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The Brucellin skin test as a tool to discriminate false positive serological reactions in bovine brucellosis

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Summary — Three experiments were performed in order to assess the diagnostic value of the Brucellin allergic skin test (AST) in a brucellosis false positive serological reactions (FPSR) context. First, 1259 cattle from 20 Brucella-free herds in a FPSR area were tested twice with AST to estimate its specificity. Secondly, AST and serological tests (complement fixation test [CFT], tube agglutination test, dithiothreitol-microagglutination test and ELISA) sensitivities were evaluated on 111 cattle positive to the Rose Bengal test (RBT) belonging to 15 Brucella-infected herds. Thirdly, AST was used in a field trial to discriminate FPSR from true brucellosis reactions. AST specificity in non-vaccinated cattle was very high (99.83%; confidence interval 95% [CI95%]: 99.67-99.96%). Skin thickening 72 h post-injection was significantly higher on vaccinated cattle (1.42 vs 0.15 mm). In this sub-population, AST specificity decreased significantly to 78% (CI95%: 68-87%). Individual sensitivity of AST relative to Rose Bengal test was 64% (CI95%: 54-72%), while all infected herds were AST positive (n = 15). When associated with CFT, it detected 95% (CI95%: 90-98%) of the infected cattle. These results were consistent with the field trial. In a FPSR context, AST was more specific than RBT or CFT. Therefore, this test could be used at herd level as a confirmation test, on cattle non vaccinated against brucellosis.

bovine brucellosis / Brucellin allergic skin test / delayed type hypersensitivity / false positive serological reaction / field trial

Résumé — L’épreuve cutanée allergique à la Brucelline : un outil pour la différenciation des réactions sérologiques faussement positives en brucellose bovine. Trois expérimentations ont été menées dans le but de valider l’utilisation de l’épreuve cutanée allergique (ECA) à la Brucelline dans un contexte de réactions sérologiques faussement positives en brucellose (RSFP). Afin d’en estimer la spécificité, 1259 bovins issus de 20 cheptels indemnes de brucellose dans une région à forte
prévalence en RSFP ont subi deux ECA. L’ECA et les tests sérologiques classiques (test de fixation du complément [FC], séroagglutination lente, microagglutination au dithiothreitol et ELISA) ont été ensuite comparés en tant que tests de confirmation sur 111 bovins positifs dans l’épreuve à l’anti-gène tamponné (EAT) issus de cheptels infectés. Enfin, l’utilisation de l’ECA a été validée à grande échelle, sur le terrain. La spécificité de l’ECA sur des animaux non vaccinés contre la brucellose était excellente (99,83 % ; intervalle de confiance 95 % [IC95 %] : 99,67-99,96 %). L’épaississement de la peau à 72 h a été significativement plus important chez les animaux vaccinés (1,42 vs 0,15 mm). Sur cette sous-population vaccinée, la spécificité de l’ECA a diminué significativement jusqu’à 78 % (IC95 % : 68-87 %). La sensibilité relative de l’ECA par rapport à l’EAT était de 64 % (IC95 % : 54-72 %), mais l’ECA a détecté tous les troupeaux infectés (n = 15). Associée à la FC, l’ECA a permis de confirmer 95 % des animaux infectés (IC95 % : 90-98 %). Ces résultats ont été confirmés lors de l’essai terrain. La spécificité de l’ECA est supérieure à celle de l’EAT et de la FC dans le contexte des RSFP. Ce test pourrait être utilisé comme test de confirmation, à l’échelle du troupeau, dans les populations non vaccinées antérieurement contre la brucellose.

**INTRODUCTION**

Bovine brucellosis is a major zoonosis, leading to great economic losses. Its eradication in the European Union (EU) is based on ‘test and slaughter’ programmes. Brucellosis has been eradicated in most of the northern part of EU and its prevalence in other European states has decreased significantly during the last 10 years (Garin-Bastuji, 1995).

