Seroprevalence of *Brucella* in an Elderly Population in Mid-Anatolia, Turkey

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**ABSTRACT**

Brucellosis is a widespread infectious disease in the mid-Anatolia regions of Turkey. This study investigated the prevalence of *Brucella* infection in elderly people of this region. In total, 750 elderly subjects were chosen by a simple random-sampling method. Blood samples were evaluated by the *Brucella* Wright agglutination test. *Brucella* seropositivity was detected in 24 (3.2%) of the 750 subjects. Although there was no statistical correlation between *Brucella* seropositivity and sex or educational level (p>0.05), seropositivity was statistically significant with subjects exposed to risk factors (p<0.001). It is concluded that *Brucella* infection is still an important public-health problem in the cities of mid-Anatolia.

**Key words:** *Brucella; Brucellosis; Brucella melitensis; Elderly; Turkey*

**INTRODUCTION**

*Brucella*, one of the major zoonotic pathogens in the world, is responsible for enormous economic losses and considerable human morbidity in endemic areas (1). *Brucella* infection is a disease that humans acquire either from animals or from animal products. Goats, sheep, cattle, swine, dogs, and buffalo may be infected with *Brucella*. Human brucellosis is a significant public-health problem in Sivas city located in mid-Anatolia, Turkey. *Brucella melitensis* is most commonly found in humans in the Mediterranean and also in Turkey (2). In this region, farming is one of the most common activities, and people usually consume dairy products. The records of the Public Health Laboratory, Sivas, revealed that 5,316 persons were diagnosed with *Brucella* infection in 2002, and the cumulative incidence was 7.8% (3).

In this study, we investigated the prevalence of human brucellosis in an elderly population in Sivas city. The elderly population was chosen to establish the frequency of infections encountered in their whole life in an endemic region. Seropositivity intended to identify exposure rather than active infection. *Brucella*-specific antibodies can be determined 2-3 weeks following the exposure to *Brucella*.

**MATERIALS AND METHODS**

**Study design and data collection**

The study, conducted during January-March 2002, was designed to detect exposure to *Brucella* in the lifetime of an elderly population of Sivas city. In total, 750 subjects were chosen by a simple random-sampling method from the elderly population (n=11,023) living in Sivas city centre. The starting point was selection using a table of random numbers, and every other person on the list was selected. Trained interviewers recorded information on gender, age, location, educational level, contact with animals, and consumption of non-hygienic dairy products. None of the members of this elderly population had active brucellosis.
Brucellosis assays

All the sera were tested by the Brucella Wright agglutination test as described by Bilgehan (4). Serum was harvested from blood collected from the peripheral venous vessels and was stored at -20 °C until used in serological tests. The presence of antibodies against Brucella was used as a proxy for exposure to Brucella infection. A sample was considered positive for infection with B. melitensis if agglutinins formed within 18 hours. The sensitivity and specificity of the Brucella Wright agglutination test was 90.0% and 96.0% respectively (5). Briefly, the test antigen (sterile killed B. melitensis whole-cell suspension) was diluted in the ratio of 1:100 in phenolized saline (i.e. 5 g phenol in 1 L of sterile normal saline) and allowed to stand for at least 12 hours before use. All sera were routinely diluted from 1:20 to 1:1280. Each batch of tests included a positive 1:1280 control and a negative saline control. A definite agglutination of the suspension was read as a positive reaction. The antigen and serum were covered with a rubber stopper, gently mixed in the tube and then incubated at 37 °C for 48 hours. After the incubation period, tubes were examined against a dull black background. Samples were considered positive if there was clearing of the suspension and agglutination at the bottom of the tube before or after shaking gently. Negative samples remained milky, and agglutination could not be seen. For positive samples, the lowest positive titre was determined. A titration of 1/80 was taken as an indication of exposure to Brucella and one of 1/160 as an indication of brucellosis. Therefore, titration of 1/80 and over were accepted as positive (4).

Statistical analysis

Data on various individuals, including Brucella antibody status, were analyzed using the SPSS 9.0 software (S.A.S. Institute, 1998). Gender, age groups, educational status, and risk factors were assessed independently with the chi-square test, and these factors were also compared with each other with multiple logistic regression analyses using the above programme.

RESULTS

Of the 750 subjects, 368 (49.1%) were males, and 382 (50.9%) were females. The mean age of males, females, and the whole population was 70.4±5.2, 69.5±5.0, and 69.9±5.1 years respectively. Four hundred eighty-seven (60.9%) subjects were illiterate, i.e. people who had never gone to primary school were called ‘illiterate’, and 230 (30.7%) had primary school education. The ratio of the married, widowed and unmarried subjects was 553 (73.7%), 189 (25.2%), and 8 (1.1%) respectively. While 340 (45.3%) subjects were retired, 370 (49.3%) were housewives. Brucella seropositivity was detected in 24 (3.2%) of the 750 subjects.

