Comparison of serological methods for the
detection of \textit{B. abortus} antibodies in sera from vaccinated and
non-vaccinated cattle*

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SUMMARY

A total of 4551 sera from 863 Strain 19 vaccinated and non-vaccinated adult
cattle, independent of disease status, were tested by five serological methods to
detect the presence of antibodies to \textit{B. abortus}. Results from Standard Agglutin-
ation Tube (SAT), Buffered Brucella Antigen or card (CT), Complement Fixation
(CF), Enzyme Linked Immunosorbent Assay (ELISA) and Rivanol (Riv) methods
were compared.

There was a 95\% probability for agreement among CT negative sera, between
serological methods, for all groups of vaccinated and non-vaccinated cattle. The
agreement between tests with Riv Positive sera, excluding the calfhood and adult
vaccinated group tested by the CF method, was 91–100\%. The probability of a
serum which was serologically negative by other methods being Riv negative
was 98 \%. The usefulness of serological results from Riv ($\geq 1/50$) tests for classify-
ing the reactor status of cattle are of doubtful supplemental value to confirm
card test positive results.

Vaccination history is an important consideration when evaluating serological
data on cattle sera particularly from SAT and CF methods.

INTRODUCTION

Evaluation of results from serological methods plays an integral role in diagnosis
of disease and management of herds and individual cattle exposed to or infected
with \textit{Brucella abortus} (\textit{B. abortus}). Data from Standard Agglutination Tube (SAT)
Buffered Brucella Antigen or card (CT) and Complement Fixation (CF) tests have
been compared to determine agreement among tests, and suitability of serological

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methods, to evaluate the health status of the animal or herd exposed to *B. abortus* (Alton *et al.* 1975; Chappel *et al.* 1978; Morgan, MacKinnon & Cullen, 1969; Morgan & Richards, 1974; Nicoletti, 1967; Rose & Roepke, 1957). Most cattle infected with *B. abortus*, except those which are recently infected, can be identified by the SAT test (Davies, 1971). However, it has been reported that other supplemental serological methods were superior to SAT for detecting *Brucella* antibodies in sera from culture positive cows (Nicoletti, 1969). Recent evidence, suggesting that negative SAT results on specific sera cannot always be confirmed by plate agglutination or CF tests, was interpreted (Morgan & Richards, 1974) as being indicative of the insensitivity of the SAT test.

The immunologic response to antigenic stimulus by *B. abortus* is characterized by the synthesis of different classes and subclasses of immunoglobulins (Allan *et al.* 1976; Patterson, Deyoe & Stone, 1976; Timbs, Digby & Doe, 1978; O'Reilly & Cunningham, 1971). The immune response of individual cattle varies with regard to natural infection and vaccination, and it has been shown that certain immunoglobulins may not be present in serum at specific times, or in proper concentration, to allow for simultaneous positive reactions in different tests (Allan *et al.* 1976).

The purpose of this study was to determine the agreement between serological methods in detecting antibodies to *B. abortus* by comparing results from five different methods, Rivanol (Riv), Enzyme Linked Immunosorbent Assay (ELISA), CF, SAT and CT, on sera from cows under varying vaccination regimens and non-vaccinated cattle. Data were analysed independent of disease status and length of time between vaccination and serum collection.

**MATERIALS AND METHODS**

**Sera**

Blood samples were collected at 30- or 60-day intervals from the coccygeal vessels. A total of 8564 sera were obtained from 910 adult dairy cows beginning October 1976 and ending in February 1978. Only individual serum samples from which five serological test results were available were used. There were 4551 sera from 863 cows which satisfied this requirement. Although several serum samples were collected from each cow within 17 months, sera were tested and analysed as independent samples. Some sera obtained from AV cattle were collected 30 days after vaccination.

**Experimental groups**

The 863 adult cows were divided into four experimental groups: 139 cows were calfhood and adult vaccinated (CVAV), 272 cows were only adult vaccinated (AV), 178 cows were only calfhood vaccinated (CV) and 274 cows were not vaccinated (NV).

