Coombs Antiglobulin Test Using *Brucella abortus* 99 as Antigen To Detect Incomplete Antibodies Induced by *B. abortus* RB51 Vaccine in Cattle

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This study showed that vaccination of cattle with *Brucella abortus* rough strain RB51 induces incomplete antibodies that can be detectable by a Coombs antiglobulin test using the *B. abortus* 99 smooth strain.

*Brucella abortus* strain RB51 is a laboratory-derived rough mutant of the standard virulent *B. abortus* strain 2308 (15). This strain lacks the O side chain of the lipopolysaccharide (LPS) characteristic of smooth *Brucella* strains. Therefore, vaccination of cattle with RB51 does not produce antibodies that can be detected by routine surveillance tests, such as complement fixation (CF) and Rose Bengal Plate (RBP) tests, which especially identify antibodies to LPS.

To monitor antibody responses following RB51 vaccination in cattle, a CF test has been developed with *B. abortus* RB51 as a homologous antigen that is able to specifically measure antibodies to *B. abortus* RB51 (1–3). Presumably, in this *B. abortus* RB51-based CF test, CF antibodies have been found that are directed to the outer membrane proteins (OMPs), which are accessible for binding in *B. abortus* RB51 strain, but not in *B. abortus* strain 99, because of the steric hindrance due to the presence of LPS in smooth brucellae (7, 11).

Studies have shown that both *B. abortus* S19 and 45/20, widely used as vaccines, produce nonagglutinating antibodies (13), the function of which is probably to delay the bacterial clearance and increase chronic *B. abortus* infections (4, 5, 12, 13). The agglutinating activity of incomplete antibodies is markedly reduced by the inadequate extension of Fab regions that prevents the effective bacterial agglutination (13, 14). However, *B. abortus* 99 cells sensitized with the incomplete antibodies can be agglutinated by adding the Coombs’ antiglobulin reagent (8, 9).

The aim of the present trial was to develop a Coombs antiglobulin test to ascertain whether *B. abortus* RB51-vaccinated cattle produce incomplete antibodies in addition to the CF antibodies detected by a RB51-based CF test. The results of the Coombs test were compared with those obtained by serum agglutination test (SAT), CF, and RBP tests, performed with standard *B. abortus* 99 antigen, and by the *B. abortus* RB51-based CF test.

For serological reactions, the following serum samples and antigens were used: three positive sera collected from cattle experimentally vaccinated with RB51 and boosted 30 days later, showing antibody titers of 1:128, 1:32, and 1:4, respectively, as measured by RB51-based CF test; a pool of 10 negative sera from brucellosis-free cattle as a negative control, and the OIE 2nd international standard anti-*B. abortus* serum (ISaBS) at 1,000 IU/ml, supplied by the Veterinary Laboratories Agency (VLA) of Weybridge, United Kingdom; S-type *B. abortus* 99 international and national standard antigens produced by the VLA and by the Istituto Zooprofilattico Sperimentale (IZS) of Brescia (Italy), respectively, for use in SAT and CF tests to detect antibodies against *B. abortus*; the buffered S-type *B. abortus* 99 international and national standard antigens produced by the VLA and by the IZS—Teramo (Italy), respectively; the R-type *B. abortus* RB51 antigen for use on the CF test, produced by the Istituto Superiore di Sanità of Rome, Italy (ISS—Roma), as previously described for the detection of antibodies to *B. abortus* strain RB51 (1–3). All serological tests were performed in microtiter 96-well plates.

The CF test with *B. abortus* RB51 as antigen and the CF and RBP tests with standard smooth antigens were performed as previously described (1–3).

The Coombs test was performed in two steps. In the first step, serum samples diluted twofold in saline (0.15 M NaCl [pH 7.2]) were tested for the presence of antibodies to *B. abortus* 99 by an SAT, and the agglutination titers were evaluated after incubation at 37°C overnight. In the second step, following three washes with saline, the supernatant of each well was replaced with 25 μl of saline and 25 μl of goat antiovine whole serum (VMRD, Inc., Pullman, Wash.), previously diluted 1:7 in saline.

After incubation at 37°C overnight in a humidified atmosphere with gentle stirring, Coombs results were compared with data obtained from conventional CF and RBP tests and from the RB51-based CF test (Table 1). All reactions were performed twice.

