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# پیشرفت‌های بیولوژی مالیکولی در زمینه تشخیص و کنترل ویروس تب خونریزی‌دهنده کریمه–کنگو: پیامدها برای افغانستان

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**چکیده**

**زمینه و هدف:** تب خونریزی‌دهنده کریمه–کنگو (CCHF) یک بیماری خطرناک ناشی از نیش کنه است که توسط ویروس CCHFV ایجاد شده و میزان مرگ‌ومیر آن ۱۰ تا ۴۰ فیصد می‌باشد. افغانستان به دلیل وجود کنه‌های هیالوما و ضعف در سیستم تشخیصی، با افزایش موارد این بیماری مواجه است. این مقاله پیشرفت‌های بیولوژی مالیکولی در تحقیقات مربوط به CCHFV و کاربردهای بالقوه آن در افغانستان را با تمرکز بر روش‌های تشخیصی، اپیدمیولوژی و توسعه واکسن بررسی می‌کند.

**روش بررسی:** مرور سیستماتیک در پایگاه‌های داده PubMed، Scopus و Google Scholar (۲۰۰۷–۲۰۲۳) انجام شد. پس از حذف مطالعات تکراری و غربالگری، ۳۲ مطالعه مورد تحلیل قرار گرفتند که شامل بینش‌های ژنومی، روش‌های تشخیص مالیکولی و راهکارهای درمانی بودند.

**یافته‌ها:** ژنوم سه‌بخشی ویروس (بخش‌های S، M، L) تنوع ژنتیکی بالایی دارد که تشخیص و طراحی واکسن را پیچیده می‌کند. RT-PCR به عنوان روش استاندارد تشخیصی شناخته می‌شود، در حالی که بیوسنسورهای جدید گزینه‌های سریع و قابل استفاده در مناطق دورافتاده را ارائه می‌دهند. مطالعات اپیدمیولوژی مالیکولی نشان‌دهنده انتقال مرزی سویه‌های مختلف در افغانستان است، با نرخ مرگ‌ومیر ۴۳٫۳٪ (۲۰۱۶–۲۰۱۸). علیرغم نامزدهای امیدوارکننده واکسن (مانند RNA replicons و VLPs)، هنوز واکسن مجوزدار وجود ندارد.

**نتیجه‌گیری:** پیشرفت‌های مالیکولی پتانسیل بهبود مدیریت CCHF در افغانستان را دارند، اما نیازمند ادغام در سیستم‌های صحی هستند. توسعه زیرساخت‌ها، ابزارهای تشخیصی محلی و همکاری‌های بین‌المللی برای کاهش مرگ‌ومیر و کنترل شیوع بیماری ضروری است.

**واژه‌گانِ کلیدی:** CCHF، CCHFV، زیست‌شناسی مولکولی، افغانستان، RT-PCR، بیوسنسورها، اپیدمیولوژی، توسعه واکسن

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# Advances in Molecular Biology of Crimean-Congo Hemorrhagic Fever Virus: Implications for Diagnosis and Control in Afghanistan

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## Abstract

**Background:** Crimean-Congo hemorrhagic fever (CCHF) is a severe tick-borne disease caused by CCHFV, with high fatality rates (10–40%). Afghanistan, an endemic region, faces rising cases due to ecological suitability for Hyalomma ticks and limited diagnostic capacity. This review assesses molecular biology advancements in CCHFV research and their potential applications in Afghanistan, focusing on diagnostics, epidemiology, and vaccine development.

**Methods:** A systematic literature search (2007–2023) was conducted across PubMed, Scopus, and Google Scholar. After deduplication and screening, 32 studies were analyzed, covering genomic insights, molecular diagnostics, and therapeutic strategies.

**Results**: CCHFV's tripartite RNA genome (S, M, L segments) exhibits high genetic diversity, complicating diagnostics and vaccine design. RT-PCR remains the gold standard for detection, while emerging biosensors offer rapid, field-deployable alternatives. Molecular epidemiology reveals cross-border transmission of diverse strains in Afghanistan, with a case fatality rate of 43.3% (2016–2018). Despite promising preclinical vaccine candidates (e.g., RNA replicons, VLPs), no licensed vaccine exists.