Vaccination was accomplished with 1 ml of strain 19 vaccine diluted with sterile peptone solution to contain $3 \times 10^9$ viable cells/ml (Crawford, Heck & Williams, 1978).
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Serological methods

Card tests were accomplished according to the standard procedure described in the Brucellosis Card Test Kit with accompanying reagents prepared for Veterinary Services, APHIS, U.S.D.A.

Procedures for SAT and Riv were done according to the standard U.S.D.A. procedure. Complement fixation tests were done according to a method previously described (Jones, Hendricks & Berman, 1963), and Enzyme Linked Immunosorbent Assay (ELISA) was done according to the method of Saunders (1977).

CARD, SAT and RIV tests were completed at the State-Federal Brucellosis Laboratory, Austin, Texas. Complement fixation and ELISA tests were completed in the Department of Microbiology and Parasitology, College of Veterinary Medicine, Texas A & M University, College Station, Texas.

Test results from five serological methods on all sera were compared with each other in all possible combinations to determine their relative agreement (%) within and between experimental groups.

Positive reactions were determined by a Riv titre $> 50$, CF titre $> 40$, SAT titre $> 100$. A positive ELISA was determined by an extinction value (EV) $> 4$.

$$EV = [(100) - (%T \text{ unknown})] - [(100) - (%T \text{ of serum control})].$$

RESULTS

Comparison of results between serologic tests and experimental groups

Card test positive

Agreement between 1401 card test positive sera was between 43 and 93 % when compared with results by other serological methods (Table 1). The agreement was 43–79 % for vaccinated and 82–93 % for non-vaccinated cattle.

Card test negative

Agreement between 3150 card test negative sera ranged between 90 and 100 % (Table 2). The agreement between sera from non-adult vaccinated cattle was 98–100 %.

Rivanol positive

Agreement between 754 Rivanol positive sera was between 84 and 100 % when compared with results by other serological methods (Table 3). Except for the 84 % agreement between Riv and CF in the CVAV group, the agreement between all tests for all experimental groups was 91–100 %.

Rivanol negative

Agreement between 3797 Rivanol negative sera between all tests was from 64 to 99 % (Table 4). Agreement in the CV and NV groups ranged from 96 to 99 % while the agreement in CVAV and AV groups, that is any cow receiving adult vaccination, ranged between 64 and 83 %.
### Table 1. Serological distribution of card test positive sera

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>ELISA</th>
<th>CF</th>
<th>Riv</th>
<th>SAT</th>
<th>Total card test positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calfhood and adult vaccinated</td>
<td>70</td>
<td>30</td>
<td>63</td>
<td>43</td>
<td>71</td>
</tr>
<tr>
<td>Calfhood vaccinated</td>
<td>70</td>
<td>30</td>
<td>74</td>
<td>26</td>
<td>83</td>
</tr>
<tr>
<td>Adult vaccinated</td>
<td>73</td>
<td>27</td>
<td>65</td>
<td>35</td>
<td>79</td>
</tr>
<tr>
<td>No vaccination</td>
<td>93</td>
<td>7</td>
<td>88</td>
<td>12</td>
<td>95</td>
</tr>
<tr>
<td>All groups</td>
<td>75</td>
<td>25</td>
<td>68</td>
<td>32</td>
<td>77</td>
</tr>
</tbody>
</table>

### Table 2. Serological distribution of card test negative samples

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>ELISA</th>
<th>CF</th>
<th>Riv</th>
<th>SAT</th>
<th>Total card test negative samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calfhood and adult vaccinated</td>
<td>10</td>
<td>90</td>
<td>8</td>
<td>92</td>
<td>3</td>
</tr>
<tr>
<td>Calfhood vaccinated</td>
<td>2</td>
<td>98</td>
<td>2</td>
<td>98</td>
<td>3</td>
</tr>
<tr>
<td>Adult vaccinated</td>
<td>9</td>
<td>91</td>
<td>6</td>
<td>94</td>
<td>6</td>
</tr>
<tr>
<td>No vaccination</td>
<td>2</td>
<td>98</td>
<td>&lt; 1</td>
<td>&gt; 99</td>
<td>2</td>
</tr>
<tr>
<td>All groups</td>
<td>5</td>
<td>95</td>
<td>3</td>
<td>&gt; 99</td>
<td>2</td>
</tr>
</tbody>
</table>

