Association between *Brucella melitensis* DNA and *Brucella* spp. antibodies

*Brucella* spp. antibodies, despite falling to low levels, can remain measurable after recovery from acute brucellosis (1). Recently, several studies have shown the persistence of *Brucella* spp. DNA in both chronic brucellosis patients and asymptomatic subjects with a previous history of brucellosis (2-5). However, to our knowledge, the association between serum antibodies and *Brucella* spp. DNA has not been investigated.

We screened a cohort of 38 subjects with a well-documented history of brucellosis for the presence of *Brucella melitensis* DNA and *Brucella* antibodies. For that purpose we tested both a quantitative real time PCR (QPCR) assay (2) and an immunocapture-agglutination test (Brucellacapt®, Vircell SL, Granada, Spain) that was performed as specified by the manufacturer. It has been described that the Brucellacapt® test offers results comparable to the Coombs anti-*Brucella* test, the most often used technique for the diagnosis of chronic brucellosis (6).

Twenty-seven (71%) subjects were men and 11 (29%) were women. The mean age was 49 ± 14 years (range, 26-83 years). The diagnosis of acute brucellosis was made between 3 to 33 years previously, according to one or both of the following criteria: isolation of *Brucella* spp. from blood or any sample of body fluid or tissue, and, the presence of a compatible clinical picture together with the demonstration of specific antibodies at significant titers (Wright test \( \geq 1:160 \) or Coombs anti-*Brucella* test \( \geq 1:320 \)) or seroconversion. According to their clinical evolution after the initial episode, subjects were divided into three groups. Group A consisted of 10 (26%) focal disease subjects. Group B comprised 8 (21%) non-focal disease subjects complaining of non-specific symptoms, such as fatigue, malaise, arthralgia and/or myalgia. The remaining 20 (53%) subjects were asymptomatic (Group C). Chronic brucellosis patients included
all patients diagnosed of focal disease and those whose symptoms had persisted for more than one year after the initial episode. Results are expressed as means ± standard deviations. *P* values less than 0.05 were considered statistically significant.

We found association between being *B. melitensis* DNA and antibody positive. Among the 22 subjects with detectable *B. melitensis* DNA, 19 (86%) subjects had *Brucella* antibodies, whilst among the 16 subjects without *B. melitensis* DNA, *Brucella* antibodies were detected in 7 (44%) (*P* = 0.005; Chi-square test). In the case of the asymptomatic subjects group, the DNA-antibody concordance was not statistically significant (*P* = 0.264; Two-tails Fisher’s exact test). The distribution of DNA-antibody results by group is shown in Table 1.

The chronic brucellosis patients harbouring *B. melitensis* DNA are more likely to show a seropositive sample than the remaining subjects. These findings suggest that after the initial infection, either the viable *Brucella* or its antigenic and structural components persist in the host and may have diagnostic and pathogenic implications.
Table 1. Distribution of the QPCR and Brucellacapt® results of the 18 patients with chronic brucellosis and the 20 asymptomatic subjects

<table>
<thead>
<tr>
<th>QPCR blood/serum</th>
<th>Focal disease patients (n=10)</th>
<th>Non-focal disease patients (n=8)</th>
<th>Asymptomatic subjects (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>+/-</td>
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<td>-/+</td>
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<td></td>
<td>9</td>
</tr>
<tr>
<td>≥640</td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

Brucellacapt® titers:

- 40: 1 1 1 1 1 5
- 80: 1 1 1 2
- 160: 2 1 1
- 320: 1 4 1 1
- ≥640: 1 1

* Reciprocal titers; +: positive; -: negative
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References


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