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Original Article

Serologic Survey of Crimean-Congo Haemorrhagic Fever among Sheep in Ardabil Province, Northwest Iran

Ehsan Mostafavi[1*], Fahimeh Bagheri Amiri[2], Sahar Khakifirouz[3], Saber Esmaeili[1,4], Fatemeh Kazemi-Lomedasht[5]

[1]Department of Epidemiology and Biostatistics, Research Centre for Emerging and Reemerging Infectious Diseases, Pasteur Institute of Iran, Tehran, Iran;
[2]Urology and Nephrology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran;
[3]Department of Arboviruses and Viral Hemorrhagic Fevers (National Reference Laboratory), Pasteur Institute of Iran, Tehran, Iran;
[4]Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran;
[5]Biotechnology Research Center, Venom and Biotherapeutics Molecules Laboratory, Pasteur Institute of Iran, Tehran, Iran.

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Introduction: Livestock is a known source of Crimean-Congo Hemorrhagic Fever (CCHF) virus infection in humans. Although CCHF is endemic in Iran, limited human cases of CCHF are reported from northwest of Iran. Considering the lack of complete and updated information on the status and distribution of CCHF infection among domestic animals in Ardabil province, this study was conducted to investigate the CCHF status among sheep in this area. **Methods:** In this study, 256 sera from sheep were collected from various geographical regions of Ardabil in 2011, and tested for specific CCHF IgG antibodies by ELISA. **Results:** The Seroprevalence of CCHF in this area was 27.34%. The seropositivity rate of CCHF in northern regions (36.36%) was higher than in central (26.27%) and southern (20%) regions. The highest and lowest seropositivity of CCHF were seen in Parsabad (45.57%) and Khalkhal counties (17.78%), respectively. **Conclusions:** In this study, a relatively high seroprevalence of CCHF was seen among livestock in the province. Therefore, health care system should increase its surveillance for the detection of CCHF patients. Molecular studies to compare the virus strains circulating in this province and those in the eastern regions of the country can shed more light on the epidemiology of the disease. *J Med Microbiol Infec Dis, 2016, 4 (1-2): 16-19.*

Keywords: CCHF, Seropositivity, Epidemiology, Zoonosis, Iran.

INTRODUCTION

Crimean-Congo Hemorrhagic Fever (CCHF) is a vector-borne viral zoonotic disease, which causes acute febrile illness in humans. The disease in humans is often accompanied by hemorrhage, with the fatality rate ranging 5 to 30%. The causing virus is usually transmitted to humans by tick bites or direct contact with the blood or tissues of a viremic animal [1, 2]. *Hyalomma* spp. ticks are considered to be the most important vector of the virus, determining the distribution of CCHF virus worldwide [3]. A wide variety of livestock (sheep, cattle, and goats) and wild animals can be infected with this virus, but sheep are considered to be the most important domestic host for the virus in nature [1]. The infection is usually asymptomatic and subclinical in animals [2]. Slaughterhouse workers, butchers, farmers, veterinarians, and shepherds are at higher risk of infection [4]. Human cases of CCHF have been reported from different parts of Asia, Africa, Europe and the Middle East [2].

In Iran, the existence of CCHF among animals was first reported in 1970 [5]. In 1999, the first cases of human infection with CCHF were reported in Shahrekord City (central Iran) and subsequently in other provinces of the country [6]. Human infections of CCHF has been reported from most of the provinces [7], and Human cases of CCHF are very few in some western and northwestern provinces of Iran [8, 9].

There is not much data on the status and distribution of CCHF infection among domestic animals in Ardabil Province. The data on livestock in this area is limited to a single study of animals (cows, sheep, and goats) in Meshkin-Shahr County (central Ardabil) in 2002-2005, in which the seroprevalence of CCHF was 39.3% [7]. The present study was conducted to investigate CCHF status among sheep in different geographical regions of Ardabil Province.

***Correspondence:** Ehsan Mostafavi

Department of Epidemiology and Biostatistics, Research Centre for Emerging and Reemerging Infectious Diseases, Pasteur Institute of Iran, No. 69, Pasteur Ave, Tehran, Iran, 1316943551.

Email: mostafavi@pasteur.ac.ir

Tel/Fax: +98 (21) 64112121

MATERIAL AND METHODS

Study area and sampling. This study was carried out in 2011. Ardabil Province is located in northwestern Iran with an area of approximately 17953 Km^2. It borders the Republic of Azerbaijan in north and northeast, East Azerbaijan in the west, Zanjan in the south, and Gilan in east. Based on a report by the Iranian veterinary organization (2009, unpublished data), this province has 2,316,990 sheep, 262,190 goats and 348,150 head of cattle.

