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Seroepidemiological and molecular investigations of infections with Crimean–Congo haemorrhagic fever virus in Kazakhstan

Karlygash Abdiyeva[a], Nurkeldi Turebekov[a], Andrey Dmitrovsky[b], Nur Tukhanova[a], Anna Shin[a,b], Lyazat Yeraliyeva[b], Norbert Heinrich[c], Michael Hoelscher[c], Ravilya Yegemberdiyeva[b], Zhanna Shapiyeva[d], Zulfiya Kachiyeva[b], Aliya Zhalmagambetova[e], Josef Montag[f], Gerhard Dobler[g], Josua Zinner[g], Edith Wagner[g], Stefan Frey[g], Sandra Essbauer[g,*]

[a] *Centre for International Health, Ludwig-Maximilian-University, Munich, Germany*
[b] *Kazakh National Medical University, Almaty, Kazakhstan*
[c] *Division of Infectious Diseases and Tropical Medicine, Ludwig-Maximilians-University, German Centre for Infection Research, Munich Partner Site, Munich, Germany*
[d] *Scientific Practical Centre for Sanitary Epidemiological Expertise and Monitoring, Almaty, Kazakhstan*
[e] *German Association for International Cooperation, Almaty, Kazakhstan*
[f] *Faculty of Law, Charles University, Prague, Czech Republic*
[g] *Bundeswehr Institute of Microbiology, Department Virology and Intracellular Agents, German Centre for Infection Research, Munich Partner Site, Munich, Germany*

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ABSTRACT

Objectives: The aim of this study was to detect the seroprevalence of Crimean–Congo haemorrhagic fever virus (CCHFV) in patients with fever of unknown origin (FUO) in endemic (Kyzylorda) and non-endemic (Almaty) oblasts of Kazakhstan.
Methods: Paired serum samples from 802 patients with FUO were collected. Serum samples were investigated by ELISA to detect IgG and IgM antibodies against CCHFV. Sera with suspected acute infection were further investigated by RT-PCR to detect the viral RNA.
Results: IgG antibodies were detected in 12.7% of the sera from both oblasts. Acute infection was shown by IgM ELISA in four patients from Kyzylorda, with only one developing severe CCHF. Viral RNA was found by RT-PCR in the other three patients' sera. Phylogenetic analysis of partial L and S segments revealed CCHFV genotype Asia 2 and a possible reassortment between the genotypes Asia 1/Asia 2. Animal husbandry, such as working with cattle and horses, was significantly associated with CCHFV seropositivity.
Conclusions: The antibodies and viral RNA detected in sera indicate that mild or even asymptomatic CCHFV infections are presented in Kazakhstan. This study describes the circulation of CCHFV in the so far non-endemic Almaty oblast for the first time. In conclusion, physicians treating patients with FUO in Kazakhstan should be aware of mild CCHF.

Introduction

Crimean–Congo haemorrhagic fever (CCHF) is an acute viral tick-borne zoonotic disease caused by the Crimean–Congo haemorrhagic fever virus (CCHFV). Recently, the taxonomy of the former family *Bunyaviridae* was revised, and CCHFV is now grouped as a member of the genus *Orthonairovirus*, family *Nairoviridae*, order *Bunyavirales* (ICTV, 2017). The genome of CCHFV consists of three segments of RNA, with the large (L) segment encoding the RNA-dependent RNA polymerase, the medium (M) segment encoding two surface glycoproteins, and

* Corresponding author at: Bundeswehr Institute of Microbiology, Department Virology and Intracellular Agents, Neuherbergstr. 11, 80937 Munich, Germany.
E-mail addresses: karla.abdi@yandex.ru (K. Abdiyeva), Nurik_1976@mail.ru (N. Turebekov), am_dimitr@mail.ru (A. Dmitrovsky), tukhanovavur@gmail.com (N. Tukhanova), annashin86@gmail.com (A. Shin), elt.niifpm@kaznmu.kz (L. Yeraliyeva), heinrich@lrz.uni-muenchen.de (N. Heinrich), hoelscher@lrz.uni-muenchen.de (M. Hoelscher), regemberdieva@rambler.ru (R. Yegemberdiyeva), z.shapiyeva@gmail.com (Z. Shapiyeva), kachieva@gmail.com (Z. Kachiyeva), aliya.zhalmagambetova@giz.de (A. Zhalmagambetova), josef.Montag@gmail.com (J. Montag), gerharddobler@bundeswehr.org (G. Dobler), josuazinner@bundeswehr.org (J. Zinner), edithwagner@bundeswehr.org (E. Wagner), stefan1frey@bundeswehr.org (S. Frey), sandraessbauer@bundeswehr.org (S. Essbauer).