### Table 3. Serological distribution of rivanol positive samples

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>CARD</th>
<th>CF</th>
<th>ELISA</th>
<th>SAT</th>
<th>Total Rivanol positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calfhood and adult vaccinated</td>
<td>&gt; 99</td>
<td>&lt; 1</td>
<td>84</td>
<td>16</td>
<td>91</td>
</tr>
<tr>
<td>Calfhood vaccinated</td>
<td>96</td>
<td>4</td>
<td>92</td>
<td>8</td>
<td>92</td>
</tr>
<tr>
<td>Adult vaccinated</td>
<td>&gt; 99</td>
<td>&lt; 1</td>
<td>91</td>
<td>9</td>
<td>95</td>
</tr>
<tr>
<td>No vaccination</td>
<td>100</td>
<td>0</td>
<td>95</td>
<td>5</td>
<td>&gt; 99</td>
</tr>
<tr>
<td>All groups</td>
<td>&gt; 99</td>
<td>&lt; 1</td>
<td>90</td>
<td>10</td>
<td>95</td>
</tr>
</tbody>
</table>

**SAT positive**

Agreement between 1141 SAT positive sera ranged between 55 and 98% (Table 5). However, agreement of the card test positive results with SAT positive sera among all experimental groups was 94–98%, while the agreement between CF, Riv and ELISA on sera from vaccinated cattle ranged between 55 and 88%. Agreement with results from CF, Riv and ELISA for sera from non-vaccinated cattle was 91–98%. 

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Table 4. Serological distribution of rivanol negative samples

Distribution of test results as a percentage of total samples

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>CARD</th>
<th>CF</th>
<th>ELISA</th>
<th>SAT</th>
<th>Total Rivanol negative samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calfhood and adult vaccinated</td>
<td>34</td>
<td>21</td>
<td>25</td>
<td>20</td>
<td>635</td>
</tr>
<tr>
<td>Calfhood vaccinated</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>&lt;1</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Adult vaccinated</td>
<td>36</td>
<td>17</td>
<td>23</td>
<td>4</td>
<td>1078</td>
</tr>
<tr>
<td>No vaccination</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>1236</td>
</tr>
<tr>
<td>All groups</td>
<td>17</td>
<td>10</td>
<td>13</td>
<td>11</td>
<td>3797</td>
</tr>
</tbody>
</table>

Table 5. Serological distribution of SAT positive samples

Distribution of test results as a percentage of total samples

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>CARD</th>
<th>CF</th>
<th>Riv</th>
<th>ELISA</th>
<th>Total SAT positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calfhood and adult vaccinated</td>
<td>95</td>
<td>68</td>
<td>55</td>
<td>76</td>
<td>282</td>
</tr>
<tr>
<td>Calfhood vaccinated</td>
<td>97</td>
<td>88</td>
<td>78</td>
<td>84</td>
<td>32</td>
</tr>
<tr>
<td>Adult vaccinated</td>
<td>94</td>
<td>69</td>
<td>61</td>
<td>77</td>
<td>666</td>
</tr>
<tr>
<td>No vaccination</td>
<td>98</td>
<td>93</td>
<td>91</td>
<td>98</td>
<td>161</td>
</tr>
<tr>
<td>All groups</td>
<td>95</td>
<td>73</td>
<td>64</td>
<td>80</td>
<td>1141</td>
</tr>
</tbody>
</table>

