Seroepidemiological Survey of Brucellosis Among Animal Farmers of Yazd Province

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ABSTRACT
Brucellosis has remained a great problem of health in most of countries, which have failed in control of zoonosis infections. This disease is caused by species of Brucella and usually is transferred from animals to humans. The purpose of this study was to investigate the seroprevalence of brucellosis among animal farmers of Yazd province. In this descriptive study, 933 animal farmers were investigated by serological tests and blood culture method. Then the data were analyzed by SPPS statistical program. The results showed that the frequency distribution of seropositive cases by MAT and STAT were 35 (3.2%); 25 (2.7%) males and 10 (1.1%) females. The highest and lowest incidences of seropositive cases were among age group of 21-30 (1%) and more than 60 (0.3%) years old, respectively. Of the 35 seropositive cases, 2MET positive were 5 (0.5%), while all blood cultures were negative.

INTRODUCTION
Brucellosis is a major cause of Zoonoses and an important public health problem in many parts of the world (27). The incidence of the disease has decreased markedly in industrialized countries (9,15). However, it remains a major public health problem in many developing countries (1,24). Diagnosis of human brucellosis relies on blood culture and serological tests, such as the standard tube agglutination test (STAT), Coombs test, and enzyme-linked immuno – sorbent assay (ELISA) (6,7).

Transmission of brucellosis can be the result of ingestion, direct contact via skin abrasion and mucous membranes (including the conjunctiva), and inhalation (in abattoirs and laboratories). Risk factors for infection include the handling of infected animals, ingestion of contaminated animal products such as unpasteurized milk and milk products (including cow, goat, and camel milk), meat, and handling of cultures of Brucella spp. in laboratories. This has significantly decreased the risk of this infection in individuals, who historically have been at increased risk, including veterinarians, farmers, meat inspectors and abattoir workers (8,10,20).

The purpose of this study was to investigate the seroprevalence of brucellosis among animal farmers of Yazd province.

MATERIALS AND METHODS
Yazd province, central Iran, covers an area of about 74214 square kilometers and has an estimated population of about 800000 with almost 75% living in urban areas. In this descriptive study 933 peripheral blood samples were collected from animal farmers of Yazd province from 1997 to 1999. For isolation and identification of Brucella species, biphasic blood culture medium (Hemolin, Biomerieux, France) which was incubated in an atmosphere of 5% to 10% carbon dioxide for 30 days has been used (16). Serum specimens were analyzed in three phases, using suspension of B. abortus and B. melitensis (Wellcome Laboratories, UK). In the first phase, all specimens were screened by the microplate agglutination test (MAT). A titer of 1:80 or greater was considered to represent the presence of specific agglutination Brucella antibodies (seropositive). In the second phase, seropositive specimens were analyzed by the standard tube agglutination test (STAT). A titer of 1:160 or greater was taken as an index of seropositivity (6,7).

In the third phase, Brucella antibody of animal farmers were investigated by 2-mercapthoethanol test (2 MET) (13,26). The collected data and the results of laboratory tests were analyzed by statistical pakage for social science (SPSS), to determine those variable that were significantly associated with seropositivity to Brucella.

RESULTS
Table 1 shows some demographic characteristic of animal farmers who provided blood samples for this study. Distribution of 35 seropositive cases by MAT and STAT were 25 (2.7%) males and 10 (1.1%) females. The highest and lowest incidence of brucellosis were observed among age group of 21-30 (1%) and more than 60 (0.3%) years old, respectively (Table 2 and Fig1). Statistical analysis showed significant differences between sexes ($\chi^2 = 9.32, P<0.001$). There were no significant differences between age groups ($\chi^2 = 3.22, P>0.5$). Findings show that 5 (0.5%) of seropositive cases were 2 MET positive, and all blood cultures were negative.

The most common presenting clinical symptoms with active brucellosis were back pain, headache, fever, chill, night sweats weakness, myalgia, arthralgia and bone pain (Table 3).

