Phylogenetic Analysis of Crimean-Congo Hemorrhagic Fever Virus in Inner Mongolia, China

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Abstract

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne arbovirus that can cause bleeding and death in humans. The mortality rate in humans is between 5 to 30%. The pathogen is prevalent in more than 30 countries in the world. In China, reports of strains of CCHFV have been concentrated in Xinjiang province. However, the CCHFV strain has never been reported in Inner Mongolia, China. This study reports new CCHFV strains, HANM-18, from *Hyalomma asiaticum* and *Hyalomma dromedarii* collected in Alxa Left Banner and Alxa Right Banner in Inner Mongolia. Complete sequences of CCHFV were obtained by the nested PCR technique and used for analyzing the identity and evolutionary relationship with other CCHFV strains. Interestingly, our results showed that the S and L fragments of the HANM-18 strain had high degrees of identity with Xinjiang isolate strains, and the M fragment had significant identity with South African isolates. These analyses also indicate that the HANM-18 strain may have been prevalent in northwest Inner Mongolia for many years. This discovery will be helpful in CCHF prevention and control in Inner Mongolia, and it also adds new evidence to the epidemiology of CCHF in China.

Keywords: Inner Mongolia; Crimean Congo hemorrhagic fever virus; phylogenetic analysis

1. Introduction

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne single-stranded RNA virus. The virus belongs to the *Nairoviridae* family of *Bunyavirales* (Garrison et al., 2020). The genome of CCHFV is composed of three fragments (Garrison et al., 2019). The S segment encodes nucleocapsid proteins, the M segment encodes two structural glycoproteins (Gn and Gc), and the L segment encodes an RNA-dependent RNA polymerase (Deyde et al., 2006; Garrison et al., 2019). As for other segmented RNA viruses, rearrangement is also significant in the evolution and migration of CCHFV. Compared with S and L fragments, the rearrangement of the M fragment is more frequent (Deyde et al., 2006). According to phylogenetic analysis, CCHFV can be divided into seven genetic categories related to geographic origin: two from Asia, two from Europe, and three from Africa (Umair et al., 2020).

CCHF caused by CCHFV is a zoonotic disease. It was first reported in Crimea in 1944, and then found in Congo in 1956, which gives it its name (Bente et al., 2013; Spengler et al., 2019). Most infected people are bitten by ticks carrying the pathogen, while others are infected by contact

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with diseased animals or human body fluids (Garrison et al., 2019; Nabeth et al., 2004; Negredo et al., 2019). The clinical symptoms in humans include fever, headache, vomiting, diarrhea, and muscle pain (Ergönül, 2006). In severe cases, there are signs of bleeding and multiple organ dysfunction (Bente et al., 2013; Ergönül, 2006). Ticks (mainly from the genus *Hyalomma*) are the main vectors of CCHFV; they are not only carriers of the virus, but they are also the natural reservoir of the virus (Spengler et al., 2019; Serretiello et al., 2020). There are no obvious symptoms in mammals infected with CCHFV (Negredo et al., 2019; Spengler et al., 2016). Meanwhile, birds are resistant to CCHFV infection (Negredo et al., 2019). Although some infected animals are usually without infection symptoms, they can still act as hosts in pathogen transmission routes (Serretiello et al., 2020; Spengler et al., 2016; Sorvillo et al., 2020).

The geographical distribution of CCHFV covers most countries and regions in west Asia, the Middle East, Europe, and Africa (Bente et al., 2013; Sorvillo et al., 2020). In China, CCHF infection was first reported in Bachu County of southwest Xinjiang in 1965 (Gao et al., 2010; Wu et al., 2013). Subsequently, there were cases and seropositive reports in the Tarim Basin and surrounding areas (Saijo et al., 2005; Sun et al., 2009). These case reports showed that most of the infected people had close contact with livestock (Gao et al., 2010; Zhang et al., 2018). So far, CCHF cases have only been reported in Xinjiang. However, CCHFV antibodies were detected in livestock and human sera in Qinghai, Inner Mongolia, and Yunnan provinces (Gao et al., 2010; Wu et al., 2013; Xia et al., 2011). This indicates that CCHF may exist in other parts of the Chinese mainland. In previous studies, we reported the detection of CCHFV antibodies in camels and sheep in the Alxa Left Banner and Alxa Right Banner of Inner Mongolia (Li et al., 2020). Nevertheless, the prevalence of CCHFV in this area is not clear. At the same time, the epidemic strain of CCHFV has not been reported in Inner Mongolia.

In this study, we report on the Crimean-Congo hemorrhagic fever virus detected in ticks collected in the Alxa Left Banner and Alxa Right Banner of Inner Mongolia from April to May 2015. The full sequence of the virus was obtained by genome-wide amplification. Phylogenetic analysis and investigation of CCHFV in sheep and camel parasitic ticks were carried out to explore the evolutionary relationship between strains, and the status of local ticks carrying CCHFV was investigated.

2. Materials and Methods

2.1. Sample collection

On the basis of previous studies, a total of 627 ticks were collected from sheep and camels in the Alxa Left Banner and Alxa Right Banner of Inner Mongolia, China, from April to May 2015 (Figure 1) (Li et al., 2020). For each sampling site, three flocks of sheep or camels were selected, and more than 50 animals in each flock were randomly selected for sampling. Ticks found on the skin surface of the animals were removed and collected in a container. The ticks were initially identified to species by morphological characteristics, and then the species were confirmed based on PCR amplification and sequencing of the cytochrome c oxidase subunit 1 gene (Zahler and Gothe, 1997; Apanaskevich and Horak, 2010; Gou et al., 2018). All samples were frozen and stored at -80°C . The collected ticks were pooled according to the hosts they were collected from and the tick species. Herdsmen were orally informed about the aims and process of this study. All animal owners agreed and allowed us to collect ticks from their camels or sheep.