Table 6. Serological distribution of SAT negative samples

Distribution of test results as a percentage of total samples

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>CARD</th>
<th>CF</th>
<th>Riv</th>
<th>ELISA</th>
<th>Total SAT negative samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calfhood and adult vaccinated</td>
<td>21</td>
<td>15</td>
<td>2</td>
<td>17</td>
<td>515</td>
</tr>
<tr>
<td>Calfhood vaccinated</td>
<td>1</td>
<td>2</td>
<td>&lt;1</td>
<td>3</td>
<td>842</td>
</tr>
<tr>
<td>Adult vaccinated</td>
<td>20</td>
<td>13</td>
<td>1</td>
<td>16</td>
<td>827</td>
</tr>
<tr>
<td>No vaccination</td>
<td>2</td>
<td>2</td>
<td>&lt;1</td>
<td>4</td>
<td>1226</td>
</tr>
<tr>
<td>All groups</td>
<td>9</td>
<td>7</td>
<td>&lt;1</td>
<td>9</td>
<td>3410</td>
</tr>
</tbody>
</table>

SAT negative

Agreement between 3410 SAT negative sera ranged between 79 and 99% (Table 6). The agreement within CV and NV groups ranged from 96 to 99%. Agreement within AV and CVAV groups ranged from 79 to 87% for ELISA, CF and CT while the agreement for Riv negative results was 98–99% among all groups.
Table 7. Serological distribution of ELISA positive samples

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>CARD (Pos.)</th>
<th>CARD (Neg.)</th>
<th>CF (Pos.)</th>
<th>CF (Neg.)</th>
<th>Riv (Pos.)</th>
<th>Riv (Neg.)</th>
<th>SAT (Pos.)</th>
<th>SAT (Neg.)</th>
<th>Total ELISA positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calfhood and adult vaccinated</td>
<td>87</td>
<td>13</td>
<td>69</td>
<td>31</td>
<td>49</td>
<td>51</td>
<td>71</td>
<td>29</td>
<td>303</td>
</tr>
<tr>
<td>Calfhood vaccinated</td>
<td>60</td>
<td>40</td>
<td>68</td>
<td>32</td>
<td>48</td>
<td>52</td>
<td>54</td>
<td>46</td>
<td>59</td>
</tr>
<tr>
<td>Adult vaccinated</td>
<td>90</td>
<td>10</td>
<td>73</td>
<td>27</td>
<td>61</td>
<td>39</td>
<td>79</td>
<td>21</td>
<td>647</td>
</tr>
<tr>
<td>No vaccination</td>
<td>86</td>
<td>14</td>
<td>81</td>
<td>19</td>
<td>74</td>
<td>26</td>
<td>78</td>
<td>22</td>
<td>292</td>
</tr>
<tr>
<td>All groups</td>
<td>87</td>
<td>13</td>
<td>73</td>
<td>27</td>
<td>60</td>
<td>40</td>
<td>75</td>
<td>25</td>
<td>1202</td>
</tr>
</tbody>
</table>

Table 8. Serological distribution of ELISA negative samples

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>CARD (Pos.)</th>
<th>CARD (Neg.)</th>
<th>CF (Pos.)</th>
<th>CF (Neg.)</th>
<th>Riv (Pos.)</th>
<th>Riv (Neg.)</th>
<th>SAT (Pos.)</th>
<th>SAT (Neg.)</th>
<th>Total ELISA negative samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calfhood and adult vaccinated</td>
<td>23</td>
<td>77</td>
<td>12</td>
<td>88</td>
<td>3</td>
<td>97</td>
<td>14</td>
<td>86</td>
<td>494</td>
</tr>
<tr>
<td>Calfhood vaccinated</td>
<td>2</td>
<td>98</td>
<td>1</td>
<td>99</td>
<td>&lt; 1</td>
<td>&gt; 99</td>
<td>&lt; 1</td>
<td>&gt; 99</td>
<td>824</td>
</tr>
<tr>
<td>Adult vaccinated</td>
<td>25</td>
<td>75</td>
<td>11</td>
<td>89</td>
<td>2</td>
<td>98</td>
<td>18</td>
<td>82</td>
<td>846</td>
</tr>
<tr>
<td>No vaccination</td>
<td>1</td>
<td>99</td>
<td>&lt; 1</td>
<td>&gt; 99</td>
<td>&lt; 1</td>
<td>&gt; 99</td>
<td>&lt; 1</td>
<td>&gt; 99</td>
<td>1185</td>
</tr>
<tr>
<td>All groups</td>
<td>11</td>
<td>89</td>
<td>5</td>
<td>95</td>
<td>1</td>
<td>99</td>
<td>7</td>
<td>93</td>
<td>3349</td>
</tr>
</tbody>
</table>

