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Epidemiology and Phylogenetic Analysis of Crimean-Congo Hemorrhagic Fever Viruses in Xinjiang, China▽

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In 2004 and 2005, an epidemiological survey of Crimean-Congo hemorrhagic fever virus (CCHFV) was conducted in Xinjiang, China. A total of 5,629 serum samples of human and livestock were collected and tested for the CCHFV antibody, and 17,319 ticks were collected for viral identification. Reverse passive hemagglutination inhibition assays showed that the average prevalence of CCHFV antibody was 1.7% for the humans and 12.7% for the livestock. A relatively high antibody prevalence, ranging from 19.1% to 23.4%, was found in the livestock of the northwest, southwest, and northeast parts of the Tarim Basin. When the ticks were pooled to inoculate suckling mice, followed by reverse transcription-PCR (RT-PCR) to detect CCHFV RNA, the average RT-PCR-positive rates for *Hyalomma asiaticum kozlovi* and *H. asiaticum asiaticum* were 12.9% and 2.6%, respectively. A significant correlation was found between the antibody prevalence in the livestock and the CCHFV prevalence in *H. asiaticum* of the same geographic region. No CCHFV RNA was detected in *Dermacentor nivenus*, *Rhipicephalus turanius*, or *Rhipicephalus sanguineus*. A total of 27 partial S segments of CCHFVs were sequenced and used for phylogeny analysis. All but one Chinese isolate grouped into the Asia 1 clade, which contains the strains from Xinjiang and Uzbekistan, while the other strain, Fub90009, grouped with strains from the Middle East.

Crimean-Congo hemorrhagic fever virus (CCHFV) is an RNA virus that belongs to the genus *Nairovirus* of the family *Bunyaviridae*. The virus has a tripartite genome composed of a small (S), a medium (M), and a large (L) RNA segment (39). Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne disease with a mortality rate of 10% to 50% (16, 35) and has been reported in more than 30 countries in Africa, Europe, and Asia (16, 20, 26, 36, 38, 43). The potential use of CCHFV as a terrorist agent is a threat to public health (5, 10). Some genera of the family *Ixodidae* (hard ticks) transmit vectors and reservoirs of this virus (10). Humans can be infected by tick bites and interaction with infected people or animals, which may cause CCHF outbreaks in some regions (1, 9, 17, 19, 24, 27, 33). In China, the first case of CCHF was reported in Bachu county of Xinjiang in 1965 (30), and since then, there have been several outbreaks in that area (3, 4, 21, 23, 37). Several regions in the Tarim Basin, such as the Tarim River and the Yeerqiang River, and the Junggar Basin were identified as natural epidemic foci of CCHF (2, 14). So far, the phylogenetic data for CCHFV in China all relate to the western part of the Tarim Basin (Bachu county and surrounding areas) (22, 28, 37), but the geographic distribution of *Hyalomma asiaticum* (the local major vector) in Xinjiang appears to occupy a much larger area (40).

In this study, the epidemiology of CCHFV in the Tarim Basin, the Junggar Basin, the Turpan-Hami Basin, and the Ili Valley, which are the habitats of ticks, was studied. A total of 5,629 serum samples from livestock and humans living in these areas were collected and tested for antibodies against CCHFV, and 17,319 ticks belonging to five species/subspecies were collected for viral isolation. Partial sequences of the S segment were amplified and used for phylogenetic analyses. Our results revealed the geographic distribution and phylogeny of CCHFV in Xinjiang, China.

MATERIALS AND METHODS

Investigation areas and sampling. According to the geographic distribution of ticks in Xinjiang, China (40), four geographic areas (the Tarim Basin, the Junggar Basin, the Turpan-Hami Basin, and Ili Valley), including 37 counties (or cities), were investigated from 2004 to 2005 (Fig. 1). The sample collecting spots were virgin droughty deserts which have a distribution of shrubbery, livestock or other herbivores, rodents, and ticks. A total of 3,175 livestock serum samples were collected from animals pastured in the investigation spots, and 2,454 human serum samples were collected from the people who were engaged in stockbreeding or agricultural activities in these spots (see Table 1). A total of 17,319 ticks belonging to five species/subspecies were also collected from these spots.

CCHFV antibody detection in serum samples from livestock and humans. Reverse passive hemagglutination inhibition assays (RPHIA) were used to detect CCHFV antibodies in the serum samples as described previously (12). The antigen used for RPHIA was prepared with Xinjiang hemorrhagic fever standard strain 66019, which was isolated from patients with hemorrhagic fever in Bachu County, Xinjiang, in 1966 (13). Briefly, the serum samples (25 μl) were serially diluted (1:4 to 1:32) in twofold steps in phosphate-buffered saline containing 1%