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Table 1. Distribution of animal farmers on the basis of age and sex

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>Male (n = 811)</th>
<th>Female (n = 122)</th>
<th>Total (n = 933)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>&lt; 10</td>
<td>4</td>
<td>(0.4)</td>
<td>0</td>
</tr>
<tr>
<td>10 – 20</td>
<td>194</td>
<td>(20.8)</td>
<td>18</td>
</tr>
<tr>
<td>21 – 30</td>
<td>142</td>
<td>(15.2)</td>
<td>11</td>
</tr>
<tr>
<td>31 – 40</td>
<td>117</td>
<td>(12.5)</td>
<td>25</td>
</tr>
<tr>
<td>41 – 50</td>
<td>116</td>
<td>(12.4)</td>
<td>39</td>
</tr>
<tr>
<td>51 – 60</td>
<td>123</td>
<td>(13.2)</td>
<td>23</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>115</td>
<td>(12.3)</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 2. The results of serological tests and blood culture method

<table>
<thead>
<tr>
<th>Tests</th>
<th>MAT</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody titers</td>
<td>1/160 1/320 1/640 1/1280</td>
<td>≤1/80</td>
<td>≥1/160</td>
</tr>
<tr>
<td>Number</td>
<td>35</td>
<td>898</td>
<td>17</td>
</tr>
<tr>
<td>Percentage</td>
<td>3.8</td>
<td>96.2</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Table 3. Distribution of seropositive animal farmers on the basis of clinical signs and symptoms

<table>
<thead>
<tr>
<th>Clinical Signs and symptoms</th>
<th>Back pain</th>
<th>Headache</th>
<th>Fever</th>
<th>Chill</th>
<th>Night sweats</th>
<th>Weakness</th>
<th>Myalgia</th>
<th>Arthralgia</th>
<th>Bone pain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>3</td>
<td>14</td>
<td>27</td>
<td>21</td>
<td>23</td>
<td>18</td>
<td>11</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>Percentage</td>
<td>0.3</td>
<td>1.5</td>
<td>2.9</td>
<td>2.3</td>
<td>2.5</td>
<td>1.9</td>
<td>1.2</td>
<td>1.8</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Fig. 1. Distribution of seropositive animal farmers on the basis of age
DISCUSSION
Brucellosis is diagnosed either by isolation of Brucella organism in culture, or by a combination of serological tests and clinical findings consistent with brucellosis. Isolation of the Brucella organism is the definitive means of diagnosis, but in practice it is difficult due to the early tissue localization and the exacting culture requirements of the organism. In practice, blood cultures are positive in 10 -30% of brucellosis and the remainder is diagnosed serologically (28). Although no single test provides 100% sensitivity and specificity, STAT still remains the test of choice in diagnosis. In the presence of appropriate signs and symptoms, a presumptive diagnosis of brucellosis is usually defined serologically as a standard tube agglutination titer of 1 : 160 or greater (7).

Recent serological data on brucellosis in developed countries are not available. Indeed, brucellosis has been brought under control in these countries through rigorous diagnostic and control procedures at the animal production level, as well as through elimination of Brucella in livestock and proper pasteurization of milk (2). Therefore, there are very few reports of indigenously acquired human cases of brucellosis, while acute imported human infections continue to occur, often associated with the consumption of raw cheese or milk (5,25).

Data from developing countries in the Mediterranean basin, particularly the Middle East, report seroprevalence rates ranging from 8% in Jordan (12) to 12% in Lebanon and Kuwait (4,17). Even higher seroprevalence rates have been reported in some Saharan countries, with percentages of 18% in Uganda (21) and 13% in Nigeria (24). According to the our findings most of patients had a history of infected cheese and milk consumption or direct contact with domestic animal. However, rate of seropositive cases among Yazd animal farmers population was very lower than above mentioned reports; 3.8% or 4.3 cases per 100000. The prevalence rate of brucellosis increases with age, a result that is consistent with observations made in Iran, Jordan, Lebanon, and Kuwait (4,11,17,18,19,22,29). In contrast to other studies, no significant difference was found in prevalence rate between age groups.

In an analysis of 104 cases of brucellosis in Saudi Arabia, 1288 cases in United States, and 60 cases in Iran, the most common symptoms and physical findings were reported fever, chills, weakness, malaise, sweating, backache, headache, lymphadenopathy, splenomegaly and arthritis (12,18,23). These results are nearly the same as our findings.

Brucellosis in animals remains a major public health hazard due to its transmissibility to man (2). The only effective way to control the disease in man is by elimination of the infected animals and vaccination of healthy ones in order to reduce the risk of those in regular contact with animals, and to produce brucellosis free animal products (3).

The effectiveness of vaccination programs can be evaluated by investigating incidence rate in humans, especially people at high risk, before and after vaccination. It is recommended that surveillance of brucellosis be strengthened. Cooperation and joint supervision between the Ministries of Health and Veterinary Organization, and cooperation with neighboring countries, should also be encouraged. Furthermore, health education programs should be adopted that aim at stopping the spread of infection among animals and then to humans, and also adoption of hygienic measures among high-risk population.

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