2.2. Sample Processing and RNA Extraction

12-15 ticks were pooled in one sample based on the species and life stage for processing and analysis. To prepare samples, ticks from each pool were put into a 2 mL centrifuge tube containing 1 mL of Dulbecco's modified Eagle's medium (DMEM; Gibco, Carlsbad, CA, USA) prior to being homogenized in a frozen grinder (Jingxin, Shanghai, China). The homogenized samples were then centrifuged at 4 °C and $12,000 \times g$ for 10 min. The supernatants were collected and stored at -80°C . Total RNA was extracted from each tick pool with the RNeasy Mini Kit (QIAGEN, Hilden, Germany). According to the manufacturer's instructions, cDNA was synthesized using M-MLV reverse transcriptase (TaKaRa Biotechnology, Dalian, China). In brief, 0.5 μL dNTP mixture, 1 μL Oligo (dT) 18 primer, 1 μL template RNA (100 ng/ μL), and 5 μL RNase-free water were mixed and incubated at 65 °C for 5 min. Next, 2 μL 5x reverse transcriptase M-MLV Buffer, 0.25 μL RNase inhibitor (40 U/ μL), and reverse transcriptase M-MLV (200 U/ μL) were added to the tube, sequentially incubated at 42 °C for 1 h, and then at 70 °C for 15 min. The cDNA was then stored at -40°C .

2.3. CCHFV detection and whole-genome amplification

To detect whether CCHFV was present in the tick pools, part of the S fragment was amplified by specific nested PCR for detection. The PCR reaction system was a 50 μL mixture, including 25 μL 2x Taq PCR master mix, 2 μL template cDNA, 19 μL ddH₂O and 20 pmol of each forward and reverse primer. For the first round of PCR, a pair of primers (CCHFV-F 5'-TGGACACYTTCACAACTC-3', CCHFV-R 5'-GRYRAAYTCCCTRCACCA-3') was used to amplify a 536 bp fragment. The PCR amplification was performed in a power cycler (Analytikjena, Jena, Germany) using the following procedure: pre-denaturation at 94 °C for 3 min, then denaturation at 94 °C for 30 s, annealing at 54 °C for 40 s, and extension at 72 °C for 40 s. This cycle was repeated for a total of 35 cycles, followed finally by maintaining at 72 °C for 7 min. For the second round of PCR, another pair of primers (CCHFV-F' 5'-GAATGTGCATGGGTTAGCTC-3', CCHFV-R' 5'-GACATCACAATTTACCAGG-3') was used to amplify a 260 bp fragment. The amplification was performed using the following program: pre-denaturation at 94 °C for 3 min, denaturation at 94 °C for 30 s, annealing at 54 °C for 40 s and extension at 72 °C for 30 s. This cycle was repeated for a total of 35 cycles, followed finally by holding at 72 °C for 7 min. The PCR products were analyzed by agarose nucleic acid gel electrophoresis and sent to ComateBio (Changchun, China) for Sanger sequencing.

Genomic amplification was carried out in tick pools where CCHFV was detected. The semi-nested PCR primers (Table S1) were designed to amplify the whole genome sequence of CCHFV. The full sequences were amplified by LA Taq DNA polymerase (TaKaRa Biotechnology, Dalian, China) under the following conditions: pre-denaturation at 94 °C for 1 min, then denaturation at 98 °C for 10 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30-90 s. This cycle was repeated for a total of 35 cycles, followed finally by holding at 72 °C for 10 min.

2.4. Phylogenetic analysis

Viral sequences were assembled and aligned using Clustal W and compared with the sequences of other CCHFV strains in GenBank (Thompson et al., 2003). Respectively, the best-fit models (the Kimura 2-parameter model and the JTT matrix-based model) of nucleotide and amino acid sequence evolution were estimated using MEGA 7.0 (Kimura 1980; Jones et al., 1992; Kumar et al., 2016).

Phylogenetic trees were constructed using the maximum-likelihood method by MEGA 7.0 software (<http://www.megasoftware.net/>). The reliability of the branches of the tree was assessed using a bootstrap analysis with 1000 replicates. The phylogenetic trees were edited and visualized with FigTree v.1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree>). The MegAlign program implemented in the Lasergene software package v5.0 (DNASTar, Madison, WI, USA) was used to calculate the identity between nucleic acid and amino acid sequences.

2.5. Data analysis

The positive rate was determined by calculating the minimum infection rate (MIR), which was the ratio of the number of positive tick pools to the total number of analyzed ticks (Walter et al., 1980).

3. Results

3.1. Detection of CCHFV in Ticks

According to the tick species, sampling site, and host animal species, 73 ticks (divided into five tick pools) were collected in Alxa Left Banner, and 554 ticks (divided into 36 tick pools) were collected in Alxa Right Banner (Table 1). Nested PCR was used to test for presence of CCHFV in these 41 tick pools. By PCR amplification, CCHFV was detected in only one tick pool in Alxa Left Banner, consisting of *Hyalomma asiaticum*. The minimum infection rate (MIR) of CCHFV was 2.2% in Alxa Left Banner. Meanwhile, CCHFV was detected in one-sixth of the tick pools in Alxa Right Banner, including four *H. asiaticum* pools and two *Hyalomma dromedarii* pools. In this area, the MIRs of CCHFV were 1.2% and 1.1%, respectively (Table 1). CCHFV was not detected in *Dermacentor marginatus* from Alxa Left Banner or *Dermacentor nuttalli* from Alxa Right Banner.

