. Although 19mer peptides may underestimate responses compared to optimal 9mers, this did not affect the objectives. $CD8^+$ T cells were confirmed as the main responders in depletion assays, though broader phenotypic confirmation is needed. Most epitopes were located in NP, with only two in GC and none in GN. Memory T cell responses persisted up to 13 years post-infection, similar to findings in Puumala virus, suggesting potential for durable vaccine-induced protection. The exclusively male cohort reflected past South African epidemiology, with high-risk groups including farmers, veterinarians, and individuals exposed to ticks or infected animal materials. The NP epitopes described here are the first identified after natural CCHFV infection and provide valuable targets for vaccine design and immunogenicity assessment [27].

Table 6: Epitopic regions

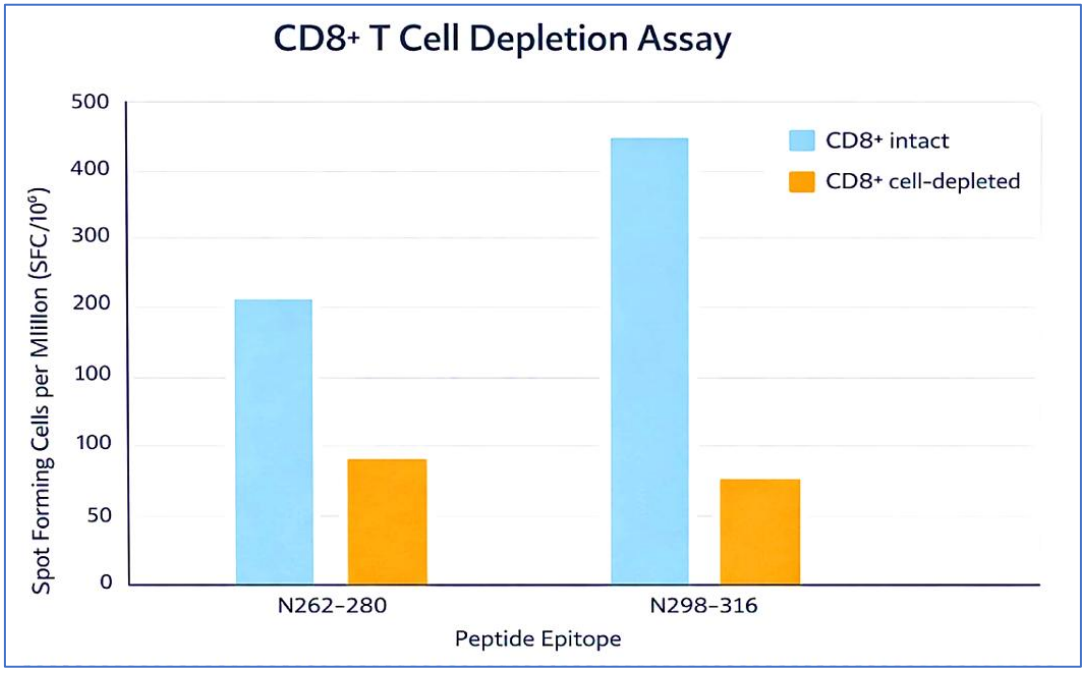
Peptide	Number of positive responses	Amino acid sequence	Range of magnitude of responses (SFC/10^6)
N19–37	3	EFKKGNGLVDTFTNSYSFC	55–110
N28–46	3	DTFTNSYSFCESVPNLDRF	55–65
N136–154	1	DIGFRVNANTAALSNKVLA	65
N145–163	1	TAALSNKVLAEYKVPGEIV	>500
N262–280	3	DKHKDEVDARASADSMITNL	65–>500
N271–289	1	ASADSMITNLLHKIAKAQE	235
N298–316	5	RAQGAQIDTAFSSYYWLYK	55–>500
N343–361	1	KMKKALLSTPMKWGKKLYE	>500
N397–415	2	VANPDDAAQGSGHTKSILN	65–250
N408–424	1	GSGHTKSILNLRTNTETNN	85
N442–460	1	NIQDMDIVASEHLLHQSLV	>500
N451–469	2	SEHLLHQSLVGKQSPFQNA	70–>500
N460–478	1	VGKQSPFQNAYNVKGNATS	>500
N464–482	1	SPFQNAYNVKGNATSANII	60
G1280–1298	1	TLHPRIIEEGFFDLHVQKV	115
G1352–1370	1	DGCDLDYYCNMGDWPSCTY	110

Figure 3: Detection of CCHFV-specific T cell responses by IFN-γ ELISpot assay.