In this study, the blood samples of sheep were collected randomly from the regions in the north (Parsabad and Bilasavar counties), center (Ardabil, Nir, Meshkin-Shahr counties) and south (Khalkhal and Kowsar counties) of the province. We included 29 herds in the study and recorded their specifications including age, gender, and the habitats. Samples were sent immediately to the laboratory, and the sera were separated and stored in -20ºC until used.

Serology. Serum samples were analyzed by a specific ELISA for detection of IgG antibodies. The process involved coating the ELISA plates with mouse hyperimmune ascitic fluid (diluted at 1:1000) in phosphate-buffered saline (PBS 1x) and incubation overnight at 4°C. Following the washing step, the native or recombinant antigen in PBS (diluted at 1:500) containing 0.5% Tween (PBST) and 3% skimmed milk (PBSTM) was added, and the plates were incubated for 3 h at 37°C.

The serum dilutions in PBSTM (1:100) were added, and the plates were incubated for 1 h at 37°C. Peroxide-labelled anti-animal immunoglobulin diluted at 1:350 in PBSTM was added, and the plates were incubated for 1 h at 37°C. The plates were washed 3 times with PBST after each incubation. Finally, hydrogen peroxide (H2O2) and 3, 3`, 5, 5` tetramethylbenzidine (TMB) were added, and the plates were incubated for 15 min at room temperature. The enzymatic reaction was stopped by the addition of 4 N H2SO4. The plates were read by the ELISA reader (Anathos 2020) at 450 nm [10, 11].

Data Analysis. The data were analyzed using the SPSS for Windows statistical package (SPSS Inc., Chicago, IL, 16th version). Chi-square test was used for the comparison of variables in the analysis and the *P-values* less than 0.05 were considered statistically significant. ArcGIS software (ESRI, version 9.3) was used for mapping the results.

RESULTS

In this study, 256 sheep sera belonging to 36 (14.1%) rams, and 220 (83.9%) ewes were obtained from the northern (66), central (120) and southern (70) regions of Ardabil Province. The average (±SE) age of the animals was 4.38±0.16 years.

The seroprevalence of anti-CCHF IgG antibodies in sheep was 27.34% (95% CI: 22.25% - 33.11%) (Table 1). Although the seropositivity rates of CCHF in the northern regions (36.36%) was higher than in the central (26.27%) and southern (20%) regions, the difference was not statistically significant (p=0.10). The highest seropositivity for CCHF was detected in Parsabad County (45.57%), and the lowest was in Khalkhal (17.78 %) (Figure 1).

No significant difference in seroprevalence rate of the CCHF was observed between rams (36.11%) and ewes (25.90%) (p=0.20). Neither, no significant difference was observed between different age groups and the seroprevalence rate of CCHF (p=0.54).

DISCUSSION

In this study, 27.35% of sheep from various regions of Ardabil Province showed a history of CCHF infection. In Iran, CCHF seroprevalence rate in sheep showed a broad range in different areas, 77.5% in three Khorasan Provinces (northeastern Iran, 2003-2005) [12], 3.7% in Mazandaran Province (northern Iran, 2010-2011) [13], 76.9% in Isfahan Province (central Iran, 2002) [14], 27.4% in Bahar County of Hamadan Province (western Iran, 2006) [15], and 15.6% in Eastern Azerbaijan Province (northwestern Iran, 2010) [16]. In a study conducted between 2004 and 2005 in Meshkin-Shahr County of Ardabil Province, 39.3% of livestock (41.9% sheep, 33.3% goats, 30.0% cattle) had a history of CCHF infection [7], this rate of seropositivity was higher than what we observed in this study (25%).

It has been shown that CCHF antibodies (IgG) can persist for more than 5 years in animals blood [17] and various studies have observed a positive association between the age of the animals and the disease seroprevalence [13, 16, 18]. In this study, however, no association between age and the rate of seropositivity was noted. Furthermore, in agreement with previous studies carried out in Eastern Azerbaijan [16] and Mazandaran [13], no significant relationship between gender and seropositivity was noticed in this study.

The relatively high CCHF seroprevalence among sheep in this study will strengthen the possibility that further cases of human disease might have occurred in this province without being diagnosed. One other probable hypothesis is that the CCHF virus strain circulating in these regions does not have a high pathogenicity for humans.