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the small (S) segment encoding the nucleocapsid protein (ICTV, 2017; Schmaljohn and Nichol, 2007).

The main vectors of CCHFV are ticks of the genus *Hyalomma*, thus the geographical distribution of the virus follows that of Hyalomma ticks (Hoogstraal, 1979; Ergönül, 2006; Messina et al., 2015). Presently CCHF is recognized in more than 30 countries of Asia, Southeast Europe, and Africa, as well as in the Middle East, with an emerging trend in the Balkans (Ergönül, 2006; Shayan et al., 2015; Elevli et al., 2010; Whitehouse, 2004). CCHF has a serious impact on public health and threatens the health system globally, as the disease manifests in severe haemorrhagic forms with a mortality rate ranging between 5% and 30% (Whitehouse, 2004; Ertugrul et al., 2009).

CCHF is an endemic infection in Kazakhstan, with approximately 10 clinically apparent cases registered annually; the average lethality rate between 1948 and 2013 was 14.8% (Onishchenko et al., 2005). The high density of the population in the southern parts of Kazakhstan, the favourable climate for vectors, and the migration of animals and wild birds carrying infected ticks from neighbouring areas of Uzbekistan and Tajikistan that are endemic for CCHFV, make the south of Kazakhstan favourable for CCHF (Onishchenko et al., 2005; Nurmakhanov et al., 2015). The main endemic areas in the south of Kazakhstan are the oblasts of Zhambyl and Kyzylorda, from where 119 clinically apparent cases of CCHF were registered during 2000–2013, and the Asia 1 and Asia 2 strains are the subtypes circulating in the country (Nurmakhanov et al., 2015; Knust et al., 2012; Atkinson, 2016; Yashina et al., 2003). CCHFV can also cause unspecific fever without any pathognomonic signs (Christova et al., 2013; Bodur et al., 2014), and this presentation has not yet been investigated in Kazakhstan.

The aim of this study was to investigate, for the first time, patients suffering from fever of unknown origin (FUO) to identify infection with CCHFV. The investigation was undertaken in the known endemic region of Kyzylorda oblast as well as in the non-endemic Almaty oblast in Kazakhstan.

Materials and methods

Paired serum samples (day 0, day 10–14) were collected from adult patients suffering from FUO in nine hospitals of Almaty oblast and four hospitals of Kyzylorda oblast in Kazakhstan, from April to October 2014 and 2015, during the season of tick activity (Figure 1). Patients with FUO were defined as having a temperature >37.5 °C (ear) for more than 3 days and by exclusion of rheumatic diseases. Participants completed an interview-based questionnaire consisting of 47 questions on socio-demographic characteristics, living conditions, livestock and vector habitat factors, and clinical symptoms. The recruitment, enrolment, questionnaire, sampling methods, and laboratory investigations were reviewed and approved by the ethics committees in Kazakhstan and Germany.

Blood was centrifuged and serum samples were stored in two aliquots, one at −20 °C and one at −80 °C, until use. The sera were screened for the presence of IgG and IgM antibodies against CCHFV using commercial ELISA tests (Vector-Best, Novosibirsk, Russia) according to the manufacturer's instructions. Briefly, in a first step, the second serum samples of all participants were screened for IgG by ELISA (dilution 1:100, as indicated by the kit instructions). Next, for the IgG-positive subset, the second sample was retested and the first sample was tested for IgG. First and second serum samples with an optical density (OD) difference of $\leq \pm 0.3$ were considered to indicate IgG from a previous infection. All other sera were titrated in $\log_2$ steps (1:100, 1:200, up to 1:3200). A four-fold titre change was considered to indicate acute infection. Following this, the samples for which the first serum tested IgG-negative were investigated by IgM ELISA. Lastly, IgM-negative and all IgM-positive first serum samples were further investigated by molecular methods.

