

# Serological Investigation of Occupational Exposure to Zoonotic Crimean-Congo Hemorrhagic Fever Infection

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**Presented in:** Data from the study were previously presented at the 2<sup>nd</sup> International Congress on Applied Biological Sciences, Sarajevo, Bosnia and Herzegovina.

**Cite this article as:** Aydin H, Uyanik MH, Karamese M, et al. Serological Investigation of Occupational Exposure to Zoonotic Crimean-Congo Hemorrhagic Fever Infection. *Eurasian J Med* 2020; 52(2): 132-5.

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Received: May 20, 2019

Accepted: September 24, 2019

Available Online Date: June 4, 2020

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DOI 10.5152/eurasianjmed.2020.19176



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## ABSTRACT

**Objective:** Crimean-Congo hemorrhagic fever (CCHF) is an acute and highly fatal disease. In this study, our aim was to compare and evaluate the prevalence of CCHF virus (CCHFV) antibody among occupational high-risk groups by using the enzyme-linked immunosorbent assay and draw attention to the occupational groups that are at high risk for CCHF infection in an endemic region for this zoonotic infection in Erzurum, Turkey.

**Materials and Methods:** The antibody levels against CCHFV were surveyed among slaughterhouse workers, animal breeders, and veterinarians. The study population was composed of 72 participants having direct contact with animals and 19 blood donors who were not in direct contact with animals.

**Results:** The overall rate of CCHF immunoglobulin G positivity in risk groups was found to be 6.94% (5/72). CCHFV antibodies were found in 4 (12.5%) individuals of the animal breeder group. This ratio was considered significantly higher compared with the healthy control group. CCHFV antibodies were found in only one person (4.0%) who was an abattoir worker. In the veterinarian group, all people were found negative.

**Conclusion:** In our study, the variables showing important associations with the prevalence of anti-CCHFV antibodies were livestock breeding, rural areas, and age. It was concluded that our region is endemic with regard to CCHF infection and persons who had direct contact with animals are at high risk. Thus, these participants must take necessary measures to protect themselves from CCHF and should be trained by health authorities.

**Keywords:** Animal breeder; Crimean-Congo hemorrhagic fever; seroprevalence, slaughterhouse worker; veterinarian

## Introduction

Crimean-Congo hemorrhagic fever (CCHF) is an important zoonotic acute-hemorrhagic viral infection and affects people from different parts of the world. CCHF was first detected clinically during the Second World War, in 1944, in Crimea. CCHF virus (CCHFV) belongs to the genus *Nairovirus* which is in the family *Bunyaviridae*. The geographic range of CCHF is one of the most extensive out of all the medically important arboviruses. Transmission of CCHF primarily occurs via infected ticks or direct contact with the blood, secretions, and meat of infected animals or humans. Transmission may sometimes also occur via aerosol. The virus infects mammals (sheep, goats, and cattle) and birds. Unlike humans, animals do not have specific clinical symptoms [1, 2]. CCHF is considered a serious and fatal disease and has a fatality rate of approximately 30%. Infected people show serious symptoms such as febrile illness, myalgia, petechial rash, and hemorrhage. Due to the absence of specific treatments and vaccine, CCHF infections usually result in death. All these conditions increase the biosafety level of this virus. CCHF is found in many parts of the world such as Africa, Asia, and Europe. The continuous increase of CCHF cases in Balkan countries and Russia draws attention to predisposing factors. These factors are likely to be multifactorial, which consist of changes in land use, spread of ticks, weather patterns, and wild bird migration [3, 4].

Direct contact with infected animals or animal products increases the risk of infection, and human to human transmission is also possible. Workers of lamb and beef slaughterhouses, ani-