3.2. Homology and Phylogeny Analysis

The new strain of CCHFV genome which was a genetic sequence recovered from tick pools, named HANM-18, consisted of three fragments: S (1,672 nt), M (5,368 nt), and L (12,155 nt) (Genbank Accession Numbers: MN832721–MN832723). Phylogenetic analysis of the HANM-18 strain showed that there were three complete sequences.

3.2.1 S fragment phylogenetic analysis

According to their geographical distribution, S fragments were divided into eight genotypes, namely Asia 1 and 2, Africa 1–3, and Europe 1–3 (Guo et al., 2017). The typing of M and L fragments was described previously (Deyde et al., 2006; Morikawa et al., 2007; Carroll et al., 2010). Most of the previously reported Chinese strains were clustered in the Asia 2 group, while WJQ16206 and FK16116 were clustered in Asia 1 (Zhang et al., 2018). In this study, HANM-18 and Chinese isolates were clustered into the Asia 2 group. According to Figure 2A, the evolutionary relationship between HANM-18 and the currently reported Chinese isolates shows that they have the same ancestral strain, while the evolutionary relationship between HANM-18 and the ancestral strains seems to be closer. In the homologous relationship, table S2 shows that the S fragment of the HANM-18 strain in this study had the highest identity with the CCHFV isolate in Xinjiang, China, ranging from 95.5–96.6%. Among these strains, HANM-18 had the highest nucleic acid identity with CCHFV discovered in Xinjiang in the 1960s and 1970s.

3.2.2 M fragment phylogenetic analysis

As for the M tree, it showed a different evolution from the currently reported Chinese strains. The M fragment of HANM-18 was clustered with an isolate from South Africa (Figure 2B). The Chinese CCHFV strains demonstrated different aggregation phenomena on the phylogenetic tree. The identity between the HANM-18 strain and South African strains (SPU97/85, SPU415/85, and SPU556/87) was 91.1% (Table S3). The homology of HANM-18 with South African strains such as SPU103/87, SPU187/90, SPU48/90, SPU130/89, SPU44/08, and SPU431/85 ranged from 80.8%-90.8%. The identity with some strains (8402, 88166, BA8402, BA88166, and C-68031) from Xinjiang in China was 87%, and the identity with the Oman strain was 86%. Compared with the amino acid sequence identity of the M fragment, it was observed that the amino acid identity of HANM-18 and South Africa was the highest, which was 91.8–91.9% (Table S3). Simultaneously, the identity with some Chinese isolates, Oman strains, and Pakistani strains was over 89%. To further determine the evolutionary relationship of the M fragment in the HANM-18 strain, the sequences with more than 85% amino acid identity were selected to construct the ML phylogenetic tree. In this amino acid tree, the HANM-18 strain was also clustered with South African isolates, and HANM-18 had a distant genetic relationship with Chinese isolates (Figure 3).

3.2.3 L fragment phylogenetic analysis

In the L tree, the strain was clustered with YL04057 and 79121M18, discovered in China in 2004, and YL16070 and YL16204 discovered in 2016 (Figure 2C). However, it was far away from the C-68031 strain discovered in 1968. The homology of HANM-18 with YL04057, 79121M18, YL16070, and YL16204 was the highest (93.7%), while that with c-68031 was 87.5% (table S3).

4. Discussion

Crimean-Congo hemorrhagic fever virus is a tick-borne virus in many countries in Asia, Africa, and Europe. In China, CCHFV is mainly distributed in Xinjiang province, and no cases have been reported in other provinces. In order to investigate the distribution of CCHFV in northwestern China, ticks were collected and tested in the western region of Inner Mongolia adjacent to Xinjiang.

In the current study, all ticks found to carry CCHFV were of the *Hyalomma* genus. *Hyalomma asiaticum* has been confirmed to be the main vector of CCHFV in Xinjiang, China (Guo et al., 2017; Moming et al., 2018). *Hyalomma dromedarii*, as a tick species mainly parasitic to camels, has also been demonstrated to carry CCHFV and infect the host (Camp et al., 2020; Moshaverinia and Moghaddas 2015). Strangely enough, *H. dromedarii* was found in both Left and Right Banner, while CCHFV was detected only in *H. dromedarii* of females from Alxa Right Banner. In the previous CCHFV survey in Saudi Arabia, CCHFV was also detected in *H. dromedarii* of females (Camp et al., 2020).

The identity and phylogeny analysis of CCHFV demonstrated that CCHFV, as a segmented RNA virus, showed high genetic diversity (Deyde et al., 2006). In the phylogenetic tree of S fragments, the isolates of HANM-18 from Xinjiang were grouped into Asia 2, and China is the origin of Asia 2 (Figure 2A) (Mild et al., 2010). And the homology with the previously reported strains in Xinjiang were the highest (Table S2). For the L fragment, the homology of HANM-18 with some Xinjiang strains (YL04057, 79121M18, YL16070, and YL16204) were the highest. HANM-18 was clustered with the Xinjiang strains in Asia 3 group on the L tree (Figure 2C). It is far away from some strains found in the Middle East. For the M fragment, HANM-18 was clustered with

strains from South Africa (SPU97/85, SPU415/85, SPU556/87, and SPU556/87), but is far away from Chinese isolates (Figure 2B.). The identity between HANM-18 and South African strains is also the highest. The amino acid tree of the M fragment and amino acid identity analysis also reached the same conclusion (Figure 3).