The magnitude of responses in spot-forming cells per million (SFC/10^6) is shown for each peptide to which study participants responded positively. Figure reproduced/adapted from Goedhals et al., 2017 [27].

Figure 4: Confirmation of $CD8^+$ T cell–mediated responses by depletion assay.



IFN-ɣ ELISpot responses ($SFC/10^6$) are shown before and after CD8+ T cell depletion for peptides N262-280 and N298-316. Figure adapted from Goedhals et al., 2017 [27].

LIMITATIONS AND DIRECTIONS FOR FUTURE STUDIES

Despite the significant potential of NP-based CCHFV diagnostics and vaccine development, many technical challenges remain to be addressed before these methods can be translated into practical clinical tools.

First, CCHFV is genetically heterogeneous, driven by an error-prone RNA polymerase and frequent recombination, which pose a major challenge and may undermine the antigenic conservation of NP epitopes across strains. This heterogeneity makes it more difficult to develop broadly protective NP-based vaccines and diagnostic tests and requires constant monitoring of circulating strains and subsequent adaptation of target epitopes [8,18].

Second, additional obstacles arise from the complexity of manufacturing emerging NP-based platforms, especially VLPs. Although VLPs are highly immunogenic, they are subject to complex production and purification processes and can be unstable during process development, aspects that can affect both vaccine consistency and regulatory approval [4,20].

Third, only a limited number of NP-targeted vaccine candidates currently have extensive in vivo efficacy data or clinical trials. Despite encouraging preclinical results, these vaccines still need to be tested in lethal, clinically relevant animal models and then in humans to verify safety, immunogenicity, and long-term protection [16,19,22].

Fourth, safety remains a priority, especially when using live vector platforms that express NP, which may raise concerns regarding neurovirulence or unwanted host responses. Moreover, regulatory pathways for innovative NP-based vaccine technologies have not yet been streamlined [17,22,26].

Future research should focus on multi-epitope and multi-antigen vaccine platforms that integrate NP with other viral proteins to boost efficacy. Recent developments in immunoinformatics, bioengineering, and nanotechnology promise to optimize NP antigen presentation and vaccine delivery [24,25,32,33]. At the same time, advances in highly sensitive, rapid NP-based diagnostics can facilitate early detection and outbreak control [6,11,12]. Addressing these limitations through multidisciplinary efforts is essential to realize the full potential of NP in controlling CCHFV and reducing the global burden of CCHF.

CONCLUSION

CCHF is a severe tick-borne disease with a high case fatality rate, ranging from 10–50% for tick-borne transmission and up to 80% for nosocomial transmission, affecting over 30 countries across Europe, Asia, and Africa [35]. Recent diagnostic advances, including a

novel SYBR Green-based one-step real-time RT-PCR assay, have successfully detected CCHFV in clinical samples with high sensitivity, capable of detecting fewer than 20 copies of viral RNA per reaction [36].NP of CCHFV is highly conserved, abundantly expressed, and strongly immunogenic, making it a promising candidate for subunit vaccines. Partial efficacy up to 78% has been reported using a prime-boost vaccination strategy targeting NP [19]. NP can self-assemble into VLPs and stimulate both humoral and cellular immune responses, highlighting its potential as a core vaccine component. Moreover, rNP has proven useful in serosurveillance and diagnostic applications by detecting IgG and IgM antibodies, serving as a safe alternative to whole-virus antigens [3,37]. However, focusing exclusively on NP may be insufficient to induce sterilizing immunity or fully block transmission. Multivalent vaccine strategies incorporating additional conserved antigens, such as the L protein, could provide broader protection. Critical challenges remain in defining which NP epitopes elicit the most protective versus non-neutralizing immune responses, optimizing antigen folding and purity for large-scale production, and establishing correlates of protection in humans and animal models. Ethical and technical limitations hinder direct human challenge studies, further complicating efficacy assessment [5,19].