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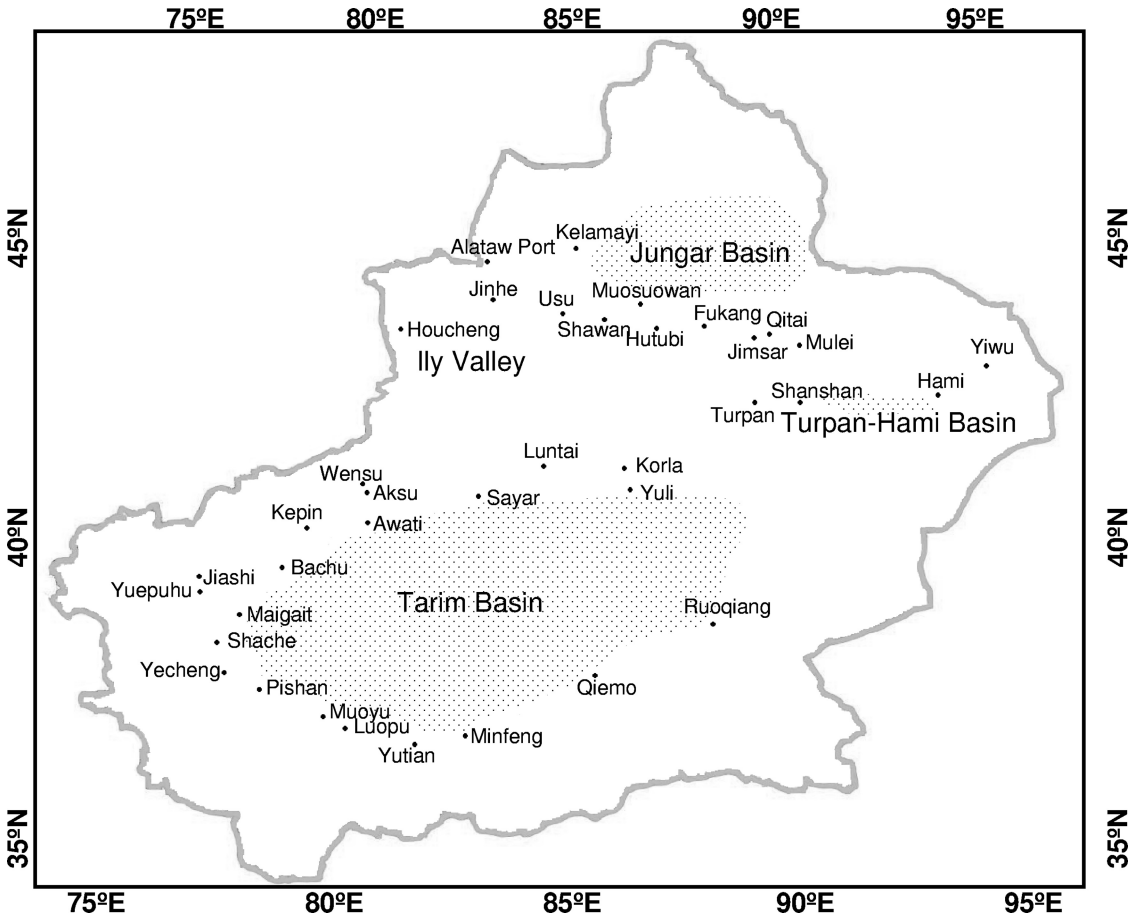

FIG. 1. Map of the Xinjiang area showing the locations of the sampling spots for the current study. The small dots indicate the sampling spots, and the names of the sampling counties are shown. The longitudes (E) and latitudes (N) are indicated on the edges of the map.

heat-inactivated normal rabbit serum in V-shaped microplates. The same amount (25 μl) of CCHFV antigen, with a hemagglutination value of 4, was added to each well. After the mixing step, the plates were incubated at 4°C overnight and then transferred to 37°C for 30 min. Twenty-five microliters of 1% sheep erythrocytes coated with a CCHFV monoclonal antibody (43E5) was added to each well. After being shaken, the plates were allowed to stand at 37°C. The hemagglutination pattern of each well was observed after 1 h. Samples with complete hemagglutination inhibition at a dilution of 1:8 or higher were chosen as being positive for the CCHFV antibody.

Virus isolation and identification. Viral isolation was done by inoculating samples into suckling mice (Kunming white mice) as described previously (12). About 50 ticks of the same species or subspecies collected at the same spot were grouped into one pool for viral isolation. Each pool of ticks was ground with Dulbecco's modified Eagle's medium (Invitrogen Corporation, China) to prepare 10% suspensions. The suspensions were centrifuged at 3,000 rpm for 20 min. The supernatant liquid (0.25 ml) was inoculated into the brain of a newborn (age 24 to 48 h) suckling mouse. The samples that caused typical symptoms at 5 to 9 days postinoculation for at least two passages in suckling mice were regarded as pathogenic. The typical symptoms of the suckling mice included becoming sensitive to stimuli, becoming easily startled, having an arched back, losing balance, and refusing to be nursed. After two to four blind passages in the brains of suckling mice, the brain tissues of the typical symptomatic mice were tested by PCR for partial sequencing of CCHFV S segments.

Sequencing and phylogenetic analyses. Total RNA from brain tissues of the typical symptomatic mice was extracted by using an SV total RNA isolation system kit (Promega), and normal mouse brain tissues were used as the negative control. About 0.1 g of the homogenized mouse brain was used for RNA extraction. An AMV reverse transcription-PCR (RT-PCR) kit (TaKaRa, Japan) was used for RT-PCR. Specific primer PCM-Tag (5′-CCGAGAATAAAATCGAG GTGAATCTCAAAGAAAT-3′) (22) and random primers (TaKaRa, Japan) were used to amplify cDNA. Primers F2 (5′-TGGACACTTTCACAA ACTC-3′) and R3 (5′-GACAAATTCCCTGCACCA-3′) were used for the first round of nested PCR, while primers F3 (5′-GAGTGTGCCTGGGTTAGTTC-3) and R2 (5′-GACATTACAATTTCACCAGG-3′) were used for the second round nested-PCR for partial sequence of the S segment (31). The PCR products were purified and sequenced by the Shanghai Sangon Company. In addition to samples obtained from 2004 to 2005, seven strains isolated earlier from the same areas were also included in this study to sequence the partial S segments (see Table 3).

The phylogeny analysis of CCFHV was conducted using the sequence data in this study as well as 25 other CCHFV S segment sequences available from GenBank (see Table 4). The S partial segment nucleotide sequence alignment was performed by CLUSTALW (version 1.83) (18), and maximum likelihood analysis with PHYLIP (version 3.67) (11) was used to construct phylogenetic trees.

Statistic analyses. The correlation of RT-PCR-positive rates for ticks to the CCHFV antibody prevalence in serum samples from different places was estimated by the Pearson product-moment correlation coefficient, and the *t* test was used to determine the significance of the correlation (34).

