of REBOV in cynomolgus monkeys resulted in higher mortality. REBOV-infected monkeys died within 8–14 days after infection, while ZEBOV and SEBOV-infected monkeys died more rapidly, within 8 days after infection [24]. These results indicated that REBOV was less pathogenic compared to African EBOVs.

5. Pathogenesis of EBOV and factors contributed to lower virulence of REBOV

Many studies on pathogenesis of ZEBOV illustrated EBOV pathogenesis which were precisely reviewed by Hoene et al. [1]. Briefly, monocytes and tissue macrophages, one of the primary target cells for EBOV infection, was activated and produced a variety of proinflammatory cytokines, nitric oxide and tissue factors, while the other primary target cells, dendric cells, were impaired in their function. Lymphocytes were depleted by apoptosis even they were not susceptible for EBOV infection, and the apoptosis is likely to be due to impairment of dendric cells, proapoptic factors from activated monocytes/macrophages and direct effect of viral glycoproteins. Impairment of dendric cell function and depletion of lymphocytes were thought to result in impairment of adaptive immune response. Endothelial cells were infected with EBOV and increased endothelial permeability was noted. The virus infection to endothelial cells may have some effect on their function, but the cells were thought to be mainly affected by proinflammatory cytokines, such as TNFα released by macrophages/monocytes. Other factors might contribute to disseminated intravascular coagulation (DIC) which was often observed in human patients and infected primates, however, tissue factor released from virus-infected macrophages/monocytes was thought to play a crucial role in induction of DIC. Infection and apoptosis of hepatocytes and adrenal cortical cells may also contribute to EBOV pathogenesis.

A majority of these studies were focused on pathogenesis of ZEBOV, however, several studies implicated the reason why REBOV was less virulent compared to African EBOV. Significant differences in virus growth in cultured African green monkey cells, Vero cells, between REBOV and ZEBOV may reflect the difference in pathogenesis [25]. REBOV replication and transcription was also shown to be less efficient compared to ZEBOV using reconstituted minigenome replication/transcription systems [25].

Earlier study showed that a cleavage of viral GP to mature G_1 and G_2, which are disulfide linked to form a mature spike protein, $G_{1,2}$. The GP was shown to be cleaved by furin or furin-like endoprotease at C-terminal of consensus furin recognition motif of R-X-K/R-R to generate G_1 and G_2. REBOV GP, however, has a suboptimal sequence of K-Q-K-R and has been shown to be cleaved at reduced level, thus the difference in GP processing between REBOV and other three EBOV was thought to be responsible for different virulence among EBOVs [26]. However, this hypothesis has been contradicted to the observation that furin-cleavage was not essential for EBOV infectivity [27.28] and not critical for virus infectivity and virulence in nonhuman primates [29].

Many studies indicated that EBOV glycoprotein ($GP_{1,2}$) plays a crucial role in pathogenesis [1]. Earlier study showed that ZEBOV GP expression using an

adenovirus vetor caused endothelial cell damage in explanted human saphenous vein and cynomolgus monkey carotid arteries leading to enhanced permeability of the vessels, while REBOV GP expression exerted a less effect on cynomolgus monkey vessels but no effect on human vessels [30]. A mucin-like domain within ZEBOV GP was shown to be responsible for vascular injury [30]. This may be caused by down regulation of adhesion molecules such as integrin $\beta1$ but not due to endothelial cell death [31,32]. The down regulation of such molecules was significantly weaker in REBOV GP expressed cells compared to ZEBOV GP expressed cells.

Several cellular proteins [1], such as $\beta1$-integrin receptor, variety of C-type lectins (DC-SIGN, L-SIGN, MGL), DC-SIGN-related factors, and Tyro3 receptor tyrosine kinase family [33], are thought to be involved in EBOV infection. Among these proteins, human MGL which is expressed on macrophages and monocyte-derived immature dendric cells, has shown to promote EBOV infection through of mucin-like domain within GP, however, infectivity of REBOV GP pseudotyped virus was significantly lower than that of ZEBOV, SEBOV, and ICEBOV GP pseudotyped viruses [34]. Since EBOV infection in monocytes/macrophages plays a crucial role in hemorrhage and DIC through high level of expressions of proinflammatory cytokines and tissue factor [1], lower level of REBOV infection utilizing hMGL may partly contribute to lower virulence of REBOV.

Antibody-dependent enhancement of ZEBOV infection in macrophages though Fc-receptor and in endothelial/epithelial cells through C1q/C1q-receptor was also demonstrated, but significantly weak antibody-dependent enhancement was demonstrated for REBOV [35–37]. These may also contribute different pathogenesis between REBOV and African EBOVs.

Lymphocytes were shown to refractory to EBOV infection, however depletion of both CD4 and CD8 cells were observed in human patients and nonhuman primates exposed to EBOV. Recent study showed that a 17 amino acid domain within ZEBOV GP, which is similar to an immunosuppressive motif of retrovirus glycoprotein, is responsible for apoptosis of these cells, inhibition of progression of CD4 and CD8 cell cycles, decreased expression of interleukin-2, interferon-γ and interleukin-12-p40, and increased expression of interleukin-10. Corresponding peptide of REBOV GP, however, caused similar effect on rhesus T cells but not on human cells [38].

Recent study using microarray technique showed that ZEBOV infection to human hepatoblastoma cells, Huh7 cells resulted in down regulation of interferon-stimulated gene expression and also down regulation of type I interferon stimulated gene expression, while REBOV-infected cells showed reduced ability to down regulate these gene expression [39]. This may be related to no or lower virulence of REBOV in human.

Even though, many other factors may also be involved in difference in virulence between African EBOVs and REBOV, these studies strongly indicated that several factors at least contributed to the lower virulence of REBOV in human. In this regard, REBOV may not be a threat to humans even though we cannot rule out the possibility to cause severe disease in humans when accidental laboratory infection of high doses of REBOV occurs. Recent advances in reverse genetic system of

filoviruses allowed to study biology and pathogenesis of filoviruses. More precise mechanisms of lower virulence of REBOV will be clarified when the reverse genetic system is applied to REBOV in near future.

References

[1] Hoenen T, Groseth A, Falzarano D, Feldmann H. Ebola virus: unravelling pathogenesis to combat a deadly disease. Trends Mol Med 2006;12(5):206–15.

[2] Ebola virus infection in imported primates—Virginia, 1989. MMWR Morb Mortal Wkly Rep 1989;38(48):831–2, 837–8.

[3] Jahrling PB, Geisbert TW, Dalgard DW, Johnson ED, Ksiazek TG, Hall WC, et al. Preliminary report: isolation of Ebola virus from monkeys imported to USA. Lancet 1990;335(8688):502–5.

[4] Geisbert TW, Jahrling PB. Use of immunoelectron microscopy to show Ebola virus during the 1989 United States epizootic. J Clin Pathol 1990;43(10):813–6.

[5] Ebola virus infection in imported primates—United States. Can Dis Wkly Rep 1990;16(4):17–8.

[6] Dalgard DW, Hardy RJ, Pearson SL, Pucak GJ, Quander RV, Zack PM, et al. Combined simian hemorrhagic fever and Ebola virus infection in cynomolgus monkeys. Lab Anim Sci 1992;42(2):152–7.

[7] Hayes CG, Burans JP, Ksiazek TG, Del Rosario RA, Miranda ME, Manaloto CR, et al. Outbreak of fatal illness among captive macaques in the Philippines caused by an Ebola-related filovirus. Am J Trop Med Hyg 1992;46(6):664–71.

[8] Update: filovirus infections among persons with occupational exposure to nonhuman primates. MMWR Morb Mortal Wkly Rep 1990;39(16):266–7, 273.

[9] Update: filovirus infection in animal handlers. MMWR Morb Mortal Wkly Rep 1990;39(13):221.