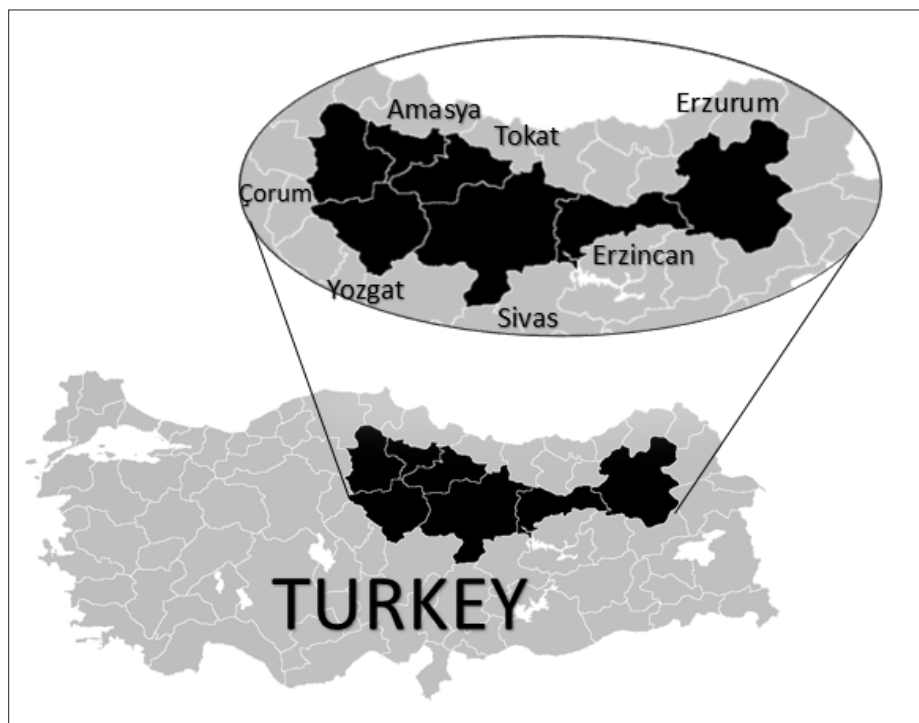
mal husbandry, and skin processors, as well as veterinarians, livestock market employees, and other workers requiring direct contact with animals and animal products are at high risk [1, 5, 6]. After the virus enters the body, anti-CCHFV immunoglobulin M (IgM) antibodies can be detected from the 6<sup>th</sup> to 14<sup>th</sup> day, and anti-CCHFV immunoglobulin G (IgG) antibodies can be detected from approximately the 10<sup>th</sup> day to 6 years after infection [5].

Risk factors are directly related with geographic and climatic conditions, human activities, tick abundance, and habitat preferences of ticks. It is an endemic disease, which is seen in various regions of Turkey. The geographical conditions of our country allow the survival of more than 30 species of tick. In Turkey, the disease was first observed in 2002 in Eastern Anatolia, Central Anatolia, and the Black Sea region, and since then, a number of fatal and nonfatal cases have been observed (Figure 1) [6, 7]. Since 2002, more than 6392 CCHF cases and 322 deaths have occurred due to CCHF in Turkey [8]. Although the CCHFV has extensive genetic diversity in different geographic areas, it shows similar characteristics serologically [9]. A variety of laboratory methods are used for the diagnosis of the viral agent. These methods include serological methods such as immunofluorescence assay and the enzyme-linked immunosorbent assay (ELISA) and molecular methods such as conventional and real-time quantitative reverse transcription polymerase chain reaction methods [10, 11].

In this study, our aim was to compare and evaluate the CCHFV antibody prevalence among occupational high-risk groups by using the ELISA method and to draw attention to occupational groups that are at a high risk for CCHF infection in an endemic region for this zoonotic infection in Erzurum, Turkey.

## Materials and Methods

The study was conducted in Erzurum in the northeast province of Turkey. In all, 91 participants (32 animal breeders, 25 abattoir workers,



**Figure 1.** Region with the most observed cases of CCHF in Turkey [11].

15 veterinarians, and 19 blood donors-the control group) were enrolled in the study. Our study team consisted of a nurse and a physician who went to the selected abattoirs, veterinary clinics, and villages in the endemic region to collect blood specimens. After the demographic surveys were completed, blood samples (5 mL) were collected from each participant. The control group was composed of blood donors who were not in direct contact with animals or animal products. After centrifugation at 3000 ×g for 10 min at 18°C, serum samples were transferred to sterile tubes and stored at -20°C until analysis. Specific CCHFV IgG antibodies were measured using a commercial ELISA kit (Vector-Best, Novosibirsk, Russia) and an automatic ELISA system (Alisei, SeacRadim Co., Rome, Italy) according to the manufacturer's recommendation. The system required 10 µL samples and 90 µL diluent suspensions. All mixtures were dispensed individual wells in the sample plate and the plate was placed in the device. The test results were automatically read and evaluated at 620-650 nm optical density (OD) and recorded by the ELISA system. Following the manufacturer's instructions, OD ≥ 0.100 was considered as positive.

## Statistical Analysis

Statistical analysis for the evaluation of data measured with ELISA was performed using the Statistical Package for Social Sciences software package, version 24.0 (IBM SPSS Corp., Armonk, NY, USA). Gender, age, history of tick bites,

occupations, and locations of the study population were compared by using the one-tailed Fisher's exact test. A statistical level of 95% ( $p < 0.05$ ) was considered as the significance limit.