The QiAmp Viral RNA Mini Kit (Qiagen, Hilden, Germany) was used for RNA extraction from 140 µl of serum, following the kit instructions. The presence of CCHFV RNA in the sera was determined by real-time reverse transcription PCR (rtRT-PCR) performed using the method of Atkinson et al. (2012) by

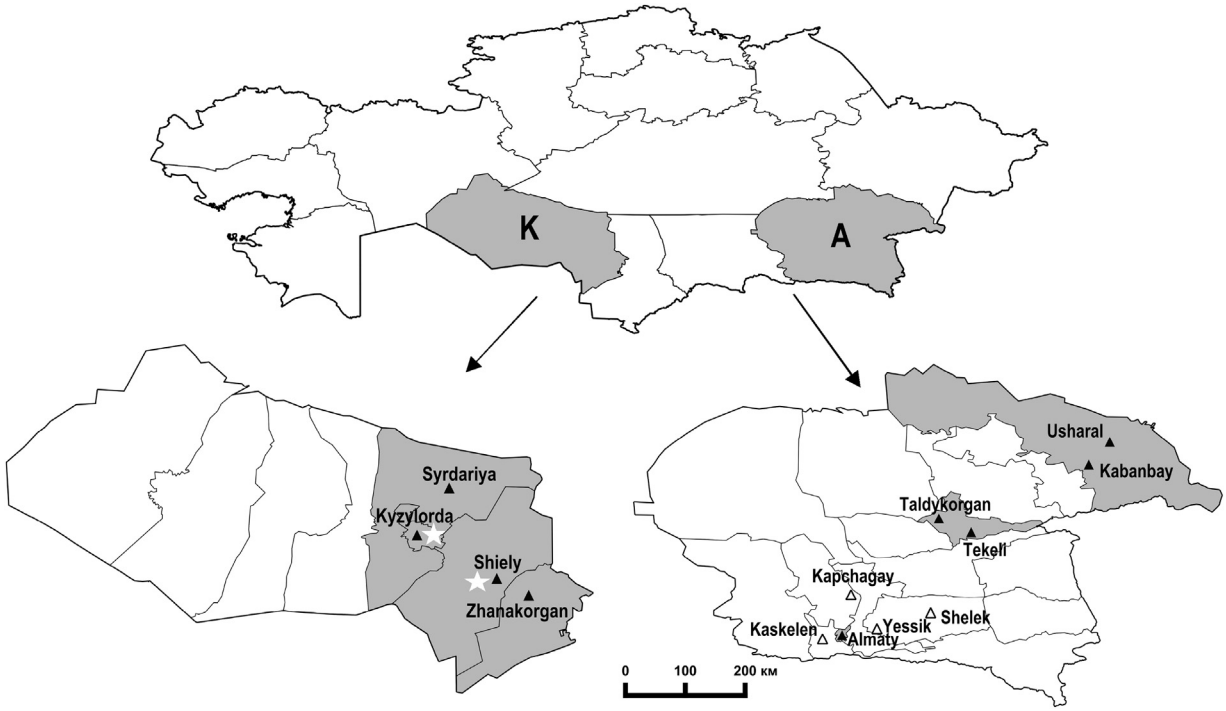

Figure 1. Crimean–Congo haemorrhagic fever antibodies in patients with fever of unknown origin investigated in the two oblasts of Almaty (A.) and Kyzylorda (K.) in Kazakhstan; regions where seropositive patients (▲), acute infections (☆), and seronegative patients (△) were found.

optimizing the protocol with the QuantiTect virus kit (Qiagen, Hilden, Germany) on a Rotor-Gene Q cycler (Qiagen, Hilden, Germany). Samples positive for viral RNA in the RT-PCR were then analyzed by CCHFV-specific RT-PCR with primers targeting the S and L segments, as described in detail previously (Woelfel et al., 2007; Filippone et al., 2013). RT-PCR products were purified and sequenced by Sanger method with the ABI Prism Big Dye Terminator V3.1 Cycle Sequencing Kit and 3500xl Genetic Analyzer machine, using the initial primers of the RT-PCR amplification. Retrieved sequences were compared with known sequences from GenBank using BLAST 2.2.32 (Zhang et al., 2000; Morgulis et al., 2008). Phylogenetic trees were constructed in MEGA 6 (Tamura et al., 2013) with the maximum likelihood method based on the Kimura two-parameter model (Kimura, 1980).

Questionnaire responses from CCHFV-positive patients were compared with those from CCHFV infection-negative participants. Statistical analyses were conducted using R 3.3.3 (R Core Team, 2013). Logistic regressions were used to evaluate the independent risk factors for seropositivity. The criterion for statistical significance was $p < 0.05$.