For CCHFV, due to its wide geographical distribution, there are significant differences among different epidemic areas, and the variation in different fragments is also dissimilar: from 10–20% for the S segment to 31% of the M segment (Deyde et al., 2006; Bente et al., 2013; Carroll et al., 2010). In general, the S segment is the slowest and most conserved, followed by the L segment, while the M segment shows great differences (Bente et al., 2013; Morikawa et al., 2007). The M fragment of HANM-18 is highly homologous with the South African isolate and is closer to the ancestor of the South African isolate in the genetic relationship (Figure S1B). This suggests that the M fragment of HANM-18 may be from an older strain. Then, its geographical isolation in China was characterized, and adaptive evolution occurred in the process of transmission. There are big differences compared with the strains prevalent in South Africa. At the same time, HANM-18 was far away from the Xinjiang strain in the phylogenetic tree (Figure S1B). This indicates that there are different epidemic types of the CCHFV M fragment in China. The reason for this phenomenon is not only mutation, but also rearrangement between different strains (Varsani et al., 2018). The S, M, and L fragments of CCHFV showed different prevalence and aggregation in China, which indicated that rearrangement was also common and played an important role in the transmission and evolution of CCHFV (Zhou et al., 2013). In previous reports, the South African isolate spu415/85 was closely related to Asian rearrangement (Goedhals et al., 2014). The strains found in South Africa have been confirmed to be prevalent and distributed in China (Zhou et al., 2013; Goedhals et al., 2014). There are many reasons for these rearrangements, either because migratory birds have carried the virus to different places, or because of the trade activities of livestock; ticks parasitized on these livestock have been taken to all parts of the world (Deyde et al., 2006; Serretiello et al., 2020; Spengler et al., 2016; Mild et al., 2010; Zhou et al., 2013; Palomar et al., 2013; Mancuso et al., 2019). In addition, the evolutionary relationship of the M fragment in the CCHFV strain found in Mongolia showed the same phenomenon as that of HANM-18 during the last 13–14 years (Voorhees et al., 2018). Inner Mongolia borders Mongolia, and it is possible that the CCHFV strain will spread between the two regions with the migration of birds or wild animals. However, since the data have not been published, it is impossible to make more comparisons.

In summary, this study indicates the presence of CCHFV strains in Inner Mongolia in China. The genetic relationship is not only related to the strains isolated from Xinjiang, but also shows that there are different types of CCHFV in China. This is the first time that CCHFV strains have been found and reported outside Xinjiang in China, which provides a good reference to characterize the prevalence and migration of CCHFV in China and Asia.

5. Conclusions

The present study provides data on the prevalence of important zoonotic agents in ticks from sheep and camels in Inner Mongolia of China. This novel information will be useful not only for medical and veterinary practitioners, but also the public health officials, when assessing the risks associated with tick-borne zoonoses in China. In addition, the authors hope that this report will also contribute, to increasing an overall awareness of tick-borne diseases among the population of China.

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CRedit authorship contribution statement

Yunyi Kong: Methodology, Investigation, Data curation, Formal analysis, Writing-original draft. **Chao Yan:** Methodology, Investigation, Data curation. **Dongxiao Liu:** Investigation, Data curation. **Lingling Jiang:** Methodology, Investigation. **Gang Zhang:** Investigation, Data curation. **Biao He:** Formal analysis, Resources. **Yong Li:** Project administration, Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors do not have any conflicts of interest to report.

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Animal and Human Right Statement

The experiments involving sheep and camels were performed according to protocols approved by the Institutional Animal Care and Use Committee of Ningxia University (NXU-2019-007). The need for consent is deemed unnecessary according to national regulations, but an informed verbal consent was obtained from the sheep and camel owners. The ethics committee for the use of animals of Ningxia University approved this study.

Appendix A. Supplementary data

Table S1-S4

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Table 1. Molecular detection of CCHFV from ticks isolated from different regions in Inner Mongolia, China

Areas of Sampling	Host species	Tick species	Tick life stage	Number of ticks	Number of pools	Number of positive pools	Positive rate (%)
Alxa Left Banner	Sheep	<i>Hyalomma asiaticum</i>	Adult (Female)	45	3	1	2.2
		<i>Hyalomma dromedarii</i>	Adult (Male)	13	1	0	0
		<i>Dermacentor marginatus</i>	Nymph	15	1	0	0
Alxa Right Banner	Camel	<i>Hyalomma asiaticum</i>	Adult (Female)	336	22	4	1.2
		<i>Hyalomma dromedarii</i>	Adult (Female)	187	12	2	1.1
		<i>Dermacentor nuttalli</i>	Nymph	31	2	0	0

Figure 1. Information for ticks included in the study. (A) Sampling locations; (B) tick species composition, quantity, and host animals. ZQ: Alxa Left Banner, YQ: Alxa Right Banner.

Figure 2. Maximum likelihood trees of Crimean-Congo hemorrhagic fever virus strains based on the complete sequences of the S (A), M (B), and L (C) segments. The solid triangles represent the same type of strains, and the hollow triangles represent the strains of the same country. The size of the triangle has nothing to do with the number of substituting strains. The new strain in this study is highlighted with a black solid circle.

Figure 3. A phylogenetic tree for Crimean-Congo hemorrhagic fever virus based on the glycoprotein precursor was constructed using the maximum likelihood method. The blue, gray, and green areas are used to represent the strains from South Africa, Oman, and China. The new strain in this study is highlighted with a black solid circle.

