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ABSTRACT

Crimean-Congo hemorrhagic fever (CCHF) is an endemic tick-borne viral disease that affects both animals and humans. This study aims to determine seroprevalence of CCHF in Turkey’s Van province by analysis of blood samples obtained from people living in Van province. Blood specimens were taken from healthy people living in Van province and a variety of its villages between January 2012 and July 2012. Blood samples were initially tested by using a CCHF virus (CCHFV) IgM IgG kit for anti-CCHFV IgG followed by anti-CCHFV IgM determination of IgG positive blood samples. IgM-positive specimens were re-confirmed by Real Time-PCR. 107 men and 261 women were included in the study. 53 blood specimens (14.4%) were anti-CCHFV IgG positive, 2 of which were anti-CCHFV IgM positive. Two blood samples with anti-CCHFV IgM seropositivity led to negative PCR results, proving their non-acute infections. Differences amongst localities, gender, with and without a history of tick bites was not found statistically significant in terms of anti-CCHFV IgG seropositivity. Although anti-CCHFV IgG levels in blood specimens were found to be 14.4%, death cases have not been reported in Turkey’s Van province yet. It is imperative that clinical CCHFV tests be implemented for people at high risk of developing CCHFV-related complications.
INTRODUCTION

Crimean-Congo hemorrhagic fever (CCHF) is a serious disease with 3-50% mortality throughout the world, which is caused by an RNA virus from the Nairovirus species of the Bunyaviridae family (1,2). CCHF is frequently encountered in Asia, Africa and Eastern Europe. CCHF was first identified and related to tick bites in the Crimea peninsula in 1944, during World World II. CCHF virus (CCHFV) was named in 1969 after a RNA virus that was identified in Congo in 1956 was found to be identical to that found in Crimea peninsula in 1944 (3).

People acquire CCHFV by infected tick bites or contact with blood or tissue of acutely infected people or animals (1,3). Clinical signs of CCHF disease include hemorrhage, fatigue and fever. Biochemical laboratory findings usually reveal increases in liver enzymes, creatinine phosphokinase and lactate dehydrogenase, as well as prolonged bleeding time (4). One of the major pathogenesis of CCHF is endothelial impairment caused by viral infection as well as viral and host factors (3). ELISA, immunofluorescent antibody and PCR techniques are primarily used to diagnose CCHFV. Anti-CCHFV IgG and IgM can be detected seven days after the onset of illness by ELISA and immunofluorescent tests. Despite anti-CCHFV IgM level decrease to undetectable levels 4 months after the onset of infection, IgG remains in detectable levels for 5 years after the onset of infection (3). Real Time Polymerase Chain Reaction (RT-PCR) is preferred to confirm CCHFV infection due to its specificity, susceptibility and rapidness. Early diagnosis of CCHFV is essential for clinical prognosis and prevention of community and hospital infections (5,6).

Although the city of Van of Turkey have had rare incidences of CCHF cases in the past, twelve CCHF cases had been reported between 2007 and 2012, suggesting that the CCHF cases have increased recently. Fortunately, no death cases of CCHFV have been reported so far in the city of Van. The seroprevalence of CCHFV has yet not been determined.
in Turkey’s Van province. The main objective of this study is therefore to determine the seroprevalence of CCHFV on people with a high risk of possessing the CCHF disease in Turkey’s Van province.

MATERIALS AND METHODS

Blood specimens were taken between January 2012 and July 2012 from 107 men (aged between 15 and 79 with an average of 40.3±16.2) and 261 women (aged between 13 and 93 with an average age of 39±15.6), giving a total of 368 people, in Turkey’s Van province including its several villages.

As CCHF is a zoonotic disease of CCHFV carried by several domestic and wild animals and people are infected by direct contact with fluid, blood or tissue of infected animals or by tick bite, people included in this study were selected from those working in farms or dealing with stock farming in viral infection seasons.

Ethics approval was received from the Ethics Committee at Yüzüncüyl University-Van, Turkey (Approval No: 2014/1). Written informed consent for participation in the study was obtained from participants.

Sample size in this study, which was designed to be a cross-sectional epidemiologic research, was determined by cluster sampling method of an event with known prevalence (7). A value of 11%, averaged out of a reported literature value of 3-19%, that was previously determined by another study involving healthy people in Turkey, was used as a reference value for CCHFV seropositivity in this study (8-10). The magnitude of sampling was determined as 418 based on a confidence accuracy ratio of 95%, a prevalence of 11%, and a fallibility of 3% (d=0.03) (7). 368 people who accepted to be involved in this study were informed of the objective of the study by face-to-face communications and asked if they were ever bitten by ticks or any other insects previously.
Sera of the blood samples were separated by centrifugation at 10000 rpm for 10 minutes, which were then stored at -80 °C. The presence of CCHFV was determined by IgM detection on blood samples that were found to be IgG positive. Anti-CCHFV IgG and IgM levels in blood samples were determined by IgM-IgG micro-ELISA kits (Vector-Best, Russia). The CCHFV IgM positive samples were tested by Real Time PCR using the RealStar CCHFV RT-PCR kits (Altona Diagnostics, Germany) on antibody positive blood samples.