Results

In total, 950 patients were enrolled in the study, but only 802 patients with FUO met the criteria of having paired serum samples as well as completing the questionnaire; these 802 patients were therefore included in the study (Table 1). Among all samples tested by IgG ELISA, 102 (12.7%) showed a positive IgG result in the serum pair, indicating a recent or past infection. In total, 59 samples exhibited a difference in OD of $\leq \pm 0.3$ between the first and second serum sample, 17 serum pairs had a low IgG titre (1:100), 22 serum pairs had a medium titre (1.200–1:400), and four serum pairs had a high titre (1:800–1:3200) (Table 2).

Of all 802 samples included in the screening for acute infection using IgM ELISA, four samples (0.5%) were positive for IgM antibodies. PCR was performed on these four samples and the 14 samples with negative IgM but elevated IgG (>1:200) titres. Three of these samples (0.4%) from patients with FUO and that were positive for IgM were also positive for CCHFV RNA. The total seroprevalence in the oblast of Almaty was 11.6% (44/378), with seropositive samples originating from five of the nine hospitals (Tekeli, Taldykorgan, Usharal, Kabanbay, and Almaty City, ranging from 4.8% to 21%); the total seroprevalence in the oblast of Kyzylorda was 14.6% (62/424), with positive samples from all four hospitals (Kyzylorda, Syrdariya, Zhanakordan, and Shiely, ranging from 9.6% to 30%) (Figure 1). Out of the four IgM-positive patients, three only had a mild infection with CCHFV with a temperature >37.5 °C (ear) for more than 5 days (samples Syrdariya 6, Shiely 26, and Kyzylorda 121). One patient developed fever 5 days after a tick bite on her left shoulder. She had thrombocytopenia and leukocytopenia, and developed severe CCHF with haemorrhages 4 days after the onset of fever (sample Kyzylorda 43) (Table 3). This patient was hospitalized for 10 days and recovered. In the analyses of the partial S segment, all three detected CCHFV RNAs from Kazakhstan clustered in subgroup Asia 2. Analysis of the partial L segments of the samples of the patient with the severe form of CCHF, Kyzylorda 43, revealed a clustering in subgroup Asia 2 with strains from Tajikistan (**AY20893**, **KX013444**) and a strain obtained in 1971 from Kazakhstan (**KX01354453**). However, the partial L segment from sample Shiely 26 clustered in subgroup Asia 1 with strains from Iraq, Afghanistan, and India. Therefore, for sample Shiely 26, a reassortment between the two Asian subtypes of CCHFV is present (Figure 2).

Table 2
Results for the 102 samples that tested positive for CCHFV IgG by ELISA.[a]

Result ELISA (2nd/1st serum)	Number of samples
Low titre (≥1:100/≥1:100)[b]	59
Low titre (1:100/1:100)[c]	17
Medium titre (1:200–1:400/1:200–1:400)	22
High titre (1:800–1:3200/1:800–1:3200)	4
Total	102

CCHFV, Crimean–Congo haemorrhagic fever virus; ELISA, enzyme-linked immunosorbent assay; OD, optical density.
[a] Not including the four samples with positive IgM and from patients with acute infection.
[b] No titration, as the difference in OD was ±0.3.
[c] Titrated, as the difference in OD was >0.3.

The questionnaires showed that CCHFV-seropositive patients had only unspecific and mild symptoms such as headache (93.4%), fever (98.1%), weakness (76.4%), exanthema (31.1%), and diarrhoea (12.3%). Table 4 summarizes the results of the statistical analyses. Among the seropositive patients, the rate of seropositivity was higher in males (60.3%) than in females (39.6%). The mean ± standard deviation age of the seropositive patients was 35 ± 16 years. The median age of the seropositive patients was 30 years. Univariate logistic regression analysis (Table 4) did not identify a statistically significant difference in the prevalence of seropositivity between male and female patients. Seropositivity was also unrelated to age. Urban areas tended to have lower seropositivity than rural areas; however this result was only statistically significant at the 10% level. Regarding the risk factors, animal husbandry and occupation as a farm worker with animals increased the prevalence of seropositivity ($p < 0.05$). However, neither a history of tick bites nor the presence of birds affected seropositivity. Finally, a sub-analysis of the animal husbandry category identified that a higher frequency of contact with all animals for which data were collected (sheep, goats, cattle, horses, cats, and poultry) led to an increased prevalence of seropositivity, with the exception of pigs; however the effects were only statistically significant for cattle and horses (data not shown in detail). Comparable results were also found using univariate ordinary least squares (OLS) regression with robust standard errors.

Table 1
Results of CCHFV ELISA and RT-PCR in patients with FUO investigated in two oblasts in Kazakhstan (2014–2015).