Table S1. Primer used in the CCHFV whole-genome amplification

HANM-18 (L segment)		
1-528	F(L 1-528)	TCTCAAAGAYATCAATCCCC
	R(L 1-528 N)	AATGTGTTTGTCCAGAATGC
	R(L 1-528 W)	TTGCAATTGAACATGCAGTC
412-2079	F(L 412-2079)	TAYTTYGAGATTGTGCGGCAGCC
	R(L 412-2079 N)	AACTCTGGATGYARYTGRGTTCCG
	R(L 412-2079 W)	GYACTGCTCYACAGAYTCAGGC
1950-2910	F(L 1950-2910 W)	TTTGCTATAYTRCCTCCYGACTG
	F(L 1950-2910 N)	GATGACATAAGAACTCYCCAAC
	R(L 1950-2910 W)	CTGYTCYAAACTRGYRGGCAGTG
	R(L 1950-2910 N)	CACCYTCRACYARTCCTTTDCGC
2997-3968	F(L 2997-3968 W)	AGYTACYTAAARGCAGGRACTGC
	F(L 2997-3968 N)	TTRGARCAGGCWATGCGHAAAGG
	R(L 2997-3968 W)	TTCATRCAYTCWGCTGCCACTTC
	R(L 2997-3968 N)	AGAARGGTCCTTGAAAGAGYTCC
3820-5361	F(L 3820-5361 W)	AAGTGYTCTGTGCCAAGRTCTTG
	F(L 3820-5361 N)	YTRTTTGACYTRAAGGTGCCAC
	R(L 3820-5361 W)	GTTYTTTAGCTTMCCTCGAAGC
	R(L 3820-5361 N)	ATCATRTGAGTTYTVGGTGATGC
5293-7371	F(L 5293-7371 W)	AAACCATTYGAAYTAAGGTCAGG
	F(L 5293-7371 N)	AARAAAYCCYAGCCACACAATGGG
	R(L 5293-7371 N)	TCTTCACTRTRYTGCTCTACACC
	R(L 5293-7371 W)	CTTRACTGTGTCRTTYCCRTCCC
7196-9253	F(L 7196-9253 W)	TCRGGTGAYAAYACCAARTGGGG
	F(L 7196-9253 N)	YTTYTCHGGCATGATGCARCAGG
	R(L 7196-9253 N)	CYCTYAGRCCRTCYTCAAATGTG
	R(L 7196-9253 W)	CTCYARCTCAGATTCYGCAGGTC
9075-11103	F(L 9075-11103 W)	CRAACTAYCGTGAYAGTTGYGGC
	F(L 9075-11103 N)	CYTTYCTR RGCTGYTCKGYTCC
	R(L 9075-11103 N)	ACATTGTTTRAAGCCTATGTCTTC
	R(L 9075-11103 W)	TCACTTTTCACTGTGTTTCTGTC
10690-12155	F(L 10690-12155 W)	GACACCCAAACCACATATC
	F(L 10690-12155 N)	AAACGGAGCTTCTCCTTAAC
	R(L 10690-12155)	TCTCAAAGAAATCGTTCCCC
HANM-18 (M segment)		
1-1395	F(M 1-1395)	TCTCAAAGAAATACTTGCGG
	R(M 1-1395 N)	AATTAATGCGTGGCAGTTTG
	R(M 1-1395 W)	AAACCTTGCCTGCATTTAAC
306-2128	F(M 306-2128 W)	AATACTTGCGGCACGTCAGTACG

1984-3871	F(M 306-2128 N)	MAGYGRAYYAGARGGCTCRGGAG
	R(M 306-2128 N)	AGCTRAACCARAARAGRAARGCC
	R(M 306-2128 W)	CCYCARACCTGCACCATATGTGA
	F(M 1984-3871 W)	CTWTGCGAAAAYAGYGCCACAGG
	F(M 1984-3871 N)	AATCACYCARGARGGRAGGGGCC
	R(M 1984-3871 N)	RTGTGATWATYTCWGGAGGGAGC
3732-4955	R(M 1984-3871 W)	RTCTCCTGGYAYACCATGTGTGC
	F(M 3732-4955 W)	TGYAAYCCYACTTGGTGYTGGGG
	F(M 3732-4955 N)	CARTGYAGYYTGATTGARGCRGG
4955-5368	R(M 3732-4955)	CATCGTCYTTGAGGTGTCTRTAC
	F(M 4955-5368 W)	AGAGAGAGAATACTGCAAGAG
	F(M 4955-5368 N)	TGGCTAGAGTCAATCAAGAG
	R(M 4955-5368)	TCTCAAAGATATAGTGGCGG
<hr/>		
HANIM 18 / Segment 1 1-549	F(S 1-549)	TCTCAAAGAAACACGTGCCGCTC
	R(S 1-549 N)	TATCTGACAGCATTTCTTTGACGG
	R(S 1-549 W)	TGGGTTTTTCATCGCCACCTCTGT
290-545	F(S 290-545)	CATGGATCCTCTCAAAGAAACACGTGCC
	R(S 290-545)	CTAACTGCAGTCTCAAAGATATCGTT
367-1673	F(S 367-1673 W)	TTCGAAAAAAATGCAGGAATCATTAAA
	F(S 367-1673 N)	TCCATCTACGCATCAGCTCTGGTGGA
	R(S 367-1673)	TCTCAAAGATATCGTTGCCGCACAG

Table S2. Comparison of nucleotide sequence identity with other CCHFV strains' S segment

