Application Of The Enfer Chemiluminescent Multiplex ELISA System For The Detection Of Mycobacterium Bovis Infection In Goats

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Abstract:
Currently there are over 5,000 goats in Ireland farmed for the purposes of producing milk and milk products for the human food market – thus there is a need to implement a tuberculin (TB) control programme to ensure food safety and to comply with EU legislative requirements. The Enfer Scientific chemiluminescent ELISA system, currently optimised for detection of Mycobacterium bovis infection in cattle and other species, was optimised to enable the detection of M. bovis infection in goats. A total of 495 goats were recruited into the study. A group of 14 animals, that had a history of being TB free, from two different herds (17 and 44) were used to assess the specificity of the multiplex assay. To determine sensitivity of the assay a further 180 positively Single Intradermal Comparative Tuberculin Test (SICTT) reactors (90 and 120 from two different herds) were tested. The remainder of the sample tested were negative SICTT classified as potentially exposed animals from the same herds as the SICTT positive reactor animals. The multiplex assay was optimised for the sensitive detection of the infection in goats and the specificity was determined as 98.3%. Among the SICTT positive reactors the specificity of the assay was further determined as being 98.3% and 100% respectively.

Materials and Methods:

Samples
A total of 495 goats were recruited into the study. A group of 14 animals that had a history of being TB free from two different herds (17 and 44) were used to assess the specificity of the multiplex assay. To determine sensitivity of the assay a further 180 positively SICTT reactors (90 and 120 from two different herds) were tested. The remainders of the sample tested were negative SICTT classified as potentially exposed animals from the same herds as the SICTT positive reactor animals.

SICTT
The SICTT was applied to goats in this study in the same manner as it is applied to cattle. Briefly, the SICTT is conducted by separately injecting a bovine and avian purified protein derivative (PPD) intradermally into four distinct sites on the neck of goat. The test is read 72 hours later, by comparing the relative increase (in mm) of skin thickness (as an in vivo cell mediated response to each tuberculine) at each injection site. The interpretation of the skin test used in this study was the ‘severe interpretation’ and positives were called if the bovine reaction was both positive (>4mm) and exceeded the avian reaction.

IFN-γ
The Bovigam IFN-γ was performed under standard conditions as previously described (Gormley et al 2006).

Multiplex Assay

The multiplex assay was carried out as previously described (Whelan et al 2008) with the following optimisation for use with goat sera. Sera samples were diluted 1:1,000 into sample dilution buffer (Enfer Scientific) and tested. A 50µl sample dilution was added per well. The plates were incubated at room temperature with agitation for 90 minutes. The plates were washed six times and aspirated. The detection antibody (Anti goat IgG peroxide conjugate, Sigma) was prepared to a dilution of 1:40,000 in detection antibody dilution buffer (Enfer Scientific). After addition of 50µl of the detection antibody to test wells, the plates were incubated at room temperature for 30 minutes with agitation (900 rpm). The plates were washed as above and 40µl of substrate (50:50 dilution of substrate and diluent) was added per well. Signals were captured as optical density readings in a microplate reader. Signals were calculated as optical density readings and data was expressed as previously described (Whelan et al 2008).

Results:

Sensitivity and Specificity of the Multiplex Assay

The multiplex assay was optimised for the sensitive detection of the infection and the specificity was determined as 100% using the 31 animals from a herd with a history of being TB free. Among the SICTT positive reactors (PPD Bovine reaction: PPD Avian reaction >4mm) (PPD Bovarian reaction –>4mm) the multiplex assay detected 57/60 and 120/120 SICTT positive animals from the two herds investigated. The results suggest that a serum based assay for the detection of M. bovis infection in goats is a feasible alternative to the intradermal tuberculin test which may cause quite painful reactions in TB infected goats and is not always easy to administer with accuracy in younger goats with very thin skin. With further development and validation the test could aid eradication of the infection from herds.

Conclusions:

• The results suggest that a serum based assay for the detection of M. bovis infection in goats is a feasible alternative to the intradermal tuberculin test which may cause quite painful reactions in TB infected goats and is not always easy to administer with accuracy in younger goats with very thin skin.

• Using serum samples from reactors TB positive and TB true negative sera the sensitivity and the specificity of the multiplex assay were determined to be up to 98.3% and 100%, respectively.

• The Multiplex serum test can be performed with greater frequency than intradermal tuberculin testing as there is no desensitization period and the test can function identically until animals die and thus close infection from herds more efficiently. This can lead to quicker identification of infected animals and prompt removal from herds.

• With further development and validation the multiplex test could aid eradication of the infection from herds.

References:


Table 1. Sensitivity and Specificity comparison for SICTT and Multiplex Assay results

<table>
<thead>
<tr>
<th>Sample</th>
<th>SICTT</th>
<th>Multiplex</th>
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<tbody>
<tr>
<td>n=180</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>n=180</td>
<td>100.0%</td>
<td>98.3%</td>
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Figure 1. Profile of antibody activity for representative selection of positive and negative animals

Figure 2. Profile of sero-reactivity of positives on the Multiplex assay

Figure 3. Profile of antibody activity for representative selection of positive and negative animals