Interference of intradermal tuberculin tests on the serodiagnosis of paratuberculosis in cattle

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INTRODUCTION

ELISA-based methods are widely used worldwide (Marassi et al., 2005). Map shares several antigens with M. bovis, but few studies have focused on the interference of M. bovis infection in PTB tests (Paolicchi et al., 2003). This interference was recently demonstrated in a study using M. bovis-infected cows, originating from PTB-free herds (Marassi et al., 2005). In countries where TB remains to be a serious problem, periodic intradermal tests are mandatory, but the possibility that regular testing of non-infected cattle may determine false-positive reactions on PTB-ELISAs have not yet been evaluated.

MATERIALS AND METHODS

Herd – The herd selected for this experiment was confirmed as TB and PTB free using standard tests (intradermal tests for TB and ELISA and feces culturing for PTB, performed on all animals).

Cattle – Sixty-three animals were studied. Animals were divided into three groups of 17 animals, plus a control group with 12 animals. Group A was tested with single intradermal tuberculin test (ITT), using only bovine PPD; group B, tested with comparative ITT using both bovine and avian PPDs; group C, inoculated only with avian PPD, and group D, as control, inoculated with PPD diluents.

Bacterial culture of faecal samples for Map – All the animals were cultured for Map. Faecal samples were processed by the centrifugation protocol, as previously described (Ristow et al., 2006). Samples were inoculated onto slopes of Herrold’s egg yolk agar with and without mycobactin J (2mg/L- Allied Monitors - USA) and also with antibiotics (nalidixic acid 50 µg/mL, amphotericin B 50 µg/mL, vancomycin 50 µg/mL). Slopes were incubated at 37°C and observed in a two-weeks-interval for 24 weeks.

PTB ELISAs – The in-house ELISA protocol (ELISA-PPA) was performed as previously described (Marassi et al., 2007). ELISA-Pourquier is a commercial ELISA for paratuberculosis diagnosis and was performed according to the manufacturer’s instructions.

RESULTS AND DISCUSSION

All the 63 animals were negative at both ITT and Map culture. In the present study, sera of 63 animals were submitted to ELISA-PPA, and three (4.76%) were reactive. One of those animals belonged to Group A, suggesting that animals submitted to single ITT (inoculated with bovine PPD) may suffer immune interference and show false-positivity to PTB-ELISA. Single ITT is currently performed as screening test and is recommended as the main tool for the detection of infected animals in Brazil and in many other countries (Lilenbaum, 2000).

The two other false-positive animals were from Group B, tested with comparative intradermal test. No animal from Group C, inoculated only with avian PPD, was reactive. It suggests that the cross reactivity presented by the animals from Group B may probably be credited to the sensitization with bovine PPD.

It is well-established that tuberculin testing of animals alters the immune status of the animal and can affect both cellular and humoral responses. It causes a period of desensitization, usually considered as 60 days long, during which the cellular response to further tuberculin tests is depressed (Monaghan et al., 1994). The interference of M. bovis antigens in the serodiagnostic of PTB in tuberculous cattle, leading to false positive reactions, has already been described (Olsen et al., 2001; Marassi et al., 2005), as well as the influence of other mycobacteria, e.g. environmental species (Osterstock et al., 2005).
Table 1. Reactivity and Increase of S/P at two ELISAs according to the tested groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>ELISA-PPA</th>
<th></th>
<th>ELISA-commercial</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Increase</td>
<td>Positive</td>
<td>Increase</td>
</tr>
<tr>
<td>A</td>
<td>1</td>
<td>7</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>10</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>C</td>
<td>-</td>
<td>12</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>D</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TOTAL</td>
<td>3</td>
<td>29</td>
<td>5</td>
<td>22</td>
</tr>
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</table>

Beside those animals that showed cross-reactivity to PTB-ELISA, some cows had an evident increase in S/P values. From the 63 tested animals, 29 (46%) demonstrated this phenomena, which could be observed in animals from all tested groups, except for the control group. When those animals that presented an important increase in ELISA titres are considered together with those that reached the cut-off point and consequently presented reactive results, we can suggest that cross reactions in PTB-ELISA due to anti-*M. bovis* antibodies which are induced by bovine PPD inoculation may be a very frequent event in countries where both infections occur, which is being undervalued.

Cross reactivity was not observed until 15 days post-inoculation (p.i.). In spite of the detection of antibody enhanced titres until 90 days p.i. in two animals, the majority of cross reactions were observed between 30 and 60 days p.i. This finding indicates that animals routinely submitted to ITT for TB diagnosis can be under effect of PPD stimulation of antibodies and present false-positive reactions to PTB-ELISA up to 90 days after PPD inoculation.

Fig. 1. Evolution of S/P values of three positive cows at PTB-ELISA according to the days post-inoculation of PPD.

Thirty-six samples that presented sero-conversion or an important increase in antibodies titres detected by ELISA-PPA were selected to be confirmed with an accredited ELISA kit. A strong correlation ($k = 0.78$) between the results was observed. Not only the occurrence of false positive reaction was confirmed, but also the moment when those reactions were more frequent, i.e. between 30 and 60 days p.i. It confirms that the interference of the PPD inoculation as well as the occurrence of cross-reactions derived from it may also be observed in commercial tests.

Concluding, we demonstrate that intradermal tuberculin tests, both single and comparative test, widely used for the in vivo diagnosis of bovine TB, may temporarily
interfere in the immune status of the animal and determine cross reactions with other mycobacteria. That interference may lead to false-positive reactions in either ELISAs widely used for the serodiagnostic of paratuberculosis. Therefore, in order to avoid such occurrence, cattle should not be bled for PTB serodiagnostic for a period of at least 90 days after tuberculin testing.

REFERENCES