Since 1990, an abnormally high rate of false positive serological reactions (FPSR) to brucellosis has emerged in bovine herds in different EU countries (Great-Britain [MacMillan, 1990]; France [Benet et al., 1991]; Belgium [Weynants et al., 1996] and The Netherlands [Olyhoek, unpublished results]) and in bovine and deer herds in New Zealand (Hilbink et al., 1995). In some French areas, more than 10% of the herds were concerned (Garin-Bastuji et al., 1994). The positive serological reactions were characterized by a rapid decrease of antibody titres, generally in less than two months (Benet et al., 1991). They were considered as false positive on the basis of clinical, bacteriological and epidemiological criteria. This phenomenon is worrying, as it may delay the eradication of brucellosis in the EU.

Brucella are facultative intracellular bacteria, which induce both cellular and humoral responses. The classical serological tests for brucellosis diagnosis are based mainly on the detection of antibodies against the smooth lipopolysaccharide (S-LPS) of Brucella. The recent increase in FPSR is thought to be linked to cross-reactions with gram-negative bacteria (Benet et al., 1991), such as Yersinia enterocolitica O:9, Salmonella urbana N group, or Escherichia coli O:157, described as sharing epitopes with the Brucella S-LPS (for a review see Corbel, 1985). Yersinia enterocolitica O:9 was mainly isolated from humans (for a review see Carniel and Mollaret, 1990) and swine (Akkermans and Hill, 1972). It has been recently isolated on several occasions from cattle and other ruminants in areas where cases of FPSR were reported (Garin-Bastuji, 1993; Reynaud et al., 1993; Hilbink et al., 1995). A way to discriminate brucellosis from FPSR linked with the S-LPS could be to measure the cellular immune response (Chukwu, 1985a), revealed mainly by internal protein components of Brucella cells and not by the S-LPS (Bongbhibhat et al., 1970).

The Brucellin allergic skin test (AST) is based on the skin test model developed for tuberculosis diagnosis. A cytoplasmic pro-
tein extract known as Brucellin Inra, prepared by a cold extraction method from the rough strain *Brucella melitensis* B115, is accepted as the most convenient allergen (Bongbibhat et al., 1970; Jones et al., 1973). Since it contains no or little S-LPS, antibody production and antibody-mediated cutaneous hypersensitivity are avoided. As the protein components are shared by all *Brucella* species in both rough and smooth phases (Jones, 1974), a wide sensitivity for the genus *Brucella* is expected. AST has been successfully used in France in order to eradicate brucellosis more efficiently and earlier from infected herds previously identified by serological tests. In New Zealand, it has been successfully used for brucellosis mass screening of extensively managed beef herds (MacDiarmid, 1994). The purpose of this study was to determine the value of AST as a confirmation test of the positive serological reactions obtained in the brucellosis screening tests.

**MATERIALS AND METHODS**

**Population and experimental designs**

**AST specificity evaluation**

In order to evaluate AST specificity, in 1994, 1,259 Charolais cattle from 20 brucellosis-free herds were tested twice, 8 weeks apart, in January (sampling session 1) and March (sampling session 2). All these herds had been brucellosis-free for at least 10 years, and no brucellosis outbreak arose after this experiment. The vaccinal status of the animals could not be known precisely. Vaccination against brucellosis had been forbidden in this area since 1984. All the herds were vaccinated before this date, according to an official procedure (either one subcutaneous injection of a standard dose \(5 \times 10^{10} \text{CFU} \) of S19 vaccine [Corbel, 1992] to heifers aged 4 to 6 months, or two subcutaneous injections of S45/20 vaccine [Nicoletti, 1990], 1 month apart, to heifers under 12 months old). Thus, animals born before 1984 were considered as probably vaccinated.