[10] Miranda ME, White ME, Dayrit MM, Hayes CG, Ksiazek TG, Burans JP. Seroepidemiological study of filovirus related to Ebola in the Philippines. Lancet 1991;337(8738):425–6.

[11] World Health Organization: viral haemorrhagic fever in imported monkeys. Wkly Epidemiol Rec 1992;67:142–3.

[12] Miranda ME, Ksiazek TG, Retuya TJ, Khan AS, Sanchez A, Fulhorst CF, et al. Epidemiology of Ebola (subtype Reston) virus in the Philippines, 1996. J Infect Dis 1999;179(Suppl. 1):S115–9.

[13] Rollin PE, Williams RJ, Bressler DS, Pearson S, Cottingham M, Pucak G, et al. Ebola (subtype Reston) virus among quarantined nonhuman primates recently imported from the Philippines to the United States. J Infect Dis 1999;179(Suppl. 1):S108–14.

[14] Miranda ME, Yoshikawa Y, Manalo DL, Calaor AB, Miranda NL, Cho F, et al. Chronological and spatial analysis of the 1996 Ebola Reston virus outbreak in a monkey breeding facility in the Philippines. Exp Anim 2002;51(2):173–9.

[15] Walsh PD, Abernethy KA, Bermejo M, Beyers R, De Wachter P, Akou ME, et al. Catastrophic ape decline in western equatorial Africa. Nature 2003;422(6932):611–4.

[16] Leroy EM, Rouquet P, Formenty P, Souquiere S, Kilbourne A, Froment JM, et al. Multiple Ebola virus transmission events and rapid decline of central African wildlife. Science 2004;303(5656):387–90.

[17] Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, et al. Fruit bats as reservoirs of Ebola virus. Nature 2005;438(7068):575–6.

[18] Biek R, Walsh PD, Leroy EM, Real LA. Recent common ancestry of Ebola Zaire virus found in a bat reservoir. PLoS Pathog 2006;2(10):e90.

[19] Swanepoel R, Leman PA, Burt FJ, Zachariades NA, Braack LE, Ksiazek TG, et al. Experimental inoculation of plants and animals with Ebola virus. Emerg Infect Dis 1996;2(4):321–5.

[20] Pourrut X, Kumulungui B, Wittmann T, Moussavou G, Delicat A, Yaba P, et al. The natural history of Ebola virus in Africa. Microbes Infect 2005;7(7/8):1005–14.

[21] Ikegami T, Calaor AB, Miranda ME, Niikura M, Saijo M, Kurane I, et al. Genome structure of Ebola virus subtype Reston: differences among Ebola subtypes: brief report. Arch Virol 2001;146(10):2021–7.

[22] Groseth A, Stroher U, Theriault S, Feldmann H. Molecular characterization of an isolate from the 1989/90 epizootic of Ebola virus Reston among macaques imported into the United States. Virus Res 2002;87(2):155–63.

[23] Fisher-Hoch SP, Brammer TL, Trappier SG, Hutwagner LC, Farrar BB, Ruo SL, et al. Pathogenic potential of filoviruses: role of geographic origin of primate host and virus strain. J Infect Dis 1992;166(4):753–63.

[24] Jahrling PB, Geisbert TW, Jaax NK, Hanes MA, Ksiazek TG, Peters CJ. Experimental infection of cynomolgus macaques with Ebola-Reston filoviruses from the 1989–1990 US epizootic. Arch Virol Suppl 1996;11:115–34.

[25] Boehmann Y, Enterlein S, Randolf A, Muhlberger E. A reconstituted replication and transcription system for Ebola virus Reston and comparison with Ebola virus Zaire. Virology 2005;332(1):406–17.

[26] Volchkov VE, Feldmann H, Volchkova VA, Klenk HD. Processing of the Ebola virus glycoprotein by the proprotein convertase furin. Proc Natl Acad Sci USA 1998;95(10):5762–7.

[27] Wool-Lewis RJ, Bates P. Endoproteolytic processing of the Ebola virus envelope glycoprotein: cleavage is not required for function. J Virol 1999;73(2):1419–26.

[28] Neumann G, Feldmann H, Watanabe S, Lukashevich I, Kawaoka Y. Reverse genetics demonstrates that proteolytic processing of the Ebola virus glycoprotein is not essential for replication in cell culture. J Virol 2002;76(1):406–10.

[29] Neumann G, Geisbert TW, Ebihara H, Geisbert JB, Daddario-Dicaprio KM, Feldmann H, et al. Proteolytic processing of the Ebola virus glycoprotein is not critical for Ebola virus replication in nonhuman primates. J Virol 2007;81(6):2995–8.

[30] Yang ZY, Duckers HJ, Sullivan NJ, Sanchez A, Nabel EG, Nabel GJ. Identification of the Ebola virus glycoprotein as the main viral determinant of vascular cell cytotoxicity and injury. Nat Med 2000;6(8):886–9.

[31] Chan SY, Ma MC, Goldsmith MA. Differential induction of cellular detachment by envelope glycoproteins of Marburg and Ebola (Zaire) viruses. J Gen Virol 2000;81(Pt 9):2155–9.

[32] Simmons G, Wool-Lewis RJ, Baribaud F, Netter RC, Bates P. Ebola virus glycoproteins induce global surface protein down-modulation and loss of cell adherence. J Virol 2002;76(5):2518–28.

[33] Shimojima M, Takada A, Ebihara H, Neumann G, Fujioka K, Irimura T, et al. Tyro3 family-mediated cell entry of Ebola and Marburg viruses. J Virol 2006;80(20):10109–16.

[34] Takada A, Fujioka K, Tsuiji M, Morikawa A, Higashi N, Ebihara H, et al. Human macrophage C-type lectin specific for galactose and N-acetylgalactosamine promotes filovirus entry. J Virol 2004;78(6):2943–7.

[35] Takada A, Feldmann H, Ksiazek TG, Kawaoka Y. Antibody-dependent enhancement of Ebola virus infection. J Virol 2003;77(13):7539–44.

[36] Takada A, Kawaoka Y. Antibody-dependent enhancement of viral infection: molecular mechanisms and in vivo implications. Rev Med Virol 2003;13(6):387–98.

[37] Takada A, Watanabe S, Okazaki K, Kida H, Kawaoka Y. Infectivity-enhancing antibodies to Ebola virus glycoprotein. J Virol 2001;75(5):2324–30.

[38] Yaddanapudi K, Palacios G, Towner JS, Chen I, Sariol CA, Nichol ST, Lipkin WI. Implication of a retrovirus-like glycoprotein peptide in the immunopathogenesis of Ebola and Marburg viruses. FASEB J 2006;20(14):2519–30.

[39] Kash JC, Muhlberger E, Carter V, Grosch M, Perwitasari O, Proll SC, et al. Global suppression of the host antiviral response by Ebola- and Marburgviruses: increased antagonism of the type I interferon response is associated with enhanced virulence. J Virol 2006;80(6):3009–20.

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Recent progress in molecular biology of Crimean–Congo hemorrhagic fever

Shigeru Morikawa*, Masayuki Saijo, Ichiro Kurane

Department of Virology 1, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashimurayama, Tokyo 208-0011, Japan

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Abstract

Crimean–Congo hemorrhagic fever (CCHF) is a severe hemorrhagic fever in humans with a case fatality rate of up to 50%. A causative agent of CCHF is CCHF virus, which is a tick-borne virus in the family *Bunyaviridae*, genus *Nairovirus*. The virus is transmitted to humans through infected tick bites, squashed ticks or from direct contact with viremic animals or humans. Outbreaks of CCHF have been documented in Africa, the Middle East, Eastern Europe and Western Asia where the vector and/or reservoir ticks of *Hyalomma* spp. are distributed. Recent advances in molecular and biochemical analyses of CCHF virus revealed that the virus encodes larger proteins compared to other genus of Bunyavirus and the processing of viral proteins are complicated. Recent studies also showed that the CCHF viruses are relatively divergent in its genome sequence and the viruses are grouped in seven different clades. In general, these phylogenetic analyses based on sequences of S-RNA and L-RNA segment of CCHF viruses indicate that the seven clades correlate with their geographical location. The phylogenetic topology based on M-RNA segment sequences of CCHF viruses is different from those based on S-RNA and L-RNA segments. These analyses indicate that M-RNA segment reassortment events occur more frequently than those in S- and L-RNA segments.
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Keywords: Crimean–Congo hemorrhagic fever (CCHF); CCHF virus; Tick; Epidemiology; Phylogenetic analysis; Reassortment

*Corresponding author. Tel.: +81 42 561 0771; fax: +81 42 561 2039.
E-mail address: morikawa@nih.go.jp (S. Morikawa).

