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Full Length Research Paper

Seroprevalence of Crimean-Congo haemorrhagic fever (CCHF) in risk groups in Tokat Province of Turkey

Saban Tekin[1*], Sener Barut[2], Ahmet Bursali[1], Gul Aydogan[1], Onem Yuce[1], Fatma Demir[3] and Beytullah Yildirim[4]

[1] Department of Biology, Gaziosmanpa a University, Faculty of Art and Sciences, Tokat, Turkey.
[2] Department of Infectious Diseases, Gaziosmanpa a University, School of Medicine Tokat, Turkey.
[3] Intensive Care Unit, C. Aykan State Hospital, Tokat, Turkey.
[4] Department of General Medicine, Gaziosmanpasa University, School of Medicine Tokat, Turkey.

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Crimean Congo Haemorrhagic Fever (CCHF) is a fatal viral haemorrhagic fever affecting humans. Serious CCHF outbreaks with high mortality have been reported from Asia, Africa and Europe. Endemic CCHF outbreaks have been seen in Turkey between 2002 and 2009, with about 5% mortality rate. People working with animals, people having tick bites, health workers and relatives of CCHF patients may be infected by CCHF virus and therefore they have been considered as CCHF risk groups. In the present study, CCHF prevalence of a control group, people working with animals (PA), people having tick bites (PT), health workers (HW) and relatives of CCHF patients (RP) from Tokat province in Turkey was investigated. A total of 715 people in control and risk groups were tested for the presence of the anti-CCHF IgG in their sera by using anti-CCHF IgG ELISA and compared. Results showed that people working with animals and relatives of CCHF patients had significantly higher CCHF prevalence ($p < 0.001$) than other groups. The higher seroprevalence of CCHF in people working with animals and relatives of patients indicate that they might be infected with CCHFV in a way that clinical symptoms of disease did not occur or not apparent in a hyper endemic region.

Key words: CCHF, ELISA, human, ticks, Tokat, seroprevalence.

INTRODUCTION

Crimean Congo haemorrhagic fever virus (CCHFV), a member of *Nairovirus* (family; Bunyaviridae) genus, is a fatal disease affecting humans. Serious endemic CCHF outbreaks have been reported in countries from Europe, Asia and Africa (Hoogstraal, 1979; Whitehouse, 2004; Flick and Whitehouse, 2005; Ergonul, 2006; Papa et al., 2009). Although the major transmission of the virus is mediated by *Hyalomma* ticks, it can also be transmitted by squashing ticks, contact with contaminated blood, sera or other secretions of patients and viremic animals or by nasocomically (Swanepoel et al., 1987; Bosan et al., 2000; Burt et al., 1997; Whitehouse, 2004; Chinikar et al., 2004; Papa et al., 2004; Morikawa et. al., 2009). Health workers, relatives of CCHF patients, people working with animals and animal products (shepherds, animal care workers, veterinarians and slaughterhouse workers) and people that had tick bites in CCHF endemic areas are under high risk of being infected by CCHF (Whitehouse, 2004; Flick and Whitehouse, 2005).

Several endemic outbreaks of CCHF has been reported in Turkey between 2002 - 2008 with average of 5% mortality (Ergonul, 2006; Yalcin et al., 2008, 2009) and from neighbouring countries such as Albania (Papa et al., 2009), Bulgaria (Papa et al., 2004), Ukraine (Whitehouse, 2004), Iran (Chinikar et al., 2008). Beside the human cases, CCHF virus has been detected in several tick species (Papadopoulos and Koptopoulos, 1980; Papa et al., 2004; Papa et al., 2009; Chinikar et al., 2004; Tekin et al., 2009).

The presence of human antibodies to CCHF virus were shown in woodcutters, farmers health and animal care workers, slaughterhouse workers and people that had tick bites in endemic areas (Antoniadis et al., 1990; Izadi

*Corresponding author. E-mail: sabant@yahoo.com. Fax: +90356-2521616.