In order to evaluate the serological status of animals and herds, each animal was tested with Rose Bengal test (RBT) during the official brucellosis annual surveillance, and with RBT and complement fixation test (CFT) at each sampling session. Faeces were also collected from each animal, at each sampling session, for bacteriological examination. The number of animals and the number of AST performed on each studied sub-population are given in Table I. Thirty-seven animals were lost to follow up between session 1 and session 2. Thirty-nine animals, from 15 herds, showed at least one FPSR (RBT+ and/or CFT+) during the survey. *Yersinia enterocolitica* O:9 was isolated from 42 cattle, issued from 13 herds (Gerbier et al., 1997).

**AST sensitivity evaluation**

To evaluate AST sensitivity and to compare it with classical serological tests, 111 animals positive to the RBT were selected from 15 ongoing brucellosis outbreaks. Herd infection was confirmed by clinical, bacteriological, serological (Corbel, 1992) and epidemiological criteria (Garin-Bastuji et al., 1994). At least one abortion due to *Brucella abortus* (bacteriologically confirmed) and three simultaneous seropositive animals (RBT and/or CFT) took place in these herds. Sampling was undertaken independently of age, sex, breed or production (dairy or beef) of the cattle. All animals were over 1-year old and no animal had been vaccinated against brucellosis. AST, RBT, CFT, tube agglutination test (SAT), dithiothreitol-microagglutination test (DTT-MAT), and ELISA test were performed once on this population. Five DTT-MAT results were lacking.

**AST diagnostic value in a field trial**

The use of AST on a large scale, in field conditions, was evaluated in a FPSR area during an official brucellosis annual surveillance campaign. RBT was performed in 8,169 herds, on all cattle over 1-year old. No information was given on the vaccinal status of the animals.

If a single animal was positive in a given herd, CFT and AST were carried out on the whole herd.

Four herds, including 81 animals, were infected with *Brucella*. Eighty-nine herds including 1,479 animals presented FPSR.
Serological tests

RBT was performed according to the French official technique, with the antigen Bengatest (Rhône Mérieux, France). A test was considered positive when any agglutination was visible to the naked eye. CFT was performed on microplates by the cold procedure (Alton et al, 1988), with the antigen Antifix (Rhone Mérieux, France), according to the official French technique. Positivity threshold was 20 IU/mL (50% or less hemolysis at a dilution of 1/4 or higher). The SAT was performed according to Alton et al (1988) with a positivity threshold of 30 IU/mL. The DTT-MAT was performed according to Thiange et al (1992), with a positivity threshold of 30 IU/mL. The same antigen (antigène SAW, Rhône Mérieux, France) was used for these two latter tests. All antigens were standardized against the international standard anti-Brucella serum (ISaBS) according to EU regulations. The ELISA test was the Chekit Brucellotest Serum kit (Bommeli/Behring, France) standardized according to EU regulations. The test was considered positive when the optical density exceeded 75% of optical density of the positive control.

Brucellin allergic skin test

AST was performed according to Alton et al (1988) with Brucellergene OCB (Rhône Mérieux, France), a commercial preparation of Brucellin Inra. Each batch of Brucellergene OCB was standardized and controlled for biological activity in a sensitized guinea pig model (De Reviers and Fensterbank, 1984). Brucellergene OCB (0.1 mL, i.e., 200 IU) was injected intradermally into a previously shaved skin fold at one side of the neck. The skin thickness was measured with vernier callipers just before injection (initial skin thickness) and at re-examination 72 h later. Any increase in the skin thickness (skin thickening) greater than 2 mm was considered as a positive reaction.
Analysis of results

Results were analysed using the procedure FREQ and TTEST of the SAS software (SAS Institute Inc, Cary, USA).

