Vaccination to Prevent Tuberculosis in Farmed Deer; Hopes and Challenges for the Future

Frank Griffin, Jan Hesketh, *Colin Mackintosh and Glenn Buchan
Deer Research Laboratory, Department of Microbiology,
University of Otago, Dunedin, New Zealand
*Nutrition Unit, MAF(Tech), Invermay Research Centre,
Mosgiel, New Zealand.

Background

Vaccines for prevention of tuberculosis rank as the most widely used and safest prophylactics in human medicine. Throughout the past 50 years up to 4 billion humans have been inoculated with BCG, (Bacillus Calmette-Guerin) throughout developed and developing countries worldwide. Notwithstanding the widespread use of BCG there have been recent studies which raise doubt as to the efficacy of this vaccine for humans (Fine, 1989).

BCG is a live, attenuated vaccine derived by laboratory culture of an isolate of M.bovis in 1906 which was shown (Calmette, 1928) to be protective against natural infection of humans caused by M.tuberculosis or M.bovis. BCG has also been used as a vaccine to prevent tuberculosis (Tb) in cattle (Berggren, 1981) and deer (Zhou Shilang & Wu Shanzhi, 1985) and the findings concerning its efficacy have been variable. In theory, BCG should be a reasonable candidate as a vaccine for Tb in domestic animals as it is derived from the mycobacterial species (M.bovis), which is the causative agent of Tb.

In spite of doubts raised about the efficacy of BCG vaccines for humans (Fine, 1989) , and domestic livestock (Berggren, 1981), the principle of using a live attenuated vaccine for Tb prevention is sound. Killed Tb vaccine and microbial extracts have consistently failed to generate protection (Orme & Collins, 1988) against natural infection with M.tuberculosis or M.bovis. There is little doubt that the vaccine is immunogenic and it contains appropriate levels of protective antigens (Collins, 1991).

A review of the literature highlights a number of features which recommend BCG as a potential vaccine:

1. It is safe and non infectious in all hosts tested to date (Waddington & Ellwood, 1992).
2. A single dose of this live, attenuated vaccine can generate immune reactivity.
3. It has natural properties of adjuvancy which potentiate immune reactivity.

4. When used as a recombinant to express foreign genes (e.g. AIDS-HIV) it acts as a potent vaccine to induce both antibody and cellular immune reactions (Aldovini & Young, 1991).
5. It is cheap to produce ($1.00).

The Immunology of Tuberculosis

When evaluating the potential of vaccines to control Tb in animals, it is necessary to first consider the history of BCG as the prototype vaccine for Tb prevention. Failure to confirm the potential of BCG so far may be explained as follows:

1. The empirical use of a prototype attenuated culture of M.bovis (BCG) is unlikely to be totally adequate as a vaccine to control a complex disease like tuberculosis.
2. Political perception and investment in Tb research in developed countries has been poor, as Tb has been largely controlled by improved public health measures.
3. Basic mechanisms of immunity to Tb are poorly understood so it is not possible to identify the properties of an ideal vaccine.
4. Until recently there has not been appropriate scientific methodology to study basic mechanisms of cellular immunity to Tb.
5. To date there has been a failure to distinguish between immunological pathways which produce protection (**Immunity**), and disease related (**Hypersensitivity**) reactions to Tb. There has been undue emphasis on the study of patterns of disease related **Hypersensitivity** and diagnosis.
6. Mechanisms of **Hypersensitivity** found in disease are unlikely to reveal relevant pathways required for **Immunity**.
7. There has not been a good natural animal model to study Tb vaccination experimentally (Wiegeshaus & Smith, 1989).

There are data available that highlight pathways of immune reactivity which are compatible with immunity or disease. Protective immunity ('Listeria-type') is mediated by macrophages (Mackeness, 1967) and T-cells (CD4+, CD8+) (Orme & Collins, 1984 and Kaufmann, 1989). It differs qualitatively from necrotising cellular immunity ('Koch-type') found in diseased individuals which is mediated by different T-cells (CD4+) (Rook and Al Attiyah, 1991), typified by some types of intradermal skin test reactivity (Rook, 1978). All researchers in this area agree that antibodies have no role in protective immunity and are in fact key indicators of active disease.

