The use of MPB70 and MPB83 to distinguish between bovine tuberculosis (TB) and paratuberculosis (PTB)

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INTRODUCTION

In many countries both tuberculosis (TB) and paratuberculosis (PTB) occur concurrently and there is a need to understand the real interference of bovine TB in PTB-ELISAs before recommending these assays for large scale screening. This interference was recently demonstrated in a study using \textit{M. bovis}-infected cows, with no signs of PTB originating from PTB-free herds and demonstrated that following ELISA and immunoblot analysis some proteins are shared between \textit{M. bovis} and Map (Marassi et al., 2005).

In order to reduce such serological interference and increase the specificity of ELISA tests, recent studies have focused on the development of tests using new purified immunogenic and species-specific antigens. MPB70 and MPB83 are \textit{M. bovis}-specific proteins that have been evaluated with promising results. Regardless of this, novel antigens could be helpful in diminishing cross-reactions between Map and \textit{M. bovis} (Olsen et al., 2001; El-Zaatari et al., 2002; Waters et al., 2004).

The aim of this study was to evaluate the performance of two purified recombinant \textit{M. bovis}-specific proteins, MPB70 and MPB83, using an ELISA assay in order to differentiate bovine tuberculosis from paratuberculosis infection.

MATERIALS AND METHODS

\textbf{Cattle} – Two TB-free herds, comprising 340 and 150 adult crossbred autochthon dairy cattle, respectively, were studied. A history of chronic and intermittent diarrhoea, wasting and occasional deaths in the previous six years led us to investigate paratuberculosis in these herds. In addition, six herds where a TB control program is being conducted were studied in order to obtain \textit{M. bovis} – infected animals.

\textbf{Bacterial culture for }\textit{M. bovis} \textbf{and histopathology} - Lymph nodes and lung samples were collected and handled for this purpose. For histopathology fixed tissue samples were stained by both hematoxylin-eosin (HE) and Ziehl-Neelsen (ZN). For bacteriology examination, samples were refrigerated for 48 hours and inoculated in Löwenstein-Jensen with pyruvate slopes, incubated at 37\(^\circ\)C and observed weekly for 10 weeks.

\textbf{Bacterial culture of faecal and tissue samples for Map} - From the 490 animals of both herds of Group A, 130 faecal samples were randomly taken from adult cows (age >3 years) over a period of 24 months and cultured for Map. Faecal samples were processed by the centrifugation protocol (Ristow et al., 2006).

\textbf{Post mortem examination of PTB clinically affected animals} - Three adult cattle presenting clinical signs of PTB were necropsied and examined for the presence of gross lesions typical of Map infection. Tissue samples were taken from both lesioned and non-lesioned tissue and processed for both bacteriology and histopathology

\textbf{Confirmation of isolates by PCR} - Map isolates were confirmed by using PCR based on IS900. DNA extraction was described elsewhere (Ristow et al., 2006). Amplification was performed as described (Ristow et al., 2007), using primers specific for the insertion sequence IS900 (Bio Synthesis, USA) and the Taq Platinum® polymerase system (Invitrogen, USA).
ELISAs - The recombinant proteins MPB70 and MPB83 were purified and used separately as capture antigens in ELISAs as previously described (Lightbody et al., 2000; McNair et al., 2001). Cut-off points based on OD readings were calculated using ROC analysis and concordance of tests conducted using \( \kappa \) index.

RESULTS

Cattle - In order to evaluate the potential of ELISAs to distinguish between Map and \( M. \) bovis infections in cattle, two distinct populations of animals were selected. In Group A (n = 23), animals coming from TB-free herds with history of PTB were faecal culture positive for Map. In this group, six animals were in the clinical stages of Map infection while 17 were sub-clinically affected. In Group B (n = 48), animals were skin test positive to bovine tuberculin and when necropsied there was evidence of \( M. \) bovis infection seen either as the presence of lesions typical of bovine tuberculosis or by the recovery of \( M. \) bovis from tissue samples.

Evaluation of MPB70 and MPB83 by ELISA - Capture ELISAs based on either MPB70 or MPB83 were used to measure antibody responses. In Group A (Map positive), ten animals reacted to MPB70 (43%) and nine to MPB83 (39%). The mean ELISA OD value for the animals in the clinical stages of infection was 0.162 compared to 0.05 for those animals sub-clinically affected. In Group B (\( M. \) bovis positive), 37 animals reacted to both MPB70 and MPB83 (77.08%). Results of MPB70 and MPB83 ELISA are depicted in Tables 1 and 2. Our results indicate that both antigens presented very similar results, with a concordance (\( \kappa \) index) of 0.91. The difference between the mean OD value for Group A and Group B measured against the same antigen was highly significant (p<0.01). Control sera reacted as expected, i.e. very low ODs (<0.06) for both ELISAs.

Table 1: Results of MPB70 ELISA used to measure antibody responses in Map infected cattle (Group A, n = 23) and \( M. \) bovis infected cattle (Group B, n = 48).