AST specificity was calculated from results obtained in brucellosis-free herds. Data from both sampling sessions were gathered in order to increase the power of the statistical tests applied on the quantitative variables ‘initial skin thickness’ and ‘skin thickening’. The independence of the values obtained during the two sessions had been previously confirmed with a mean comparison on matched data. Therefore, 2 481 AST results were analysed, but they only concerned 1 259 cattle. AST specificity was calculated for each sub-population as:

\[
\text{specificity} = \frac{\text{number of negative } \text{AST}}{\text{number of } \text{AST performed}}
\]

The sensitivity of the AST, the serological tests, and the associations between two of these tests were evaluated from the results obtained on the 111 cattle positive to the RBT issued from infected herds. Results of the associations between two tests were interpreted either in series (ie, an animal was declared positive if both tests were positive) or in parallel (ie, an animal was declared positive if at least one out of the two tests was positive). All the animals were considered as infected on account of the very high predictive value of positive RBT in infected herds. Sensitivity of a given test or association was then calculated in the infected population as:

\[
\text{sensitivity} = \frac{\text{number of positive tests}}{\text{number of tests performed}}
\]

Confidence intervals (CI95%) were calculated for each point estimate using a method based on the binomial distribution (\(\alpha = 0.05\)).

RESULTS

AST specificity

Average initial skin thickness and average skin thickening in brucellosis-free herds according to the studied variation sources are given in table I. Initial skin thickness differed significantly according to the sampling session \((P < 0.05)\), the sex \((P < 0.01)\), the serological and the bacteriological status of the animal, and the serological status of the herd \((P < 0.05)\). The sex and the vaccinal status of the animal were the only significant variation sources for the skin thickening \((P < 0.05 \text{ and } P < 0.01, \text{ respectively})\).

The positive AST results in this population according to the variation sources are given in table II. Nineteen tests out of 2 481 (0.77%) were positive: 15 out of 69 (22%) in the vaccinated population, and four out of the 2 412 (0.17%) in the non-vaccinated population. These four latter reactions were strong, ie, 4.0, 5.2, 7.3 and 13.9 mm. None of the 39 animals showing a FPSR was positive in AST. Three positive results were obtained on animals from which Yersinia enterocolitica O:9 had been isolated, but these cases corresponded to vaccinated animals.

Overall, AST specificity was 2 408/2 412, ie, 99.83% (CI: 99.67-99.96%) in the non-vaccinated population, but decreased significantly to 54/69, ie, 78% (CI: 68-87%) in the vaccinated one.

AST sensitivity

The sensitivity of the various tests performed on samples from the 111 cattle positive to RBT issued from infected herds are shown in table III. Seventy of the 111 animals (64%) were positive to AST. All herds (15/15) were detected by at least one animal positive to AST. CFT, ELISA, SAT and DTT-MAT validated 92%, 90%, 85% and 83% of infected cattle, respectively. The sensitivity of the various associations of results including AST or CFT interpreted in series was lower than when interpreted in parallel. Associations including CFT were more sensitive than those including AST.
Table II. Estimates of the specificity of allergic skin test in a brucellosis-free cattle population.

<table>
<thead>
<tr>
<th>Population</th>
<th>Factor</th>
<th>Variable</th>
<th>No of positive tests/No of tests</th>
<th>AST specificity (%) (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole population</td>
<td></td>
<td></td>
<td>19/2,481</td>
<td>99.23 (98.87-99.55)</td>
</tr>
<tr>
<td>Whole population</td>
<td>Brucellosis</td>
<td>vaccination yes</td>
<td>15/69</td>
<td>78 (68-87)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>no</td>
<td>4/2,412</td>
<td>99.83 (99.67-99.96)</td>
</tr>
<tr>
<td>Non vaccinated population</td>
<td>Sampling session</td>
<td>January</td>
<td>1/1,224</td>
<td>99.92 (99.75-100.00)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>March</td>
<td>3/1,188</td>
<td>99.75 (99.41-100.00)</td>
</tr>
<tr>
<td>Non vaccinated population</td>
<td>FPSR animals</td>
<td>yes</td>
<td>0/75</td>
<td>99.82 (99.66-99.96)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>no</td>
<td>4/2,337</td>
<td>99.85 (99.64-100.00)</td>
</tr>
<tr>
<td>Non vaccinated population</td>
<td>FPSR herds</td>
<td>yes</td>
<td>2/1,373</td>
<td>99.90 (99.71-100.00)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>no</td>
<td>1/1,039</td>
<td>99.84 (99.53-100.00)</td>
</tr>
<tr>
<td>Non vaccinated population</td>
<td>YO:9c</td>
<td>yes</td>
<td>0/69</td>
<td>99.83 (99.66-99.96)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>no</td>
<td>4/2,343</td>
<td>99.83 (99.66-100.00)</td>
</tr>
<tr>
<td>Non vaccinated population</td>
<td>YO:9 herds</td>
<td>yes</td>
<td>1/633</td>
<td>99.83 (99.61-100.00)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>no</td>
<td>3/1,779</td>
<td></td>
</tr>
</tbody>
</table>