Table 1. Seropositivity rate of CCHF among sheep in different geographical regions of Ardabil Province

Region	Number tested (%Seropositive)	County name	Number tested (%Seropositive)
Northern	66 (36.36)	Parsabad	35 (48.57)
		Bilasavar	31 (22.58)
Central	120 (26.67)	Ardabil	54 (29.63)
		Nir	30 (23.33)
		Meshkin-Shahr	36 (25.00)
Southern	70 (20.00)	Khalkhal	45 (17.78)
		Kowsar	25 (24.00)
Total	256 (27.34)		

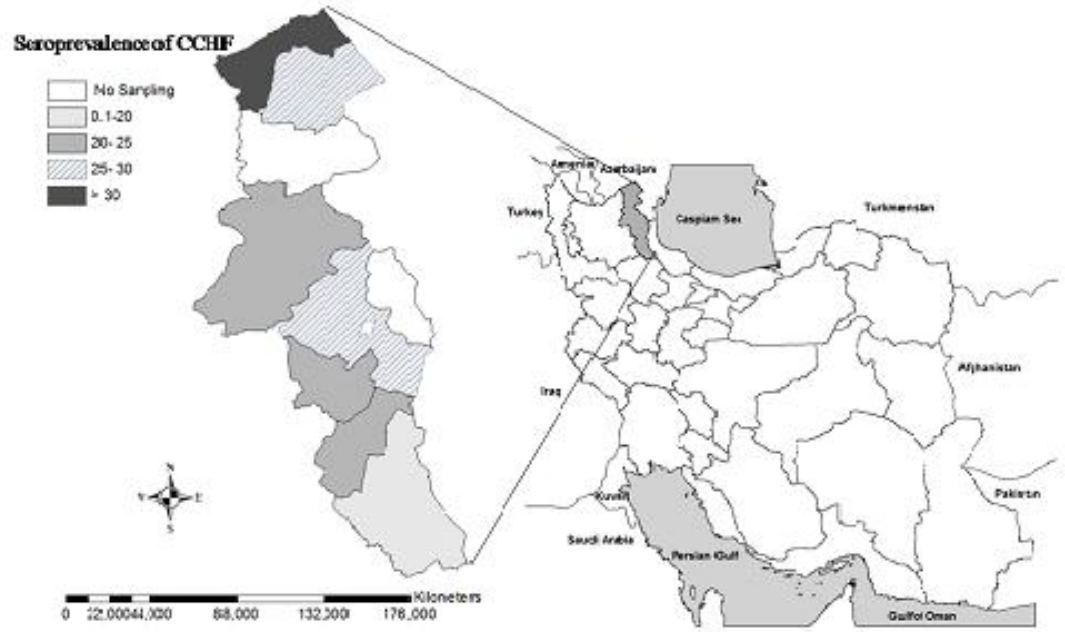

Fig. 1. Geographic distribution of CCHF seropositivity among sheep in Ardabil Province. Sampling was conducted in Parsabad (A) and Bilasavar (B) in the northern region, Meshkin-Shahr (C), Ardabil (D) and Nir (E) in the central region, Kowsar (F) and Khalkhal (G) in the southern part of the Province. Seroprevalence of CCHF is shown in different colors.

Therefore, a molecular epidemiological study is recommended to compare the virus strains circulating in this region and the virus circulating in eastern parts of the country. Complementary studies on these high-risk groups, ticks, and other livestock can delineate the status of CCHF in this province. Similar surveys in neighboring areas (Gilan, Zanjan, East-Azerbaijan Provinces) can further resolve the epidemiological features of CCHF disease in northeastern Iran.

This study demonstrated a relatively high seroprevalence of CCHF among sheep in Ardebil Province. Therefore, health care system should increase its surveillance for the detection of CCHF patients. Molecular studies to compare the virus strains circulating in this province and those in the eastern regions of the country can shed more light on the epidemiology of the disease. Also, complementary studies on high-risk populations, ticks, and other livestock will delineate the status of CCHF in this province and neighboring areas.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

REFERENCES

1. Whitehouse CA. Crimean–Congo hemorrhagic fever. Antiviral Res. 2004; 64 (3): 145-60.

2. Ergönül Ö. Crimean-Congo haemorrhagic fever. Lancet Infect Dis. 2006; 6 (4): 203-14.

3. Vorou R, Pierroutsakos IN, Maltezou HC. Crimean-Congo hemorrhagic fever. Curr Open Ifect Dis. 2007; 20 (5): 495-500.

4. Leblebicioglu H. Crimean–Congo haemorrhagic fever in Eurasia. Int J Antimicro Ag. 2010; 36: S43-S6.

5. Chumakov M, Smirnova S. Detection of antibodies to CHF virus in wild and domestic animal blood sera from Iran and Africa. Aktual Probl Virus Profilakt. 1972: 367-8.