In conclusion, while NP demonstrates significant promise as a vaccine target and diagnostic tool, further research is needed to refine vaccine design, evaluate combinatorial strategies, and fully understand its protective potential against CCHFV. Continued preclinical and clinical studies are essential to advance safe and effective vaccines and to mitigate the public health impact of this high-consequence pathogen [37].

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The authors declare that they have no competing interests.

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Authors' contribution

Conceptualization, R.K, M.S.H., M.M.; methodology, R.K.; validation, R.K.; formal analysis, R.K.; investigation, R.K., M.S.H., M.M.; resources, M.M.; data curation, M.S.H.; writing—original draft preparation, R.K.; writing—review and editing, R.K, M.S.H., M.M.; visualization, R.K.; supervision, M.S.H.; All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

All assessments were conducted in accordance with ethical principles and under the supervision of the University's Ethics Committee (Ethic NO. IR.BMSU.BLC.1403.001).

References

1. Hawman DW, Feldmann H. Crimean–Congo haemorrhagic fever virus. Nat Rev Microbiol. 2023;21(8):463–77. doi:10.1038/s41579-023-00871-9.

2. Riccò M, Baldassarre A, Corrado S, Bottazzoli M, Marchesi F. Seroprevalence of Crimean Congo hemorrhagic fever virus in occupational settings: systematic review and meta-analysis. Trop Med Infect Dis. 2023;8(9):452. doi:10.3390/tropicalmed8090452.

3. Shayan S, Bokaean M, Shahrivar MR, Chinikar S. Crimean-Congo hemorrhagic fever. Lab Med. 2015;46(3):180–9. doi:10.1309/LMN1P2FRZ7BKZSCO.

4. Zhou ZR, Wang ML, Deng F, Li TX, Hu ZH, Wang HL. Production of CCHF virus-like particle by a baculovirus-insect cell expression system. Virol Sin. 2011;26(5):338–46. doi:10.1007/s12250-011-3209-6.

5. Omoga DCA, Tchouassi DP, Venter M, Ogola EO, Osalla J, Kopp A, et al. Transmission dynamics of Crimean-Congo haemorrhagic fever virus (CCHFV): evidence of circulation in humans, livestock, and rodents in diverse ecologies in Kenya. Viruses. 2023;15(9):1891. doi:10.3390/v15091891.

6. Shrivastava N, Shrivastava A, Ninawe SM, Sharma S, Kumar JS, Alam SI, et al. Development of multispecies recombinant nucleoprotein-based indirect ELISA for high-throughput screening of Crimean-Congo hemorrhagic fever virus-specific antibodies. Front Microbiol. 2019; 10:1822. doi:10.3389/fmicb.2019.01822.

7. Karaaslan E, Çetin NS, Kalkan-Yazıcı M, Hasanoğlu S, Karakeçili F, Özdarendeli A, et al. Immune responses in multiple hosts to nucleocapsid protein (NP) of Crimean-Congo hemorrhagic fever virus (CCHFV). PLoS Negl Trop Dis. 2021;15(12): e0009973. doi: 10.1371/journal.pntd.0009973.

8. Chinikar S, Persson SM, Johansson M, Bladh L, Goya M, Houshmand B, et al. Genetic analysis of Crimean-Congo hemorrhagic fever virus in Iran. J Med Virol. 2004;73(3):404–11. doi:10.1002/jmv.20106.

9. Raabe VN. Diagnostic testing for Crimean-Congo hemorrhagic fever. J Clin Microbiol. 2020;58(4):e01580-19. doi:10.1128/JCM.01580-19.

10. Wei PF, Luo YJ, Li TX, Wang HL, Hu ZH, Zhang FC, et al. Serial expression of the truncated fragments of the nucleocapsid protein of CCHFV and identification of the epitope region. Virol Sin. 2010;25(1):45–51. doi:10.1007/s12250-010-3067-7.