RESULTS

CCHFV antibody prevalence in serum samples from livestock and humans in different geographic regions. A total of 2,454 blood samples from humans, and 3,175 serum samples from sheep and camels, were collected from 27 counties (or townships) of the Tarim Basin, the Junggar Basin, and the Turpan-Hami Basin (Table 1). Antibodies against CCHFV

TABLE 1. Prevalence of the CCHFV antibody in serum samples collected by RPHIA from livestock and humans in different geographic regions in Xinjiang[a]

Geographic region	Counties	Human sera			Livestock sera		
		No. tested	No. positive	% positive (95% CI)	No. tested	No. positive	% positive (95% CI)
Tarim Basin							
Northwest	Aksu, Awati, Bachu, Jiashi, Yuepuhu	306	8	2.6 (2.31–2.89)	640	122	19.1 (17.59–20.54)
Southwest	Maigait, Shache, Yecheng, Pishan, Luopu, Yutian	167	1	0.6 (0.51–0.69)	363	85	23.4 (20.99–25.81)
Northeast	Sayar, Luntai, Yuli, Ruoqiang	486	2	0.4 (0.36–0.44)	534	115	21.5 (19.71–23.36)
Southeast	Minfeng, Qiemo	52	3	5.8 (4.22–7.38)	256	9	3.5 (2.08–3.95)
Junggar Basin	Jinghe, Kelamayi, Shawan, Muosuowan, Hutubi, Fukang, Mulei	853	24	2.8 (2.61–2.99)	720	26	3.6 (3.34–3.86)
Turpan-Hami Basin	Turpan, Hami, Yiwu	590	5	0.8 (0.74–0.86)	662	46	6.9 (6.37–7.43)
Total		2,454	43	1.7 (1.63–1.77)	3,175	403	12.7 (12.25–13.13)

[a] 95% CI, 95% confidence interval.

were detected by RPHIA in a total of 446 samples (Table 1). The average prevalence of CCHFV antibody in serum samples of humans and livestock was 1.7% and 12.7%, respectively. The CCHFV antibodies in human sera were at low levels (0.4 to 5.8%), with low range variations among different regions. A relatively high prevalence of the CCHFV antibody, ranging from 19.1 to 23.4%, was found in the livestock sera from the northwest, southwest, and northeast parts of the Tarim Basin. In contrast, a relatively low prevalence of the CCHFV antibody, ranging from 3.5 to 6.9%, was found in the livestock sera from the southeast part of the Tarim Basin, the Junggar Basin, and the Turpan-Hami Basin (Table 1). The antibody prevalence in the livestock from the northwest, southwest, and northeast parts of the Tarim Basin is significantly different from that of the southeast part of the Tarim Basin, the Junggar Basin, and the Turpan-Hami Basin (Table 1).

Investigation of CCHFV in different species of ticks in Xinjiang. From 2004 to 2005, a total of 10,436 *H. asiaticum kozlovi* specimens were collected from different areas of the Tarim Basin (Table 2). They were grouped into 209 pools and inoculated into sucking mice. Thirty-seven pools induced the typical symptoms in the inoculated suckling mice, of which 27 were positive by RT-PCR for the CCHFV S gene (Table 2). The RT-PCR-positive percentages ranged from 11.1 to 14.3%, with an average of 12.9%. The percentage of tick pools that caused typical symptoms in the inoculated mice (17.7%) was higher than the RT-PCR-positive percentage (12.9%), indicating that some of the CCHFVs might not be amplified by the primers used, or there might be other pathogens in the ticks which induced similar symptoms in the mice.

A total of 5,861 *H. asiaticum asiaticum* samples were collected from the southeastern part of the Tarim Basin, the Junggar Basin, the Turpan-Hami Basin, and the Ili Valley (Table 2). They were grouped into 114 pools and inoculated into sucking mice. Seven pools induced the typical symptoms in the inoculated suckling mice, of which three tested positive by RT-PCR for CCHFV (Table 2). The average RT-PCR-positive rate for *H. asiaticum asiaticum* (2.6%) was significantly lower than that for *H. asiaticum kozlovi* (12.9%) ($\chi^2 = 10.05$, $P = 0.03$).

A total of 1,022 other ticks, including 644 *Dermacentor nivenus* (12 pools), 327 *Rhipicephalus turanius* (7 pools), and 51 *Rhipicephalus sanguineus* (1 pool) specimens collected from the Tarim Basin and the Junggar Basin, were inoculated into suckling mice, but the typical symptoms did not appear in any of the inoculated mice.

Correlation of CCHFV antibody prevalence in livestock with CCHFV RNA prevalence in *H. asiaticum* ticks. When the Pearson product-moment correlation coefficient and the *t* test were used to determine the significance of the correlation (40), a significant correlation between RT-PCR-positive rates in ticks (*H. asiaticum kozlovi* and *H. asiaticum asiaticum*) (Table 2) and CCHFV antibody prevalence in serum samples of the livestock from different places (Table 1) was revealed ($r = 0.869$, $P = 0.025$).

Phylogenetic analysis of CCHFV in Xinjiang, China. A total of 27 partial S segments (220 bp long, corresponding to nucleotides 329 to 548 of the CCHFV Chinese strain 7001) from this study were analyzed by nested PCR (Table 3). The sequence analyses indicated that the Chinese isolates shared high similarity, with 86 to 99.5% nucleotide identity and 89 to 100% amino acid identity. Figure 2 shows the phylogenetic tree based on the partial sequences of the S segment of the 52 CCHFV sequences (Table 3 and Table 4). Our results showed that CCHFVs could be grouped into seven clades, including (i) Africa-Asia 1 (mainly from West Africa and Iran), (ii) Africa 2 (mainly from South and West Africa), (iii) Africa 3 (mainly from Uganda and West Africa), (iv) Europe (mainly from Eastern Europe, including Russia and Bulgaria), (v) Asia 1 (mainly from China and Uzbekistan), (vi) Asia 2 (mainly from Pakistan and Iraq), and (vii) Greece AP92. This result is very similar to those of previous studies (7, 8, 15, 29, 41, 42).