a confidence interval 95%; b animals issued from herds where at least one FPSR had been detected; c animals from which Yersinia enterocolitica O:9 had been isolated; d animals issued from herds where Yersinia enterocolitica O:9 had been isolated.

Table III. Estimates of the sensitivity of allergic skin test and serological tests used alone, in series or in parallel on cattle positive to Rose Bengal from infected herds.

<table>
<thead>
<tr>
<th>Tests</th>
<th>No of positive results/No of tests</th>
<th>Sensitivity (%) (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One test interpreted alone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>70/111</td>
<td>64 (54-72)</td>
</tr>
<tr>
<td>CFT</td>
<td>102/111</td>
<td>92 (86-96)</td>
</tr>
<tr>
<td>SAT</td>
<td>94/111</td>
<td>85 (77-90)</td>
</tr>
<tr>
<td>DTT-MAT</td>
<td>88/106</td>
<td>83 (75-90)</td>
</tr>
<tr>
<td>ELISA</td>
<td>100/111</td>
<td>90 (84-95)</td>
</tr>
</tbody>
</table>

Two tests interpreted in series (positive result if both tests are positive)

<table>
<thead>
<tr>
<th>Tests</th>
<th>No of positive results/No of tests</th>
<th>Sensitivity (%) (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST - CFT</td>
<td>67/111</td>
<td>60 (51-69)</td>
</tr>
<tr>
<td>AST - SAT</td>
<td>61/111</td>
<td>55 (46-64)</td>
</tr>
<tr>
<td>AST - DTT-MAT</td>
<td>58/106</td>
<td>55 (45-64)</td>
</tr>
<tr>
<td>AST - ELISA</td>
<td>66/111</td>
<td>59 (50-68)</td>
</tr>
<tr>
<td>CFT - SAT</td>
<td>90/111</td>
<td>81 (74-88)</td>
</tr>
<tr>
<td>CFT - DTT-MAT</td>
<td>87/106</td>
<td>82 (75-89)</td>
</tr>
<tr>
<td>CFT - ELISA</td>
<td>98/111</td>
<td>88 (82-94)</td>
</tr>
</tbody>
</table>

Two tests interpreted in parallel (positive result if at least one test is positive)

<table>
<thead>
<tr>
<th>Tests</th>
<th>No of positive results/No of tests</th>
<th>Sensitivity (%) (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST - CFT</td>
<td>105/111</td>
<td>95 (90-98)</td>
</tr>
<tr>
<td>AST - SAT</td>
<td>103/111</td>
<td>93 (87-97)</td>
</tr>
<tr>
<td>AST - DTT-MAT</td>
<td>96/106</td>
<td>91 (85-95)</td>
</tr>
<tr>
<td>AST - ELISA</td>
<td>104/111</td>
<td>94 (89-98)</td>
</tr>
<tr>
<td>CFT - SAT</td>
<td>106/111</td>
<td>95 (91-99)</td>
</tr>
<tr>
<td>CFT - DTT-MAT</td>
<td>98/106</td>
<td>92 (87-97)</td>
</tr>
<tr>
<td>CFT - ELISA</td>
<td>104/111</td>
<td>94 (89-98)</td>
</tr>
</tbody>
</table>