Résumé

La fièvre hémorragique de Crimée–Congo (FHCC) est une fièvre hémorragique foudroyante qui s'attaque à l'homme et entraîne la mort dans 50% des cas. L'agent responsable de la FHCC est un virus de type *Nairovirus de la famille des Bunyaviridae* qui se transmet par les tiques. Ce virus est transmis aux hommes lors de la morsure d'une tique infectée, par une tique écrasée ou par contact direct avec des animaux ou des personnes infecté(e)s. Les tiques *Hyalomma spp.* ont joué le rôle de vecteur et/ou de réservoir en répandant la FHCC en Afrique, au Moyen Orient, en Europe de l'Est et en Asie occidentale. Les récentes découvertes en matière d'analyses moléculaires et biochimiques sur le virus de la FHCC ont révélé qu'il encodait des protéines plus grosses que celles des autres virus de la famille des Bunyavirus, et que la fabrication des protéines virales était compliqué. De récentes études ont également montré que la séquence génomique des virus de la FHCC était assez différente, et que les virus étaient répartis en sept clades. En général, les analyses phylogénétiques, basées sur des séquences de segment d'ARN court et long des virus de la FHCC, indiquent que les sept clades sont liées à leur situation géographique. La topologie phylogénétique, basée sur les séquences du segment d'ARN moyen des virus de la FHCC, diffère de celle basée sur les segments d'ARN court et long. Ces analyses montrent que les évènements de réassortiment du segment d'ARN moyen se produisent plus fréquemment que ceux des segments d'ARN court et long.
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Mots clés: La fièvre hémorragique de Crimée–Congo (FHCC); le virus de la FHCC; tictaquer; épidémiologie; phylogenetic analyse; reassortment

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1. Introduction

Crimean–Congo hemorrhagic fever (CCHF) is a severe hemorrhagic fever in humans with a case fatality rate of up to 50%. A causative agent of CCHF is CCHF virus, which is a tick-borne virus in the family *Bunyaviridae*, genus *Nairovirus*. The virus is transmitted to humans through infected tick-bites or direct contact with the viremic animals or humans. Outbreaks of CCHF have been documented in Africa, the Middle East, Eastern Europe and western and central Asia, where the vector and/or reservoir ticks, *Hyalomma* spp., *Rhipicehpalus*, *Ornithodoros*, *Boophilus*, *Dermatocentor*, and *Ixodes* spp. are prevalent. Among these ticks, *Hyalomma* spp. is the most important vector for the virus. Clinical features of CCHF patients are characterized by a sudden onset of fever, myalgia, headache, dizziness, sore eyes, photophobia and hyperanemia. In severe cases, hemorrhagic manifestations develop several days after onset of disease. Course of CCHF virus infection and clinical features are recently reviewed more in detail [1,2]. In this review, epidemiology and ecology of CCHF, recent advances in biology of CCHF virus, molecular epidemiology of the virus, and diagnosis are reviewed.

2. Epidemiology and ecology

CCHF was first reported in the Crimean peninsula in 1940s, when a large outbreak of severe hemorrhagic fever with a case fatality rate of 10% was identified

Table 1
A major tick species associated with CCHF virus

Country or region	Tick species
West and southern Africa	*Hyalomma marginatum rufipes* and *H. turanicum*
Madagascar	*Boophilus microplus*
China	*H. asiaticum asiaticum*
Uzbekistan	*H. asiaticum asiaticum*
Tajikistan	*Dermacentor niveus*
Pakistan	*H. anatolicum*
Russia, Balkan	*H. marginatum marginatum*
Turkey	*H. marginatum marginatum, Rhipicephalus bursa*
Greece	*Rhipicephalus bursa*

[1,2]. Disease was designated as Crimean hemorrhagic fever and later the disease was reported throughout the European and central Asian republics of the former Soviet Union, and other countries. The Crimean hemorrhagic fever virus was isolated by inoculating patient specimens in newborn mice [3]. The virus was later shown to be antigenically identical to Congo virus which was isolated from a febrile patient in Democratic Republic of Congo in 1956 [4] and subsequently became named CCHF virus.

The epidemiology of CCHF reflects the geographic distribution of the ixodid ticks, particularly those of the genus *Hyalomma* [5,6] (Table 1). The virus and/or CCHF is reported in over 30 countries in Africa (Democratic Republic of Congo, Uganda, Mauritania, South Africa, Tanzania, Nigeria, Senegal, etc.), southeast Europe (Russia, Bulgaria, Kosovo, Turkey, Greece, etc.), the Middle East (UAE, Iraq, Iran, Saudi Arabia, Oman) and Asia (China, Kazakhstan, Tajikistan, Uzbekistan, Pakistan) [1,5] (Fig. 1).

CCHF virus persists in the host ticks through its life stages from larvae to nymph to adult (transstadial transmission) [7–11]. The virus can also be transmitted transovarially [1,12,13]. However, the virus generally circulates in an enzootic tick–vertebrate–tick cycle and vertebrates such as sheep, goats, cattle are thought to amplify the virus in the cycle. Smaller mammals, such as hares, hedgehogs, rodents, are known to be infested by immature stages of the ticks and may play a role in the lifecycle of the ticks [5]. Even though a variety of animals was demonstrated to be infected, the virus only causes disease in human and newborn mice [13]. In contrast, birds are generally refractory to CCHF virus infection, even though some species of birds are demonstrated to support large number of the virus infected ticks [5]. This has also been demonstrated in some birds experimentally infected with CCHF viruses [5]. However, many migrating bird species are known to be infested by immature ticks such as *H. marginatum marginatum* in Eurasia, and *H. m. rufipes*, *H. truncum* and some other *Hyalomma* spp. in Africa [5], thus the birds may play an important role in virus dissemination. Among birds, ostrich is only known to be susceptible to virus infection, and two CCHF outbreaks associated with slaughtering of Ostriches in South Africa have been reported [14,15]. Trade in livestock is shown to be associated with several outbreaks of CCHF [16–19].

Fig 1. The worldwide geographical distribution of CCHF virus and CCHF cases. Outbreaks of CCHF have been documented in Africa, the Middle East, Eastern Europe and Western and Central Asia, where the vector and/or reservoir ticks, especially *Hyalomma* spp., are prevalent.

3. Biology of CCHF virus

CCHF virus belongs to the family *Bunyaviridae*, genus *Nairovirus*. The genus *Nairovirus* is a tick-born virus and includes 34 viruses, which are grouped in seven serogroups. CCHF serogroup contains CCHF virus and Hazara virus. Recent study demonstrated the striking similarities between Nairovirus and tick phylogenies which indicate possible co-evolution of the viruses and their host ticks [20].

CCHF virus is an enveloped virus with a tripartite (small (S), medium (M), and large (L)), negative-sense single stranded RNA genome which encodes viral nucleocapsid (N), membrane glycoprotein precursor (GPC), and RNA-dependent RNA polymerase (L) proteins, respectively [21]. Basically, the structure and replication strategy of CCHF virus is indistinguishable from other Bunyaviruses [21].