Approval by the ethic committee for research with Non-Drug Clinical Trials Ethical Committee of Ataturk University, Medical Faculty with case number B.30.2.ATA.0.01.00/203. In addition, informed consent was obtained from all participants.

## Results

The state of Erzurum has continental climate; the altitude is 1853 m and average annual temperature is 13.35°C. Winters are long and harsh, and summers are short and hot. Specific testing for CCHF of risk groups was carried out according to their occupation. As expected, the prevalence of CCHFV antibodies was high in the occupational high-risk groups. The overall rate of CCHFV IgG was found to be 6.94% (72/5) in high-risk experimental groups. The CCHFV antibodies were found in four animal breeders (12.5%). This ratio was considered significantly higher compared with the control group. CCHFV antigen was found in only one abattoir worker (4.0%). In the veterinarian group, all people were found to be CCHFV antigen negative. This unexpected situation emphasizes that the risk of CCHF is associated with professional experience and educational status. All blood donors were found to be CCHFV IgG negative. The median age of all the participants was 40.6 years, while that of IgG seropositive participants was 39.8 years.

### Main Points

- CCHF infection is a potential occupational hazard for peoples who work in jobs directly related to animals or animal products.
- Several regions of the globe including Turkey is endemic with regard to CCHF infection and persons who had direct contact with the animals are under a high-risk.
- Thus, these persons must take the necessary measures to protect themselves from the CCHF and should be trained by health authorities.

**Table 1.** Demographics and CCHFV seroprevalence in this study

Variable	Seropositive, n (%)	Seronegative, n (%)	P
<b>Gender</b>			
Male	5 (5.9)	80 (94.1)	0.705
Female	0 (0.0)	6 (100)	
<b>Age</b>			
21-30	0 (0.0)	6 (100.0)	0.790
31-40	4 (6.7)	56 (93.3)	
41-50	1 (6.3)	15 (93.8)	
51-70	0 (0.0)	9 (100.0)	
<b>History of tick bites</b>			
Yes	1 (50.0)	1 (50)	0.107
No	4 (4.5)	85 (95.5)	
<b>Occupation</b>			
Animal breeder	4 (12.5)	28 (87.5)	5.109
Slaughterhouse worker	1 (4.0)	24 (96.0)	
Veterinarian	0 (0.0)	15 (100.0)	
Blood donor	0 (0.0)	19 (100.0)	
<b>Location</b>			
Rural	4 (8.7)	42 (91.3)	0.187
Urban	1 (2.2)	44 (97.8)	

CCHFV: Crimean-Congo hemorrhagic fever virus

**Table 2.** Scientific data from around the world on anti-CCHFV IgG positivity

Author	Year	Seropositive number/Sample size	(%)	Country
Mardani et al. [16]	2007	5/129	3.87	Iran
Chinikar et al. [5]	2012	16/108	14.80	Iran
Gergova et al. [17]	2014	24/751	3.20	Bulgaria
Sidira et al. [19]	2012	68/1611	4.20	Greece
Sidira et al. [22]	2013	6/277	2.20	Greece
Sargianou et al. [18]	2013	7/207	3.40	Greece
Lwande et al. [23]	2012	96/517	23	Kenya
Al-Abri et al. [21]	2014	2/38	5.20	Tunisia
Ergonul et al. [9]	2007	1/62	1.60	Turkey
Ertugrul et al. [12]	2012	84/429	19.60	Turkey
Yagci-Caglayik et al. [15]	2014	25/1066	2.30	Turkey
Koksal et al. [20]	2014	85/625	13.60	Turkey

CCHFV: Crimean-Congo hemorrhagic fever virus; IgG: immunoglobulin G

ing, and bleeding from various organs [9]. On the other hand, seropositivity, especially IgG positivity, is an important predictor of preexisting infectious diseases [12]. CCHF was first detected in Crimea in 1944 and then reported in more than 30 countries, including Bulgaria, Kosovo, Albania, Northern Greece, Russia, Iraq, Pakistan, Turkey, and China [13]. Additionally, reports from Turkey about this fatal disease were first reported in 2004 [9, 14]. We have shown the seropositivity for anti-CCHFV IgG among a small group of workers who have contact with in Erzurum-an endemic region located in the eastern part of Turkey. Our aim was to draw attention to the occupational groups that are at high risk for CCHF infection, in the endemic region for this zoonotic infection, by detecting IgG seropositivity among slaughterhouse workers, animal breeders, and veterinarians.