All but one Chinese isolate were clustered in the Asia 1 clade, which contains the Xinjiang strains from China and the strains from Uzbekistan (Fig. 2). It is interesting that the Fub90009 strain isolated from Fukang County in the Junggar Basin (14) is located in the Asia 2 clade, which contains mainly strains from the Middle East (the Matin strain and JD206 strain from Pakistan and the Baghdad12 strain from Iraq).

TABLE 2. Results of CCHFV detection from *H. asiatium* collected from different regions in Xinjiang[a]

Geographic region	County(ies)	*H. asiaticum kozlovi*					*H. asiaticum asiaticum*				
		No. of ticks	No. of pools	No. of pools pathogenic to mice	No. of RT-PCR-positive pools	% RT-PCR-positive (95% CI)	No. of ticks	No. of pools	No. of pools pathogenic to mice	No. of RT-PCR-positive pools	% RT-PCR-positive rate (95% CI)
Tarim Basin											
Northwest	Aksu, Wensu, Awati, Kepin, Bachu, Yuepuhu	3,155	63	12	9	14.3 (10.8–17.8)	0				
Southwest	Maigait, Shache, Yecheng, Pishan, Moyu, Luopu, Yutian	1,754	36	7	4	11.1 (7.5–14.7)	0				
Northeast	Sayar, Luntai, Korla, Yuli, Ruoqiang	5,527	110	18	14	12.7 (10.3–15.1)	0				
Southeast	Minfeng, Qiemo	0					3,452	68	4	1	1.5 (1.1–1.8)
Junggar Basin	Alataw Port, Usu, Kelamayi, Muosuowan, Hutubi, Jimsar, Qitai, Mulei	0					1,769	36	3	2	5.6 (3.7–7.4)
Turpan- Hami Basin	Turpan, Shanshan, Hami, Yiwu	0					590	12	0	0	0
Ili Valley	Houcheng	0					50	1	0	0	0
Total		10,436	63	37	27	12.9 (11.2–14.7)	5,861	117	7	3	2.6 (2.1–3.0)

[a] 95% CI, 95% confidence interval.

TABLE 3. Xinjiang CCHFV strains sequenced in the present study

Region	Strain	County	Origin	Year	GenBank accession no.
Northwest Tarim	Aw04318	Awati	*H. asiaticum kozlovi*	2004	EU715264
	Ba66063	Bachu	*H. asiaticum kozlovi*	1966	EU715257
	Ba68038	Bachu	Human	1968	EU715258
	Hf7403	Bachu	Human	1978	EU715259
	Ba8002	Bachu	Human	1980	EU715261
	Ba8004	Bachu	Human	1980	EU715262
	Ba04005	Bachu	*H. asiaticum kozlovi*	2004	EU715265
	Ba04203	Bachu	*H. asiaticum kozlovi*	2004	EU715266
	Ba05108	Bachu	*H. asiaticum kozlovi*	2005	EU715277
Southwest Tarim	Lp04224	Luopu	*H. asiaticum kozlovi*	2004	EU715271
	Yt05093	Yutian	*H. asiaticum kozlovi*	2005	EU715285
	Yt05099	Yutian	*H. asiaticum kozlovi*	2005	EU715286
Northeast Tarim	Bz002	Bazhou	Human	1979	EU715260
	Lt04035	Luntai	*H. asiaticum kozlovi*	2004	EU715267
	Lt05146	Luntai	*H. asiaticum kozlovi*	2005	EU715279
	Yl04032	Yuli	*H. asiaticum kozlovi*	2004	EU715273
	Yl04033	Yuli	*H. asiaticum kozlovi*	2004	EU715274
	Yl04038	Yuli	*H. asiaticum kozlovi*	2004	EU715275
	Yl04041	Yuli	*H. asiaticum kozlovi*	2004	EU715276
	Yl05034	Yuli	*H. asiaticum kozlovi*	2005	EU715283
	Yl05035	Yuli	*H. asiaticum kozlovi*	2005	EU715284
	Rq04219	Ruoqiang	*H. asiaticum kozlovi*	2004	EU715270
	Rq04244	Ruoqiang	*H. asiaticum kozlovi*	2004	EU715272
Southeast Tarim	Qm04222	Qeimuo	*H. asiaticum asiaticum*	2004	EU715269
Junggar Basin	Fub90009	Fukang	*H. asiaticum asiaticum*	1990	EU715263
	Ml05225	Mulei	*H. asiaticum asiaticum*	2005	EU715280
	Ml05232	Mulei	*H. asiaticum asiaticum*	2005	EU715281

DISCUSSION

Geographic distribution of CCHFV in Xinjiang, China. Since the first report of CCHF in Bachu County in 1965, a greater number of CCHFV samples have been isolated from *H. asiaticum*, suggesting that it is the major vector and reservoir of CCHFV in this region (2, 14). By combining historical survey data (14, 6, 43) and our current data, it is clear that CCHFV is distributed widely in Xinjiang.