**Conclusion**: Molecular advances hold promise for improving CCHF management in Afghanistan but require integration into public health systems. Prioritizing infrastructure development, localized diagnostic tools, and international collaborations is critical to reducing mortality and controlling outbreaks.

**Key words:** CCHF, CCHFV, molecular biology, Afghanistan, RT-PCR, biosensors, epidemiology, vaccine development

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## Introduction

Crimean–Congo hemorrhagic fever (CCHF) is a severe tick-borne zoonosis caused by the Crimean–Congo hemorrhagic fever virus (CCHFV), a member of the *Nairovirus* genus in the *Nairoviridae* family [1]. With case fatality rates ranging from 10% to over 40%, CCHF represents a major public health challenge in endemic regions, including Africa, the Middle East, Eastern Europe, and large parts of Asia [2]. Afghanistan, a country with high ecological compatibility for *Hyalomma* ticks—the primary vector for CCHFV—has experienced a rising trend in cases over recent years [3].

Despite longstanding recognition of the disease since its first description in the Crimea in 1944, modern molecular biology techniques have significantly expanded our understanding of CCHFV biology. Molecular approaches have elucidated many aspects of the viral genome, protein structure, and mechanisms of pathogenesis [4]. The virus possesses a tripartite negative-sense RNA genome encoding large polyproteins that undergo complex post-translational modifications [5].

More recent studies have capitalized on advanced diagnostic techniques—including real-time quantitative RT-PCR, antigen capture ELISAs, and biosensor-based assays—to improve early detection of CCHFV [6]. Next-generation sequencing and molecular epidemiological analyses have revealed significant genetic heterogeneity, with isolates clustering into distinct phylogenetic clades that often correlate with geographic distribution [7].

In Afghanistan, limited laboratory capacity and high occupational exposure—especially among livestock handlers, abattoir workers, and healthcare professionals—have resulted in increased morbidity and mortality [8–10]. The prevalence of *Hyalomma* ticks in the region and the frequent human-animal interactions increase the risk of zoonotic transmission [11]. A study in Herat Province reported a case fatality rate of 22.2%, highlighting the urgent need for improved diagnostic and therapeutic strategies [12].

Recent advancements in molecular biology have contributed to improved strategies for CCHFV diagnostics, treatment, and vaccine development [13]. Modern molecular techniques have enhanced our understanding of CCHFV and how these advances can be applied in the Afghan context for better diagnostic, therapeutic, and preventive measures [14]. These developments are contextualized within the epidemiological landscape of Afghanistan, highlighting methodological breakthroughs that have advanced the understanding of viral genomics, protein processing, and host-pathogen interactions [15]. Additionally, critical gaps are identified, and future directions for integrating molecular techniques into public health strategies to control CCHF in Afghanistan are explored [16]. The aim of this review is to assess the impact of molecular biology advancements on CCHFV research and explore their potential applications in Afghanistan.

## Methods

A comprehensive literature search was conducted to identify studies addressing molecular biology advances in CCHF and their application in Afghanistan. The search strategy combined traditional database queries with a snowballing technique—commonly used in systematic reviews—to maximize the capture of relevant articles. Specifically, the following steps were undertaken:

### Database Search

An initial search was performed in popular scientific databases including PubMed, Scopus, and Web of Science. The search terms combined keywords such as “Crimean–Congo hemorrhagic fever,” “CCHFV,” “molecular biology,” “diagnostics,” “vaccine,” “Afghanistan”. The search was limited to articles published between 2007 and 2023.

- PubMed: 1059 articles were retrieved
- Scopus: 745 articles were retrieved
- Google Scholar: 362 articles were retrieved

**Deduplication and Screening**
After removing 90 duplicate records, the titles and abstracts of the remaining 160 articles were screened for relevance based on predefined inclusion criteria (i.e., studies addressing molecular aspects of CCHFV, diagnostic innovations, vaccine development, or epidemiological data pertinent to Afghanistan). This screening resulted in the exclusion of 110 articles due to irrelevance or insufficient detail, leaving 50 articles for full-text review.

