A study of the chikungunya virus in humans in Turkey

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1. Introduction
The chikungunya virus (CHIKV) is a single-stranded, positive sense RNA virus that is a member of the genus Alphavirus of the family Togaviridae. The CHIKV is transmitted primarily by Aedes aegypti and Aedes albopictus mosquitoes (1). CHIKV infection led to more than 6 million confirmed cases worldwide (2). CHIKV is a mosquito-borne disease and has recently been causing explosive outbreaks. The CHIKV has spread throughout all continents. Although the first chikungunya case imported from India to Turkey was reported in 2012, there is no detailed epidemiologic study in Turkey yet. The aim of this study was to investigate the seroprevalence of the CHIKV in Turkey.

2. Materials and methods
2.1. Samples
Blood samples were taken from 500 healthy, randomly selected volunteer blood donors who live in Kırıkkale, which is located in central Anatolia in Turkey. The results were verified by indirect immunofluorescence test (IIFT).

Results: The results showed that 0.4% samples were positive for CHIKV. In the verification study with IIFT, CHIKV IgG type antibodies were defined as negative. To the best of our knowledge, this is the first serological study on the CHIKV in Turkey.

Conclusion: Further studies are needed to elucidate the epidemiological situation in patients that have fever and arthritis.

Key words: Chikungunya virus, ELISA, serology, Turkey

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2015 (Table). Samples from volunteers, who were accepted as healthy blood donors after the assessment of a donor questionnaire, were collected with their informed consent. The study was approved by the Ethic Review Committees of the Faculty of Medicine, Kirikkale University (Date 11.05.2015/Decision number: 12-02). Blood samples were collected into tubes without an anticoagulant for the separation of serum, allowing for clotting at room temperature, and centrifuged at 2000 rpm for 10 min. Sera were collected and kept at −80 °C until use.

2.2. Detection of anti-CHIKV IgG
A semiquantitative anti-chikungunya IgG ELISA test (Catalog number EI 293a-9601G Euroimmun) was performed according to the manufacturer’s instructions. Reference controls included positive, negative, and blank samples. The Ab% values were calculated using the following formula: Ratio = Extinction of the control samples or patient samples / Extinction calibrator. A serum sample was considered positive if Ab% was ≥1.1, while a sample was considered negative if Ab% was ≤0.8. An Ab% value of 0.8–1.1 indicated a borderline sample. Blood samples were investigated in terms of IgG type antibodies by IIFT with a commercial kit (Catalog number FI293a-1010G, Euroimmun). The manufacturer confirms the CHIKV IgG IIFT test’s sensitivity as 96.7% and specificity as 100%.

3. Results
A total of 500 serum samples from healthy people were tested by ELISA. Two out of the 500 (0.4%) sera were positive for the CHIKV. The positive results were obtained from a woman aged 79 who is a housewife from Keskin District (OD value 1.385) and a man aged 55 who is a farmer from Balışeyh District (OD value 1.242). Four out of the 500 (0.8%) sera were borderline. In the validation study with IIFT, CHIKV IgG type antibodies were defined as negative. To the best of our knowledge, this is the first serological study on the CHIKV in Turkey.

4. Discussion
The CHIKV causes mosquito-borne disease of key public health importance in tropical and subtropical countries and the health and economic burden due to this virus is enormous. The La Reunion outbreak cost the French authorities millions in lost productivity, and over €43 million in direct medical costs (14).

CHIKV probably first emerged as a human pathogen in the 18th century and certainly reemerged periodically with relatively low prevalence in Africa but also in Asia, with the earliest outbreak in the Philippines in 1954. CHIKV has been reported in many countries in Asia, America, and Africa after an epidemiologic silent period as a neglected tropical disease (2). The reemergence of CHIKV as an urban epidemic was described in Kinshasa, Democratic Republic of the Congo, in 2000 (15). A chikungunya epidemic occurred in several states of India in 2005–2006, affecting about 1.3 million people. The Pan American Health Organization recorded 776,000 cases and 152 deaths attributed to CHIKV infection in 33 countries in 2014 (http://www.paho.org/chikungunya). CHIKV infection has been identified in nearly 80 countries across 5 continents and caused more than 6 million confirmed cases (2). While CHIKV imported cases into Northern Italy were very low between 2011 and 2013, the number of imported cases has increased significantly since 2014 (16). Besides Italy, Turkey also has a coast on the Mediterranean Sea. Chikungunya infection is transmitted between humans by Aedes mosquitoes. Presence of Aedes mosquitoes was reported in Turkey as well (17–19). The first chikungunya case imported from New Delhi, India,

### Table. Kirikkale and its population and sampling data for Kirikkale.

<table>
<thead>
<tr>
<th>Province</th>
<th>Number of samples</th>
<th>Male</th>
<th>Female</th>
<th>≤18 year olds</th>
</tr>
</thead>
<tbody>
<tr>
<td>City center</td>
<td>364</td>
<td>134</td>
<td>230</td>
<td>37</td>
</tr>
<tr>
<td>Bahşılı</td>
<td>13</td>
<td>7</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Balışeyh</td>
<td>11</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Celebi</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Delice</td>
<td>16</td>
<td>7</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Karakeçili</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Keskı̇n</td>
<td>33</td>
<td>6</td>
<td>27</td>
<td>9</td>
</tr>
<tr>
<td>Sulakyurt</td>
<td>13</td>
<td>4</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Yahşi̇han</td>
<td>39</td>
<td>18</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>187</td>
<td>313</td>
<td>62</td>
</tr>
</tbody>
</table>
to Ankara, Turkey, was reported in 2012. However, this is the first seroepidemiological study in Turkey and CHIKV specific antibodies were reported for the first time in Kırıkkale in Turkey.

A concern was that the commercial kit employed may detect the antibodies fighting against other Semliki Forest virus antigenic complex viruses. The CHIKV is part of the Semliki Forest virus antigenic complex that also includes O’Nyong Nyong, Mayaro, and Ross River viruses. It was evaluated, including four CHIKV serologic diagnostic tests. The Euroimmun ELISA, which was used this study, had a specificity of 95% (IgG) and a sensitivity of 88% (IgG). The Euroimmun ELISA test was evaluated as to whether it might detect Mayaro and O’Nyong-Nyong virus and positivity was found (20). Until day 7 after disease onset genome detection by RT-PCR is recommended for diagnosis. IgM and IgG antibodies can be detected as early as 3–6 days after the onset of clinical symptoms. It was reported that IIFT is more sensitive than ELISA. For the commercial IgG assay the specificity was 100% and the sensitivity 95.4% (10).

In conclusion, this research is the first CHIKV seroprevalence study in Turkey. Later studies should focus on viruses in mosquitoes. In addition, the proximity of mosquito vector breeding areas in the Kızılırmak is an important risk factor for other diseases transmitted by the same vector as well as CHIKV. For the prevention and control of vector transmissions, municipalities need to be mobilized. CHIKFV fever should be kept in mind when clinicians encounter patients who have fever and arthralgia and a history of visits to endemic places.

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References