11. Saijo M, Qing T, Niikura M, Maeda A, Ikegami T, Prehaud C, et al. Recombinant nucleoprotein-based enzyme-linked immunosorbent assay for detection of immunoglobulin G antibodies to Crimean-Congo hemorrhagic fever virus. J Clin Microbiol. 2002;40(5):1587–91. doi:10.1128/JCM.40.5.1587-1591.2002.

12. Samudzi RR, Leman PA, Paweska JT, Swanepoel R, Burt FJ. Bacterial expression of Crimean-Congo hemorrhagic fever virus nucleoprotein and its evaluation as a diagnostic reagent in an indirect ELISA. J Virol Methods. 2012;179(1):70–6. doi: 10.1016/j.jviromet.2011.10.024.

13. Tipih T, Burt FJ. Crimean-Congo hemorrhagic fever virus: advances in vaccine development. Biores Open Access. 2020;9(1):137–50. doi:10.1089/biores.2020.0026.

14. Shi YZ, Xiong S, Zhang Y, Chin LK, Chen Y, Zhang JB, et al. Sculpting nanoparticle dynamics for single-bacteria-level screening and direct binding-efficiency measurement. Nat Commun. 2018;9(1):815. doi:10.1038/s41467-018-03156-5.

15. Hu YL, Zhang LQ, Liu XQ, Ye W, Zhao YX, Zhang L, et al. Construction and evaluation of DNA vaccine encoding Crimean Congo hemorrhagic fever virus nucleocapsid protein, glycoprotein N-terminal and C-terminal fused with LAMP1. Front Cell Infect Microbiol. 2023; 13:1121163. doi:10.3389/fcimb.2023.1121163.

16. Kortekaas J, Vloet RP, McAuley AJ, Shen X, Bosch BJ, de Vries L, et al. Crimean-Congo hemorrhagic fever virus subunit vaccines induce high levels of neutralizing antibodies but no protection in STAT1 knockout mice. Vector Borne Zoonotic Dis. 2015;15(12):759–64. doi:10.1089/vbz.2015.1855.

17. Aligholipour Farzani T, Földes K, Hanifehnezhad A, Yener Ilce B, Bilge Dagalp S, Amirzadeh Khiabani N, et al. Bovine herpesvirus type 4 vector delivering nucleocapsid protein of Crimean-Congo hemorrhagic fever virus induces comparable protective immunity against lethal challenge in IFNα/β/γR-/- mice models. Viruses. 2019;11(3):237. doi:10.3390/v11030237.

18. Zivcec M, Scholte FE, Spiropoulou CF, Spengler JR, Bergeron É. Molecular insights into Crimean-Congo hemorrhagic fever virus. Viruses. 2016;8(4):106. doi:10.3390/v8040106.

19. Zivcec M, Safronetz D, Scott DP, Robertson S, Feldmann H. Nucleocapsid protein-based vaccine provides protection in mice against lethal Crimean-Congo hemorrhagic fever virus challenge. PLoS Negl Trop Dis. 2018;12(7): e0006628. doi: 10.1371/journal.pntd.0006628.

20. Scher G, Bente DA, Mears MC, Cajimat MNB, Schnell MJ. GP38 as a vaccine target for Crimean-Congo hemorrhagic fever virus. NPJ Vaccines. 2023;8(1):73. doi:10.1038/s41541-023-00663-5.

21. Mousavi-Jazi M, Karlberg H, Papa A, Christova I, Mirazimi A. Healthy individuals' immune response to the Bulgarian Crimean-Congo hemorrhagic fever virus vaccine. Vaccine. 2012;30(44):6225–9. doi: 10.1016/j.vaccine.2012.08.003.

22. Dowall SD, Buttigieg KR, Findlay-Wilson SJ, Rayner E, Pearson G, Miloszewska A, et al. A Crimean-Congo hemorrhagic fever viral vaccine expressing nucleoprotein is immunogenic but fails to confer protection against lethal disease. Hum Vaccin Immunother. 2016;12(2):519–27. doi:10.1080/21645515.2015.1078045.

23. Behboudi E, Kakavandi E, Hamidi-Sofiani V, Ebrahimian A, Shayestehpour M. Crimean-Congo hemorrhagic fever virus vaccine: past, present, and future. Rev Res Med Microbiol. 2022;33(2):109–116. doi:10.1097.