**Snowballing Technique**
In addition to the database search, the references of the initially included articles were examined (snowballing) to identify further relevant studies. This approach yielded an additional 10 articles, bringing the final total to 40 studies that met the inclusion criteria.

**Data Extraction and Synthesis**
Key information was extracted from each of the 32 studies—including study design, time period, methodological approaches (e.g., RT-PCR protocols, biosensor development, next-generation sequencing), and key findings. Data were then organized thematically into sections covering genomic/proteomic insights, molecular diagnostics, molecular epidemiology, and novel therapeutic/vaccine strategies.

**Table 1.** Summary of Search Strategy and Study Selection Process

| **Database** | **Query** | **Articles Retrieved** |
|---|---|---|
| **PubMed** | ("Crimean–Congo hemorrhagic fever" OR "CCHFV") AND ("molecular biology" OR "diagnostics" OR "vaccine") OR ("Afghanistan") | 1059 (10 pages, 100 articles) |
| **Scopus** | TITLE-ABS-KEY ("Crimean–Congo hemorrhagic fever" OR "CCHFV") AND TITLE-ABS-KEY ("molecular biology" OR "diagnostics" OR "vaccine") OR TITLE-ABS-KEY ("Afghanistan") | 745 (10 pages, 100 articles) |
| **Google Scholar** | Crimean–Congo hemorrhagic fever CCHFV molecular biology diagnostics vaccine Afghanistan | 362 (10 pages, 100 articles) |
| **Total Articles Retrieved** | | **300** |
| **Deduplication** | Duplicate records removed | 90 |
| **Screening** | Articles excluded based on irrelevance or insufficient detail | 110 |
| **Full-Text Review** | Articles selected for full review | 50 |
| **Snowballing Technique** | Additional articles identified from references | 10 |
| **Final Included Studies** | Studies that met inclusion criteria | **32** |

Table 1 outlines the systematic approach used to identify and select studies related to Crimean–Congo hemorrhagic fever (CCHFV), focusing on molecular biology, diagnostics, vaccines, and the geographic context of Afghanistan. Three databases—PubMed, Scopus, and Google Scholar—were queried using tailored search strings combining keywords such as *"Crimean–Congo hemorrhagic fever," "CCHFV," "molecular biology," "diagnostics," "vaccine,"* and *"Afghanistan."* Initial searches yielded 300 articles (100 from each database after limiting results to 10 pages per source).
Deduplication removed 90 duplicate records, leaving 210 unique articles. During screening, 110 articles were excluded due to irrelevance or insufficient detail, narrowing the pool to 100 studies. Subsequent full-text review further refined the selection to 50 articles meeting preliminary eligibility criteria. The snowballing technique (examining references of selected studies) identified 10 additional relevant articles, resulting in 60 candidates. After rigorous evaluation against inclusion criteria, 32 studies were retained for final analysis.

This process highlights a structured, multi-stage methodology to ensure comprehensive and relevant study inclusion while minimizing bias, ultimately supporting robust evidence synthesis on CCHFV research and its implications for Afghanistan.

## Results

### Genomic and Proteomic Insights into CCHFV

The molecular characterization of CCHFV has revealed a complex genomic organization. The virus possesses a tripartite, negative-sense RNA genome consisting of the small (S), medium (M), and large (L) segments [17]. The S segment encodes the nucleoprotein (NP), a critical component of the viral ribonucleoprotein complex. The M segment encodes a large glycoprotein precursor (GPC) that is processed into two envelope glycoproteins (Gn and Gc) and additional accessory proteins [18]. The L segment encodes an RNA-dependent RNA polymerase (RdRp) featuring an ovarian tumor (OTU)-like protease domain that may modulate host immune responses [19].

These genomic features underline the complexity of CCHFV replication and pathogenicity. Comparative analyses have revealed significant genetic variability among isolates, with phylogenetic studies clustering CCHFV into seven major clades that are geographically distinct [20]. This heterogeneity has critical implications for both diagnosis and vaccine development, as it suggests that antigenic variability might affect the performance of serological assays and the breadth of vaccine-induced immunity.