Strain	County	GenBank accession number	S segment of HANM-18
			nt (%)
75024	China	AF362080	95.1
7001	China	AF415236	93.3
9553/2001	Kosovo	AF428144	88.6
Uzbek/TI10145	Uzbekistan	AF481799	94.7
STV/HU29223	Russia	AF481802	89.4
Matin	Pakistan	AF527810	89.9
66019	China	AJ010648	95.2
8402	China	AJ010649	94.7
Baghdad 12	Iraq	AJ538196	89.5
Pakistan	Pakistan	AJ538198	89.8
88166	China	AY029157	94.9
TAJ/HU8966	Tajikistan	AY049083	93.0
Hodzha	Uzbekistan	AY223475	93.1
ROS/TI28044	Russia	AY277672	88.1
Bul/HU517	Bulgaria	AY277676	87.9
TAJ/HU8975	Tajikistan	AY297692	95.5
Semunya	Uganda	DQ076413	81.7
SPU128/81/7	South Africa	DQ076415	87.6
SPU4/81	South Africa	DQ076416	87.9
Hoti	Kosovo	DQ133507	88.6
ROS/HUVLV-100	Russia	DQ206447	88.2
AP92	Greece	DQ211638	82.0
ArD8194	Senegal	DQ211639	83.1
ArD15786	Senegal	DQ211640	83.1
ArD39554	Mauritania	DQ211641	87.3
C-68031	China	DQ211642	95.1
Drosdov	Russia	DQ211643	87.9
Kashmanov	Russia	DQ211644	88.2
Oman	Oman	DQ211645	89.8
SPU97/85	South Africa	DQ211646	87.3
SPU103/87	South Africa	DQ211647	87.7
SPU415/85	South Africa	DQ211648	87.6
Turkey200310849	Turkey	DQ211649	88.0
UG3010	Congo	DQ211650	85.3
CYL/TI05035	China	DQ217602	93.4
CYT/TI05099	China	DQ227495	93.6
Iran-52	Iran	DQ446212	88.3
Iran-53	Iran	DQ446213	88.3
Iran-56	Iran	DQ446214	88.2
66/08-Rodopi	Greece	EU871766	87.8
YL04057	China	FJ562093	93.9
Turkey-Kelkit06	Turkey	GQ337053	87.7
Sudan_AI-Fulah_3-2008	Sudan	GQ862371	87.6
Sudan_AI-Fulah_4-2008	Sudan	GQ862372	87.6
V42/81	Bulgaria	GU477489	88.0
79121M18	China	GU477494	93.4
Afg09-2990	Afghanistan	HM452305	90.0
Sudan_AB1-2009	Sudan	HQ378179	85.8
Beruwe 2008	Congo	HQ849545	77.2
NIV_1040532	India	JF922679	90.8
NIV_10405051	India	JF922681	91.3

TAJUK_*7	Tajikistan	JN086996	82.6
Dubai_616	United Arab Emirates	JN108025	82.2
NIVA_118594	India	JN572087	92.5
NIV_112143	India	JN572089	92.0
HSADL	India	JX051650	93.0
SCT	Afghanistan	JX908640	89.7
Zahedan	Iran	KC867274	90.3
Iran-Gilan69	Iran	KJ027521	89.7
Iran-Isfahan78	Iran	KJ027522	88.1
Iran-Kerman43	Iran	KJ196326	90.1
Iran-Zahedan19	Iran	KJ676542	93.2
SPU_431/85	South Africa	KJ682815	87.6
SPU_383/87	South Africa	KJ682816	87.9
SPU_556/87	South Africa	KJ682817	87.2
SPU_18/88	South Africa	KJ682818	87.3
SPU_45/88	South Africa	KJ682819	87.1
SPU_497/88	South Africa	KJ682820	87.2
SPU_130/89	South Africa	KJ682821	87.8
SPU_48/90	South Africa	KJ682822	87.1
SPU_187/90	South Africa	KJ682823	87.5
SPU_44/08	South Africa	KJ682824	87.0
59-TK-2012	Russia	KR814833	79.0
128-TK-2012	Russia	KR814834	79.0
37-ST-2009	Russia	KR814835	79.2
73-ST-2013	Russia	KR814836	85.4
24-R-2012	Russia	KR814837	87.8
36-ST-2014	Russia	KR814838	87.9
37-R-2013	Russia	KR814839	87.8
44-ST-2010	Russia	KR814840	88.0
46-ST-2012	Russia	KR814841	88.2
47-ST-2013	Russia	KR814842	88.2
52-R-2014	Russia	KR814843	87.9
75-R-2012	Russia	KR814844	88.2
75-ST-2010	Russia	KR814845	88.0
81-R-2014	Russia	KR814846	87.9
3756-ST-2007	Russia	KR814847	85.9
3809-ST-2007	Russia	KR814848	88.0
3820-ST-2007	Russia	KR814849	87.9
4495-ST-2008	Russia	KR814850	86.0
4574-ST-2008	Russia	KR814851	88.0
4691-ST-2009	Russia	KR814852	87.9
4701-ST-2009	Russia	KR814853	87.9
5170-R-2011	Russia	KR814854	87.7
5180-R-2011	Russia	KR814855	87.8
CHH	Iran	KU707898	90.2
GRH	Iran	KU707900	90.3
SRR	Iran	KU707901	92.8
Gaib	Tajikistan	KX013446	91.6
Hodzha	Uzbekistan	KX013449	90.9
K128_76	Kazakhstan	KX013455	93.1
K168_40	Turkmenistan	KX013458	91.4
K168_125	Turkmenistan	KX013461	90.7
SK_2015/human	Kazakhstan	KX129738	88.1
YL16070	China	KY354080	93.5
WJQ16206	China	MG659724	88.9
FK16116	China	MG659727	81.6