Oblast	Number of samples tested	Number of seronegative samples	Number of IgG-positive samples (%)	Number of IgM-positive samples		Number of RT-qPCR-positive samples
				1st serum	2nd serum	
Almaty	378	334	44 (11.6)	0	0	0
Kyzylorda	424	362	58 (13.6)	1	3	3
Total	802	696	102 (12.7)	4[a]		3[a]

CCHFV, Crimean–Congo haemorrhagic fever virus; ELISA, enzyme-linked immunosorbent assay; RT-PCR, reverse transcription PCR; RT-qPCR; reverse transcription quantitative PCR; FUO, fever of unknown origin.
[a] Three samples had IgM and RNA.

Table 3
CCHFV identified by sequencing of the partial S and L segments in patients with FUO in Kyzylorda oblast in Kazakhstan (2014–2015).

Patient number	Age (years)/sex	Development of haemorrhagic fever	S segment genotype	Accession number	L segment genotype	Accession number
Shiely 26	73/M	–	Asia 2	MG974102	Asia 1	MG974103
Kyzylorda 121	17/M	–	Asia 2	MG974101	NA[a]	–
Kyzylorda 43	22/F	+	Asia 2	MG974100	Asia 2	MG974104

CCHFV, Crimean–Congo haemorrhagic fever virus; FUO, fever of unknown origin; M, male; F, female.
[a] NA = no amplification in several different L-gene RT-PCRs.

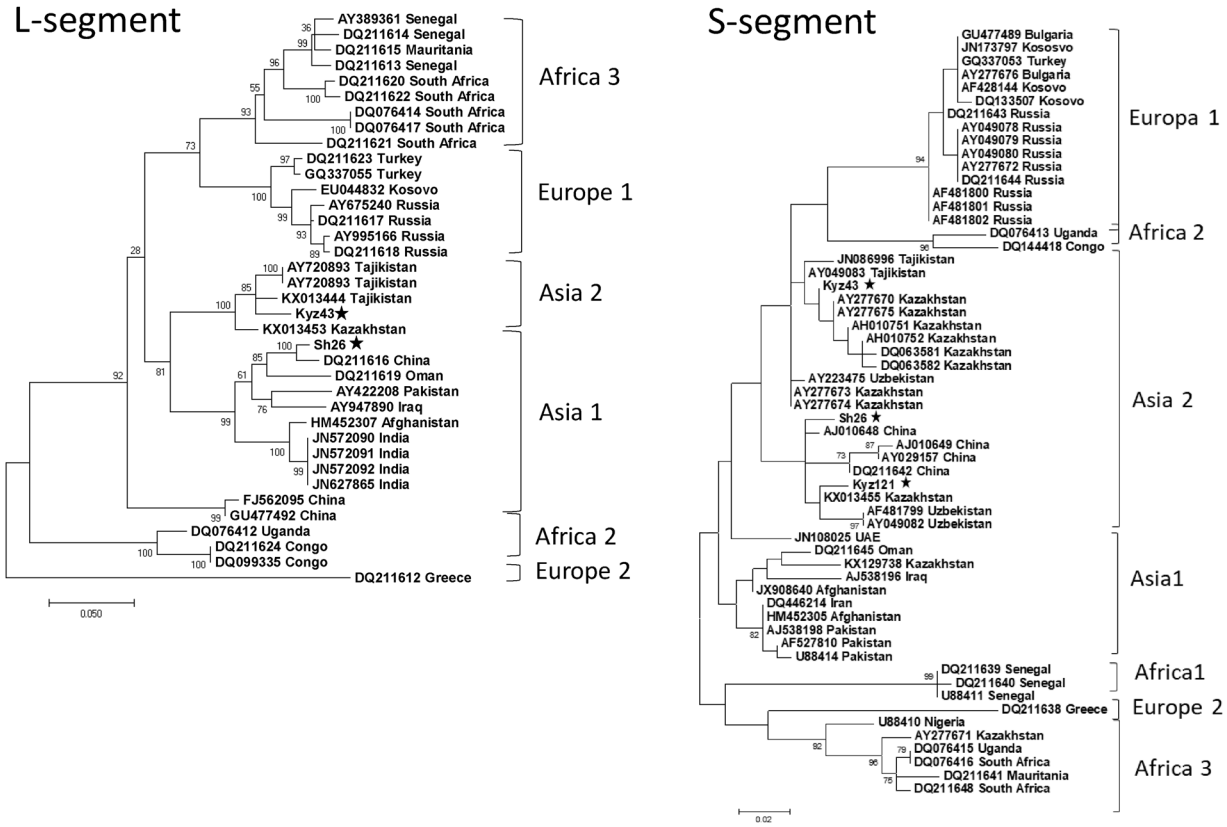

Figure 2. Phylogenetic analysis of the partial small (S, 180 nt) and large (L, 220 nt) gene fragments obtained from Crimean–Congo haemorrhagic fever virus strains analyzed in this study (★). Reference strains belong to different genogroups, as retrieved from GenBank. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Numbers next to branches indicate the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates).

