Short Communication

Seroprevalence of Crimean-Congo Hemorrhagic Fever in Southeastern Bulgaria

Ivanka Gergova¹ and Bozhin Kamarinchev^{1*}

¹Department of Military Epidemiology and Hygiene, Military Medical Academy, Sofia, 1606, Bulgaria

(Received February 3, 2014. Accepted March 25, 2014)

SUMMARY: Crimean-Congo hemorrhagic fever (CCHF), which is endemic in Bulgaria, is caused by the Crimean-Congo hemorrhagic fever virus (CCHFV). The seroprevalence of CCHFV in southeastern Bulgaria was examined in this study. For this purpose, a total of 751 human blood samples were collected and examined by indirect immunofluorescence assay. In addition, a questionnaire was completed for every participant. Anti-CCHFV antibodies were detected in 3.20% (24/751) of the tested sera. None of the seropositive individuals had a history of CCHF. The results indicate that the proportion of positive findings increase with age. The significant risk factors for CCHFV infection are tick bites (18.85%, 23/122), livestock breeding (6.15%, 16/260), and residing in rural areas (6.20%, 21/339).

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne disease recognized in humans by the severe hemorrhagic fever and caused by Crimean-Congo hemorrhagic fever virus (CCHFV, genus *Nairovirus*, family *Bunyaviridae*) (1). CCHF was first described in Bulgaria in 1952, and it became a reportable disease in 1953 (2). Over 1600 CCHF cases were registered from 1953 to 2013 (as reported by the Bulgarian Ministry of Health), and the country has been considered an endemic region for CCHF. However, systematic research on the seroprevalence of CCHFV in Bulgaria has not been performed from the late 70s until 2010.

This study determined the seroprevalence of CCHF in southeastern Bulgaria from 28 locations previously described as endemic (within the borders of 3 governmental administrative regions—Kardzhali, Haskovo, and Stara Zagora, which included 5, 9, and 14 locations, respectively), and the risk factors for CCHF infection were assessed. These regions are found along the border of Turkey, where several cases of CCHF are reported annually. A total of 751 blood samples were collected from the general population from 2011 to 2012. Children (<10 years) were excluded because of difficulties associated with drawing blood. Participants previously vaccinated against CCHFV were also excluded from the tested group. The research team explained the objectives of the study and asked for written informed consent from the participants; an interview-based questionnaire was then provided, which included the following variables: age, sex, living environment, livestock breeding, history of tick bites, and occupation. Blood samples (10 mL each) were serologically tested for antibodies to CCHFV by an in-house assay of indirect immunofluorescence, which was performed as described in a previous study (3). Data was analyzed using Statistical Package for Social Scientists (SPSS) version 13. Chi-square and Fisher's exact tests were employed for comparing categorical variables. Statistical significance was

defined as a *P* value of ≤ 0.05 .

This study was designed to determine the seroprevalence of CCHFV in endemic areas of Bulgaria. The established seroprevalence was 3.20% (24/751), which corresponded to the levels of anti-CCHFV antibodies documented in other regions of the country (4). Moreover, the results obtained showed a correlation with the number of cases reported from these regions, the seroprevalence of CCHFV among livestock, and the levels of CCHFV infestation among *Hyalomma marginatum marginatum* (the main vector of CCHFV in the country) (5). None of the seropositive participants had a history or any clinical indications of CCHF, suggesting that these seropositive participants probably experienced subclinical infection. According to a previous study, in epidemic situations, the expected seroprevalence is approximately 10% (6), which is much higher than the observed seroprevalence in the present study. In a survey conducted in endemic regions, the rate of seropositivity was significantly lower (1.26%) (7) than that of those in this study. Therefore, in the present study, the estimated seroprevalence of CCHFV was found to be lower than expected for an epidemic situation but similar to the levels of seropositivity in other endemic regions.

The characteristics of seropositive participants were compared with those of the seronegative ones (Table 1). In the present study, a history of tick bites, livestock breeding, and residence in rural areas were found to be significantly associated with CCHFV seropositivity. The overall frequency of tick bites among the participants was 16.25% (122/751), but 23 of 24 (96.00%) seropositive participants reported a history of tick bites, whereas the only remaining participant (1/24, 4.17%) failed to recall the same. Therefore, history of tick bites appears to be the most important risk factor for CCHF ($P < 0.001$). An association was also discovered between the seroprevalence of CCHFV and livestock breeding ($P = 0.026$); the rate of seropositivity among breeders of livestock was 6.15% (16/260), whereas the seroprevalence among other participants was only 1.63% (8/491). Similar observations of increased levels of seropositivity associated with livestock breeding has

*Correspondence author: Mailing address: Department of Military Epidemiology and Hygiene, Military Medical Academy, Georgi Sofiysky blvd. 3, 1606 Sofia, Bulgaria. Tel: +359 885 837 602, E-mail: kamarinchev.b@abv.bg