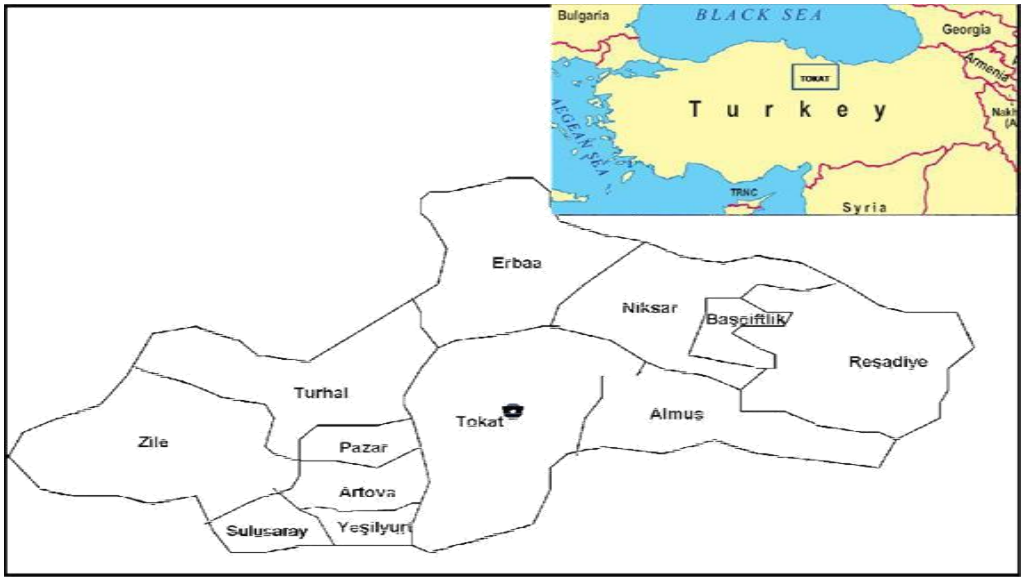

Figure 1. Map of Tokat Province of Turkey showing the location of 12 districts where the present study was conducted.

et al., 2004). Nosocomial outbreaks have been reported in recent years in Pakistan, Iraq, Dubai, and South Africa (Bosan et al., 2000; el-Azazy and Scrimgeour, 1997; Burt et al., 1997; Van Eeden et al., 1985; Swanepoel et al., 1987). As shown in the previous studies, people in various risk groups may be infected with CCHF by various routes. Although CCHF cases was reported in Turkey, the disease potential of people in different risk groups has not been documented well enough to date, especially in relatives of CCHF patients in a hyper endemic region. In the present study, the seroprevalence of CCHF in various risk groups was investigated.

METHODS

Location and People

Study was conducted on people in Tokat province (Figure 1) where sporadic cases and outbreaks of CCHF have been reported between 2002 and 2009. The mild climate, year- long vegetation and enriched wild life of Tokat province, provide suitable habitat for ixodid ticks which are main vector of Crimean-Congo haemorhagic fever virus (CCHFV) (Hoogstraal, 1956; Whitehouse, 2004) and are considered to be responsible for transmission of the virus to humans during these outbreaks (Ergonul, 2006; Yilmaz et al., 2008, 2009).

A control (C) group (240 people) from general population and several risk groups; health workers (HW: 150), people working with animals (PA: 26), relative of patients (RP: 193) and people that had tick bites (TB: 106) were selected randomly for the prevalent study in 2006 and 2007. Health workers group were composed of Doctors, Nurses and other medical personnel. The shepherds were dominant in PA group. The PR group was composed of first or second degree relatives who either directly or indirectly contacted with the patients during visits or caring for the patient before or after hospitalization, or relatives who shared the same house or room with the patients.