Discussion

CCHFV is endemic in many regions such as Africa, parts of Eastern and Southern Europe, and Central Asia (Ergönül, 2006; Shayan et al., 2015; Elevli et al., 2010; Whitehouse, 2004). Clinically apparent moderate and severe haemorrhagic forms of CCHF have been described for approximately 70 years in the south of Kazakhstan, and official recording of clinically apparent cases of CCHF was implemented in 1965. Despite awareness of the moderate and severe haemorrhagic forms of CCHF, no accurate data exist on the burden of disease in the south of Kazakhstan or in so far non-endemic regions of the country (Onishchenko et al., 2005). The clinical pattern of CCHFV infection can differ from unspecific symptoms such as FUO to fatal haemorrhagic fever (Ergönül, 2006; Elevli et al., 2010; Ertugrul et al., 2009; Christova et al., 2013; Bodur et al., 2014). FUO is a significant public health issue as it is rarely properly diagnosed and the treatment of patients is therefore insufficient. There is a need to distinguish CCHF from other infections in the course of infection and to determine the burden of disease for preventive strategies (Christova et al., 2013; Bodur et al., 2014; Karimov et al., 1975).

This study provides serological and molecular evidence of mild forms of CCHF for the first time, with a prevalence of 12.7% (n = 102/802; Table 1) in patients with FUO investigated in two oblasts in Kazakhstan. The oblast Kyzylorda in which 13.6% seropositivity (IgG, together with IgM 14.1%) was found for samples originating from four regional hospitals, is a well-known endemic region for clinically apparent CCHF infections (Nurmakhanov et al., 2015; Knust et al., 2012; Atkinson, 2016; Yashina et al., 2003). However, the study results also showed that mild CCHFV infections with moderate and non-specific symptoms were present in patients with FUO in Kyzylorda, as well as in those presenting to five hospitals in the non-endemic Almaty oblast, including the city of Almaty. These data cannot be explained by immigration, as only individuals residing in the same oblast for the last 10 years were included and three-fourths (n = 31/40) of the patients were also born in Almaty oblast (data not shown in detail). Previous seroprevalence studies with complement fixation were performed in the 1970s in humans and animals in southern Kazakhstan and indicated that CCHFV infection might be present in southern Kazakhstan, in Kyzylorda and eventually in Almaty oblast, causing

Table 4
Characteristics of CCHFV seropositivity in patients with FUO in Kazakhstan (2014–2015).[a]

Variables	Seronegative IgG ($n = 696$)	Seropositive ($n = 106$)	Univariate logistic regression explaining seropositivity	
			Logistic (Change in OR)	OLS (Change in probability)
Sex			−0.071 ($p > 0.05$)	−0.008 ($p > 0.05$)
Male	427	64		
Female	261	42		
NA	8	0		
Age (years)			0.005 ($p > 0.05$)	0.001 ($p > 0.05$)
Mean	35	35		
SD	16	15		
History of tick bites			−0.253 ($p > 0.05$)	−0.028 ($p > 0.05$)
Yes	159	20		
No	531	86		
NA	6	0		
Animal husbandry			0.508 ($p < 0.05$)*	0.059 ($p < 0.05$)*
Yes	290	48		
No	399	58		
NA	7	0		
Birds or nests at home			0.061 ($p > 0.05$)	0.008 ($p > 0.05$)
Yes	97	19		
No	399	97		
NA/does not know	409	0		
House location			−0.364 ($p > 0.05$)	−0.042 ($p > 0.05$)
Rural area	328	60		
Urban area	362	46		
NA	6	0		
Occupation				
Student/pupil	97	17	0.446 ($p > 0.05$)	0.048 ($p > 0.05$)
Farmer/farm worker				
Plants	11	2	0.495 ($p > 0.05$)	0.186 ($p < 0.05$)
Animals	10	4	1.283 ($p < 0.05$)*	0.186 ($p < 0.05$)*
Forestry	8	1	0.063 ($p > 0.05$)	0.006 ($p > 0.05$)
Housekeeper	27	6	0.665 ($p > 0.05$)	0.079 ($p > 0.05$)
Unskilled labourer	40	8	0.579 ($p > 0.05$)	0.066 ($p > 0.05$)
Driver	20	5	0.813 ($p > 0.05$)	0.100 ($p > 0.05$)
Administration/academic professional	55	8	0.264 ($p > 0.05$)	0.027 ($p > 0.5$)
Business man/woman	17	2	0.005 ($p > 0.05$)	0.0009 ($p > 0.05$)
Nurse/physician/pharmacist	19	1	−1.089 ($p > 0.05$)	−0.085 ($p > 0.05$)
Other profession	124	14	0.013 ($p > 0.05$)	0.001 ($p > 0.05$)
Unemployed	137	24	0.443 ($p > 0.05$)	0.048 ($p > 0.05$)