a confidence interval 95%.
Field trial

The results of the serological and allergic tests obtained in field conditions are given in table IV. In the four infected herds (81 animals), serological tests (RBT or CFT) detected 25 (78%) and AST detected 23 (72%) of the 32 positive animals (AST+ or RBT+ or CFT+). AST sensitivity relative to serological results (RBT+ or CFT+) was 64% (16/25; IC: 44-84%). However, the four infected herds were detected with AST.

In the FPSR herds (1,479 animals), 185 animals were seropositive (RBT and/or CFT), seven were positive in the AST. No significant difference in skin thickening was found between seropositive and seronegative animals in these herds. 181 (97.8%) out of the 185 FPSR animals, and 85 (96%) out of the 89 FPSR herds were negative in the AST. Thus, in FPSR herds, the individual specificity of the AST was 1,472/1,479, ie, 99.53% (CI: 99.12-99.86%), while the individual specificity of RBT/CFT was only 1,294/1,479, ie, 87.49% (CI: 85.80-89.18%).

The herd specificity of AST was 85/89, ie, 96% (CI: 91-99%).

DISCUSSION

In areas with a low brucellosis prevalence, serological tests have a low positive predictive value. Thus, one or more confirmation tests should be applied to samples positive in the screening tests. However, in some EU areas, no classical serological test can actually invalidate a false positive result. This study gave the first evaluation of an allergic test specificity in such areas.

The absence of a significant difference in skin thickening between two tests performed 8 weeks apart implied that AST did not induce a long-lasting sensitisation or anergy. Blasco et al (1994) showed that an anergic state could be observed in sheep 6 days after a first allergen inoculation, but that allergic reactivity was recovered after 24 days. Therefore, there was little likelihood of inducing an anergic state in this study.

The skin thickness before injection was higher in January (sampling session 1), in FPSR cattle, in FPSR herds, and in Yersinia enterocolitica O:9 carriers. These initial skin thickness variations might be due to body conditions variations. Nevertheless, the skin thickening due to the injection was not influenced by these factors: initial variations were cancelled by using each animal as its own control.

The skin thickening was significantly higher in male than in female, but this result was not meaningful as no skin thickening was higher than the positivity threshold (ie, 2 mm) in this population. This result might be due to the higher initial skin thickness, implying higher measurement errors. On
the other hand, the skin thickening and the number of animals positive to AST were significantly higher in vaccinated than in non-vaccinated cattle. The vaccinated cattle tested in this study were vaccinated for at least 10 years because vaccination became illegal in France in 1984. Therefore, a sampling bias occurred as all vaccinated cows were older than 10 years and conversely. It is not possible to discriminate whether these positive AST results were derived from exposure to a vaccinal or a natural Brucella antigen. On one hand, brucellosis herd prevalence rate in France was higher in 1983 than in 1994 (3.12% vs 0.35%; Dufour, 1984; Garin-Bastuji et al, 1994), leading to a possible infection of this population. On the other hand, vaccination produces long-lasting responses in AST, either with vaccine strain 45/20 (Chukwu, 1985b; Sutherland, 1983) or with S19 (Fensterbank and Pardon, 1977; Chukwu, 1985b; MacDiarmid and Hellstrom, 1986). Thus, AST should not be recommended for cattle populations vaccinated against brucellosis.

Four positive allergic reactions occurred in non-vaccinated animals. These four reactions were unmistakable: the probability of such occurrence was very low according to the normal distribution of the skin thickening. Thus, these animals probably belonged to another sub-population. These positive reactions could be attributed to previous contacts with a wild strain of Brucella, as latent carriers are often revealed only by AST (Bercovich et al, 1992). A possible presence of animals born from infected dams, who were then potentially sensitised against Brucella antigens (Plommet et al, 1973), could not be excluded in our study. The possibility of cross-reactions cannot be excluded either: Chukwu (1987) obtained one positive reaction (out of five animals) on cows experimentally infected with Yersinia enterocolitica O:9 and tested using a Brucella allergen prepared from Brucella abortus strain 45/20.

