Comparison of the specificity of cervical and caudal fold tuberculin tests applied to bovines in Uruguay

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Summary: In order to evaluate their specificity, the simple and comparative cervical tests and the caudal fold test were employed simultaneously, with bovine and avian PPD's from different sources, in 60 animals located in a tuberculosis-free area in Uruguay.

Responses to bovine PPD's, measured by the increase in skin thickness, ranged between 0 and 2.2 mm in the cervical test, and between 0 and 1.7 mm in the caudal fold test. According to interpretation criteria, all animals proved to be tuberculin-negative. The interval between reactions achieved and the minimal positive value was less for the simple cervical than for the caudal and comparative cervical tests.

By means of the comparative cervical test, reactions larger than 2.5 mm to avian PPD were observed in 4 of 60 animals. These four animals were killed and found free of tuberculous lesions at autopsy. Nevertheless, mycobacteria belonging to the Mycobacterium avium and M. terrae complexes were isolated in two of them.

On the basis of these results and bearing in mind the operating conditions and cost, the use of the caudal test, the sensitivity of which has also been confirmed in other studies, is recommended for epidemiological surveillance in situations such as those under study. In the case of doubtful interpretation, or in herds with a history of sensitisation due to environmental mycobacteria, the comparative cervical test may be employed as a second test.

KEYWORDS: Bovine and avian PPD tuberculin - Bovine tuberculosis - Specificity - Tuberculin tests - Uruguay.

INTRODUCTION

Bovine tuberculosis control is based on the identification and subsequent slaughter of animals reactive to the tuberculin test. Some reactors are observed to be free of...
tuberculous lesions at autopsy. This finding is relatively more frequent when tuberculosis prevalence is very low, during the final stages of a control and eradication programme (1, 8).

When an animal reactive to bovine PPD tuberculin presents no lesions resembling tuberculosis and *Mycobacterium bovis* cannot be cultured from tissue samples, the tuberculin reaction is considered "non-specific". Such reactions are commonly attributable to sensitisation by other mycobacteria sharing common antigens with *M. bovis*, thus inducing a crossed response to bovine PPD. When the relative frequency of such crossed reactions tends to increase, the comparative tuberculin test with bovine and avian PPD tuberculins is recommended (13, 14).

On employing the comparative test, in cases of infection due to *M. avium* or *M. johnei*, or else owing to sensitisation by other mycobacteria with low pathogenicity such as those included in group III of Runyon's classification, responses to avian PPD are greater than to bovine PPD. This affords a higher reliability in the identification of truly positive animals (infected by *M. bovis*).

In Uruguay, mycobacteria belonging to the *M. avium* Complex (MAC) have been isolated from bovines and swine with granulomatous lesions, and also from lesion-free swine lymph node samples (4, 5, 15).

PPD tuberculins are prepared in Uruguay, as well as in several other Latin American countries, by standardised methods, and the potency of each batch is controlled in guinea pigs (3). Test specificity, i.e. the capacity to provide a negative response in animals not infected by *M. bovis*, has become even more crucial in Uruguay where, as a result of a programme organised on a voluntary basis, infection prevalence in dairy herds dropped from 4.9% in 1965 to 0.01% in 1984, while the figure for beef cattle is 0.005% (2).

The goal of this study was to determine the specificity of PPD and of different tuberculin test methods applied to bovines in a tuberculosis-free area.

**MATERIALS AND METHODS**

**PPD tuberculins**

PPD's produced at the "M.C. Rubino" Centre for Veterinary Research (CIVET), Uruguay, and at CEPANZO (CPZ) were employed as follows: bovine PPD batches 1-86 CIVET and 1-85 CPZ, and avian PPD's 1-86 CIVET and 1-85 CPZ, which were prepared from *M. bovis* AN5 strain (source: Centraal Diergeneeskundig Instituut, CDI, Netherlands), and from *M. avium* D4, according to methods already described (3). Protein concentration for bovine PPD was 1 mg/ml and for avian PPD 0.5 mg/ml.

**Sensitivity of tuberculin PPD's**

The potency of a bovine PPD as tested in guinea pigs is an indicator of its sensitivity for detecting tuberculosis in cattle. In this case CIVET PPD potency has been adjusted to that of CPZ PPD according to results from guinea pig assay (3). The CPZ bovine PPD batch, in turn, had presented a relative potency of 0.89 against the standard
M. bovis PPD of the European Economic Community (ECS). The latter was taken as reference (16). Potency of the CPZ avian PPD batch, quantified against the international avian PPD standard, was 20,000 IU/ml.