6. Chinikar S, Mazaheri V, Mirahmadi R, Nabeth P, Saron MF, Salehi P, Hosseini N, Bouloy M, Mirazimi A, Lundkvist A. A serological survey in suspected human patients of Crimean-Congo hemorrhagic fever in Iran by determination of IgM specific ELISA method during 2000–2004. Arch Iran Med. 2005; 8 (1): 52-5.

7. Telmadarraiy Z, Ghiasi SM, Moradi M, Vatandoost H, Eshraghian MR, Faghihi F, Zarei Z, Haeri A, Chinikar S. A survey of Crimean-Congo haemorrhagic fever in livestock and ticks in Ardabil Province, Iran during 2004-2005. Scand J Infect Dis. 2010; 42 (2): 137-41.

8. Chinikar S, Mojtaba Ghiasi S, Moradi M, Goya M, Reza Shirzadi M, Zeinali M, Mostafavi E, Pourahmad M, Haeri A. Phylogenetic analysis in a recent controlled outbreak of Crimean-Congo haemorrhagic fever in the south of Iran, December 2008. Euro Surveill. 2010; 15 (47).

9. Chinikar S, Ghiasi SM, Moradi M, Goya MM, Shirzadi MR, Zeinali M, Meshkat M, Bouloy M. Geographical distribution and

surveillance of Crimean-Congo hemorrhagic fever in Iran. Vector Borne Zoonotic Dis. 2010; 10 (7): 705-8.

10. Chinikar S, Goya M, Shirzadi M, Ghiasi S, Mirahmadi R, Haeri A, Moradi M, Afzali N, Rahpeyma M, Zeinali M. Surveillance and Laboratory Detection System of Crimean Congo Haemorrhagic Fever in Iran. Transbound Emerg Dis. 2008; 55 (5-6): 200-4.

11. Garcia S, Chinikar S, Coudrier D, Billecocq A, Hooshmand B, Crance J, Garin D, Bouloy M. 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.

12. Bokaie S, Mostafavi E, Haghdoost A, Keyvanfar H, Gooya M, Meshkat M, Davari A, Chinikar S. Crimean Congo Hemorrhagic fever in northeast of Iran. J Animal Vet Adv. 2008; 7 (3): 343-50.

13. Mostafavi E, Chinikar S, Esmaeili S, Bagheri Amiri F, Tabrizi AMA, KhakiFirouz S. Seroepidemiological Survey of Crimean-Congo Hemorrhagic Fever Among Sheep in Mazandaran Province, Northern Iran. Vector Borne Zoonotic Dis. 2012; 12 (9): 739-42.

14. Ataei B, Touluei HR, Chinikar S, Darvishi M, Jalali N, Izadi M, Eilami O, Mirkhani M, Mardani M. Seroepidemiology of Crimean-Congo hemorrhagic fever in the local and imported sheep in Isfahan Province, Iran, 2002. Iran J Clin Infect Dis. 2006; 1 (1): 19-23.

15. Telmadarraiy Z, Moradi A, Vatandoost H, Mostafavi E, Oshaghi M, Zahirnia A, Haeri A, Chinikar S. Crimean-Congo hemorrhagic fever: a seroepidemiological and molecular survey in Bahar, Hamadan Province of Iran. Asian J Anim Vet Adv. 2008; 3 (5): 321-7.

16. Rezazadeh F, Chinikar S, Bagheri Amiri F. A seroprevalance Survey of Anti-CCHFV IgG by ELISA in Sheep from Some Area in Northwest of Iran. World Appl Sci J. 2013; 28 (11): 1757-60.

17. Wilson ML, LeGuenno B, Guillaud M, Desoutter D, Gonzalez J-P, Camicas J-L. Distribution of Crimean-Congo hemorrhagic fever viral antibody in Senegal: environmental and vectorial correlates. Am J Trop Med Hyg. 1990; 43 (5): 557-66.

18. Mohamed M, Said A-R, Murad A, Graham R. A serological survey of Crimean-Congo haemorrhagic fever in animals in the Sharkia Governorate of Egypt. Vet Ital. 2008; 44 (3): 513-7.