<table>
<thead>
<tr>
<th>M. bovis culture positive</th>
<th>Map positive (faeces)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA positive</td>
<td>37</td>
<td>10</td>
</tr>
<tr>
<td>ELISA negative</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>23</td>
</tr>
</tbody>
</table>

Table 2: Results of MPB83 ELISA used to measure antibody responses in Map infected cattle (Group A, n = 23) and \( M. \) bovis infected cattle (Group B, n = 48). The difference between serum samples taken from \( M. \) bovis or Map culture positive and which were positive to MPB83 was statistically significant (p <0.01).

<table>
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ELISA cut-off values - Cut-off points were determined from OD readings and analysed by ROC analysis using isolation and recovery of \( M. \) bovis as an indicator of infection in tested cattle. At a cut-off point of 0.06, for both antigens (MPB70, MPB83) each ELISA test had a sensitivity of 52.2% and a specificity of 77.1%.

DISCUSSION

Map shares several antigens with other \textit{mycobacteria}, including \( M. \) bovis. Reports show that PTB can compromise the specificity of bovine tuberculosis diagnostic tests, and natural infection with Map was demonstrated to lead to false-positive reactions in TB skin tests (Buddle et al., 2003). Bovine TB also interferes on the efficacy of diagnostic tests, but this phenomenon has not yet been widely evaluated (Olsen et al., 2001). More recently, a cross reactivity between \( M. \) bovis and Map antigens in PTB-ELISAs was demonstrated using sera of \( M. \) bovis- infected and Map-free cows (Marassi et al., 2005).
Several recombinant proteins have been tested as antigens in order to improve ELISA specificity. AhpC, AhpD and 14kDa proteins were used in an ELISA in order to discriminate PTB from TB infected-cattle, with promising results (Olsen et al., 2001). A fusion protein comprising ESAT-6 and CFP-10 was used in an ELISA system with the purpose of improving the detection of specific antibodies to bovine TB, detecting even early stages of the infection (Waters et al., 2004).

Immune responses to MPB70 and MPB83, proteins derived from pathogenic strains of *M. bovis* culture filtrates, are both representative of a strong antigen-induced CMI response in the early stages of the tuberculosis infection. MPB70 is the major secreted antigen of *M. bovis*, while MPB83 is a cell wall lipoprotein (Juarez et al., 2001). MPB70 is specific to *M. bovis* since cattle infected with Map exposed to *M. avium* presented no detectable serum antibodies to it (Lightbody et al., 2000).

In the present study, ELISAs based on either MPB70 or MPB83 were used to identify the antibody status of animals from either *M. bovis* or Map infected herds. Using these ELISAs, more *M. bovis* infected cattle were positive to both MPB70 and MPB83 compared to Map infected cattle, and the mean OD values for each group were statistically different. Using a cut-off value of 0.06, sensitivity was defined at 52.2% and specificity at 77.1%.

A relatively high proportion of Map infected cattle were also ELISA positive to MPB70 and MPB83 (43% and 39% respectively). This contrasted strongly with those animals which were infected with *M. bovis*, where 77% were antibody positive to both MPB70 and MPB83. In addition to a greater number of antibody positives, the mean OD values in the *M. bovis* infected group was greater than that for the Map infected group, with the difference between the mean values statistically significant. While these higher recognition rates within the *M. bovis* infected group indicate the usefulness of these proteins as diagnostic reagents, they lack total specificity.

There was a much higher antigen recognition frequency in the *M. bovis* compared to the Map infected group. The mean OD values for each antigen were significantly higher than that for the Map infected group (*P* ≤ 0.01) and in addition, there was no significant difference between the mean values for MPB70 or MPB83 (κ = 0.91). These data indicate that either antigen could be used in an ELISA to detect antibodies to *M. bovis*.

In group A, six animals were in the late stage of paratuberculosis infection, presenting clinical symptoms, while 17 were sub-clinically infected. Mean OD of the six ill animals was 0.162, while for the sub-clinically infected cows was 0.050. This difference is highly significant (*P* ≤ 0.01) and demonstrates that high ODs are more common in clinically infected animals. Consequently, cross reactions are more probable to occur in those animals since at this stage humoral responses to Map are more detectable, a feature of mycobacterial infections.

In conclusion, our results indicates that MPB70 and MPB83 clearly detect *M. bovis* specific antibodies more often in tuberculous cows than in paratuberculous cattle, and therefore can be considered as valuable tools to differentiate between these two infections using serological assays. The confusion caused by antigenic cross-reactivity between bovine paratuberculosis and tuberculosis may be resolved using purified recombinant antigens derived from each micro-organism, in order to increase the specificity of serological assays. The future combination of assays, each based on different antigens, has therefore potential as a diagnostic tool, taking into account the evolution of these infections, the individual variation of immune responses and herd history.

REFERENCES