N protein encoded in the S-RNA segment comprises 482 amino acids and a major component of nucleocapsid, however, mechanism of interaction of the N protein to

viral RNAs is poorly understood. In the virus infected mammalian cells, the N protein is mainly localized in the perinuclear region, but not associated with Golgi apparatus. Recent analysis showed that the N protein is targeted to the perinuclear region without viral glycoproteins and native viral RNAs. It has been also demonstrated that the depolymerization of actin filaments by Cytochalasin D resulted in disruption of N protein localization in both CCHF virus infected cells and the N protein expressing cells, indicating that actin filaments are involved in the targeting of the N protein [22]. Human MxA protein has been shown to inhibit replication of CCHF virus by interacting with the N protein [23] and inhibition of the growth of CCHF virus in human cells by interferon-alpha is mediated by the interferon-induced MxA GTPase [24].

Recent research clarified the unique coding strategy of the GPC protein in the M-RNA segment and the complex processing of the GPC into mature viral glycoproteins, Gn and Gc. CCHF virus M-RNA segment encodes an unusually large polyprotein compared to that of other genera of the *Bunyaviridae* family, in which GPCs are co-translationally processed into Gn and Gc [21]. The processing of CCHF virus GPC is more complicated in that further post-translational cleavage of the glycoprotein precursor products is required for production of the mature glycoproteins, Gn and Gc. In CCHF virus infected cells, M-RNA derived mRNA translates a large precursor protein, which is thought to be co-translationally cleaved into 140 kDa PreGn and 85 kDa PreGc at the N-terminal and the fifth hydrophobic stretch of the precursor by signalase in the endoplasmic reticulum (ER). The PreGn and PreGc give rise to the two mature envelope proteins, 37 kDa Gn and 75 kDa Gc [25–27]. The PreGn is processed at the consensus motif, RRLL, by SKI-1 protease in the ER/*cis* Golgi network to give rise to the Gn and the N-terminal region consisting the hypervariable mucin-like domain and GP38 [26,27]. The latter is heavily O-glycosylated in its mucin-like domain and some of them are further processed by furin to generate GP38 and GP85/GP160 in the *trans* Golgi network [27]. Function of these three proteins is unknown but they are secreted in significant quantity [27]. The N-terminal regions of GP85/GP160 comprising 243–259 amino acids are mucin-like, highly variable (amino acid identity ranging 15.2–100%, similarity ranging 67.5–100%) [25,28] and extremely rich in serine/threonine/proline residues (43.1–51.8%) [25]. The C terminus of the PreGn is thought to be processed at the R(R/K)LL by unknown protease [26,27]. The PreGc is further processed into mature Gc at the motif of RKPL by unidentified SKI-1-like protease in ER/*cis* Golgi network [26,27]. The mature Gn and Gc are localized to the Golgi where assembly and release of the virion occur. The Golgi targeting/retention signal resides within the ectodomain of Gn and its N-glycosylation is important for its localization and transport [29,30].

Early study has shown only minor antigenic differences among some CCHF virus strains originated in widely separated areas of the world by virus neutralization assay, but a slight difference has been observed between two strains, one isolated in Senegal and the other in Pakistan [31]. Recent analysis using a panel of monoclonal antibodies (mAbs) to Gn and Gc of CCHF virus IbAr10200 strain demonstrated broadly reactive and group-specific neutralizing and/or protective epitopes on Gn

and Gc [32]. Interestingly, any of mAbs against Gn has no virus neutralizing in *in vitro* plaque reduction assays, many of them confer protection to CCHF virus challenge in suckling mice [29,32]. Passive immunization of the plasma of convalescent patients was shown to be effective for the treatment of CCHF in seven patients, indicating that an inactivated or subunit vaccine may be effective [33]. Actually, CCHF inactivated vaccine was produced from mice brain tissues and used in Russia in 1970s [5].

The complete nucleotide sequence of L-RNA segment has been recently determined [34,35]. The data showed that L-RNA is nearly twice the size (12164 nucleotides) of those of other genus of Bunyavirus. The L-RNA segment of the CCHF virus encodes a large protein with 3944 amino acids in which an ovarian tumor (OTU)-like protease motif is found at N terminus followed by zinc finger motif and helicase domain, and RNA polymerase catalytic domain locates in the central region, indicating that the large protein is autoproteolytically cleaved, even though such a processing is not yet proved in the virus infected cells or in recombinant L protein expressing cells.

The processing of viral proteins is more complicated compared with other Bunyaviruses as described above, thus an establishment of a reverse genetics system for CCHF virus may help to understand biology of CCHF virus more in detail. In the case of Bunyaviruses, Bunyamwera virus was the first generated from cloned cDNA using T7 RNA polymerase system [36]. Recently, a reverse genetics approach has been made for CCHF virus using RNA polymerase I system [37]. In this study, S-RNA segment based minigenomes were transcribed, replicated and encapsidated upon infection of helper CCHF virus infection. Further development of a reverse genetics system for CCHF virus to generate an infectious CCHF virus from entirely cloned cDNA will allow remarkable progress in understanding biology of CCHF virus and also to develop therapeutic and prophylactic measures against CCHF virus infections.

4. Phylogenetic relationship and geographic distribution of the CCHF viruses

Complete nucleotide sequence of S-RNA segment of Chinese isolate C68031 of CCHF virus was determined in 1992 [38]. Thereafter, nested reverse transcriptase polymerase chain reaction (RT-PCR) amplifying the partial S-RNA segment of CCHF virus was developed and used for analysis of CCHF viruses at 1994–1995 CCHF outbreak in the United Arab Emirates (UAE) [16,39]. Phylogenetic analyses based on the sequence data of the amplicons have revealed genetic diversity. The outbreak was indicated to be a multisource outbreak associated with importation of the virus infected livestock and ticks. The genetic diversity in S-RNA segment sequence was also demonstrated for many CCHF virus isolates from different regions of the world [40–55]. A partial sequence of S-RNA segment was often used for phylogenetic analyses. However, recent studies indicated the possibility of recombination of the S-RNA segments [55–57], although the recombination is relatively rare. Thus, it would be better to use the full sequence data of the S-RNA segment in a comprehensive phylogenetic analysis. The analysis showed that CCHF

viruses were grouped in seven different clades; African clade 1 comprising isolates in Senegal, African clade 2 comprising those in Uganda and some in South Africa, African clade 3 comprising those in South and Western Africa, European clade 1 comprising those in Russia, Turkey, and Balkan region (Bulgaria, Kosovo), European clade 2 composed of a single Greek isolate AP92, Asian clade 1 comprising those in Middle East, Pakistan, Iran and Asian clade 2 comprising those in China, Uzbekistan, Kazakhstan. Among these clades, European clade 2 is most distantly related to other clades including European clade 1. In general, these phylogenetic analyses based on S-RNA segment sequences indicate that the seven clades correlate with their geographical location. It is worth mentioning that AP92 isolate was isolated from *Rhipicephalus bursa* ticks and there were no CCHF cases associated with this clade of the CCHF virus in Greece even though seroepidemiological survey demonstrated asymptomatic infection in human [58]. The genetic difference between European clades 1 and 2 may be due to genetic isolation of Greek isolate by adjacent mountain ranges [59] but not to the different tick species since an extensive survey of ticks in Turkey demonstrated that the CCHF viruses of European clade 1 were detected by RT-PCR in both *H. marginatum marginatum* and *R. bursa* [44]. In several cases (UAE [16], Oman [17], Saudi Arabia [18] and Madagascar [5,48]), CCHF viruses are thought to be introduced through import of the virus infected and/or tick-infested livestock such as sheep and cattle as mentioned above. Recently, it has been shown that two genetic lineages of CCHF viruses, Asian clade 1 and African clade 1, exist in Iran [42] and the latter is thought to be introduced by livestock trade [48]. Within the Asian clade 2, the viruses are clustered in two subclades, the first subclade including the viruses in China and Uzbekistan where *H. asiaticum* tick is a major vector, and the second in Tajikistan and Kazakhstan where *Dermatocentor niveus* is a major vector, indicating that a long-term association with a particular tick species plays a crucial role in genetic diversity among the clade [43]. Recent phylogenetic analyses based on L-RNA segment sequences showed that the L tree topology was similar to the S tree topology [55,60], however, L-segment reassortment has been suggested for some isolates in Senegal [55].