**Statistical Analysis**

Once the sample size was determined, people included in this study were selected by the proportional random sampling method. Statistical variables were given as averages with standard deviations while categorical variables were expressed as numbers and percentages. The Z-test was used to compare proportions between categorical variables. Statistical computations were implemented by MINITAB v.14 software, by which a result obtained within a statistical significance level of 5% was considered meaningful.

**RESULTS**

Among 368 people tested, 53 (14.4%) were found to be anti-CCHFV IgG seropositive. Two of those people were also anti-CCHFV IgM seropositive. When they were tested by Real-Time RT-PCR no CCHFV was detected, suggesting that they were not acute infections.

In terms of researching CCHFV seropositivity, a total of 368 blood specimens (one specimen per a case) taken from farmers and people dealing with agriculture were initially analyzed for the presence of anti-CCHFV IgG, whose results are outlined and sorted out in Table 1 for men and women according to different age groups, including age groups 13-20, 21-30, 31-40, 41-50, and those for older than 61. As seen in Table 1, 53 (14.4%) of the blood samples were found to be anti-CCHFV IgG seropositive. Analysis of the 53 anti-CCHFV IgG
seropositive blood samples revealed two blood samples, 3.8%, that were anti-CCHFV IgM seropositive. Further analysis of the two anti-CCHFV IgM seropositive blood samples by RT-PCR using a RealStar CCHFV RT-PCR kit (Altona Diagnostics, Germany) did not confirm an acute CCHF disease. Table 2 lists anti-CCHFV IgG seropositivity versus athropoda bites. It was determined that only 14.4% (53 out of 368) of the cases possessed a history of tick bite, a finding that disables making a significant correlation. Therefore, considering the data in Tables 1 and 2 as well as case locality, anti-CCHFV IgG seropositivity of the cases is neither correlated to sex nor to athropoda bite history nor to locality (p>0.05).

DISCUSSION AND CONCLUSION
Life cycle of CCHFV continues amongst vertebrates and ticks. The highest risk group of people that may possess the CCHF disease substantially include people dealing with animals (farmers, butchers, herdsmen, etc.), veterinarians, and travellers in endemic areas (8,11). CCHFV appears to have drawn substantial attention considering its wider geographical and ecological distribution, infectiousness amongst people, increasing mortality rates as well as debates on treatment (12-14).

Crimean-Congo hemorrhagic fever (CCHF) with prevalent hemorrhage and high mortality rates has recently came into prominence. It has been recently known that CCHFV causes epidemic outbreaks in the Balkan Penninsula, Pakistan, Iran, as well as countries around the Black Sea costs (3,5,8,15). For instance, a 14.4% anti-CCHFV IgG seropositivity was reported in Greece in a study involving farmers living in rural areas, in which case the anti-CCHFV IgG seropositivity is the greatest for elderly people who had contacted with sheeps (16). However, no human case has been reported in that region, and the high seroprevalence was attributed to the circulation of CCHFV lineage Europe 2 strains, which seem to be of low pathogenicity (16). It was reported in another study conducted in
Afganistan that 37 out of 330 people (11.2%) who had contacted with cows were found to be anti-CCHFV IgG seropositive (17). Anti-CCHFV IgG seropositivity in Saudi Arabia was reported to be 1.3% (18).

As for Turkey, the first epidemic outbreak of a disease with symptoms of hemorrhaging and fever upon tick bites was officially reported in the spring and summer of 2002 for people living in a wide area including the northern anatolia and southern blacksea provinces of Turkey, a disease that was later identified as CCHF in 2003 (8,19,20). CCHFV later spread over other cities in Turkey including Kastamonu, Bartın, Ankara, Çankırı, Bolu, Balıkesir, etc. as well as the Kelkit Valley area including the cities of Tokat, Sivas, Gümüşhane, Amasya, Yozgat and Çorum. Recently CCHFV was reported to have spread over cities in the eastern part of Turkey (21,22). Death cases due to CCHFV have been recently reported in many areas of Turkey, with an average mortality rate of 5% as determined by several epidemic studies in Turkey (20,23). Likewise, the CCHFV seroprevalence has been reported in literature to be quite high according to certain epidemic studies implemented in Turkey. For instance; Ertuğrul et al. (9) reported that the CCHFV IgG seropositivity is %19.6 in Turkey, which was found to be significantly related to tick bites. In another study conducted in 2009 in Turkey, anti-CCHFV IgG seropositivity was reported to be 10%, determined out of 3557 sera samples collected from people living in the Kelkit Valley area in Turkey (10).