CCHFV, Crimean–Congo haemorrhagic fever virus; FUO, fever of unknown origin; OR, odds ratio; OLS, univariate ordinary least squares; NA, not available; SD, standard deviation.
[a] p-Values based on heteroscedasticity-robust standard errors.
* Significant, $p < 0.05$.

moderate symptoms in humans, i.e. a mild clinical presentation of acute febrile illness with no clinical hallmark, or asymptomatic infection (Gaeta et al., 2006). The results of the present study strengthen the assumptions made in this earlier study.

The rate of seropositivity of 4.8% up to 30% found in FUO patients presenting to the 13 hospitals included in this study corresponds with previously reported rates for CCHFV endemic regions in Eastern and Southern Europe, Africa, and other parts of Asia (Ergönül, 2006; Shayan et al., 2015; Elevli et al., 2010; Ertugrul et al., 2009; Yashina et al., 2003; Christova et al., 2013; Grekova and Kamarinchev, 2014; Izadi et al., 2006). Typical signs of mild CCHFV infection are moderate fever, headache, weakness, backache, exanthema, and diarrhoea (Yashina et al., 2003; Christova et al., 2013; Grekova and Kamarinchev, 2014; Izadi et al., 2006). Most of these symptoms were also detectable in seropositive patients in the present study. According to the clinical classification in Kazakhstan, CCHF with moderate symptoms is considered the non-haemorrhagic form of CCHF. However, CCHF cases with moderate symptoms are not diagnosed, and only critical cases with the severe haemorrhagic form have been registered during the last years (Leshinskaya, 1967; N.N., 2014; Yegemberdiyeva, 2012). The results of this study showed that a certain proportion of the population (Table 2; 59 samples) had CCHFV-specific IgG antibodies in both investigated sera without any titre change. It is supposed that this could be evidence of a previous CCHFV infection in the past that was either asymptomatic or mild, as also shown in previous studies in other countries (Ergönül, 2006; Shayan et al., 2015; Elevli et al., 2010; Ertugrul et al., 2009; Yashina et al., 2003; Christova et al., 2013; Grekova and Kamarinchev, 2014; Izadi et al., 2006).

Four acute infections were detected in patients from Kyzylorda with only one developing a severe haemorrhagic form of CCHF. In the patient with acute CCHF, the disease also started with mild atypical symptoms. IgM is usually detectable at the time of infection, between day 7 and day 10. The study findings are consistent with those of previous studies in other endemic countries that have reported the detection of CCHFV-specific IgM or viral RNA and clinical manifestations of CCHF as mild

disease with moderate fever but without any haemorrhagic syndrome or asymptomatic disease, in a certain proportion of patients (Yashina et al., 2003; Christova et al., 2013; Grekova and Kamarinchev, 2014; Izadi et al., 2006; Fajs et al., 2014; Sargianou et al., 2013). As nosocomial infections have already been described in Kazakhstan (Pshenichnaya et al., 2017), hospital personnel should also be aware that mild forms or even asymptomatic forms of CCHF with viremia exist, and early diagnostic tools must be implemented in hospitals and laboratories.