Collection of human sera

Peripheral blood was collected from total of 715 people in 5 groups (C: 240, HW: 150, PA: 26, RP: 193 and TB: 106) described above, using Vacutainer tubes (BD Scientific, USA) . Blood samples were centrifuged at 1500xg for 30 min, serum was separated, transferred into 2 ml eppendorf microcentrifuge tubes and stored at -20°C until used for the ELISA. In addition, peripheral blood samples from 11 patients who were confirmed CCHF positive were collected during acute phase and 3 to 12 months after recovery, processed as above and used for Reverse Transcription-Polimerase Chain Reaction (RT-PCR) and ELISA respectively to validate the tests.

Viral RNA Isolation and RT-PCR

Viral RNA samples were extracted from 200 µl serum samples from 11 CCHF patients by using High Pure Viral Isolation Kit (Roche, USA) according to manufacturers' procedure. In all cases, RNA samples were eluted in 50 µl PCR grade water and stored at -86°C. RT-PCR tests were performed by using Ctherm One Step RT-PC kit (Roche, USA) according to kits' protocol.

Presence of CCHFV in patient's sera was tested by one step RT-PCR using Ctherm One Step RT-PCR kit (Roche, USA) according to manufacturer's protocol using 2-4 µl RNA. In all RT- PCR re-actions, the following cycling conditions were used; 30 min at 55°C, 10 min 95°C followed by 35 cycle with 30 s at 94°C, 1 min at 55°C, 10 s at 72°C, and a final elongation step 5 min at 72°C. In all test, reaction volume was 25 µl. A PCR product of 536 bp was amplified by using 0.2 µM CCHFV S segment specific forward (5'-TGGACACCTTCACAAACTC-3') and 0.2 µM reverse (5'-GACAAATTCCCTGCACCA- 3') primers (Chinikar et al., 2004). In all tests, a sequence confirmed CCHFV RNA isolated from a tick was used as positive control and ddH2O used as negative control. PCR products of 4 µl were analyzed on a 1% agarose gel containing ethidium bromide and visualized on a jel documentation system

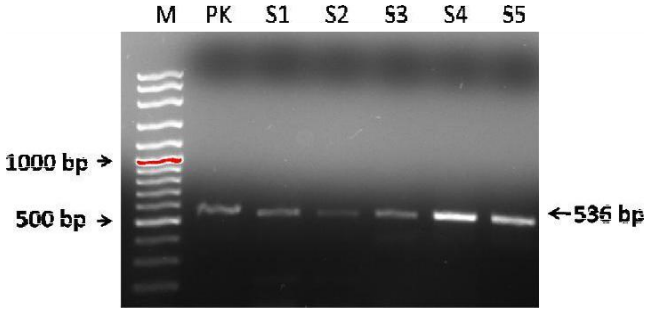

Figure 2. Detection of CCHFV in patient sera by one step RT-PCR. M; molecular weight standard, PK; positive control, S1-5; patient sera.

(UVP, UK) (Figure 2). These samples were also positive with real-time RT-PCR by using CCHFV specific primer-probe set (not shown).

ELISA procedure

Presence of human anti-CCHF IgG in human sera was determined using a commercial anti-human IgG ELISA kit (BSDL, Scotland, UK) according to manufacturer's protocol using 100 l undiluted serum sample. ELISA test plates were read at 405 nm using a microplate reader (Ryto, India). Only ELISA tests that passed internal quality control limits determined by the producer of kit were evaluated. Sera from 11 recovered CCHF patients who were confirmed as CCHF clinically and by RT-PCR were CCHF positive using the kit. Dilution of sera 1:2 with phosphate buffered saline did not affect the results.

Statistical analysis

The CCHF seroprevalence of control and the risk groups were presented using percentages. The statistical association between CCHF prevalence and risk groups was analysed using ^{2} test. Human anti- CCHF IgG antibody prevalence estimates were calcula-ted for each risk group, compared and contrasted. A p value < 0.05 was considered significant.