The Tarim Basin (approximately 37° to 42°N, 77° to 89°E), which occupies the center of South Xinjiang, with an area of 560,000 square kilometers, is surrounded by mountains that block the southern warm wet air and the cold air of Siberia, causing extreme drought in the region. The snow from the mountains melts into the rivers and creates oases, but the area where rivers cannot reach remains a barren desert. The relatively isolated oases in the Tarim Basin provide habitats for wild and domestic animals as well as ticks. Our recent study indicated that there are two geographic subspecies of *H. asiaticum* in the Tarim Basin, *H. asiaticum kozlovi* and *H. asiaticum asiaticum* (44). The distribution and epidemiology of CCHFV in the Tarim Basin appeared to be related to the distribution of the two geographic subspecies of *H. asiaticum*. In the northwestern, southwestern, and northeastern parts of the Tarim Basin, *H. asiaticum kozlovi* is the main species and accounts for 66.9 to 92% of the tick population (44). The rates of CCHFV antibody-positive serum samples from livestock were 19.1 to 23.4% (Table 1). This result is consistent with the relatively high prevalence of CCHFV (11.1 to 14.3%) in the ticks collected in these regions (Table 2). In the southeastern part of the Tarim Basin, *H. asiaticum asiaticum* was the main species and accounted for 72.6% of the tick population (44). The CCHFV antibody-positive rate in the serum of livestock in the southeast Tarim Basin was 3.5% (Table 1), which is consistent with the relatively low CCHFV prevalence (1.5%) for the ticks collected in this region (Table 2). Our current results indicate that the correlation of CCHFV antibody prevalence in the livestock with CCHFV RNA prevalence in *H. asiaticum* ticks of the same geographic region is significant.

The landscape and ecology of the Junggar Basin (approximately 44° to 47°N, 82° to 92°E) are quite different from those of the Tarim Basin. In the Junggar Basin, plants are widely distributed in much greater quantity and at a much higher density than those of the Tarim Basin. Wild animals and ticks are also widely distributed. Like the southeast part of the Tarim, *H. asiaticum asiaticum* is the main tick species of the Junggar Basin. Likewise, the CCHFV antibody-positive rate in the serum of livestock in the Junggar Basin was 3.6% (Table 1), which is consistent with the relatively low CCHFV prevalence (5.6%) of the ticks collected in this region (Table 2).

The Turipan-Hami Basin (approximately 42° to 42°40′N, 88° to 94°E) located in east Xinjiang. Although CCHFV-positive antibodies were identified from the serum samples from human and livestock in this region (Table 1), we were unable to isolate CCHFV from the ticks (Table 2). Whether this region is a natural focus of CCHFV remains to be further investigated.

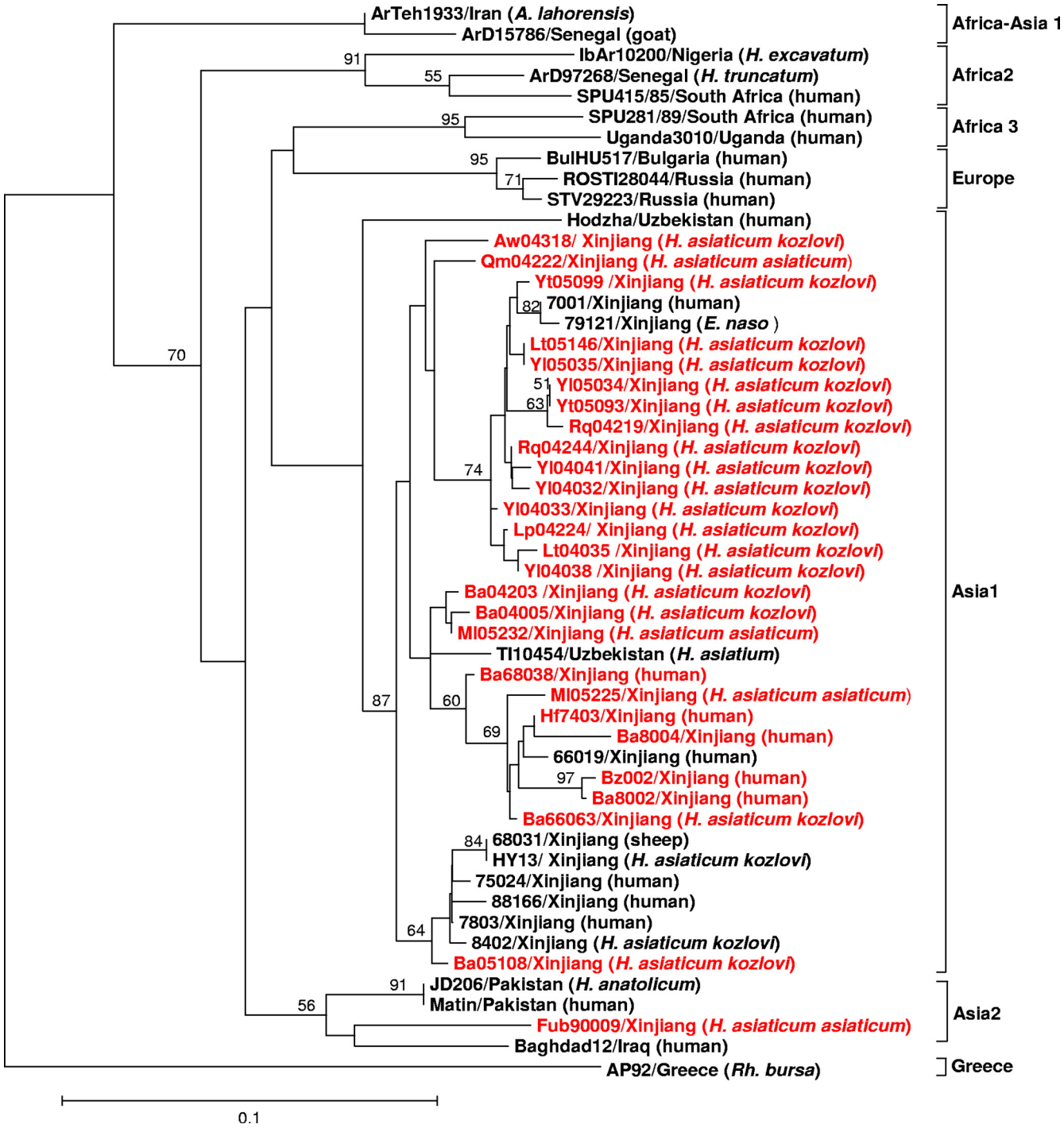

FIG. 2. Phylogenetic tree of CCHFVs based on the 220-nucleotide S RNA sequences. The tree was constructed by using the maximum likelihood method with PHYLIP. The sequences obtained from this study are shown in red. The numbers above the branches indicate the bootstrap values in percentages (of 100 replicates). Values lower than 50% are not shown. The scale bar indicates 10% nucleotide sequence divergence.