On the other hand, the phylogenetic topology based on M-RNA segment sequences of CCHF viruses is different from that based on S-RNA segments [28,32,55,60–66]. These analyses show that CCHF viruses are likely to be grouped in six different phylogenetic clades based on M-RNA sequences; clade M1 comprising isolates in China, Pakistan (Matin isolate), Oman, and South Africa (SPU97/85 and SPU415/85 isolates); clade M2 comprising those in Uzbekistan, Tajikistan, China, Pakistan, Iran, Iraq, South Africa and Nigeria; clade M3 comprising those in Congo (UG3010 isolate), Senegal, China (7001 and 79 121 isolates) and maybe Uzbekistan (Uzbek/TI10145 isolate which sequence is only partially determined (GenBank Acc. AY093627)); clade M4 comprising those in Russia, Kosovo and Turkey; other two clades are composed of Greek isolate AP92 and Mauritanian isolate ArD39554, respectively (Fig. 2). Essentially identical M tree can be obtained in phylogenetic analyses using partial sequence data of the M-RNA segment even when the extremely variable mucin-like domain is used [32,60], indicating that recombination
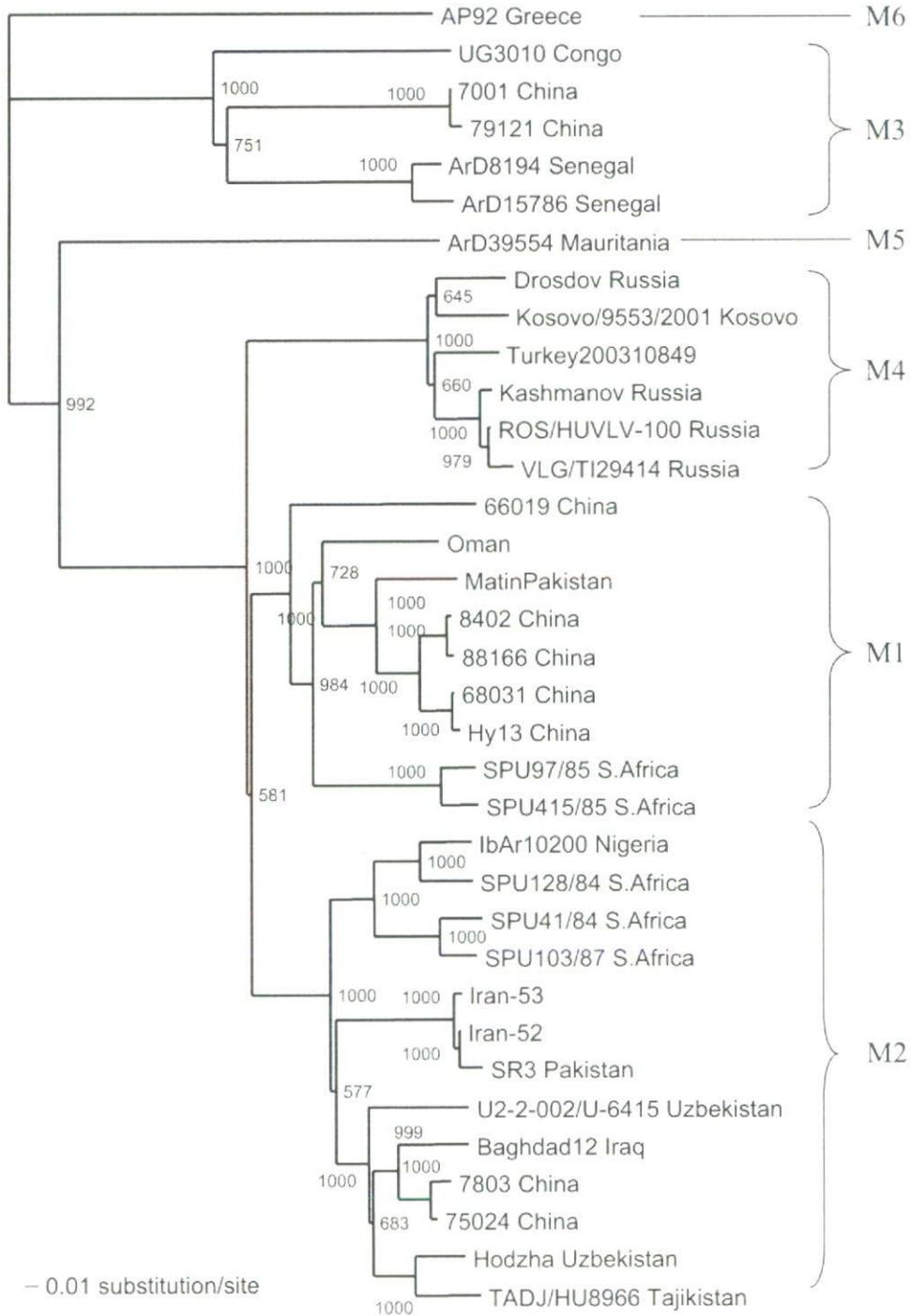
Fig 2. Phylogenetic relations of CCHF virus isolates based on the entire amino acid sequences of the M-RNA segment encoded protein. Phylogenetic analysis of CCHF virus isolates based on the entire amino acid sequences of the M-RNA segment encoded protein available on the GenBank using neighbor-joining method with Kimura's parameter was performed using the ClustalW program. The tree was drawn using the TreeView program. The numbers at the nodes are the bootstrap confidence level for 1000 replicates. The scale indicates the number of substitutions per site. The analyses based on M-RNA sequences indicate that CCHF viruses are likely to be grouped in six different phylogenetic clades, and tentatively denoted as M1 to 6.

within the M-RNA segments did not occur during evolution of the CCHF viruses. These data strongly indicate that M-RNA segment reassortment event occur frequently in CCHF viruses. The genetic reassortment may occur in ticks co-infected with different types of CCHF viruses, since the virus persists for long periods in ticks. The reason why M-RNA segment reassortment is more frequently observed is not clear, however, strong interrelation between N protein encoded in the S-RNA segment and RNA polymerase encoded in the L-RNA segment may be required to produce viable virus [67]. In addition, the virus is thought to be highly adapted to a particular species of host ticks in endemic region, and the S- and L-RNA segments may have evolved together in a particular tick. In contrast, the M-RNA segment sequence may not be restricted in a particular tick species, thus the reassortment event is frequently observed in the M-RNA segment. The possible M-RNA segment reassortment events between geographically distant regions are frequently observed as illustrated in Fig 3. Such an M-RNA segment reassortment between most geographically distant regions is observed in Chinese isolates, 7001 and 79 121, which were isolated from a patient in 1970 and from a jerboa in 1979, respectively, in Bachu region of the Xinjiang Autonomous Region in Western China. The M-RNA segments of these isolates are closely related to those of Senegal isolates within the clade M4. Birds are refractory to CCHF virus viremia except ostriches, but many migrating birds are known to be infested by immature ticks, such as *H. marginatum*, thus the virus was likely to be introduced to China by intercontinental migration of birds and then the genetic reassortment of M-RNA segment occurred between the African clade 1 and the Asian clade 2 type viruses.

5. Diagnosis, treatment and vaccine

Early diagnosis is important in terms of treatment of patients and prevention of nosocomial infections. Differential diagnosis is also necessary for other infectious diseases showing similar symptoms. Laboratory diagnosis includes demonstration of virus in the blood specimens and detection of virus-specific antibodies. The demonstration of virus in the specimens are carried out by isolation of the virus in tissue culture or suckling mice, by detection of viral RNA in RT-PCR including more sensitive one-step real-time RT-PCR [39,68-74], and/or by detection of virus antigens in antigen-capture ELISA using a recombinant virus N protein [75]. The CCHF virus specific antibodies were previously detected by complement fixation assay, gel diffusion assay, but recently immunofluorescence assay and ELISA are used to detect virus specific IgM and IgG antibodies including virus antigens or a recombinant virus N protein [72,73,76-82]. In general, viremia is demonstrated in first 9 days from onset of diseases, while antibodies are detected 7 days from onset of disease [2].

