DEPARTMENT for ENVIRONMENT, FOOD and RURAL AFFAIRS **CSG 15**

Research and Development

Final Project Report

(Not to be used for LINK projects)

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Project title	Improved diagnostics for cattle
DEFRA project code	**SE3005**
Contractor organisation and location	Veterinary Laboratories Agency Weybridge New Haw Addlestone, Surrey KT15 3NB
Total DEFRA project costs	£ 511,347
Project start date	01/04/99
Project end date	31/03/02

Executive summary (maximum 2 sides A4)

Current tuberculosis control in cattle is based on the tuberculin skin test to identify infected animals and the subsequent slaughter of such tuberculin-positive animals. This has dramatically reduced tuberculosis in cattle in countries where such *test and slaughter* strategies have been implemented. However, attempts to eradicate the disease have not been equally and universally successful, especially in countries with wildlife reservoirs and in Great Britain the test can also lack specificity. Further problems with tuberculin testing are that the test may not detect animals in the early stages of infection. Tuberculin skin testing also results in inconclusive reactions. Resolving inconclusive reactions is a costly and time-consuming process because, due to the de-sensitization caused by the initial tuberculin skin-test, animals can only be re-tested after a period of 60 days, followed by another 60-day waiting period if the first re-test remains inconclusive. This increases the risk of the disease spreading from cattle to cattle within the herd. The BOVIGAM interferon-gamma (IFN-γ) test has shown promise in New Zealand to resolve doubtful skin-test results earlier (albeit using the caudal fold tuberculin test), since a re-test using BOVIGAM is possible as early as 7 days after the initial skin-test. Nevertheless, our own studies and studies elsewhere indicate that this test using tuberculin as antigen, has the risk of a greater number of false-positive reactions compared to tuberculin skin testing. Thus, use of defined antigens and alternative immunological measurements could offer improvements in terms of both sensitivity and specificity, and could reduce overall costs.

The main objectives of this project were to develop a more specific blood based test, based on defined antigens or synthetic peptides, as an adjunct to the tuberculin skin test to detect infected animals with higher specificity, that does not require prolonged intervals between re-tests, and that can help to resolve inconclusive reactors. This assay was based on the BOVIGAM IFN-γ kit.

The main findings of this report are summarized below:

- Diagnostic reagents based on *M. tuberculosis* antigens not expressed by BCG can improve the specificity of PPD in blood based assays not only in relation to BCG vaccinated animals but also to un-vaccinated, un-infected controls (objective 01, 02 and 08).

- Diagnostic reagents containing synthetic peptides are a practical, cheap, and safe alternative to recombinant proteinsand a prototype diagnostic reagents based on peptides derived from ESAT-6 and CFP-10 has shown a high degree of specificity compared to PPD, and is exhibiting comparable sensitivity levels (objectives 01, 02 and 08).
- Our data suggests that the IFN-γ test using tuberculin, or the peptide or protein cocktail used in this study, can detect infected cattle at an earlier stage of disease compared to the tuberculin skin test (objective 02).
- Specific and defined protein or peptide-based reagents (and to a lesser degree PPD) when used in IFN-γ tests might resolve inconclusive reactors earlier than with tuberculin skin testing (objective 02).
- An MPB70 and MPB83-based anamnestic ELISA system imparts no or only a very limited additional benefits in resolving inconclusive reactor cattle compared to the application of the IFN-γ test alone(objective 03).
- Recombinant MPB83 expressed and purified from *E. coli* was comparable to the *M. smegmatis* produced MPB83 in its capacity to stimulate immune responses in *M. bovis* infected guinea pigs and cattle (objective 04).
- The protein component of bovine tuberculin PPD constitutes the the principal antigenic constituent contributing to its *in vitro* and *in vivo* immunogenicity in *M. bovis* infected hosts (objective 05).
- The modifying glycans of MPB83 can modulate immune responses in *M. bovis* infected hosts: *In vivo* stimulation of DTH responses to MPB83 may be enhanced by the presence of glycans (in guinea pigs), whereas *in vitro* recognition of MPB83 in cattle is enhanced following the removal of the modifying glycans supporting the use of a non-glycosylated form of the antigen in diagnostic studies utilising blood based assays (objective 06).
- Adsorption of an immunogenic peptide to hydroxylapatite microparticles with high surface area improved its presentation to T cells ca. 25fold. However, since the costs of peptide synthesis have decreased substantially over the last year, and in the light of the amount of quality control and standardisation that would be required to produce peptide-loaded particles to a uniform and reproducible standard, we do not envisage any benefit in using them in large-scale, routine test settings and recommend the use of free synthetic peptides (objective 07.
- The skin test reactivity of recombinant proteins in cattle can be enhanced by the addition of a synthetic lipopeptide ($Pam_3CysSer(Lys)_4$ (objective 07).
- A study undertaken in New Zealand confirmed (i) that sensitivity levels approaching those of tuberculin can be achieved with cocktails composed of defined synthetic peptides and (ii) their high degree of specificity compared to tuberculin. In addition, these peptide cocktails performed comparably to cocktails composed of the respective recombinant proteins (objective 08).
- Bovine γδ T cells recognize mycobacterial protein antigens and peptides and contribute to the overall IFN-γ response upon antigenic stimulation. Bovine γδ T cells also produce TGF-β after stimulation with mycobacterial antigens and have immunomodulatory properties on other T cell subsets. However, addition of decorin to block TGF-β activity to PBMC cultures had no beneficial effect (objective 11).

Further studies to identify more specific antigens to improve test sensitivity to a level approaching or exceeding that of tuberculin are needed and are now conducted under DEFRA-funded project SE3028.

Publication output:
The findings in these report have been published so far in six peer-reviewed journals (average impact factor: 4.08), 4 more manuscripts are in preparation or have been submitted. The data have also been presented at a large number of international and national scientific conferences.

Scientific report (maximum 20 sides A4)

See attachment:
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