Sensitivity of bovine PPD CIVET 1-86 has been controlled by its current use in diagnosing infected herds, where positive reactors are sacrificed and autopsied, according to the Uruguayan control programme regulations.

**Specificity test**

Sixty female bovines identified with ear tags, of the Hereford and Polled Hereford breeds, were employed. Ages ranged from 4 to 9 years and weights from 300 to 600 kg. The animals belonged to DILFA and were raised on islands and peninsulas of Rincón del Bonete Lake, Uruguay, the only area in this country which has been kept entirely free of bovine tuberculosis for more than ten years. The following tests were performed simultaneously on each animal:

a) **Caudal fold**: 0.1 ml of each bovine PPD was inoculated by the intradermal route, the CIVET PPD in the middle third of the right caudal fold and the CPZ PPD in the left. Prior to inoculation, skin thickness was measured with a 0.5 mm precision calipers. At 72 (± 6) hours, skin thickness was again measured, and the increase was recorded. The same person carried out all readings. Interpretation criteria for this test in Uruguay are as follows: an increase in skin thickness of 5 mm or greater is considered positive; from 3.0 to 4.9 mm, doubtful; and less than 3 mm, negative.

b) **Comparative cervical**: hair was clipped in two areas of 3 cm in diameter, located in the middle third of the neck, 10 cm from one another, both on the right and left cervical side. In these areas the thickness of a skin fold was measured with the calipers.

On the right side, 0.1 ml of avian PPD (upper area) and 0.1 ml of bovine PPD (lower area) produced by CIVET were inoculated intradermally. The same was done on the left side with avian and bovine PPD’s produced by CPZ. Readings were made as for the ano-caudal test. In Uruguay, this test is considered positive when the reaction to bovine PPD is 5 mm or greater than that to avian PPD; doubtful when the reaction to bovine PPD is from 3.0 to 4.9 mm greater than that to avian PPD; and negative when the reaction to bovine PPD is less than 3 mm.

c) **Simple cervical**: reactions to bovine PPD’s, obtained by cervical inoculation, were taken into account. An increase of 3 mm or more in skin thickness is considered a positive reaction and one smaller than 3 mm is regarded as negative.

**Pathological and bacteriological studies**

Animals selected according to their reactions were killed and autopsied. Searches were conducted for lesions compatible with tuberculosis and cultures were made on media suitable for isolating mycobacteria from samples of submaxillary, retropharyngeal, mediastinal, bronchial, retrohepatic and mesenteric-iliac lymph nodes. Methods already described for culture and identification of isolated mycobacteria were employed (11, 12).

**Statistical analysis**

Comparison of responses to each PPD in caudal or cervical tests was made by Student’s test for paired samples.
RESULTS

Tuberculin tests

Results are presented in Table I. In the cervical test, reactions to bovine PPD were smaller than 2 mm, except for one case (2.2 mm to CIVET PPD). In the caudal test, reactions were smaller than 1.5 mm throughout, again except for one case (1.7 mm to CIVET PPD).

**TABLE I**

Results of cervical tuberculin tests
(with bovine and avian PPD tuberculins) and caudal tests
(with bovine PPD), applied to 60 bovines in a tuberculosis-free area in Uruguay

<table>
<thead>
<tr>
<th>Test</th>
<th>PPD</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Range</th>
<th>No. of non-reactor animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical</td>
<td>Bovine CPZ(^{(a)})</td>
<td>0.74</td>
<td>0.60</td>
<td>0-1.8</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Bovine CIVET</td>
<td>0.69</td>
<td>0.61</td>
<td>0-2.2</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Avian CPZ(^{(b)})</td>
<td>0.89</td>
<td>0.82</td>
<td>0-3.5</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Avian CIVET</td>
<td>0.50</td>
<td>0.58</td>
<td>0-3.1</td>
<td>22</td>
</tr>
<tr>
<td>Caudal</td>
<td>Bovine CPZ(^{(c)})</td>
<td>0.46</td>
<td>0.42</td>
<td>0-1.4</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Bovine CIVET</td>
<td>0.34</td>
<td>0.39</td>
<td>0-1.7</td>
<td>21</td>
</tr>
</tbody>
</table>

(a) non-significant difference
(b) \(P < 0.001\)
(c) \(0.1 > P > 0.05\)

Mean responses to cervical and caudal fold tuberculin tests are presented in Figure 1. Two standard deviations have been added (mean + 2SD) in order to show the safety/reliability margins between actual responses and minimal positivity criteria. These margins are high for both the cervical and caudal test though higher for the latter.