In a CCHF seroprevalence study which involved 40 veterinarians living in the city of Tokat, one of the cities of Turkey in which CCHFV was firstly identified, and 43 veterinarians living in the city of Aydın where no CCHFV cases had ever been reported before, giving a total of 83 veterinarians, 3% of CCHFV IgG seropositivity was identified for the city of Tokat while no CCHFV IgG/IgM seropositivity was identified for the city of Aydın (5). The first epidemic CCHF case ever reported in Turkey was studied by Kartı et al. (5)
between the years of 2002 and 2003, in that 6 sera samples tested anti-CCHFV IgM positive out of 19 sera samples collected from 19 patients (including 18 farmers and 1 nurse working in the hospital), who were suspected of having either the CCHF virus or a kind of a viral infection. Genome analysis of the CCHF virus isolated from 2 of the aforementioned CCHFV IgM positive sera gave rise to high resemblance with those of isolated from the former Yugoslavia and Southeastern Russia (5). In that case, the death rate was 11% (2 death reports out of 19 infected patients) (5).

In general, the CCHF virus is genetically grouped into seven subtypes according to geographical locations. These groups are Africa-1 (Senegal), Africa-2 (Congo Republic and South Africa), Africa-3 (Southern and Western Africa), Europe-1 (Russia, Turkey, Bulgaria, Kosovo, and Croatia), Europe-2 (Greece), Asia-1 (Middle-East, Pakistan and Iran), and Asia-2 (China, Uzbekistan, Tajikistan and Kazakhstan) (24). There are about 32 species identified in the tick fauna of Turkey, amongst which *Haemaphysalis concinna, Hyalomma anatolicum, Hyalomma detritum, Hyalomma marginatum, Hyalomma turanicum, Rhipicephalus bursa and Rhipicephalus turanicus* are the most frequently encountered ones throughout Turkey (25,26). On the other hand, Ornithodorus type of ticks are the most commonly seen ticks in the vicinity of the city of Van, the far Eastern side of Turkey (25).

Although Turkey is a part of Europe-1 area, the city of Van closely neighbors the Asia-1 area, where tick bites rarely culminate in deaths (24). Therefore, the tick fauna of the city of Van is thought to be rather genetically similar to those where CCHFV lineage Asia-1 is present. Despite increased recent incidences of CCHF cases, to the best of our knowledge, there has been no published reports addressing the current seroprevalence of CCHF in the vicinity of the city of Van of Turkey. This is the first time that CCHF seropositivity results are published here for the city of Van.
As a result, we report in this study that the CCHFV IgG seropositivity in the vicinity of the city of Van is %14.4, whose CCHF-IgM seropositivity was determined to be 3.7%, which are comparatively high and consistent values with other endemic locations in Turkey, and yet no death cases have been reported for that area of interest. It is imperative that farmers and stock breeders living in rural areas as well as health care workers be informed and educated for the presence and risks of CCHF. It is also required that the outcome of this study be supplemented with additional epidemiologic seroprevalance studies in places neighboring the city of Van in order to keep CCHF under control and diminish the risks of acquiring CCHF.

CONFLICT OF INTERESTS
The work presented in this article was supported by the Scientific Research Council of the Yüzüncü Yıl University at Van, Turkey (Project No: 2011-TF-B018). There is no conflict of interest.

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REFERENCES


Table 1. Variation of anti-CCHF IgG seropositivity with age groups

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Men</th>
<th>Women</th>
<th>Total</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (%)</td>
<td>n</td>
<td>Positive (%)</td>
<td>n</td>
</tr>
<tr>
<td>13-20</td>
<td>1 (7.1)</td>
<td>14</td>
<td>2 (7.1)</td>
<td>28</td>
</tr>
<tr>
<td>21-30</td>
<td>1 (4.5)</td>
<td>22</td>
<td>5 (7.9)</td>
<td>63</td>
</tr>
<tr>
<td>31-40</td>
<td>4 (17.4)</td>
<td>23</td>
<td>8 (10.4)</td>
<td>77</td>
</tr>
<tr>
<td>41-50</td>
<td>2 (11.1)</td>
<td>18</td>
<td>8 (20.0)</td>
<td>40</td>
</tr>
<tr>
<td>51-60</td>
<td>2 (11.1)</td>
<td>18</td>
<td>4 (16.7)</td>
<td>24</td>
</tr>
<tr>
<td>&gt;61</td>
<td>4 (33.3)</td>
<td>12</td>
<td>12 (41.4)</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>14 (13.1)</td>
<td>107</td>
<td>39 (14.9)</td>
<td>261</td>
</tr>
</tbody>
</table>

*: Statistical significance between the sex groups of an age group
Table 2. Distribution of anti-CCHF IgG seropositivity based on arthropoda bites

<table>
<thead>
<tr>
<th>Insect/tick bite</th>
<th>IgG Positive</th>
<th>%</th>
<th>Total samples</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insect bite</td>
<td>18</td>
<td>11.8</td>
<td>152</td>
<td></td>
</tr>
<tr>
<td>Tick bite</td>
<td>1</td>
<td>100</td>
<td>1</td>
<td>&gt;0.05</td>
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<tr>
<td>Insect and tick bites</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>34</td>
<td>15.9</td>
<td>214</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>53</strong></td>
<td><strong>14.4</strong></td>
<td><strong>368</strong></td>
<td></td>
</tr>
</tbody>
</table>