Apart from intensive supportive therapy, antiviral drug, ribavirin, is a choice of specific treatment of severe case of CCHF, even though no controlled studies have been performed to confirm its efficacy for CCHF treatment. However, its efficacy was shown in *in vitro* study [83,84] and in mice model [85] and ribavirin treatment was shown to be effective in CCHF patients [41,78,86-92].

Fig 3. The possible M-RNA segment reassortment events between geographically distant regions. Phylogenetic analyses indicated that M-RNA segment reassortment events occurred frequently between CCHF viruses of geographically distant regions. Genetically distinct CCHF virus is likely to be introduced in geographically distant regions by migrating birds infested with ticks carrying the virus.

In 1970s, CCHF inactivated vaccine was produced from mice brain tissues and used in Russia [5], but it is not known if it is effective and/or safe in humans. Recently, DNA vaccine of CCHF expressing Gn and Gc genes was shown to elicit neutralizing antibodies in mice, however, its protective efficacy is not demonstrated because of a lack of challenge animal model for CCHF virus [93].

References

[1] Whitehouse CA. Crimean–Congo hemorrhagic fever. Antivir Res 2004;64(3):145–60.
[2] Ergonul O. Crimean–Congo haemorrhagic fever. Lancet Infect Dis 2006;6(4):203–14.

[3] Chumakov MP, Butenko AM, Shalunova NV, Mart'ianova LI, Smirnova SE, Bashkirtsev Iu N, et al. New data on the viral agent of Crimean hemorrhagic fever. Vopr Virusol 1968;13(3):377.

[4] Casals J. Antigenic similarity between the virus causing Crimean hemorrhagic fever and Congo virus. Proc Soc Exp Biol Med 1969;131(1):233–6.

[5] Hoogstraal H. The epidemiology of tick-borne Crimean–Congo hemorrhagic fever in Asia, Europe, and Africa. J Med Entomol 1979;15(4):307–417.

[6] Swanepoel R, Shepherd AJ, Leman PA, Shepherd SP, McGillivray GM, Erasmus MJ, et al. Epidemiologic and clinical features of Crimean–Congo hemorrhagic fever in southern Africa. Am J Trop Med Hyg 1987;36(1):120–32.

[7] Logan TM, Linthicum KJ, Bailey CL, Watts DM, Moulton JR, et al. Experimental transmission of Crimean–Congo hemorrhagic fever virus by *Hyalomma truncatum* Koch. Am J Trop Med Hyg 1989;40(2):207–12.

[8] Okorie TG. Comparative studies on the vector capacity of the different stages of *Amblyomma variegatum* Fabricius and *Hyalomma rufipes* Koch for Congo virus, after intracoelomic inoculation. Vet Parasitol 1991;38(2-3):215–23.

[9] Shepherd AJ, Swanepoel R, Shepherd SP, Leman PA, Mathee O. Viraemic transmission of Crimean–Congo haemorrhagic fever virus to ticks. Epidemiol Infect 1991;106(2):373–82.

[10] Gordon SW, Linthicum KJ, Moulton JR. Transmission of Crimean–Congo hemorrhagic fever virus in two species of Hyalomma ticks from infected adults to cofeeding immature forms. Am J Trop Med Hyg 1993;48(4):576–80.

[11] Dohm DJ, Logan TM, Linthicum KJ, Rossi CA, Turell MJ. Transmission of Crimean–Congo hemorrhagic fever virus by *Hyalomma impeltatum* (Acari:Ixodidae) after experimental infection. J Med Entomol 1996;33(5):848–51.

[12] Gonzalez JP, Camicas JL, Cornet JP, Faye O, Wilson ML. Sexual and transovarian transmission of Crimean–Congo haemorrhagic fever virus in *Hyalomma truncatum* ticks. Res Virol 1992;143(1):23–8.

[13] Nicol ST. *Bunyaviruses*. Fields virology, (4th ed) In: Howley DMKaPM, editor. Philadelphia, PA: Lippincott-Raven 2001.

[14] Swanepoel R, Leman PA, Burt FJ, Jardine J, Verwoerd DJ, Capua I, et al. Experimental infection of ostriches with Crimean–Congo haemorrhagic fever virus. Epidemiol Infect 1998;121(2):427–32.

[15] Shepherd AJ, Swanepoel R, Leman PA, Shepherd SP. Field and laboratory investigation of Crimean–Congo haemorrhagic fever virus (*Nairovirus*, family *Bunyaviridae*) infection in birds. Trans R Soc Trop Med Hyg 1987;81(6):1004–7.

[16] Rodriguez LL, Maupin GO, Ksiazek TG, Rollin PE, Khan AS, Schwarz TF, et al. Molecular investigation of a multisource outbreak of Crimean–Congo hemorrhagic fever in the United Arab Emirates. Am J Trop Med Hyg 1997;57(5):512–8.

[17] Scrimgeour EM, Zaki A, Mehta FR, Abraham AK, Al-Busaidy S, El-Khatim H, et al. Crimean–Congo haemorrhagic fever in Oman. Trans R Soc Trop Med Hyg 1996;90(3):290–1.

[18] el-Azazy OM, Scrimgeour EM. Crimean–Congo haemorrhagic fever virus infection in the western province of Saudi Arabia. Trans R Soc Trop Med Hyg 1997;91(3):275–8.

[19] Khan AS, Maupin GO, Rollin PE, Noor AM, Shurie HH, Shalabi AG, et al. An outbreak of Crimean–Congo hemorrhagic fever in the United Arab Emirates, 1994–1995. Am J Trop Med Hyg 1997;57(5):519–25.

[20] Honig JE, Osborne JC, Nichol ST. The high genetic variation of viruses of the genus *Nairovirus* reflects the diversity of their predominant tick hosts. Virology 2004;318(1):10–6.

[21] Schmaljohn CS, Hooper JW, *Bunyaviridae*: the viruses and their replication. Fields virology, 4th ed. In: Howley DMKaPM, editor. Philadelphia, PA: Lippincott-Raven 2001.

[22] Andersson I, Simon M, Lundkvist A, Nilsson M, Holmstrom A, Elgh F, et al. Role of actin filaments in targeting of Crimean Congo hemorrhagic fever virus nucleocapsid protein to perinuclear regions of mammalian cells. J Med Virol 2004;72(1):83–93.

[23] Andersson I, Bladh L, Mousavi-Jazi M, Magnusson KE, Lundkvist A, Haller O, et al. Human MxA protein inhibits the replication of Crimean–Congo hemorrhagic fever virus. J Virol 2004;78(8):4323–9.

[24] Andersson I, Lundkvist A, Haller O, et al. Type I interferon inhibits Crimean–Congo hemorrhagic fever virus in human target cells. J Med Virol 2006;78(2):216–22.

[25] Sanchez AJ, Vincent MJ, Nichol ST. Characterization of the glycoproteins of Crimean–Congo hemorrhagic fever virus. J Virol 2002;76(14):7263–75.

[26] Vincent MJ, Sanchez AJ, Erickson BR, Basak A, Chretien M, Seidah NG, et al. Crimean–Congo hemorrhagic fever virus glycoprotein proteolytic processing by subtilase SKI-1. J Virol 2003;77(16):8640–9.

[27] Sanchez AJ, Vincent MJ, Erickson BR, Nichol ST. Crimean–congo hemorrhagic fever virus glycoprotein precursor is cleaved by Furin-like and SKI-1 proteases to generate a novel 38-kilodalton glycoprotein. J Virol 2006;80(1):514–25.