Thus, the AST specificity in non-vaccinated brucellosis-free cattle was high (99.83%). Garin-Bastuji (1993) attained a similar specificity of 99.33% in a FPSR area (eight false positive reactions out of 1 222 AST). MacDiarmid and Hellstrom (1986) obtained a higher rate (99.99%) on a very large sample (one false positive reaction out of 5 064 tests), while other authors found no false positive reactions on smaller samples (Fensterbank, 1983, Sutherland, 1983, Chukwu, 1985b). Differences between these results cannot be evaluated because of the differences in allergen and the different epidemiological situations with regards to brucellosis history. AST performed on unvaccinated animals was more specific than classical serological tests in this FPSR area. The positive AST results did not occur more often in FPSR herds or in herds containing animals from which Yersinia enterocolitica O:9 was isolated. Despite the low number of cases tested, FPSR or Yersinia enterocolitica O:9 isolation and positive AST do not seem to be linked. The use of cellular immunity response as a way to distinguish FPSR from brucellosis is thus supported.

The sensitivity of AST relative to RBT was quite low (64%), but in agreement with previous results (Fensterbank, 1977, 1983; Nicoletti, 1983; Chukwu, 1985b; MacDiarmid, 1987). The sensitivity of AST has been reported to be higher (about 85%) when Brucella infection was confirmed by culture (Fensterbank, 1983) or after experimental infections (Sutherland, 1983). Discrepancies between serological and allergic tests in infected areas are frequently observed, because these two kinds of tests do not measure the same immunological response: agreement between the allergic and serological tests does not exceed 75% (Nicoletti, 1983). Animals may develop an allergic reaction during a long seronegative period (Nicoletti, 1983) and others may be anergic, particularly chronically infected animals (Cunningham et al, 1980). Nevertheless, as 36% of the animals detected by
RBT in infected herds were not confirmed by AST, this test should not be used alone as a confirmation test at the individual level. However, all infected herds were detected by AST in this study. As more than one animal is classically infected in a brucellosis outbreak, it would be more convenient to use AST as a confirmation test at the herd level.

Interpretation of two or more serological tests results in series is currently proposed as a testing strategy. This procedure is risky, because of the dramatic decrease in the sensitivity (Dohoo et al., 1986). Conversely, the use of AST with other serological tests interpreted in parallel allowed a good sensitivity, because both allergic and humoral responses were tested. The association CFT-AST is comparable to the association CFT-ELISA or CFT-SAT. Nevertheless, these results are biased by the high sensitivity of CFT, as this test alone detected almost the whole population positive in RBT. The results are not adequate when AST is used in parallel with any serological test, because almost all FPSR animals would be considered as positive.

The specificity (99.53%) and the relative sensitivity of AST (64%) obtained in the field trial were very consistent with those obtained in brucellosis-free and infected herds. In infected herds, disagreement between the serological and the allergic tests rose 20%, but the total number of positive animals detected by these two kinds of tests was similar. Moreover, the use of AST made it possible to invalidate 181/185 FPSR animals (97.8%), and 85/89 FPSR herds (96%). A higher value could have been obtained by testing only the unvaccinated population.

In conclusion, if a positive serological reaction is detected in a given herd, AST should be performed on all the non-vaccinated cattle and any AST positive reaction should then be considered as a confirmation of Brucella infection in the herd. The main inconvenience behind this strategy is that the AST is time consuming, expensive, and requires a careful application procedure. Additional research has to be conducted to develop more specific and sensitive tests to differentiate FPSR from those due to brucellosis.

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