The recently published 'Krebs' Report' on bovine tuberculosis in cattle and badgers recommended the development of vaccines to protect cattle against infection with *Mycobacterium bovis* as a long term strategy to control the disease in cattle. It was also recommended that the option of badger vaccination should be retained and could be pursued if immunological reagents and a challenge model become available for the badger. The aim of this proposal was to apply recent advances in mycobacterial genetics and vaccinology to the development of TB vaccine candidates

Since it cannot be predicted with certainty which approach to vaccine development will yield the best candidate vaccine against *M. bovis* two of the most promising avenues of vaccine research were pursued. The first avenue was to produce live attenuated vaccines based on *M. bovis* and *M. bovis* BCG. For a successful vaccine of this type the disabled micro-organism should be sufficiently robust to induce a protective immune response but be contained or eliminated such that the disease process is self-limited. In this project key genes essential for the growth and survival of *M. bovis* within the host were inactivated by transposon mutagenesis. Techniques were also developed to identify further targets for disabling *M. bovis* namely those genes that are expressed by *M. bovis* inside its host.

The second approach to generating candidate vaccines for *M. bovis* was based on one of the most promising areas of subunit vaccine development, DNA vaccination. This approach involved the introduction of *M. bovis* DNA encoding proteins that stimulate protective immunity into appropriate target cells within the host. Transient expression of the DNA inside the host cell was found to trigger a protective response.

The development of a TB vaccine is an ambitious programme and it was therefore essential that a mechanism be established to co-ordinate R & D efforts between the bovine and human vaccine programmes. A further objective of this project was to establish such a mechanism.

Main Findings of the project:

- Transposon mutagenesis using phage delivery systems was found to be more efficient for *M. bovis* BCG than for wild type *M. bovis* (AF2122/97).
- A library of BCG transposon mutants was produced and screened for strains that had been disabled in key metabolic pathways.
- Techniques for the rapid genetic characterisation of transposon mutants were developed.
- One mutant in particular, which required leucine for growth, showed much promise as a vaccine candidate. This strain conferred significant protection against *M. bovis* infection in guinea pigs and did not compromise tuberculin skin testing in either guinea pigs or cattle.
- DNA vaccines based on the *M. bovis* antigens MPB83 and MPB70 were produced which gave significant protection against *M. bovis* in mice and guinea pigs and stimulated strong T cell responses in immunised cattle without compromising tuberculin skin testing. These vaccines were more effective than a DNA vaccine based on Ag85 that had shown much promise against *M. tuberculosis* in mice and guinea pigs.
- A DNA vaccine encoding MPB70 was shown to exert a therapeutic effect on mice heavily infected with *M. tuberculosis*, especially in the lungs.
- The vaccines produced or identified in this project rank among the most effective so far developed against *M. bovis* and *M. tuberculosis*.
- An *in vitro* model was developed which induced the expression of genes that are usually expressed inside bovine macrophages and was used to identify other proteins that are upregulated under such conditions.
- A technique for harvesting *M. bovis* directly from the lungs of guinea pigs was developed.
- A mechanism was established to facilitate co-ordination of R & D efforts between the bovine and human TB vaccine programmes *via* the Animal Models Task Force of the WHO Steering Committee on the Immunology of Mycobacterial Diseases (IMMYC).

VLA Weybridge incorporated the main implications of this research into their CSG 1999-2000 Research Proposals.