In this study, only one sample site (Houcheng) belongs to the Ily Valley, and no human or livestock sera were collected there. Therefore, the data are too preliminary to draw any conclusions about the region.

Historically, CCHF cases have been reported from around the Bachu region (Bachu, Awati, Jiashi, Kepin, Kuche, and Maigait) in Xinjiang (3, 4, 21, 23, 30, 32, 37), and CCHFVs have been isolated mainly from that region (14, 22, 28, 37). Our results indicate that CCHFV is likely to be distributed over a much larger area in Xinjiang. The isolates Ml05225 and Ml05232 (Table 3), collected from Mulei (43°55′N, 90°25′E) in this study, to our knowledge, are the easternmost isolates to be reported so far.

TABLE 4. The 25 CCHFV isolates obtained from GenBank and used for phylogeny analysis

Strain	Region	Origin	Year	GenBank accession no.
66019	Bachu, China	Human	1966	AJ010648
68031	Bachu, China	Sheep	1968	DQ211642
7001	Aksu, China	Human	1970	AF415236
75024	Bachu, China	Human	1975	AF362080
7803	Bachu, China	Human	1978	AF354296
79121	Bachu, China	*Euchoreutes* naso	1979	AF358784
8402	Bachu, China	*H. asiaticum kozlovi*	1984	AJ010649
88166	Bachu, China	Human	1988	AY029157
AP92	Greece	*Phipicephalus bursa*	1976	DQ211638
ArD97268	Senegal	*H. truncatum*	1993	U15091
ArD15786	Senegal	Goat	1973	U15020
ArTeh1933	Iran	*Alectorobius lahorensis*	1978	U15022
Baghdad 12	Iraq	Human	2000	AJ538196
Bul/Hu517	Bulgaria	Human	1978	AY277676
HY13	Bachu, China	*H. asiaticum kozlovi*	1968	AY900145
Hodzha	Uzbekistan	Human	1967	AY223475
IbAr10200	Nigeria	*H. excavatum*	1966	U75674
JD206	Pakistan	*H. anatolicum*	1965	U88414
Matin	Pakistan	Human	1976	AF527810
ROS/TI28044	Rostov, Russia	*H. marginatum*	2000	AY277672
SPU415/85	South Africa	Human	1985	DQ211648
SPU281/89	South Africa	Human	2005	AY905637
STV/HU29223	Stavropol, Russia	Human	2000	AF481802
Uganda3010	Uganda	Human	1956	U88416
Uzbek/TI10145	Uzbekistan	*H. asiaticum*	2000	AF481799

No significant correlation between CCHFV antibody prevalence in humans with CCHFV prevalence in the ticks of the same geographic region was identified. This may be due to the different lifestyles of the people and/or to differences in our sample population. For example, the human samples of the southeast Tarim were mainly from herders or butchers, while human samples from other areas include not only herders or butchers but also people involved in other agriculture activities. This may partially explain why the CCHFV antibody prevalence was relatively high in samples from southeast Tarim compared to those from other regions (Table 1).

Phylogenetic analyses of CCHFVs in Xinjiang, China. There have been several genetic analyses of CCHFVs isolated from Xinjiang (22, 25, 28, 37); however, all the reported Xinjiang strains were isolated from the area around Bachu County (at the western part of the Tarim Basin) from the 1960s to the 1980s. In this study, 27 CCHFV strains isolated from different geographic regions (the Tarim Basin and the Junggar Basin) in Xinjiang were partially sequenced (Table 3). Together with the previously published CCHFV data (Table 4), a phylogenetic tree with more information on Chinese isolates is presented (Fig. 2). The phylogeny analysis reveals that all but one Chinese isolate (Fub90009) belong to one clade, Asia 1 (Fig. 2), regardless of their origin (different tick species, humans, or animals). The Xinjiang isolates grouped with the isolates from Uzbekistan, indicating that the virus circulating in Xinjiang and Uzbekistan are genetically related, consistent with previous reports (37, 41). However, the Fub90009 strain, which was isolated from the Junggar Basin, was grouped into another clade (Asia 2) with Iraq, Pakistan, and other Central Asian strains (Fig. 2). Xinjiang has a geographical location and environmental climate similar to those of Central Asian countries. The migration of virus-infected birds or tick-infected birds may contribute to the spread of CCHFV (16, 39). In addition, frequent livestock trading and economic and cultural exchanges could also cause the spread of CCHFV. Our results indicate that the CCHFVs in Xinjiang may have multiple origins.

It has been shown that although phylogenetic analyses based on S and L segments generate similar results, M segments often show greater diversity, which could lead to a better-resolved phylogenetic tree (7). Morikawa et al. (25) previously sequenced M segments of 7 CCHFV isolates around Bachu County, and their results indicated that there is a multisource virus population in that region. At the moment, we are focusing on sequencing more M segments to further reveal the evolutionary history of CCHFV in Xinjiang.