Table 9. Serological distribution of CF positive samples

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>CARD (Pos.)</th>
<th>CARD (Neg.)</th>
<th>ELISA (Pos.)</th>
<th>ELISA (Neg.)</th>
<th>Riv (Pos.)</th>
<th>Riv (Neg.)</th>
<th>SAT (Pos.)</th>
<th>SAT (Neg.)</th>
<th>Total CF positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calfhood and adult vaccinated</td>
<td>88</td>
<td>12</td>
<td>78</td>
<td>22</td>
<td>51</td>
<td>49</td>
<td>71</td>
<td>29</td>
<td>269</td>
</tr>
<tr>
<td>Calfhood vaccinated</td>
<td>71</td>
<td>29</td>
<td>76</td>
<td>24</td>
<td>53</td>
<td>47</td>
<td>62</td>
<td>38</td>
<td>45</td>
</tr>
<tr>
<td>Adult vaccinated</td>
<td>92</td>
<td>8</td>
<td>83</td>
<td>17</td>
<td>67</td>
<td>33</td>
<td>82</td>
<td>18</td>
<td>564</td>
</tr>
<tr>
<td>No vaccination</td>
<td>94</td>
<td>6</td>
<td>95</td>
<td>5</td>
<td>83</td>
<td>17</td>
<td>87</td>
<td>13</td>
<td>173</td>
</tr>
<tr>
<td>All groups</td>
<td>90</td>
<td>10</td>
<td>83</td>
<td>17</td>
<td>65</td>
<td>35</td>
<td>79</td>
<td>21</td>
<td>1051</td>
</tr>
</tbody>
</table>

**ELISA positive**

Agreement between 1202 ELISA positive sera was between 48 and 90% (Table 7). These data vary within groups such that no pattern can be established.

**ELISA negative**

Agreement between 3349 ELISA negative sera ranged between 75 and 99% (Table 8). There was 98–99% agreement between tests on sera from NV or CV.
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Table 10. Serological distribution of CF negative samples

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>CARD Pos.</th>
<th>CARD Neg.</th>
<th>ELISA Pos.</th>
<th>ELISA Neg.</th>
<th>Riv Pos.</th>
<th>Riv Neg.</th>
<th>SAT Pos.</th>
<th>SAT Neg.</th>
<th>Total CF negative samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calfhood and adult</td>
<td>27</td>
<td>73</td>
<td>18</td>
<td>82</td>
<td>5</td>
<td>95</td>
<td>17</td>
<td>83</td>
<td>528</td>
</tr>
<tr>
<td>vaccinated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calfhood vaccinated</td>
<td>1</td>
<td>99</td>
<td>2</td>
<td>98</td>
<td>&lt; 1</td>
<td>&gt; 99</td>
<td>&lt; 1</td>
<td>&gt; 99</td>
<td>829</td>
</tr>
<tr>
<td>Adult vaccinated</td>
<td>30</td>
<td>70</td>
<td>19</td>
<td>81</td>
<td>4</td>
<td>96</td>
<td>22</td>
<td>78</td>
<td>929</td>
</tr>
<tr>
<td>No vaccination</td>
<td>2</td>
<td>98</td>
<td>3</td>
<td>97</td>
<td>&lt; 1</td>
<td>&gt; 99</td>
<td>&lt; 1</td>
<td>&gt; 99</td>
<td>1214</td>
</tr>
<tr>
<td>All groups</td>
<td>13</td>
<td>87</td>
<td>9</td>
<td>91</td>
<td>2</td>
<td>98</td>
<td>9</td>
<td>91</td>
<td>3500</td>
</tr>
</tbody>
</table>

animals and 97–99 % agreement with Riv negative results between experimental groups.

**CF positive**

Agreement between 1051 CF positive sera was between 51 and 95 % (Table 9). Agreement among tests in the NV group ranged from 83 to 95 %, while agreement between tests on sera from vaccinated cattle ranged from 51 to 92 %.