Contact exposure to infected animals, animal products, or infected patients' secretions plays a key role. There are many cases of CCHF infections due to occupational exposure. Slaughterhouse workers, veterinarians, healthcare personnel, butchers, animal breeders, and shepherds at high risk for CCHF. In our study, the rate of CCHF infection among animal breeders, slaughterhouse workers, and veterinarians in the Eastern provinces of Turkey was 6.94% (5/72). Of the 5 positive samples, 4 belonged to animal breeders and 1 belonged to a slaughterhouse worker. Various studies performed worldwide over the past 5 years have identified animal breeders, butchers, and slaughterhouse workers as high-risk groups for CCHF infection (Table 2) [5, 9, 12, 15-23].

CCHF infection is a potential occupational hazard for people whose jobs are directly related to animals or animal products. The data obtained from the different regions of our country revealed the same [15]. Occupational exposure is very important for this disease. Other demographic relations are shown in Table 1.

In the literature, the highest prevalence of CCHF was reported in central and southwestern Kosovo, with a rate of 24.3% [24]. In Iran, another endemic area, two different studies performed by Chinikar et al. [5] and Mardani et al. [16] indicated that the seropositivity rate was found to be 14.8% and 3.87%, respectively. Gergova et al. [17] found the IgG seropositivity rate in 75 human blood samples was 3.20% in Bulgaria. Two studies from Greece showed that the overall seroprevalence was 3.40% and 4.20% [18, 19].

Most of the participants were male (85 males, 6 females). When CCHFV seroprevalence was statistically evaluated, no significant difference was found between genders and mean age groups ( $p>0.05$ ). Only one participant had tick-bite history. Nearly half of the participants were living in urban areas, while a large proportion of IgG seropositive participants were living in rural areas. CCHF seroprevalence did not show a statistically

significant difference in terms of tick-bite history and living environments ( $p>0.05$ ). We think that this situation is related to the insufficient size of the study population. All demographic data can be seen in Table 1.

## Discussion

CCHF is a viral hemorrhagic disease that shows serious symptoms such as fever, nausea, vomit-

In Turkey, seroprevalence of CCHFV was found was 1.6% to 19.6% in different studies [9, 12, 20]. A multicenter study performed in 2014 with 1066 blood samples, which were obtained from seven different provinces of Turkey (Adana, Aydin, Erzurum, Gaziantep, Istanbul, Samsun, and Yozgat), showed that IgG positivity rate was 2.3% [15]. Other seroprevalence ratios can be seen in Table 2.

Most of the studies showed that increased age is an important risk factor which may be a result of the increased possibilities for transmission [5, 12, 17, 19, 21]. In our study, the highest seropositivity was found in the 31-40-year age range (39.75 years, range 21-69 years).

It is shown that the CCHF IgG seropositivity rate varied according to the location where the study was performed (rural or urban). Living in rural areas was found to be a risk factor for exposure to tick bites and CCHF infection. In our study, 4 of the 5 seropositive individuals lived in rural areas. Another study performed in our country also showed that the rate of seropositivity was related with the living location (rural 4.8% and urban 1.8%) [15].

Tick-bite history and gender were not effective study parameters in our study because of the sample size. Among the seropositive individuals, only one individual had tick-bite history. The major part of our experimental group consisted of male individuals (n=85), so the role of gender could not be assessed. However, tick-bite history and gender are important risk factors according to other studies [15, 17, 19, 25, 26].

In conclusion, CCHF infection is a potential occupational hazard for people whose jobs are directly related to animals or animal products. In our study, the variables showing important association with the prevalence of anti-CCHFV antibodies were identified as livestock breeding, rural areas, and age. Usage of gloves, masks, and other protective equipment by veterinarians provided an advantage to prevent infection. It was concluded that our region is endemic with regard to CCHF infection and people who had direct contact with animals are at high risk. Thus, these people must take necessary measures to protect themselves from CCHF and should be trained by health authorities. Furthermore, more comprehensive studies are recommended to clarify the condition of the disease among high-risk groups.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of Ataturk University School of Medicine. (B.30.2.ATA.0.01.00/203)

**Informed Consent:** Informed consent was obtained from volunteers who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept - H.A., M.H.U.; Design - H.A., M.H.U.; Supervision - H.A., O.A., M.O.T.; Resources - H.A., M.H.U., I.S.; Materials - H.A., M.H.U., E.O.; Data Collection and/or Processing - H.A., M.K., M.O.T., A.G.; Analysis and/or Interpretation - H.A., M.O.T., M.K., M.H.U.; Literature Search - H.A., M.K., O.A.; Writing Manuscript - H.A., M.K., E.O.; Critical Review - H.A., M.H.U., M.K., I.S., M.O.T., A.G., E.O., O.A.

**Conflict of Interest:** Authors have no conflicts of interest to declare.

**Financial Disclosure:** The authors declared that this study has received no financial support.

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