As shown in Table 1, unlike the ISaBS, the serum samples from RB51-vaccinated cattle, as expected, didn’t react when tested with RBP and CF tests against the *B. abortus* 99 standard antigen. To the contrary, these sera scored positive in the Coombs antiglobulin test by using the same smooth strain *B. abortus* 99 as an antigen. No reaction was observed with negative sera.

This study shows that the Coombs antiglobulin test can be performed with a buffered antigen routinely used in the RBP test and that international and national antigens give comparable results. Our results indicate that the vaccination with *B.
**TABLE 1.** Comparative analysis of results obtained by Coombs antiglobulin, serum agglutination, CF, and RBP tests

<table>
<thead>
<tr>
<th>National or international standard antigen</th>
<th>Serum agglutination</th>
<th>CF</th>
<th>Coombs antiglobulin</th>
<th>RBP</th>
<th>Antibody titer in sera from RB51-vaccinated cattle</th>
<th>Result with negative bovine serum&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Antibody titer in 2nd IsBS of Weybridge&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-type <em>B. abortus</em> 99 of Weybridge</td>
<td>Serum agglutination</td>
<td>CF</td>
<td>Coombs antiglobulin</td>
<td>RBP</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>S-type <em>B. abortus</em> 99 of IZS—Brescia</td>
<td>Serum agglutination</td>
<td>CF</td>
<td>Coombs antiglobulin</td>
<td>RBP</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Buffered S-type <em>B. abortus</em> 99 of Weybridge</td>
<td>Serum agglutination</td>
<td>CF</td>
<td>Coombs antiglobulin</td>
<td>RBP</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Buffered S-type <em>B. abortus</em> 99 of IZS—Teramo</td>
<td>Serum agglutination</td>
<td>CF</td>
<td>Coombs antiglobulin</td>
<td>RBP</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>S-type <em>B. abortus</em> 99 of Weybridge</td>
<td>CF</td>
<td>CF</td>
<td>Coombs antiglobulin</td>
<td>RBP</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>S-type <em>B. abortus</em> 99 of IZS—Brescia</td>
<td>CF</td>
<td>CF</td>
<td>Coombs antiglobulin</td>
<td>RBP</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>R-type <em>B. abortus</em> RB51 of ISS—Roma</td>
<td>CF</td>
<td>CF</td>
<td>Coombs antiglobulin</td>
<td>RBP</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Buffered S-type <em>B. abortus</em> 99 of Weybridge</td>
<td>RBP</td>
<td>CF</td>
<td>Coombs antiglobulin</td>
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<td>Buffered S-type <em>B. abortus</em> 99 of IZS—Teramo</td>
<td>RBP</td>
<td>CF</td>
<td>Coombs antiglobulin</td>
<td>RBP</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
</tbody>
</table>

<sup>a</sup> Pool of 10 sera from brucellosis-free cattle.

<sup>b</sup> Second international standard anti-*B. abortus* serum, supplied by the VLA, New Haw, Addlestone, Surrey, United Kingdom.

*abortus* RB51 induces the production of antibodies, directed against epitopes of the RB51 rough strain, which are able to fix the complement when an RB51 homologous strain is used as an antigen (1–3). In addition, after a booster vaccination with the same strain, incomplete antibodies are evident, directed against epitopes of smooth *Brucella* strains, which are not able to fix the complement as already observed when another rough strain, *B. abortus* 45/20, has been used for vaccination of cattle (12). In addition, according to previous data (6), these incomplete antibodies can be demonstrated only after a booster vaccination (R. Adone, unpublished results). To verify that different antibodies are involved in the different tests used, a serum sample from RB51-vaccinated cattle was previously tested against RB51 and then incubated overnight with a suspension of *B. abortus* 99. After centrifugation at 4,000 rpm for 10 min, the pellet was discarded to eliminate the antibodies directed against the *B. abortus* 99 strain, and the supernatant was retested with the CF test against RB51. No reduction of the reactivity against RB51 was evident following incubation with *B. abortus* 99, thus confirming that different antibodies are involved in the CF and Coombs tests (data not shown).

In conclusion, our results indicate that Coombs antiglobulin test with smooth strain *B. abortus* 99 as an antigen can be successfully used to identify incomplete antibodies following RB51 vaccination in cattle. Studies are in progress in our laboratory to evaluate the specificity of these antibodies and their role in the course of RB51 infection.

**REFERENCES**