**CF negative**

Agreement between 3500 negative sera ranged from 70 to 99 % (Table 10). Agreement among tests within the NV group and CV group ranged from 97 to 99 %, while agreement among sera from the AV and CVAV groups ranged from 70 to 96 %. The agreement between groups with Riv negative results ranged from 95 to 99 %.

**DISCUSSION**

The disparity of results on card test positive sera (Table 1) when tested by other methods was greater between groups of vaccinated cattle than was apparent on sera from non-vaccinated cattle.

Data in Table 2 indicate that if the serum was CT negative, there was at least a 95 % chance, for all groups, that the serum would be negative by other methods. If the animal was not adult vaccinated there was 98 to 100 % probability that the serum was serologically negative by other methods. If the cows were adult vaccinated, there was a 90–99 % probability that the sera were negative by other methods. This agreement between tests for negative animal sera suggests that if the CT is used as the initial herd screening method, those sera which are serologically positive should be tested by supplemental methods to clarify the reactor status of the cattle.

The agreement with rivanol positive sera between methods for all groups is 84–100 %. Excluding the CVAV group sera tested by the CF method, the agreement between methods is 91–100 %. If a serum was positive by any serological
method, a Riv positive result did not increase the basic knowledge relative to reactor status. If a serum was serologically negative by any other method (Tables 2, 6, 8, and 10) the probability, on the average, of it being Riv negative was 98\%.

Therefore, the value of the Riv procedure as a supplemental method to the card test for the detection of Brucella antibodies is of doubtful value when results from CF or SAT tests are known.

Of the 1401 sera that were CT positive, 68\% were also CF positive, and of the 1051 sera that were CF positive, 90\% were card test positive; therefore, the 32\% which were CT positive and CF negative may represent sera which contained immunoglobulins more capable of agglutination (Morgan & Richards, 1974; Allan et al. 1976).

Sera from non-adult vaccinated cattle which are negative by the SAT test have a 96–99\% probability of being negative by the other serological methods. Twenty per cent of the sera from AV cattle which were SAT negative were positive by the CT, 16\% were positive by ELISA and 13\% were positive by CF (Table 6). Vaccination history was critical when SAT was considered as the initial screening test for AV cattle, because the variation in results between methods suggest that the SAT did not detect immunoglobulins detectable by the CT and CF methods. However, sera which were positive by SAT have a 94–98\% probability of being positive by the CT regardless of vaccination history.

There is a 74\% probability that CT positive sera will be positive by CF if the sera were collected from CV cattle (Table 1). These data were in agreement with the conclusions of others (Timbs et al. 1978), who indicated that 78.8\% of card test positive sera from CV cattle were also CF positive. The agreement of CF negative results on sera from CV and NV cattle was between 97 and 99\%, compared with 70 and 96\% agreement if the sera originated from AV cows when Riv was included, and 70–83\% agreement when Riv test results were excluded (Table 10). These data show the importance of vaccination history when evaluating CF test results on sera from vaccinated cattle.

There was a disagreement between serological test results for sera which were ELISA positive. The variance among all serological test results was so great as to indicate that ELISA reflects specific and non-specific reactions of immunoglobulins not measured by the other methods or, as indicated by others, ELISA results may be affected by affinity characteristics of these immunoglobulins (Butler et al. 1978).

The effect of adult vaccination upon the serologic results was apparent when sera which were negative by Riv, SAT, ELISA and CF were compared between methods. Agreement among negative sera from non-adult vaccinated cattle for all methods was between 96 and 100\%. Disparity between tests on sera from vaccinated cattle which were positive by any method may reflect serological differences in immunoglobulin classes. Conceivably, soluble immune complexes could influence in vitro reactions between standardized reagents and contribute to apparent variances between serologic methods. The best correlation between methods in sera positive by any test was achieved with sera from non-vaccinated cattle. If sera are card test positive they should be tested by supplemental methods and the

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Detection of *B. abortus* antibodies

results should be evaluated with consideration being given to vaccination history of the herd.

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