Oman_2012-40S	Oman	MH037279	90.2
Oman_2013-116S	Oman	MH037280	90.4
Oman_2014-828P	Oman	MH037281	90.2
2013_16_44	Kosovo	MH133289	88.8
2013_22_319	Kosovo	MH133298	88.8
2014-C-K14-1C	Kosovo	MH133307	88.9
NIV1733666	India	MH396675	95.4
61T	Pakistan	MN135942	87.3
MCL-19-T-1812	India	MN866192	93.2
19-H-3075	India	MN930427	93.3
19-H-3068	India	MN930428	93.3
19-H-3081	India	MN930429	93.0
UGANDA_3010	Ugandan	U88416	85.0

Table S3. Comparison of amino acid and nucleotide sequence identity with other CCHFV strains' M segment

Strain	Country	GenBank accession number	M segment of HANM-18	
			nt (%)	aa (%)
66019	China	AB069669	84.4	87.4
7001	China	AB069670	69.8	72.6
75024	China	AB069671	81.2	85.7
7803	China	AB069672	80.9	85.5
79121	China	AB069673	69.7	72.3
8402	China	AB069674	87.5	89.3
88166	China	AB069675	87.5	89.4
BA88166	China	AF338470	87.4	89.4
BA66019	China	AF350448	84.3	87.4
BA8402	China	AF350449	87.2	88.7
IbAr10200	Nigeria	AF467768	81.5	85.1
Matin	Pakistan	AF467769	87.8	90.1
Baghdad 12	Iraq	AJ538197	81.0	85.8
Pakistan	Pakistan	AJ538199	80.5	84.1
VLG/TI29414	Russia	AY179961	80.5	84.2
TADJ/HU8966	Tajikistan	AY179962	80.0	84.4
Hodzha	Uzbekistan	AY223476	81.1	85.2
Kosovo/9553/2001	Yugoslavia	AY675511	80.9	84.4
3010	Congo	DQ019222	68.0	72.3
Semunya	Uganda	DQ094832	67.7	71.6
SPU128/81/7	South Africa	DQ157174	81.5	85.7
SPU4/81	South Africa	DQ157175	81.6	85.7
ROS/HUVLV-100	Russia	DQ206448	81.5	84.8
AP92	Greece	DQ211625	70.3	72.8
ArD8194	Senegal	DQ211626	67.9	72.5
ArD15786	Senegal	DQ211627	67.8	72.4
ArD39554	Mauritania	DQ211628	70.5	76.5
C-68031	China	DQ211629	87.6	89.4
Drosdov	Russia	DQ211630	81.2	84.0
Kashmanov	Russia	DQ211631	81.7	84.7
Oman	Oman	DQ211632	86.7	89.7
SPU97/85	South Africa	DQ211633	91.1	91.9
SPU103/87	South Africa	DQ211634	80.8	84.4
SPU415/85	South Africa	DQ211635	91.1	91.9
Turkey200310849	Turkey	DQ211636	81.7	84.3
UG3010	Congo	DQ211637	68.0	72.3
Iran-52	Iran	DQ446215	79.6	84.9
Iran-53	Iran	DQ446216	80.8	84.8
Kosova Hoti	Yugoslavia	EU037902	81.7	84.6
YL04057	China	FJ562094	69.7	72.5
Turkey-Kelkit06	Turkey	GQ337054	81.6	83.8
V42/81	Bulgaria	GU477490	80.8	83.5
79121M18	China	GU477493	69.8	72.4
Afg09-2990	Afghanistan	HM452306	80.9	84.8
Al-Fulah 3-2008	Sudan	HQ378184	80.8	85.1
Al-Fulah 9-2008	Sudan	HQ378185	80.8	85.1
AB1-2009	Sudan	HQ378187	81.1	85.2
NIVA118594	India	JN572084	81.4	85.2
NIV112143	India	JN572085	81.4	85.2
NIV11703	India	JN572086	81.5	85.2
SCT ex Afghanistan	Afghanistan	KC344856	83.6	86.2
Zahedan	Iran	KC867273	80.8	84.8
SPU44/08	South Africa	KJ682805	86.1	88.7
SPU130/89	South Africa	KJ682807	85.9	88.2

SPU45/88	South Africa	KJ682809	86.5	87.9
SPU18/88	South Africa	KJ682810	86.1	88.4
SPU556/87	South Africa	KJ682811	91.1	91.8
SPU431/85	South Africa	KJ682812	90.8	91.9
SPU48/90	South Africa	KJ682813	81.3	85.2
SPU187/90	South Africa	KJ682814	81.3	85.7
Haskovo-V46/13	Bulgaria	KR092379	81.4	84.2
24-R-2012	Russia	KR814856	81.7	84.8
37-R-2013	Russia	KR814858	81.6	84.7
46-ST-2012	Russia	KR814860	81.5	85.0
52-R-2014	Russia	KR814862	81.6	84.6
75-R-2012	Russia	KR814863	81.5	84.5
75-ST-2010	Russia	KR814864	81.7	84.9
3809-ST-2007	Russia	KR814867	81.6	84.6
4495-ST-2008	Russia	KR814869	81.4	84.9
4574-ST-2008	Russia	KR814870	81.7	84.8
4691-ST-2009	Russia	KR814871	81.1	84.4
5180-R-2011	Russia	KR814874	81.6	84.7
Oman-199809166	Oman	KR864901	86.7	89.5
1-CR/HU-2015	Russia	KU161584	81.1	84.2
Gaib	Tajikistan	KX013445	83.1	87.2
K128_76	Kazakhstan	KX013454	80.8	85.7
K168_40	Turkmenistan	KX013457	80.9	85.7
K168_125	Turkmenistan	KX013460	80.8	85.5
K229_243	Russia	KX013466	81.2	84.7
Min	Russia	KX013481	81.2	84.7
Saf	Russia	KX013487	81.1	84.8
Ast133	Russia	KX056060	81.1	84.6
YL16070	China	KY354081	69.6	72.3
Oman_812956	Oman	KY362518	86.7	89.5
UCCR4405	Russia	KY484029	81.2	84.1
UCCR4459	China	KY484032	87.5	89.4
UCCR4401	Nigeria	KY484035	81.6	85.2
UCCR4448	South Africa	KY484042	81.6	85.6
UCCR4417	Uganda	KY484045	81.6	85.7
Tur_2004_813139	Turkey	MF511227	81.6	84.5
Tur_2004_813151	Turkey	MF511233	81.4	84.4
SPUD8_81_7_813051	South Africa	MF511236	81.2	85.7
2013_6_180	Kosovo	MH133278	81.3	84.7
2013_14_79	Kosovo	MH133287	81.7	84.5
2014-C-K14-1C	Kosovo	MH133308	81.0	84.5
2015-A-K15-1A	Kosovo	MH133311	81.2	84.4
NIV1311122	India	MH396643	81.2	85.2
NIV149247	India	MH396650	81.2	85.0
NIV1511559	India	MH396656	80.8	85.0
NIV164392	India	MH396662	81.3	85.1
61T/Pakistan	Pakistan	MN135943	80.6	84.7
19-H-3116	India	MN930412	80.7	85.0