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REFERENCES

1. **Altaf, A., S. Luby, A. J. Ahmed, N. Zaidi, A. J. Khan, S. Mirza, J. McCormick, and S. Fisher-Hoch.** 1998. Outbreak of Crimean-Congo haemorrhagic fever in Quetta, Pakistan: contact tracing and risk assessment. Trop. Med. Int. Health **3:**878–882.
2. **Chai, J.** 2004. The preliminary investigation report on geographical distribution of Xinjiang hemorrhagic fever in upper and middle reach of Tarim River in Xinjiang in 1968. Endemic Dis. Bull. **19**(Suppl.)**:**34–36.
3. **Chai, J., Y. Han, C. Feng, Y. Zhang, Y. Nuer, Y. Liu, Y. Zhang, Y. Qiao, and H. Li.** 2004. Report of investigation on hemorrhagic fever in Bachu County, Xinjiang in 1966. I. Clinical observation on 5 cases with hemorrhagic fever. Endemic Dis. Bull. **19**(Suppl.)**:**1–5.
4. **Chai, J., Y. Nuer, C. Feng, G. Li, and J. Liu.** 2004. Hemorrhagic fever in Bachu: two case reports. Endemic Dis. Bull. **19**(Suppl.)**:**23–25.
5. **Charrel, R. N., H. Attoui, A. M. Butenko, J. C. Clegg, V. Deubel, T. V. Frolova, E. A. Gould, T. S. Gristsun, F. X. Heinz, M. Labuda, V. A. Lashkevich, V. Loktev, A. Lundkvist, D. V. Lvov, C. W. Mandl, M. Niedrig, A. Papa, V. S. Petrov, A. Plyusnin, S. Randolph, J. Süss, V. I. Zlobin, and X. de Lamballerie.** 2004. Tick-borne virus diseases of human interest in Europe. Clin. Microbiol. Infect. **10:**1040–1055.
6. **Dai, X., A. Muhtar, C. H. Feng, R. Sun, X. P. Tai, X. H. Wang, K. Burenmind, W. W. Meng, R. Azat, and Y. J. Zhang.** 2006. Geography and host distribution of Crimean-Congo hemorrhagic fever in the Tarim Basin. Zhonghua Liu Xing Bing Xue Za Zhi **27:**1048–1052.
7. **Deyde, V. M., M. L. Khristova, P. E. Rollin, T. G. Ksiazek, and S. T. Nichol.** 2006. Crimean-Congo hemorrhagic fever virus genomics and global diversity. J. Virol. **17:**8834–8842.
8. **Drosten, C., D. Minnak, P. Emmerich, H. Schmitz, and T. Reinicke.** 2002. Crimean-Congo hemorrhagic fever in Kosovo. J. Clin. Microbiol. **40:**1122–1123.
9. **Engin, A., A. Yildirim, T. Kunt, M. Bakir, I. Dokmetas, and L. Ozdemir.** 2008. Clinical investigation of the transient evoked otoacoustic emission test in Crimean-Congo hemorrhagic fever. Int. J. Infect. Dis. **12:**162–165.
10. **Ergonul, O.** 2006. Crimean-Congo hemorrhagic fever. Lancet Infect. Dis. **6:**203–214.
11. **Felsenstein, J.** 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39:**783–791.
12. **Feng, C.** 2004. Laboratory assays applied for diagnosis of Xinjiang hemorrhagic fever. Endemic Dis. Bull. **19**(Suppl.)**:**101–116.
13. **Feng, C., J. Chai, Y. Han, and H. Li.** 2004. Report of investigation on