Table 1. Demographics and CCHFV seroprevalence in southeastern Bulgaria

Variable	Seropositive ¹⁾ <i>n</i> = 24	Seronegative ¹⁾ <i>n</i> = 727	Total ¹⁾ <i>n</i> = 751	CCHFV seroprevalence ¹⁾
Gender				
Male	18 (75.00)	350 (48.14)	368 (49.00)	18/368 (4.89)
Female	6 (25.00)	377 (51.86)	383 (51.00)	6/383 (1.57)
Age, (yr)				
10–20	0 (0)	1 (0.14)	1 (0.13)	0/1 (0)
21–30	0 (0)	37 (5.09)	37 (4.93)	0/37 (0)
31–40	1 (4.17)	10 (14.17)	104 (13.85)	1/104 (0.96)
41–50	2 (8.33)	153 (21.05)	155 (20.64)	1/155 (1.29)
51–60	6 (25.00)	218 (29.99)	224 (29.83)	6/224 (2.68)
61–70	10 (41.67)	161 (22.15)	171 (22.77)	10/171 (5.85)
>70	5 (20.83)	54 (7.43)	59 (7.85)	5/59 (8.48)
Living environment				
In rural area	21 (87.50)	318 (43.74)	339 (45.14)	21/339 (6.20)
In urban area	3 (12.50)	409 (56.26)	412 (54.86)	3/412 (0.73)
Breeding livestock				
Yes	16 (66.67)	244 (33.56)	260 (34.62)	16/260 (6.15)
No	8 (33.33)	483 (66.44)	491 (65.38)	8/491 (1.63)
History of tick bite				
Yes	23 (95.83)	99 (13.62)	122 (16.25)	23/122 (18.85)
No	1 (4.17)	628 (86.38)	629 (83.75)	1/629 (0.16)

¹⁾: No. positive sera (%).

been previously reported (7–9). Residing in a rural area is a risk factor for exposure to the tick vector and for acquiring CCHFV infection (10). The current study was performed in CCHF-endemic regions in rural and urban areas of the country. The CCHFV seroprevalence among participants residing in rural areas was significantly higher (21/339, 6.20%, $P = 0.021$) than that among participants in urban areas (3/412, 0.73%); this observation parallels the results of a survey conducted in Turkey, where the rate of seropositivity among individuals residing in rural and urban areas was 12.8% and 2%, respectively (8). In contrast, another study reported the absence of significant differences in the seroprevalence of CCHFV between rural (3.2%) and urban (1.92%) areas (9). The sex ratio of the participants was approximately 1:1 (368 males, 383 females), but the rate of seropositivity was higher among men than among women ($P = 0.061$). This is attributable to the involvement of men from these regions of Bulgaria in livestock breeding, and consequently, to greater exposure to livestock and ticks. The mean age of seropositive participants was slightly higher (58.34 years, range 36–74 years) than that of the total participants (56.60 years, range 11–83 years), suggesting that the seroprevalence of CCHFV increased with age. An age of >50 years showed significant association with CCHFV seropositivity among the participants ($P < 0.001$). According to a previous study, increased the seroprevalence of CCHFV with age may be a result of increased opportunities for contact with ticks, livestock breeding, and farming (6).

In conclusion, the variables showing significant association with the prevalence of anti-CCHFV antibodies were identified as a history of tick bites, livestock breeding, residing in rural areas, and age of >50 years. In rural areas, there is often a combination of several of

these factors and this is probably the reason for the high seroprevalence of CCHFV among the population of these settlements.

Acknowledgments We thank Prof. Z. Kalvatchev and Prof. I. Christova for the useful comments and support.

Conflict of interest None to declare.

REFERENCES

1. Bishop DH. Biology and molecular biology of bunyaviruses. In: RM. Elliot, editor. The Bunyaviridae. NY: Plenum Press; 1996. p. 19–61.
2. Nekliudov M. A case of hemorrhagic fever (Crimean). *Savremenna Medicina*. 1952;5:92–5.
3. Gergova I, Kamarinchev B. Comparison of the prevalence of Crimean-Congo haemorrhagic fever virus in endemic and non-endemic Bulgarian locations. *J Vector Borne Dis*. 2013;50:265–70.
4. Christova I, Gladnisha T, Taseva E, et al. Seroprevalence of Crimean-Congo hemorrhagic fever virus, Bulgaria. *Emerg Infect Dis*. 2013;19:177–9.
5. Gergova I, Kunchev M, Kamarinchev B. Crimean-Congo hemorrhagic fever virus-tick survey in endemic areas in Bulgaria. *J Med Virol*. 2012;84:608–14.
6. Hoogstraal H. The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. *J Med Entomol*. 1979;15:307–417.
7. Fisher-Hoch SP, McCormick JB, Swanepoel R, et al. Risk of human infections with Crimean-Congo hemorrhagic fever virus in a South African rural community. *Am J Trop Med Hyg*. 1992;47:337–45.
8. Gunes T, Engin A, Poyraz O, et al. Crimean-Congo hemorrhagic fever virus in high-risk population, Turkey. *Emerg Infect Dis*. 2009;15:461–4.
9. Izadi S, Halakouie-Naieni K, Majdzadeh SR, et al. Seroprevalence of Crimean-Congo hemorrhagic fever in Sistan-baluchestan province of Iran. *Jpn J Infect Dis*. 2006;59:326–8.
10. Saidi S, Casals J, Faghih MA. Crimean haemorrhagic fever-Congo (CHF-C) virus antibodies in man, and in domestic and small mammals, in Iran. *Am J Trop Med Hyg*. 1975;24:353–7.