[28] Morikawa S, Qing T, Xinqin Z, Saijo M, Kurane I. Genetic diversity of the M RNA segment among Crimean-Congo hemorrhagic fever virus isolates in China. Virology 2002;296(1):159–64.

[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.

[30] Haferkamp S, Fernando L, Schwarz TF, Feldmann H, Flick R. Intracellular localization of Crimean–Congo hemorrhagic fever (CCHF) virus glycoproteins. Virol J 2005;2:42.

[31] Tignor GH, Smith AL, Casals J, Ezeokoli CD, Okoli J. Close relationship of Crimean hemorrhagic fever-Congo (CHF-C) virus strains by neutralizing antibody assays. Am J Trop Med Hyg 1980;29(4): 676–85.

[32] Ahmed AA, McFalls JM, Hoffmann C, Filone CM, Stewart SM, Paragas J, et al. Presence of broadly reactive and group-specific neutralizing epitopes on newly described isolates of Crimean–Congo hemorrhagic fever virus. J Gen Virol 2005;86(Pt 12):3327–36.

[33] Vassilenko SM, Vassilev TL, Bozadjiev LG, Bineva IL, Kazarov GZ. Specific intravenous immunoglobulin for Crimean–Congo haemorrhagic fever. Lancet 1990;335:791–2.

[34] Kinsella E, Martin SG, Grolla A, Czub M, Feldmann H, Flick R. Sequence determination of the Crimean-Congo hemorrhagic fever virus L segment. Virology 2004;321(1):23–8.

[35] Honig JE, Osborne JC, Nichol ST. Crimean-Congo hemorrhagic fever virus genome L RNA segment and encoded protein. Virology 2004;321(1):29–35.

[36] Bridgen A, Elliott RM. Rescue of a segmented negative-strand RNA virus entirely from cloned complementary DNAs. Proc Natl Acad Sci USA 1996;93(26):15400–4.

[37] Flick R, Flick K, Feldmann H, Elgh F. Reverse genetics for Crimean–Congo hemorrhagic fever virus. J Virol 2003;77(10):5997–6006.

[38] Marriott AC, Nuttall PA. Comparison of the S RNA segments and nucleoprotein sequences of Crimean–Congo hemorrhagic fever, Hazara, and Dugbe viruses. Virology 1992;189(2):795–9.

[39] Schwarz TF, Nsanze H, Longson M, Nitschko H, Gilch S, Shurie H, et al. Polymerase chain reaction for diagnosis and identification of distinct variants of Crimean–Congo hemorrhagic fever virus in the United Arab Emirates. Am J Trop Med Hyg 1996;55(2):190–6.

[40] Iashina LN, Petrov VS, Petrova ID, Gutorov VV, Kazakov SV, Ospanov KS, et al. Genetic identification of the Crimean–Congo hemorrhagic fever virus during epidemic outbreak in Kazakhstan in 2000. Mol Gen Mikrobiol Virusol 2002(4):31–5.

[41] Papa A, Bozovi B, Pavlidou V, Papadimitriou E, Pelemis M, Antoniadis A. Genetic detection and isolation of Crimean–Congo hemorrhagic fever virus, Kosovo, Yugoslavia. Emerg Infect Dis 2002; 8(8):852–4.

[42] 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.

[43] Yashina L, Petrova I, Seregin S, Vyshemirskii O, Lvov D, Aristova V, et al. Genetic variability of Crimean-Congo haemorrhagic fever virus in Russia and Central Asia. J Gen Virol 2003;84(Pt 5): 1199–206.

[44] Tonbak S, Aktas M, Altay K, Azkur AK, Kalkan A, Bolat Y, et al. Crimean–Congo hemorrhagic fever virus: genetic analysis and tick survey in Turkey. J Clin Microbiol 2006;44(11):4120–4.

[45] Tumanova I, Seregin SV, Vyshemirskii OI, Gutorov VV, Petrova ID, Tiunnikov GI, et al. Genetic monitoring of the Crimean–Congo Hemorrhagic Fever virus in Kazakhstan and Tajikistan in 2001–2003. Mol Gen Mikrobiol Virusol 2006(2):36–41.

[46] Tang Q, Gao D, Zhao X, Han L, Hang C. Study on the molecular biology of hemorrhagic fever virus in Xinjiang. Zhonghua Liu Xing Bing Xue Za Zhi 2002;23(6):449–52.

[47] Seregin SV, Tumanova I, Petrova ID, Iashina LN, Kuzina II, Vyshemirskii OI, et al. Genomic S segment of Crimean–Congo hemorrhagic fever virus circulating in Russia and Bulgaria. Vopr Virusol 2006;51(3):25–32.

[48] Burt FJ, Swanepoel R. Molecular epidemiology of African and Asian Crimean–Congo haemorrhagic fever isolates. Epidemiol Infect 2005;133(4):659–66.

[49] Petrova ID, Seregin SV, Petrov VS, Vyshemirskii OI, Kuzina II, L'Vov DK, et al. Genetic characteristics of the S-segment of RNA from two strains of the Crimean–Congo hemorrhagic fever virus isolated in the south of Russia and in Uzbekistan. Vopr Virusol 2003;48(2):8–11.

[50] Iashina LN, Petrov VS, Vyshemirskii OI, Aristova VA, Moskvina TM, L'Vov D K, et al. Characteristics of Crimean–Congo hemorrhagic fever virus circulating in Russia and Central Asia. Vopr Virusol 2002;47(3):11–5.

[51] Papa A, Bino S, Llagami A, Brahimaj B, Papadimitriou E, Pavlidou V, et al. Crimean–Congo hemorrhagic fever in Albania, 2001. Eur J Clin Microbiol Infect Dis 2002;21(8):603–6.

[52] Karti SS, Odabasi Z, Korten V, Yilmaz M, Sonmez M, Caylan R, et al. Crimean–Congo hemorrhagic fever in Turkey. Emerg Infect Dis 2004;10(8):1379–84.

[53] Drosten C, Minnak D, Emmerich P, Schmitz H, Reinicke T. Crimean–Congo hemorrhagic fever in Kosovo. J Clin Microbiol 2002;40(3):1122–3.

[54] Hewson R, Chamberlain J, Mioulet V, Lloyd G, Jamil B, Hasan R, et al. Crimean–Congo haemorrhagic fever virus: sequence analysis of the small RNA segments from a collection of viruses world wide. Virus Res 2004;102(2):185–9.

[55] Deyde VM, Khristova ML, Rollin PE, Ksiazek TG, Nichol ST. Crimean-Congo hemorrhagic fever virus genomics and global diversity. J virol 2006;80(17):8834–42.

[56] Chare ER, Gould EA, Holmes EC. Phylogenetic analysis reveals a low rate of homologous recombination in negative-sense RNA viruses. J Gen Virol 2003;84(Pt 10):2691–703.

[57] Lukashev AN. Evidence for recombination in Crimean–Congo hemorrhagic fever virus. J Gen Virol 2005;86(Pt 8):2333–8.

[58] Antoniadis A, Casals J. Serological evidence of human infection with Congo–Crimean hemorrhagic fever virus in Greece. Am J Trop Med Hyg 1982;31(5):1066–7.

[59] Papa A, Christova I, Papadimitriou E, Antoniadis A. Crimean–Congo hemorrhagic fever in Bulgaria. Emerg Infect Dis 2004;10(8):1465–7.

[60] Hewson R, Gmyl A, Gmyl L, Smirnova SE, Karganova G, Jamil B, et al. Evidence of segment reassortment in Crimean–Congo haemorrhagic fever virus. J Gen Virol 2004;85(Pt 10):3059–70.

[61] Yashina L, Vyshemirskii O, Seregin S, Petrova I, Samokhvalov E, Lvov D, et al. Genetic analysis of Crimean-Congo hemorrhagic fever virus in Russia. J Clin Microbiol 2003;41(2):860–2.