hemorrhagic fever in Bachu County, Xinjiang in 1966. II. A preliminary study on etiology of hemorrhagic fever in Bachu. Endemic Dis. Bull. **19**(Suppl.)**:**6–14.
14. **Feng, C. H., X. Bai, H. Liu, F. Li., and Y. Gu.** 1991. Discovery of natural foci of Xinjiang hemorrhagic fever in the southern margin area of Junggar Basin, Xinjiang. Endemic Dis. Bull. **6:**52–55.
15. **Hewson, R., J. Chamberlain, V. Mioulet, G. Lloyd, B. Jamil, R. Hasan, A. Gmyi, L. Gmyi, S. E. Smirnova, A. Lukashev, G. Karganova, and C. Clegg.** 2004. Crimean-Congo haemorrhagic fever virus: sequence analysis of the small RNA segments from a collection of viruses world wide. Virus Res. **102:**185–189.
16. **Hoogstraal, H.** 1979. The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. J. Med. Entomol. **15:**307–417.
17. **Jauréguiberry, S., P. Tattevin, A. Tarantola, F. Legay, A. Tall, P. Nabeth, H. Zeller, and C. Miachelet.** 2005. Imported Crimean-Congon hemorrhagic fever. J. Clin. Microbiol. **43:**4905–4907.
18. **Jeanmougin, F. J., D. Thompson, M. Gouy, D. G. Higgins, and T. J. Gibson.** 1998. Multiple sequence alignment with Clustal X. Trends Biochem. Sci. **23:**403–405.
19. **Joubert, J. R., J. B. King, D. J. Rossouw, and R. Cooper.** 1985. A nosocomial outbreak of Crimean-Congo haemorrhagic fever at Tygerberg Hospital. Part III. Clinical pathology and pathogenesis. S. Afr. Med. J. **68:**722–728.
20. **Khan, A. S., G. O. Maupin, P. E. Rollin, A. M. Noor, H. H. Shurie, A. G. Shalabi, S. Wasef, Y. M. Haddad, R. Sadek, K. Ijaz, C. J. Peters, and T. G. Ksiazek.** 1997. An outbreak of Crimean-Congo hemorrhagic fever in the United Arab Emirates, 1994-1995. Am. J. Trop. Med. Hyg. **57:**519–525.
21. **Liu, Y., J. Chai, C. Xiao, and W. Li.** 2004. Epidemiological analysis of 140 cases with Xinjiang hemorrhagic fever. Endemic Dis. Bull. **19**(Suppl.)**:**47–49.
22. **Ma, B., C. Hang, and A. Papa.** 2001. 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 **15:**105–111.
23. **Ma, B., and H. Wang.** 2000. Tianshan hacker: Crimean-Congo hemorrhagic fever virus. Foreign Med. Sci. **7:**8–10.
24. **Midilli, K., A. Gargili, O. Ergonul, G. Sengoz, R. Ozturrk, M. Bakar, and F. Jongejan.** 2007. Imported Crimean-Congo hemorrhagic fever cases in Istanbul. BMC Infect. Dis. **54:**1–6.
25. **Morikawa, S., T. Qing, Z. Xinqin, M. Saijo, and I. Kurane.** 2002. Genetic diversity of the M RNA segment among Crimean-Congo hemorrhagic fever virus isolates in China. Virology **296:**159–164.
26. **Morikawa, S., M. Saijio, and I. Kurane.** 2007. Recent progress in molecular biology of Crimean-Congo hemorrhagic fever. Comp. Immunol. Microbiol. Infect. Dis. **30:**375–389.
27. **Papa, A., I. Christova, E. Papadimitriou, and A. Antoniadis.** 2004. Crimean-Congo hemorrhagic fever in Bulgaria. Emerg. Infect. Dis. **10:**1465–1467.
28. **Papa, A., B. J. Ma, S. Kouidou, Q. Tang, C. S. Hang, and A. Antoniadis.** 2002. Genetic characterization of the M RNA segment of Crimean Congo hemorrhagic fever virus strains, China. Emerg. Infect. Dis. **8:**50–53.
29. **Petrova, I. D., S. V. Seregin, V. S. Petrov, O. I. Vyshemirskii, I. I. Kuzina, D. K. Lvov, E. I. Samokhvalov, G. I. Tyummikov, V. V. Gutorov, L. N. Iashina, and S. V. Netesov.** 2003. 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. **48:**8–11. (In Russian.)
30. **Region HaEPSoXUA.** 1975. Compilation of information on Xinjiang haemorrhagic fever. Health and Epidemic Prevention Station of Xinjiang Uygur Autonomous Region, Urumqi, China.
31. **Rodriguez, L. L., G. O. Maupin, T. G. Ksiazek, P. E. Rollin, A. S. Khan, T. F. Schwarz, R. S. Lofts, J. F. Smith, A. M. Norr, C. J. Peters, and S. T. Nichol.** 1997. Molecular investigation of a multisource outbreak of Crimean-Congo hemorrhagic fever in the United Arab Emirates. Am. J. Trop. Med. Hyg. **57:**512–518.
32. **Saijo, M., Q. Tang, B. Shimayi, L. Han, Y. Zhang, M. Asiguma, T. Dong, A. Maeda, and I. Kurane.** 2004. Possible horizontal transmission of Crimean-Congo hemorrhagic fever virus from a mother to her child. Jpn. J. Infect. Dis. **57:**55–57.
33. **Schwarz, T. F., H. Nsanze, M. Longson, H. Nitschko, S. Gilch, H. Shurie, A. Ameen, A. R. Zahir, U. G. Acharya, and G. Jager.** 1996. 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. **55:**90–96.
34. **SPSS, Inc.** 2003. SPSS 12.0 for Windows user's guide. SPSS, Inc., Chicago, IL.
35. **Swanepoel, R., A. J. Shepherd, P. A. Leman, S. P. Shepherd, G. M. McGillivray, M. J. Erasmus, L. A. Searle, and D. E. Gill.** 1987. Epidemiologic and clinical features of Crimean-Congo hemorrhagic fever in southern Africa. Am. J. Trop. Med. Hyg. **36:**120–132.
36. **Swanepoel, R., J. K. Struthers, A. J. Shepherd, G. M. McGillivray, M. J. Nel, and P. G. Jupp.** 1983. Crimean-congo hemorrhagic fever in South Africa. Am. J. Trop. Med. Hyg. **32:**1407–1415.
37. **Tang, Q., X. Q. Zhao, H. Y. Wang, B. Simayi, Y. Z. Zhang, M. Saijo, M. Shigeru, G. D. Liang, and I. Kurane.** 2005. Molecular epidemiology of Xinjiang hemorrhagic fever viruses. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi **19:**312–318.
38. **Tikriti, S. K., F. K. Hassan, I. M. Moslih, F. Jurji, M. I. Mahmud, and H. H. Tantawi.** 1981. Congo/Crimean hemorrhagic fever in Iraq: a seroepidemiological survey. J. Trop. Med. Hyg. **84:**117–120.
39. **Whitehouse, C. A.** 2004. Crimean-Congo hemorrhagic fever. Antivir. Res. **64:**145–160.
40. **Xin, Y., Y. Rui, and G. Zheng-Da.** 1997. The tick fauna of Xinjiang, p. 3-8. Xinjiang Scientific, Technological and Medical Publishing House, Urumqi, China.
41. **Yashina, L., I. Petrova, S. Seregin, O. Vyshemirskii, D. Lvov, V. Aristova, J. Kuhn, S. Morzunnov, V. Gutorov, I. Kuzina, G. Tyunnikov, S. Netesov, and V. Petrov.** 2003. Genetic variability of Crimean-Congon hemorrhagic fever virus in Russia and Central Asia. J. Gen. Virol. **84:**1199–1206.
42. **Yashina, L., O. Vyshemirskii, S. Seregin, I. Petrova, E. Samokhvalov, D. Lvov, V. Gutorov, I. Kuzina, G. Tyunnikov, Y. W. Tang, S. Netesov, and V. Petrov.** 2003. Genetic analysis of Crimean-Congon hemorrhagic fever virus in Russia. J. Clin. Microbiol. **41:**860–862.
43. **Yen, Y. C., L. X. Kong, L. Lee, Y. Q. Zhang, F. Li, B. J. Cai, and S. Y. Gao.** 1985. Characteristics of Crimean-Congo hemorrhagic fever virus (Xinjiang strain) in China. Am. J. Trop. Med. Hyg. **34:**1179–1182.
44. **Zhang, Y.-J., H.-L. Cao, X. Dai, M. Azaz, W. Jiang, A. Abulikm, B. Li, M. Ablimiti, G. Lei, A. Rezwan, X.-H. Liang, H.-B. Liu, X. Yu, and C.-H. Feng.** 2006. Classification and diversity of tick community in Tarim Basin. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi **24:**6–11.