24. Ahata B, Akçapınar GB. CCHFV vaccine development, current challenges, limitations, and future directions. Front Immunol. 2023; 14:1238882. doi:10.3389/fimmu.2023.1238882.

25. Leventhal SS, Meade-White K, Rao D, Haddock E, Leung J, Scott D, et al. Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge. EBioMedicine. 2022; 82:104188. doi: 10.1016/j.ebiom.2022.104188.

26. Hawman DW, Ahlén G, Appelberg KS, Meade-White K, Hanley PW, Scott D, et al. A DNA-based vaccine protects against Crimean-Congo haemorrhagic fever virus disease in a Cynomolgus macaque model. Nat Microbiol. 2021;6(2):187–95. doi:10.1038/s41564-020-00815-6.

27. Goedhals D, Paweska JT, Burt FJ. Long-lived CD8+ T cell responses following Crimean-Congo haemorrhagic fever virus infection. PLoS Negl Trop Dis. 2017;11(12):e0006149. doi: 10.1371/journal.pntd.0006149.

28. Akıncı E, Bodur H, Leblebicioglu H. Pathogenesis of Crimean-Congo hemorrhagic fever. Vector Borne Zoonotic Dis. 2013;13(7):429–37. doi:10.1089/vbz.2012.1061.

29. Bertolotti-Ciarlet A, Smith J, Strecker K, Paragas J, Altamura LA, McFalls JM, et al. Cellular localization and antigenic characterization of Crimean-Congo hemorrhagic fever virus glycoproteins. J Virol. 2005;79(10):6152–61. doi:10.1128/JVI.79.10.6152-6161.2005.

30. Sahib MM. Rapid development of optimized recombinant adenoviral vaccines for biosafety level 4 viruses [dissertation]. Sweden: Umeå University; 2010.

31. Spik K, Shurtleff A, McElroy AK, Guttieri MC, Hooper JW, Schmaljohn C. Immunogenicity of combination DNA vaccines for Rift Valley fever virus, tick-borne encephalitis virus, Hantaan virus, and Crimean-Congo hemorrhagic fever virus. Vaccine. 2006;24(21):4657–66. doi: 10.1016/j.vaccine.2005.08.034.

32. Imran MA, Islam MR, Saha A, Ferdousee S, Mishu MA, Ghosh A. Development of multi-epitope-based subunit vaccine against Crimean-Congo hemorrhagic fever virus using reverse vaccinology approach. Int J Pept Res Ther. 2022;28(4):124. doi:10.1007/s10989-022-10430-0.

33. Sette A, Livingston B, McKinney D, Appella E, Fikes J, Sidney J, et al. The development of multi-epitope vaccines: epitope identification, vaccine design and clinical evaluation. Biologicals. 2001;29(3–4):271–6. doi:10.1006/biol.2001.0297.

34. Rodriguez SE, Hawman DW, Sorvillo TE, O'Neal TJ, Bird BH, Rodriguez LL, et al. Immunobiology of Crimean-Congo hemorrhagic fever. Antiviral Res. 2022; 199:105244. doi: 10.1016/j.antiviral.2022.105244.

35. Sharti M, Hashemzadeh MS, Dorostkar R. Development of quantitative real-time RT-PCR assay for detection and viral load determination of Crimean-Congo hemorrhagic fever (CCHF) virus. Rom J Mil Med. 2019;122(3):34–40. Available from: https://api.semanticscholar.org/CorpusID:248312317.

36. Zahraei B, Hashemzadeh MS, Najarasl M, Zahiriyeganeh S, Tat M, Metanat M, et al. Novel, in-house, SYBR Green based one-step rRT-PCR: rapid and accurate diagnosis of Crimean-Congo hemorrhagic fever virus in suspected patients from Iran. Jundishapur J Microbiol. 2016;9(1): e29246. doi:10.5812/jjm.29246.

37. Mertens M, Schmidt K, Ozkul A, Groschup MH. The impact of Crimean-Congo hemorrhagic fever virus on public health. Antiviral Res. 2013;98(2):248–60. doi: 10.1016/j.antiviral.2013.02.007.