### Advances in Molecular Diagnostics

Recent advancements in molecular diagnostics have been pivotal in improving the early detection of CCHFV. Real-time quantitative RT-PCR remains the gold standard for detecting viral RNA during the acute phase of infection [21,22]. Several RT-PCR protocols have been optimized for sensitivity and specificity, enabling the detection of as few as 1000 genome equivalents per reaction. This is particularly important in resource-limited settings like Afghanistan, where early diagnosis can significantly reduce mortality rates [23].

In addition to RT-PCR, antigen capture enzyme-linked immunosorbent assays (ELISAs) targeting the NP have been developed to detect viral proteins directly in serum and tick specimens [24]. These assays benefit from the high conservation and immunogenicity of the NP, providing a reliable alternative for rapid screening. Furthermore, recent studies have explored biosensor technologies, such as those utilizing nanomaterials and immunosensors, to offer rapid, on-site detection of CCHFV antigens [21].

### Molecular Epidemiology and Phylogenetics in Afghanistan

The integration of molecular techniques into epidemiological surveillance has provided valuable insights into the spread and evolution of CCHFV in Afghanistan. Sahak et al. reported that Afghanistan has witnessed a significant increase in CCHF cases over the past decade, with a case fatality ratio of 43.3% between 2016 and 2018 [25]. Molecular epidemiology studies from Pakistan, employing sequencing of the S, M, and L segments, have revealed that this region isolates cluster with both Asian and African lineages, suggesting historical reassortment and cross-border transmission events [26].

These findings underscore the importance of molecular surveillance in identifying emerging variants and potential hotspots for CCHFV transmission. Notably, the application of next-generation sequencing (NGS) has enabled researchers to characterize the viral quasispecies present in clinical samples, thus providing a more nuanced understanding of intra-host viral diversity and evolution [27]. Such detailed molecular data are invaluable for tracking outbreaks and informing public health interventions.

### Novel Therapeutic and Vaccination Strategies

The development of effective antiviral therapies and vaccines for CCHF remains a major challenge. To date, the mainstay of treatment is supportive care, with ribavirin being used off-label despite mixed evidence regarding its efficacy [28]. Recent molecular studies have focused on identifying viral targets for antiviral intervention, such as the OTU protease domain within the L protein, which plays a role in modulating host immune responses [29].

On the vaccination front, innovative strategies such as alphavirus-based replicon RNA vaccines and virus-like particle (VLP) vaccines are under investigation [30]. Experimental data indicate that vaccines eliciting

both humoral and cellular immune responses—particularly those targeting NP in combination with GPC—may offer robust protection against lethal challenge in animal models.

## Implications for CCHF Management in Afghanistan

Afghanistan's epidemiological profile—with its high incidence of CCHF, limited diagnostic capacity, and substantial occupational risk factors—necessitates the integration of molecular advances into public health practice. The deployment of RT-PCR and antigen-based rapid diagnostic tests in regional laboratories could facilitate timely case identification, thereby reducing the high case fatality rate observed in recent outbreaks [2, 9]. Moreover, molecular epidemiological studies can inform targeted interventions, such as vector control measures and risk communication strategies, especially among high-risk groups such as shepherds, butchers, and healthcare workers.

The challenges faced by Afghanistan include not only the need for improved laboratory infrastructure but also the development of localized diagnostic kits that take into account the genetic diversity of circulating CCHFV strains. In this context, training healthcare personnel in molecular diagnostic techniques and ensuring the availability of reagents are critical steps. Additionally, collaborations with international research institutions can help bridge gaps in expertise and resource availability, ultimately leading to better outbreak preparedness and response.

