COMPARISON OF ROSE BENGAL PLATE TEST ANTIGENS PREPARED FROM *BRUCELLA ABORTUS*, *BRUCELLA MELITENSI S* AND *BRUCELLA SUIS*

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Received for publication March 07, 2005.

Abstract

Rose bengal plate test (RBPT) antigens from *Brucella melitensis* and *Brucella suis* S 2 were prepared and compared with RBPT antigen prepared from classical *Brucella abortus* S 99. A total of 54 sera samples, of which 7 were collected from humans with brucellosis and 47 from infected sheep, were studied by using RBPT and serum agglutination test (SAT). RBPT and SAT results showed that RBPT antigens prepared from *Br. melitensis* and *Br. suis* S 2 were compatible with RBPT antigen prepared from *Br. abortus* S 99. Therefore, we concluded that RBPT antigens can be prepared from *Br. melitensis* and *Br. suis* S 2 and used for epidemiological surveillance of human and sheep brucellosis.

Key word: brucellosis, rose bengal plate test, antigen.

Brucellosis is an infectious disease caused by *Brucella* strains in domestic animals and humans. It is a well-known worldwide zoonotic disease that has a high incidence in Mediterranean countries (3, 8). *Brucella abortus* is a pathogen mainly in cattle and humans, *Br. melitensis* is pathogenic for sheep, goats, and humans, *Br. suis* is isolated from swine, cattle and humans, and *Br. canis* is found in dogs. In addition to these, it was recently reported that there is an increasing occurrence of *Br. melitensis* in cattle, camel and humans (2, 5-7, 14). Brucellosis in veterinarians and farmers exposed to diseased animals or infected specimens such as blood, soft tissues and bones, called as job-related-brucellosis, have been reported in the United States (13, 15) and Turkey (1).

Diagnosis of brucellosis is performed through the isolation of the bacteria and serologic tests. Allergic skin reaction tests in sheep are also used to detect the disease (3). Serologic tests such as rose bengal plate test (RPBT), complement fixation test, serum agglutination test, indirect haemolysis test and ELISA are mostly used to detect and eradicate the disease rather than isolate the bacteria (2, 6, 9, 14).

RBPT developed by Morgan (11) for the diagnosis and detection of brucellosis in animal herds, is a cheap, rapid, and effective serologic test compared to others (2, 5, 8). Reliable results can be obtained in large herds in a short period with the least amount of equipment. RBPT antigens are mainly used for the brucellosis diagnosis in cattle, sheep and goats (2, 5, 8). However, the specificity and sensitivity of RBPT in sheep and goats are still unclear (2, 3, 6, 8).

The purpose of this study was to prepare RBPT antigens from *Br. melitensis* and *Br. suis* S 2 and compare them with classical RBPT antigens prepared from *Br. abortus* S 99.

Material and Methods

Blood serum samples. Serum samples were collected from 47 sheep, from whose aborted foetuses *Br. melitensis* was isolated. In addition, 7 serum samples were taken from positive humans. The sheep had never been vaccinated with *Br. melitensis* Rev. 1.

Preparation of RBPT antigens. RBPT antigens were prepared from *Br. abortus* S 99 (Cimenlik 99), *Br. melitensis* and *Br. suis* S 2 according to the technique of OIE (12). Then, the antigens were compared with the standard RBPT antigens prepared from *Br. abortus* S 99 (Pendik 99), produced by the Pendik Veterinary Control and Research Institute, Istanbul, Turkey.

RBPT. RBPT was performed after the technique described by OIE (12).

Serum agglutination test (SAT). SAT was also performed after the technique described by OIE (12). Serum samples were diluted as follows: 1/10, 1/20,
1/40, 1/80, 1/160, 1/320 and 1/640. SAT antigen was provided by the Pendik Veterinary Control and Research Institute, Istanbul, Turkey. Then, the SAT results were evaluated as negative or positive.

**Statistical analysis.** Data were converted from ordinal to numerical as follows: negative = 0, positive (+) = 1, (++) = 2, and (+++) = 3. Then, RBPT results were compared by Kruskal-Wallis one-way analysis of variance on ranks and Mann-Whitney rank sum test (4).

**Results**

Titres of serum samples of SAT are shown in Table 1. Ten out of 54 serum samples were found to be negative (2 of these were negative in the dilution of 1/10) and were not evaluated by RBPT. Then, using 4 kinds of RBPT antigens, 44 SAT-positive serum samples were tested. RBPT results, evaluated by the degrees of agglutination (+++, ++, +, and (-)), are shown in Table 2.

**Table 1**

<table>
<thead>
<tr>
<th>Serum titers</th>
<th>(-)</th>
<th>10</th>
<th>20</th>
<th>40</th>
<th>80</th>
<th>160</th>
<th>320</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>8</td>
<td>2</td>
<td>6</td>
<td>15</td>
<td>17</td>
<td>5</td>
<td>1</td>
<td>54</td>
</tr>
</tbody>
</table>

**Table 2**

Comparison of 4 different RBPT antigens (n=44 each)

<table>
<thead>
<tr>
<th>Antigen</th>
<th>+++</th>
<th>++</th>
<th>+</th>
<th>(-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Br. abortus (Pendik S 99)</td>
<td>23</td>
<td>9</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Br. abortus (Çimenlik S 99)</td>
<td>30</td>
<td>9</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Br. melitensis</td>
<td>32</td>
<td>10</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Br. suis S 2</td>
<td>31</td>
<td>9</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 3**

Mann-Whitney rank sum test results (n = 44)