[62] Ma B, Hang C, Papa A. Sequencing and comparative analysis of the complete glycoprotein gene of three Crimean–Congo hemorrhagic fever virus Chinese isolates. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi 2001;15(2):105–11.

[63] Papa A, Ma B, Kouidou S, Tang Q, Hang C, Antoniadis A. Genetic characterization of the M RNA segment of Crimean Congo hemorrhagic fever virus strains, China. Emerg Infect Dis 2002;8(1):50–3.

[64] Meissner JD, Seregin SS, Seregin SV, Vyshemirskii OI, Yakimenko NV, Netesov SV, et al. The complete genomic sequence of strain ROS/HUVLV-100, a representative Russian Crimean Congo hemorrhagic fever virus strain. Virus Genes 2006;33(1):87–93.

[65] Papa A, Papadimitriou E, Bozovic B, Antoniadis A. Genetic characterization of the M RNA segment of a Balkan Crimean–Congo hemorrhagic fever virus strain. J Med Virol 2005;75(3):466–9.

[66] Seregin SV, Samokhvalov EI, Petrova ID, Vyshemirskii OI, Samokhvalova EG, Lvov DK, et al. Genetic characterization of the M RNA segment of Crimean–Congo hemorrhagic fever virus strains isolated in Russia and Tajikistan. Virus Genes 2004;28(2):187–93.

[67] Chamberlain J, Cook N, Lloyd G, Mioulet V, Tolley H, Hewson R. Co-evolutionary patterns of variation in small and large RNA segments of Crimean–Congo hemorrhagic fever virus. J Gen Virol 2005;86(Pt 12):3337–41.

[68] Burt FJ, Leman PA, Smith JF, Swanepoel R. The use of a reverse transcription-polymerase chain reaction for the detection of viral nucleic acid in the diagnosis of Crimean–Congo haemorrhagic fever. J Virol Methods 1998;70(2):129–37.

[69] Duh D, Saksida A, Petrovec M, Dedushaj I, Avsic-Zupanc T. Novel one-step real-time RT-PCR assay for rapid and specific diagnosis of Crimean–Congo hemorrhagic fever encountered in the Balkans. J Virol Methods 2006;133(2):175–9.

[70] Yapar M, Aydogan H, Pahsa A, Besirbellioglu BA, Bodur H, Basustaoglu AC, et al. Rapid and quantitative detection of Crimean–Congo hemorrhagic fever virus by one-step real-time reverse transcriptase-PCR. Jpn J Infect Dis 2005;58(6):358–62.

[71] Drosten C, Kummerer BM, Schmitz H, Gunther S. Molecular diagnostics of viral hemorrhagic fevers. Antivi Res 2003;57(1-2):61–87.

[72] Saijo M, Qing T, Niikura M, Maeda A, Ikegami T, Sakai K, et al. Immunofluorescence technique using HeLa cells expressing recombinant nucleoprotein for detection of immuno-globulin G antibodies to Crimean–Congo hemorrhagic fever virus. J Clin Microbiol 2002;40(2): 372–5.

[73] 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.

[74] Drosten C, Gottig S, Schilling S, Asper M, Panning M, Schmitz H, et al. Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean–Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR. J Clin Microbiol 2002;40(7):2323–30.

[75] Saijo M, Tang Q, Shimayi B, Han L, Zhang Y, Asiguma M, et al. Antigen-capture enzyme-linked immunosorbent assay for the diagnosis of Crimean–Congo hemorrhagic fever using a novel monoclonal antibody. J Med Virol 2005;77(1):83–8.

[76] Garcia S, Chinikar S, Coudrier D, Billecocq A, Hooshmand B, Crance JM, et al. Evaluation of a Crimean–Congo hemorrhagic fever virus recombinant antigen expressed by Semliki Forest suicide virus for IgM and IgG antibody detection in human and animal sera collected in Iran. J Clin Virol 2006;35(2):154–9.

[77] Saijo M, Tang Q, Shimayi B, Han L, Zhang Y, Asiguma M, et al. Recombinant nucleoprotein-based serological diagnosis of Crimean–Congo hemorrhagic fever virus infections. J Med Virol 2005;75(2):295–9.

[78] Tang Q, Saijo M, Zhang Y, Asiguma M, Tianshu D, Han L, et al. A patient with Crimean–Congo hemorrhagic fever serologically diagnosed by recombinant nucleoprotein-based antibody detection systems. Clin Diagn Lab Immunol 2003;10(3):489–91.

[79] Marriott AC, Polyzoni T, Antoniadis A, Nuttall PA. Detection of human antibodies to Crimean–Congo haemorrhagic fever virus using expressed viral nucleocapsid protein. J Gen Virol 1994;75(Pt 9):2157–61.

[80] Burt FJ, Leman PA, Abbott JC, Swanepoel R. Serodiagnosis of Crimean–Congo haemorrhagic fever. Epidemiol Infect 1994;113(3):551–62.

[81] Shepherd AJ, Swanepoel R, Gill DE. Evaluation of enzyme-linked immunosorbent assay and reversed passive hemagglutination for detection of Crimean–Congo hemorrhagic fever virus antigen. J Clin Microbiol 1988;26(2):347–53.

[82] Saluzzo JF, Le Guenno B. Rapid diagnosis of human Crimean–Congo hemorrhagic fever and detection of the virus in naturally infected ticks. J Clin Microbiol 1987;25(5):922–4.

[83] Watts DM, Ussery MA, Nash D, Peters CJ. Inhibition of Crimean–Congo hemorrhagic fever viral infectivity yields in vitro by ribavirin. Am J Trop Med Hyg 1989;41(5):581–5.

[84] Paragas J, Whitehouse CA, Endy TP, Bray M. A simple assay for determining antiviral activity against Crimean–Congo hemorrhagic fever virus. Antiviral Res 2004;62(1):21–5.

[85] Tignor GH, Hanham CA. Ribavirin efficacy in an in vivo model of Crimean–Congo hemorrhagic fever virus (CCHF) infection. Antiviral Res 1993;22(4):309–25.

[86] Fisher-Hoch SP, Khan JA, Rehman S, Mirza S, Khurshid M, McCormick JB. Crimean Congo–haemorrhagic fever treated with oral ribavirin. Lancet 1995;346(8973):472–5.

[87] Ozkurt Z, Kiki I, Erol S, Erdem F, Yilmaz N, Parlak M, et al. Crimean–Congo hemorrhagic fever in Eastern Turkey: clinical features, risk factors and efficacy of ribavirin therapy. J Infect 2006;52(3): 207–15.

[88] Jabbari A, Besharat S, Abbasi A, Moradi A, Kalavi K. Crimean–Congo hemorrhagic fever: case series from a medical center in Golestan province, Northeast of Iran (2004). Indian J Med Sci 2006; 60(8):327–9.

[89] Ergonul O, Celikbas A, Baykam N, Eren S, Dokuzoguz B. Analysis of risk-factors among patients with Crimean–Congo haemorrhagic fever virus infection: severity criteria revisited. Clin Microbiol Infect 2006;12(6):551–4.

[90] Bakir M, Ugurlu M, Dokuzoguz B, Bodur H, Tasyaran MA, Vahaboglu H. Crimean–Congo haemorrhagic fever outbreak in Middle Anatolia: a multicentre study of clinical features and outcome measures. J Med Microbiol 2005;54(Pt 4):385–9.

[91] Ergonul O, Celikbas A, Dokuzoguz B, Eren S, Baykam N, Esener H. Characteristics of patients with Crimean–Congo hemorrhagic fever in a recent outbreak in Turkey and impact of oral ribavirin therapy. Clin Infect Dis 2004;39(2):284–7.

[92] Mardani M, Jahromi MK, Naieni KH, Zeinali M. The efficacy of oral ribavirin in the treatment of Crimean–Congo hemorrhagic fever in Iran. Clin Infect Dis 2003;36(12):1613–8.

[93] 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.