The table shows Brucella seropositivity with respect to characteristics of the population. There was no statistical difference in seropositivity between males and females (p>0.05), various age groups (p>0.05), or educational level (p>0.05). The relationship between Brucella seropositivity and exposure to risk factors, such as occupational risks (n=2, butchery), contact with animals (n=11), and consumption of non-hygienic dairy products and non-pasteurized milk (n=24), is also shown in the table. The table shows that 71.2% (534/750) of

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Seropositivity</th>
<th>Total</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>3.3</td>
<td>368</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>3.1</td>
<td>382</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65-69</td>
<td>16</td>
<td>3.9</td>
<td>413</td>
</tr>
<tr>
<td>70-74</td>
<td>7</td>
<td>3.3</td>
<td>213</td>
</tr>
<tr>
<td>75+</td>
<td>1</td>
<td>0.8</td>
<td>124</td>
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<td>Education</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>14</td>
<td>3.1</td>
<td>457</td>
</tr>
<tr>
<td>Primary school</td>
<td>7</td>
<td>3.0</td>
<td>230</td>
</tr>
<tr>
<td>Secondary school</td>
<td>3</td>
<td>4.8</td>
<td>63</td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>21</td>
<td>9.7</td>
<td>216</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>0.6</td>
<td>534</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>3.2</td>
<td>750</td>
</tr>
</tbody>
</table>
the population had no contact with animals nor were exposed to unpasteurized dairy products during their life since they spent their lifetime in urban areas. Brucella seropositivity was detected in 21 (9.7%) of 216 subjects exposed to risk factors and in 3 (0.6%) of 534 with no exposure to risk factors. The difference between these two groups was statistically significant (p<0.001).

Seropositivity was found to be affected by the positive risk factors in multiple logistics regression analysis (p<0.05).

DISCUSSION
Brucellosis is a widely-seen infectious disease in Turkey, especially in the mid-Anatolia region. The most common mode of infection is consumption of cheese and butter made from unboiled milk (6,7). Boiling is the most effective method of disinfecting milk in mid-Anatolia. Pasteurized milk is not commonly used in this region. Various studies on Brucella seropositivity have been conducted in Turkey and in other countries. Although the prevalence of Brucella is not exactly known in Turkey, seropositivity in a few studies was reported to be 2-6% (6,7). In some at-risk occupational groups (e.g. butchers), the incidence of seropositivity was observed to be 2.0-12.5% among cattle-dealers and slaughter-house workers (8-11). Brucella seropositivity was reported to be 0.8-11.9% in various occupational groups from different countries (12-14). The incidence of seropositivity in our study, 3.2%, is similar to other society-based studies, while it is less than the seropositivity ratios of some occupational groups with high risk factors (8-14).

In the present study, the male/female ratio of the seropositives was 3.1:3.3%. In similar studies, seropositivity in males was generally higher than in females (12,15-18). However, some investigators reported no difference between males and females (9,14). Although there is a gender difference in brucellosis, most cattle dealers, veterinarians, and slaughter-house workers are males. So, the studies reporting the relationship between gender and seropositivity were conducted in these occupational areas. In society-based studies, no difference between seropositivity and gender was reported.

In the present study, there was no correlation between educational level and Brucella seropositivity. Most of our subjects had a low level of education. It can be speculated that some habits, such as making cheese from unboiled milk and drinking milk without boiling, are the dominant factors rather than educational levels.

The most important risk factors in brucellosis are occupation (butcher, veterinarian, cattle-dealer, and slaughter-house worker) and widespread consumption of unpasteurized milk and fresh cheese. In our study, Brucella seropositivity was 9.7% in the subjects exposed to at least one risk factor. However, seropositivity was 0.6% in subjects having no exposure to any risk factor. The difference between these two proportions is statistically significant. In various studies that evaluated risk factors, occupation (15,17,19,20), contact with animals (16,19), and consumption of unpasteurized milk (16,18,20) were reported as important risk factors.

This study confirms that brucellosis is a potential public-health problem in the cities of mid-Anatolia. To prevent brucellosis, it is suggested that the people should be educated to boil milk, farm animals should be immunized, and farm people should be taught about the transmission of infection and pasteurization of milk products, as the most effective methods. In addition, for protection of people from coming into contact with infected animals, some personal protective materials (e.g. gloves) should be used by people working in occupations with a risk of exposure to brucellosis.

REFERENCES