Differences in responses obtained in the simple cervical test, according to the origin of bovine PPD employed (CPZ or CIVET), were not significant (\(P > 0.1\)). In the caudal test, responses to the CPZ PPD were greater than to CIVET PPD (0.1 > \(P > 0.05\)).

Comparing results of cervical and caudal tests with both bovine PPD's, responses obtained in the latter test were significantly smaller (\(P < 0.01\)).

Results of the cervical test considered as a comparative assay likewise afforded high reliability as regards discrimination between positive and negative results. None of the animals showed a reaction greater than 2.2 mm to bovine PPD.

Figure 2 shows the distribution of reactions to bovine and avian PPD's recorded on a scattergram. All points above the diagonal represent animals which gave larger responses to avian than to bovine PPD, points along the diagonal those with equal reaction to both PPD's, at the vertex those proving negative and below the diagonal those in which reaction differences favoured bovine PPD.
Responses obtained in tuberculin tests performed on 60 bovines in a tuberculosis-free area in Uruguay, using bovine and avian CPZ and CIVET PPD's

Millimeters of skin thickness increase:
mean value + two standard deviations

Employing PPD's produced at CEPANZO, responses were found to shift towards the "avian" sector: 32 cases with CPZ PPD vs 19 with CIVET PPD (p < 0.001) (Figure 2, a and b).

Pathological and bacteriological studies

Four out of the 60 bovines, marked in Figure 2 as A, B, C and D, presented reactions greater than 2.5 mm to avian CPZ PPD and one of them (D) also to avian CIVET PPD. Although the reaction level was weak, all four were killed; none showed lesions at autopsy. However, from lymph node samples taken from animals D and B, one strain of MAC and one of M. terrae (Runyon Group III) were isolated, respectively.
DISCUSSION

Although mean response to CPZ bovine PPD was somewhat greater than to CIVET PPD, the difference was scarcely significant (Table I). It might be inferred from these results that CPZ PPD specificity is rather lower than that of CIVET PPD. As regards avian PPD’s, mean response to CPZ PPD was significantly greater than that to CIVET PPD. This can hardly be attributed to higher potency when it is recalled that both products had been adjusted according to the guinea pig assay result. However, no strict parallelism has been demonstrated between PPD potency evaluated in guinea pigs sensitised with killed mycobacteria in an oil adjuvant — as employed herein — and the potency to detect natural sensitisation induced by viable mycobacteria in bovines (9).

At any rate, such differences in the PPD’s here employed failed to affect the interpretation of the test in bovines.
In agreement with the foregoing remarks on test specificity, and with previous studies on test sensitivity (10, 14), the simple tuberculin test with bovine PPD, both cervical and caudal, together with the above-mentioned interpretation criteria, afford a highly acceptable margin of reliability for differentiating between negative bovines and those infected by *M. bovis*. In this survey carried out on 60 animals in a tuberculosis-free area, there was not a single case of positivity (which would have been false), nor any which could be regarded as doubtful, according to interpretation criteria adopted in Uruguay.

Even bearing in mind that the simultaneous application of several PPD injections could have lowered response to each, the difference between observed reactions and the limit of positivity was quite ample. This difference was greater in the caudal test, which thus proved more specific than the cervical test.

Likewise, all animals were qualified as tuberculin-negative according to the results of the comparative test. This test allowed the detection of a certain reaction to avian PPD, greater with CPZ avian PPD, in 4 of the 60 animals, which did not correlate with the presence of lesions. However, mycobacteria were isolated from lymph nodes in two of the four animals, so that these strains might have induced sensitisation to avian PPD.

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In Australia (8), the simple caudal test afforded high sensitivity and specificity. In Argentina, studies performed on infected herds showed that the sensitivity of the caudal test was equal to or greater — according to PPD potency and interpretation criteria adopted — than that of the comparative cervical test (10, 14).

Furthermore, taking into account the operating conditions and cost, the simple caudal test seems at present the most appropriate for epidemiological surveillance in the area under study. Given its simplicity and lower relative cost, this test offers the advantage — for countries which have acquired experience in its use during control programmes — of not requiring any change in the tuberculin diagnostic method once a stage bordering on eradication has been reached. In addition, there is always the alternative of adopting the comparative cervical test as a second assay for cases of doubtful interpretation, or in herds with a history of non-specific sensitisation (13).

Each particular region may present its own features as regards sensitisation levels due to environmental mycobacteria. Therefore, at the time of taking decisions, it would be advisable to carry out an evaluation of diagnostic efficiency of the various tuberculin tests applicable to local herds.

We believe that both the results and conclusions of this study may find application in other countries where similar methods for the control of bovine tuberculosis are employed (1).

**

REFERENCES