Table S4. Comparison of nucleotide sequence identity with other CCHFV strains' L segment

Strain	County	GenBank accession number	L segment of HANM-18
			nt (%)
Matin	Pakistan	AY422208	87.3
TADJ/HU8966	Tajikistan	AY720893	87.4
Baghdad-12	Iraq	AY947890	87.4
IbAr10200	Nigeria	AY947891	86.8
VLV-100	Russia	AY995166	87.4
Semunya	Uganda	DQ076412	82.3
3010	Congo	DQ099335	83.4
AP92	Greece	DQ211612	77.7
ArD8194	Senegal	DQ211613	86.9
ArD15786	Senegal	DQ211614	86.7
ArD39554	Mauritania	DQ211615	86.7
C-68031	China	DQ211616	87.5
Drosdov	Russia	DQ211617	87.9
Kashmanov	Russia	DQ211618	87.6
Oman	Oman	DQ211619	86.5
SPU103/87	South Africa	DQ211621	87.3
SPU415/85	South Africa	DQ211622	87.2
10849	Turkey	DQ211623	87.9
UG3010	Congo	DQ211624	83.4
Kosova_Hoti	Yugoslavia	EU044832	87.9
YL04057	China	FJ562095	93.7
Kelkit06	Turkey	GQ337055	87.8
79121M18	China	GU477492	93.7
_Afg09-2990	Afghanistan	HM452307	87.2
Al-Fulah_3	Sudan	HQ378180	86.5
Al-Fulah_4	Sudan	HQ378181	86.5
Al-Fulah_9	Sudan	HQ378182	86.5
AB1	Sudan	HQ378183	86.7
NIVA118595	India	JN572090	86.9
NIV112143	India	JN572091	87.0
SCT_ex_Afghanistan	Afghanistan	KC344855	87.3
Zahedan	Iran	KC867272	87.1
SPU187/90	South Africa	KJ682795	87.3
SPU48/90	South Africa	KJ682797	86.7
SPU431/85	South Africa	KJ682799	87.0
SPU383/87	South Africa	KJ682801	87.0
SPU18/88	South Africa	KJ682803	87.2
SPU497/88	Namibia	KJ682804	87.0
36-ST	Russia	KR814876	87.7

37-R	Russia	KR814877	87.5
44-ST	Russia	KR814878	87.7
46-ST	Russia	KR814879	87.7
3820-ST	Russia	KR814887	87.6
4691-ST	Russia	KR814890	87.7
1-CR/HU	Russia	KU161582	87.5
1231-CR/TI-2015	Russia	KU161583	87.7
Gaib	Tajikistan	KX013444	87.3
Hodzha	Uzbekistan	KX013447	87.3
K168_40	Turkmenistan	KX013456	87.3
K229_194	Russia	KX013462	87.5
K229_243	Russia	KX013465	87.5
K315_14	Russia	KX013468	87.4
Mamon	Russia	KX013477	87.3
Saf	Russia	KX013486	87.6
Ast199	Russia	KX056050	87.3
Ast149	Russia	KX056053	87.6
Ast133	Russia	KX056059	87.7
NIV161064	India	KY213712	86.9
YL16070	China	KY354082	93.7
UCCR4413	Pakistan	KY484039	87.2
UCCR4417	Uganda	KY484043	87.2
Kalmykia_Shch_1	Russia	KY982864	87.4
Tur_2004_813048	Turkey	MF511190	87.9
Tur_2004_813135	Turkey	MF511191	87.7
Tur_2004_813137	Turkey	MF511192	87.8
SPU41_84_813060	South Africa	MF511206	86.5
2013_6_180	Kosovo	MH133279	88.1
2013_14_79	Kosovo	MH133288	88.0
2013_19_289	Kosovo	MH133297	88.0
2013_25_380	Kosovo	MH133303	87.9
A-K14-1A	Kosovo	MH133306	87.7
A-K15-1A	Kosovo	MH133312	87.6
NIV1733666	India	MH396673	87.0
YL16204	China	MH688499	93.7
MCL-18-T-305	India	MK330136	87.2
19-H-3068	India	MN930403	87.1
19-H-2856	India	MN930405	87.2
19-H-3092	India	MN930408	87.2
19-T-1989	India	MN930410	87.1