<table>
<thead>
<tr>
<th>Comparison of groups</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Br. abortus (Pendik S 99) vs Br. abortus (Çimenlik S 99)</td>
<td>0.095</td>
</tr>
<tr>
<td>Br. abortus (Pendik S 99) vs Br. melitensis</td>
<td>0.033*</td>
</tr>
<tr>
<td>Br. abortus (Pendik S 99) vs Br. suis S 2</td>
<td>0.062*</td>
</tr>
<tr>
<td>Br. abortus (Çimenlik S 99) vs Br. melitensis</td>
<td>0.619</td>
</tr>
<tr>
<td>Br. abortus (Çimenlik S 99) vs Br. suis S 2</td>
<td>0.828</td>
</tr>
<tr>
<td>Br. melitensis vs Br. suis S 2</td>
<td>0.786</td>
</tr>
</tbody>
</table>

* Statistically significant

The evaluation of 44 SAT-positive serum samples tested by 4 different RBPT antigens was as follows: Standard RBPT antigen (Pendik 99): 23 (52.2%) +++ positive, 9 (20.4%) ++ positive, 6 (13.6%) + positive, and 6 (13.6%) (-) negative. Çimenlik (S 99) RBPT antigen: 30 (68.1%) +++ positive, 9 (20.4%), and 5 (11.3%) + positive. Br. melitensis RBPT antigen: 32 (72.7%) +++ positive, 10 (22.7%) ++ positive, and 2 (4.3%) + positive. Br. suis S 2 RBPT antigen: 31 (70.4%) +++ positive, 9 (20.4%) ++ positive, and 4 (9.9%) + positive. However, there were no negative results by using the antigens of Çimenlik, Br. melitensis and Br. suis.

Kruskal-Wallis test showed that there was a difference between groups (P = 0.046), however, Student-Newman-Keuls multiple comparison test did not show significant difference. Therefore, Mann-Whitney rank sum test was performed to find out which groups were significant (Table 3). Br. abortus S 99 RBPT antigens (Pendik S 99 and Çimenlik S 99), Br. melitensis and Br. suis RBPT antigens were found to be compatible, but Pendik S 99, Br. melitensis, and Br. suis S 2 were statistically different.

**Discussion**

RBPT is a cheap, dependable and giving rapid results method and is frequently used for the diagnosis of brucellosis and detection of its prevalence (2, 5, 8).
The test is used mostly as a screening test (2, 3, 6, 10). RBPT antigens produced from Br. abortus biovar 1 and 2 strains have A-antigen. However, Br. melitensis with dominant features causes infections in sheep and goats and has M-antigen (5, 8). Moreover, Br. abortus biovar 5 strain also has predominant features for M-antigen (5). Thus, in some cases, when CFT is positive, RBPT may give negative results or vice versa (2). These conflicting results can be explained by the fact that brucellosis in sheep and goats is mainly caused by Br. melitensis. Since serological test antigens are usually prepared from Br. abortus, RBPT using antigens from Br. abortus may give negative results for brucellosis caused by Br. melitensis.

Evaluation of 15 RBPT antigens prepared from different strains with different pH and cell concentrations as compared to the standard anti-Br. abortus sera and to CFT, revealed 100% specificity with RBPT and CFT in brucella-free animals, on the other hand, varying degrees of sensitivity were found with the use of RBPT antigens with Br. melitensis infected animals (2). Mikolon et al. (10), using 17 different tests and antigens for the diagnosis of brucellosis in goats, had high sensitivity in both Br. abortus and Br. melitensis infections with the RBPT antigens at 3% cell concentration prepared from Br. abortus. Blasco et al. (3) reported that the sensitivity of RBPT was 92.1% in sheep herds with brucellosis diagnosed by the isolation of Br. melitensis; however, it was 57.6% in brucella-free sheep herds. Corbel et al. (5) used RBPT antigens prepared from Br. abortus and Br. melitensis in cattle infected with Br. abortus biovar 5 strain to detect brucellosis in England. Although Br. melitensis antigen had lower sensitivity than Br. abortus antigen, its use was advocated. The sensitivity of 100% using RBPT was also reported in goat herds with and without Br. melitensis infection. However, the sensitivity and specificity was 80% and 100%, respectively, in goats vaccinated with Br. melitensis Rev. 1 (5). Macmillan et al. (8) compared 9 different RBPT antigens prepared in different countries to determine if there was a standardization between these antigens in goat herds with and without Br. melitensis infection by using RBPT, SAT, and ELISA and found 100% specificity and sensitivity with RBPT in vaccinated and infection-free goat herds. However, the sensitivity of RBPT was lower in goat herds with a low prevalence.

In our study, the sera samples were obtained from Br. melitensis positive sheep. The sheep had never been vaccinated with Br. melitensis Rev. 1, therefore, the titres were representative of naturally occurring disease rather than a vaccine response. Only Pendik S 99 and Br. melitensis were not compatible statistically. All patients from which Br. melitensis was isolated had clinically diagnosed brucellosis. Six serum samples were negative by RBPT prepared from Pendik S 99. Thus, RBPT antigens prepared from Br. abortus may give negative results in some patients with brucellosis caused by Brucella species other than Br. abortus.

Our study showed that RBPT antigens can be prepared from Br. suis S 2 and Br. melitensis as well as from Br. abortus. In Turkey, mainly Br. melitensis causes brucellosis in humans and sheep. So, we concluded that Br. suis S 2 and Br. melitensis RBPT antigens which can easily be produced in large amounts because of their rapid and easy growth characteristics, can be used for epidemiological detection and eradication of brucella infections.

**References**