RESULTS

Seroprevalence of CCHF in risk groups

According to ELISA results, the prevalence of CCHF in Control, HW, RP, PA and TB groups were found as 11/240 (%4.6), 3/150 (%2), 44/193 (%22.8), 6/26 (%23), 11/106 (%10.4), respectively (Table 1). Analysis of the results indicated that the prevalence of CCHF was signi-fycantly higher ($p < 0.001$) in people working with animals and relatives of the CCHF patients than people in other groups, indicating that they have higher risk of having CCHF infection.

In contrast to CCHF patients, the anti- CCHF IgG positive people were asymptomatic and they had never been hospitalized. Our results showed that the prevalence of CCHF in people working with animals and relatives of the CCHF patients was very high suggesting that they might be infected with CCHFV in a way that clinical symptoms of disease did not occur or not apparent.

DISCUSSION

Endemic outbreaks of CCHF have been reported in Turkey between 2002 - 2008, with average of 5% mortality (Ergonul, 2006; Yalcin et al., 2008, 2009). The higher prevalence of CCHF in countries such as Albania, Bulgaria, the former Yugoslavia, Ukraine, Georgia, Tacikistan, Iran, and Pakistan was reported (Whitehouse 2004; Chinikar et al., 2006; Papa et al., 2004; Papa et al., 2009). Our findings was also parallel to previous studies that people working with animals such as shepherds, animal workers, butchers, and tanners have higher CCHF prevalence indicating their higher risk of infection. According to our results, the prevalence CCHF in PA was 20% in districts where the livestock breeding was a major occupation. As it was proposed by Izadi et al. (2006), it is possible that CCHFV was transmitted to people working with animal through removal of ticks from animals or occasional contact with blood and secretion of viremic animals. Relationship between tick bite and higher CCHF prevalence were reported in numerous studies (El-Azazy and Scrimgeour, 1997). Even though number of people is low in our tick bite group (TB), data clearly indicate that ticks were infected and contributed to transmission of CCHFV in the province.

Health workers are also considered as people in risk groups for CCHF (Ergonul, 2006). In contrast to some countries, the prevalence of CCHF was very low (2%) in Tokat provinces suggesting that the precautions for the protection of health workers were appropriate.

The transmission of CCHFV by close contact with the patients has been reported (Athar et al., 2005; Izadi et al., 2008). In contrast to these studies that the prevalence of CCHF in relatives was low, the present results showed that CCHF prevalence of relatives of CCHF patients was as high as people working with animals, indicating that transmission of CCHF from patients to relatives occurs in some cases in a hyper endemic region. This is the first study showing the high prevalence of CCHF in relatives of CCHF patients in an endemic location. We suggested that the relatives of people were probably transmitted with CCHFV in a way that immunity developed against the virus but symptoms of disease were did not occur. It is possible that the relatives of patients were transmitted with CCHFV either by direct contact with the blood or secretions of the patients during patient care or visits before or during hospitalization of the patient. This high prevalence was not associated with tick bite because during sera collection, only the relatives who had no tick bite history included RP group. However, in addition to contact with the patient, some of the relatives might have

Table 1. Seroprevalence of Crimean-Congo Haemorrhagic fever in humans in risk groups in Tokat province of Turkey.

Risk groups	CCHF IgG positive (%)	Human tested (column %)
Control	11(4.6)	240(33.5)
HW	3(2)	150(21)
PA	6(23)*	26(3.6)
RP	44(22.8)*	193(27)
TB	11(10)	106(15)
Total	69(14.4)	715(100)

*$p < 0.01$.

been transmitted with the virus by occasional contact with viremic animals because they live in hyper endemic rural area. Even though there is insufficient data to support transmission of CCHFV by an airborne mechanism, it is possible that some of these relatives were transmitted with CCHFV nasocomically. These results may also indicate that route of viral transmission might affect immune response against CCHFV and disease outcome. Eventually, the mechanism of developing immunity against CCHFV without any symptoms requires further investigation.

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