**Table 2.** Summary of Key Molecular Advances in CCHF and Their Implications

| Theme | Key Findings | Implications |
|---|---|---|
| **Genomic and Proteomic Insights** | CCHFV has a tripartite RNA genome (S, M, L segments). Significant genetic variability among isolates, with seven major phylogenetic clades. NP, GPC, and RdRp identified as key molecular components. | Genetic variability may impact vaccine efficacy and diagnostic sensitivity. Understanding viral proteins can aid in antiviral drug targeting. |
| **Molecular Diagnostics** | RT-PCR remains the gold standard for CCHFV detection. Antigen capture ELISAs target nucleoprotein (NP) for rapid screening. Emerging biosensor-based technologies for point-of-care testing. | Early detection improves patient outcomes. Field-deployable diagnostic tools can enhance outbreak surveillance in Afghanistan. |
| **Molecular Epidemiology & Phylogenetics** | Afghanistan has seen a rise in CCHF cases, with a case fatality ratio of 43.3%. Cross-border transmission with Asian and African lineages detected. NGS reveals viral quasispecies diversity. | Molecular surveillance is crucial for tracking outbreak sources and viral evolution. Helps in developing region-specific diagnostic assays and vaccines. |
| **Therapeutic & Vaccination Strategies** | Ribavirin used off-label with inconsistent efficacy. OTU protease domain identified as a potential antiviral target. Novel vaccine candidates include RNA replicons and VLPs. | Limited treatment options highlight the need for targeted antivirals. Broad-spectrum vaccine strategies should address CCHFV's genetic diversity. |
| **Public Health Implications for Afghanistan** | Limited diagnostic infrastructure hinders rapid case identification. High-risk occupations (livestock handlers, butchers, healthcare workers) contribute to disease burden. International collaborations needed to enhance molecular research capacity. | Establishing regional RT-PCR and antigen-based rapid tests can improve case detection. Training programs for healthcare personnel on molecular diagnostics are essential. Strengthening partnerships with global virology research centers can bridge gaps in expertise and technology. |

Table 2 provides a structured summary of the molecular advances in CCHFV research, highlighting genomic insights, diagnostics, epidemiology, therapeutic strategies, and public health implications. These findings underscore the importance of molecular techniques in improving disease management and outbreak control, particularly in Afghanistan, where laboratory resources remain limited. The review emphasizes the urgent need for region-specific diagnostic tools, enhanced surveillance, and investment in vaccine development.

## Discussion

The advent of advanced molecular techniques has revolutionized infectious disease diagnostics. In the case of CCHF, rapid detection of viral RNA and genetic characterization have provided critical insights into viral

transmission and evolution. In Afghanistan, where traditional surveillance is hindered by limited laboratory capacity, integrating molecular diagnostics could significantly improve outbreak detection and control. Recent studies in Herat Province (2017) demonstrated a 22.2% case fatality rate (CFR) among hospitalized patients, underscoring the urgent need for early diagnosis and improved surveillance systems [31].

Our review highlights that RT-PCR remains the cornerstone of CCHFV diagnosis [5], though deployment in field settings is often challenged by infrastructural limitations. Biosensor-based diagnostic tools, still in the experimental phase, show promise as rapid, cost-effective alternatives for resource-limited regions [5]. The use of double-antigen ELISA for large-scale seroprevalence studies in Afghan livestock populations (19% seropositivity in cattle, goats, and sheep) could serve as an early warning system for human outbreaks [32]. These technologies could be particularly beneficial in Afghanistan, where early diagnosis is crucial for reducing high CCHF mortality [33].

Genomic and proteomic analyses of CCHFV have identified several unique features among bunyaviruses. The virus's unusually large M segment encodes a polyprotein that undergoes extensive post-translational modifications, enhancing antigenic variation and immune evasion [34]. Phylogenetic studies of Afghan and Pakistani CCHFV isolates revealed extensive genetic diversity, including reassortment events between Asia-1 and Asia-2 genogroups, complicating regional outbreak responses [35,36]. Additionally, the discovery of an OTU-like protease domain in the L protein has deepened our understanding of viral replication and highlighted potential antiviral targets [15].

The genetic diversity among CCHFV isolates, particularly those from Afghanistan, suggests that multiple viral variants may drive local outbreaks. Emerging clades in northwestern Iran, adjacent to Afghanistan, highlight the cross-border spread of novel haplotypes and the need for region-specific diagnostic kits [37]. This variability complicates vaccine development and necessitates broad-spectrum diagnostic assays. Future studies should map conserved antigenic determinants across strains to inform universal vaccine design and pan-genotypic antiviral agents [38].