Interestingly, factors that have been described previously as significantly associated with CCHF, such as older age, male sex, history of previous tick bite, and living in a rural residence (Schmaljohn and Nichol, 2007), were found not to be significantly associated with antibodies against CCHF in the present study. The main vectors of CCHFV, Hyalomma and Dermacentor ticks, favour dry climates and vegetation where rodents are abundant (Hoogstraal, 1979; Leblebicioglu, 2010). Previous investigations have shown that both tick species are widely distributed in Kazakhstan and found in several oblasts, including Kyzylorda and Almaty oblasts (Onishchenko et al., 2005; Knust et al., 2012). Furthermore, a previous study showed that reported tick bites in South Kazakhstan oblast were significantly associated with the number of CCHF cases per week (Knust et al., 2012). CCHFV was identified by antigen ELISA in 3% ($n = 3/100$ pools from 1000 ticks) of *Dermacentor niveus* in two districts of Kyzylorda in 2001 (Yashina et al., 2003). So, the vectors of CCHF are present in some areas of Kazakhstan. Further vector studies are needed in Kazakhstan to describe the natural foci of CCHFV in ticks (Yagci-Caglayik et al. (2014).

Several domestic and wild animals, e.g., cattle, goats, and sheep, transmit CCHFV. Small mammals and birds support the transmission cycle (Hoogstraal, 1979; Ergönül, 2006; Whitehouse, 2004). This study found that the rate of seropositivity was significantly higher in people with a history of animal husbandry, such as those working with sheep, goats, cattle, horses, cats, and poultry, as well as in people with an occupation of farm worker working with animals. Previous studies have also reported that people could become infected by occasional contact with livestock. There is an overlap of seroprevalence in animals and the incidence in humans (Grekova and Kamarinchev, 2014; Izadi et al., 2006). Tick densities on a single farm animal can be high in CCHF endemic regions in southern Kazakhstan during the summer, with over 1500 adults and 2–3000 nymphs (Onishchenko et al., 2005; Nurmakhanov et al., 2015). Therefore, information on CCHF should be provided to people working with sheep, goats, cattle, horses, cats, and poultry, as well as doctors in Kazakhstan.

Phylogenetic analyses of CCHFV strains indicate at least seven different clades of CCHFV. Analyses of the S segment usually mirror the geographical distribution of the strains. So far only limited data on genetic sequences of Kazakh CCHFV strains have been published, as RT-PCR is still uncommon in Kazakhstan (Onishchenko et al., 2005). The phylogenetic analyses of sequences of all three partial S segments obtained showed that they clustered within the Asia 2 subgroup, whereas in the L segment sample, Shiely 26 grouped within the Asia 1 group. Previous studies have described the presence of these two subgroups in Kazakhstan (Christova et al., 2013; Atkinson et al., 2012; Tumanova et al., 2006; Iashina et al., 2002).

The sample Shiely 26 in this study showed a reassorted genotype Asia 2 in the S segment and Asia 1 in the L segment. Interestingly, despite the general observation that the S segments of CCHFV strains were grouped according to the geographic distribution, Kazakhstan is not close to any of the other countries with the circulating Asia 1 subgroup, which include Iraq, Afghanistan, Oman, and India. However, segmented RNA viruses often undergo genetic reassortment (Chinikar et al., 2016; Greenbaum et al., 2012). Based on sequences of all three genomic fragments, it has been shown that CCHFV genomes are highly susceptible to reassortment and that the reassortment is statistically supported (Zhou et al., 2013). However, reassortment does not completely follow geographical factors, and it is suggested to be facilitated by the dispersal of different genotypes through migratory birds. Tick distribution by birds, animal trading, and local and long distance transport routes such as the new silk road and railways, could play a significant role in the transmission of the virus, and this may explain the presence of a reassorted Asia 1/Asia 2 type in Kyzylorda oblast (Whitehouse, 2004; Tumanova et al., 2006; Chinikar et al., 2015; Leblebicioglu et al., 2014).

In summary, this article reports for the first time that CCHF may appear as a mild or asymptomatic endemic infection in two oblasts of Kazakhstan. Moreover, recent CCHF infections were detected in patients with FUO in the so far non-endemic oblast of Almaty. Animal husbandry appears to be a significant factor for infection with CCHFV. The results of this study show that the distribution of CCHFV in Kazakhstan could be wider than previously thought and that physicians treating patients with FUO should also be aware of mild or asymptomatic CCHF.

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Ethical approval

The recruitment and enrolment of patients, questionnaire, sampling methods, and laboratory investigations were reviewed and approved by the ethics committees in Kazakhstan and Germany following international standards.

Disclaimer

The opinions expressed by the authors contributing to this study do not necessarily reflect the opinions of the institutes involved.

Conflict of interest

No conflict of interest to declare.

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