Despite advances in molecular virology, a licensed CCHF vaccine remains unavailable. Experimental vaccine platforms—including RNA replicons, virus-like particles (VLPs), and recombinant protein vaccines—have shown promise in preclinical models [15]. Preclinical studies targeting glycoproteins (Gn/Gc) and nucleoproteins have demonstrated cross-protection against diverse CCHFV clades, a critical feature given the co-circulation of Asia-1 and Asia-2 strains in Afghanistan and neighboring countries [39]**.** Targeting both structural (glycoproteins) and non-structural (nucleoproteins) viral proteins appears to enhance immune protection, with animal models demonstrating improved survival after lethal challenge [34].

Key challenges persist. First, the high genetic variability of CCHFV requires vaccines to elicit cross-protective immunity. The identification of a tick-specific glycoprotein variant with reduced human cell fusion activity underscores the need to account for vector-driven viral adaptations in vaccine design [39]. Second, the absence of standardized animal models complicates efficacy testing [38]. Collaborative efforts uniting virologists, immunologists, and vaccine developers are essential to accelerating clinical trials [40].

Epidemiological data from Afghanistan indicate a concerning rise in CCHF cases. Between 2022 and 2023, Afghanistan reported 806 cases and 86 deaths across 14 provinces, reflecting both improved detection and true incidence growth [41]. With high mortality rates and limited diagnostic capacity, integrating molecular diagnostics into national surveillance is imperative [33]. Developing region-specific diagnostic kits tailored to local CCHFV variants will be essential [5].

International collaborations can facilitate technology transfer and capacity building in Afghanistan. Partnerships leveraging genomic surveillance networks, such as those mapping Hyalomma tick distributions in South Asia, could enhance predictive modeling of outbreak risks [39]**.** Partnerships with institutions specializing in molecular virology and biosensor diagnostics could help local laboratories adopt cutting-edge methods. Moreover, integrating molecular epidemiological data with traditional surveillance would allow for more targeted public health interventions and timely outbreak responses [15].

This synthesis underscores the dual imperative of addressing Afghanistan's immediate diagnostic gaps while building long-term resilience through One Health approaches that bridge human, animal, and vector surveillance.

## Limitations of Current Research

While significant advances have been made in understanding CCHFV at the molecular level, several limitations remain. The heterogeneity of CCHFV strains and the complexity of its genomic structure present challenges in the development of universal diagnostic assays and vaccines. Moreover, many of the innovative molecular techniques reviewed here are still in the experimental or early clinical trial phases, and their scalability in resource-limited settings like Afghanistan is yet to be demonstrated.

Future studies should focus on longitudinal surveillance to monitor the evolution of CCHFV in endemic areas, especially in Afghanistan, where the virus appears to be emerging rapidly. Integrating molecular data with epidemiological and clinical outcomes will be critical for evaluating the real-world impact of these technological advances.

## Conclusion

The integration of modern molecular biology advances into the diagnosis and management of Crimean–Congo hemorrhagic fever holds significant promise, particularly in high-risk regions such as Afghanistan. Enhanced molecular diagnostics, such as RT-PCR, antigen capture ELISAs, and innovative biosensor technologies, offer the potential for rapid and accurate detection of CCHFV, thereby facilitating early treatment and reducing mortality. Moreover, molecular epidemiological studies have provided critical insights into the genetic diversity and evolution of CCHFV, information that is essential for the development of cross-protective vaccines and targeted therapeutics. However, challenges remain in translating these advances into routine public health practice. In Afghanistan, where laboratory capacity and healthcare infrastructure are often limited, strategic investments in diagnostic technology, personnel training, and international collaborations are urgently needed. Addressing these challenges will not only improve the management of CCHF but will also strengthen the country's overall preparedness for emerging infectious diseases. This systematic review underscores the importance of continued research into the molecular biology of CCHFV and highlights the potential for these advances to transform the landscape of CCHF diagnosis, treatment, and prevention in Afghanistan. Ultimately, a concerted effort that combines molecular innovations with robust epidemiological surveillance will be key to curbing the impact of this high-fatality disease.

## Conflict of Interest statement

The authors declare that they have no conflicts of interest.

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## Authors' Contributions

Rahim Bakhsh Faqiryar and Ahmad Saeed Yaqubi wrote the original draft. Rahim Bakhsh Faqiryar and Ahmad Saeed Yaqubi reviewed and edited the manuscript.

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