Recent extensive diagnostic studies in our laboratory, which have looked at patterns of immune reactivity in diseased deer, have highlighted the fact that active tuberculous disease evoked a complex

array of immune reactions, characterised by T-cell responsiveness; lymphocyte transformation (LT), and antibody (ELISA) specific for M.bovis antigens. While T-cell reactivity (LT) is directed strongly to the denatured proteins found in bovine tuberculin; purified protein derivative (PPD), antibodies (ELISA) react more specifically with native antigens obtained directly from cultures of M.bovis. Another marker for disease is elevated levels of inflammatory cofactors (ICF) found in the plasma of diseased animals in the days after skin testing (Cross et.al., 1991). Haptoglobin and fibrinogen are the two main proteins used to characterise ICF. These appear to characterise the necrotising, immune reaction which is associated with tuberculous disease. The combined data obtained from LT, ELISA and ICF constitute the blood test for Tb; (BTB) (Griffin & Cross, 1989). During these diagnostic studies it was noted that a small proportion of deer from infected herds exposed to M.bovis developed lymphocyte responses specific for bovine PPD but did not have antibody or produce ICF following intradermal skin testing. The positive predictive value for disease in animals with lymphocyte reactivity (LT), antibody (ELISA) and inflammation (ICF) was >80%. By contrast, the positive predictive value of BTB for Tb in animals with LT(+), ELISA(-) and ICF(-) status was <10%. These findings indicate that some animals exposed to M.bovis may develop protective cellular immunity after exposure to natural infection by M.bovis.

BCG Studies in New Zealand Deer

Studies were begun at Invermay in 1990 to evaluate aspects of immunity to BCG, with the primary goal to chart pathways of immune reactivity in deer to known Tb vaccines. It was recognised from the outset that, whereas BCG may not necessarily be the ideal vaccine, it must from an historical perspective be the benchmark against which new generation vaccines will be evaluated. The objectives of the initial study using BCG were to:

1. Generate measurable immune reactivity to the vaccine.
2. Determine if different types of BCG vaccine (live or dead), used with or without oil adjuvants, could generate distinguishable patterns of immune reactivity.
3. Establish that the live BCG vaccine did not produce disease in vaccinated deer, or cause infection for in-contact control animals.
4. Compare patterns of immune reactivity to BCG in subadult (>1 year old) and neonatal (<3 month old) deer.

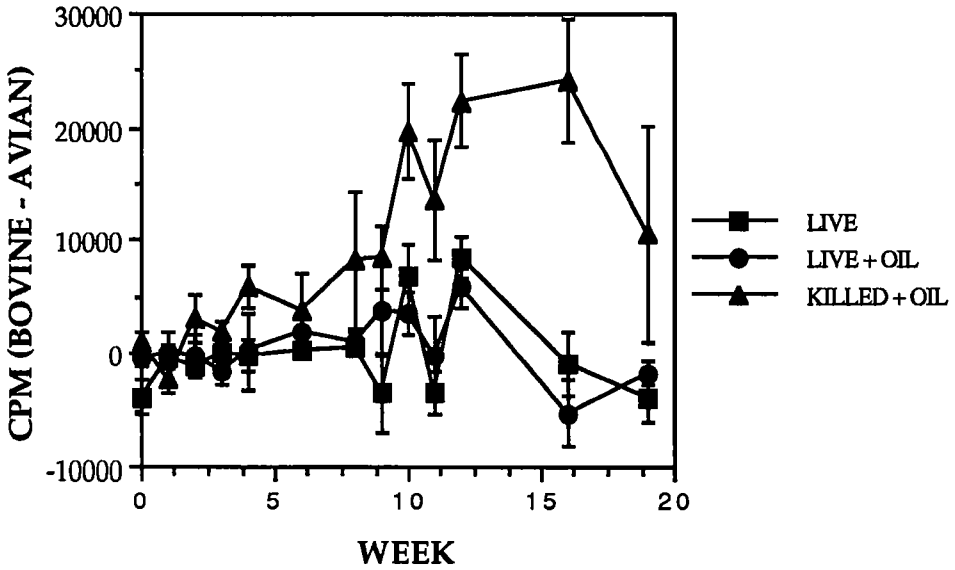
Groups of five deer were each inoculated with live or heat killed BCG (Connaught) vaccine at 5-10 times the dosage used for

human vaccination. Killed vaccine was used in combination with oil adjuvant (Span, Tween, Marcol-STM), as it was considered that killed vaccine alone would be insufficiently potent (immunogenic) to generate measurable immunity. Animals were boosted after 8 weeks with similar doses of vaccine to that used for primary stimulation. Blood samples, obtained at weekly or fortnightly intervals after vaccination, were subjected to BTB analysis.

Results of bovine specific lymphocyte transformation (Bovine minus Avian) responses to PPD are given in Figure 1. This data shows that BCG evoked modest levels of primary immune reactivity in vaccinated animals which increased significantly following boosting. LT responses to killed BCG were low after primary vaccination, but reactivity to killed vaccines in oil increased significantly following boosting, with higher specificity than was seen with live BCG in oil. Live BCG alone produced LT reactivity to mycobacterial PPD which was cross reactive (B=A), with B-A reactions which were rarely positive.

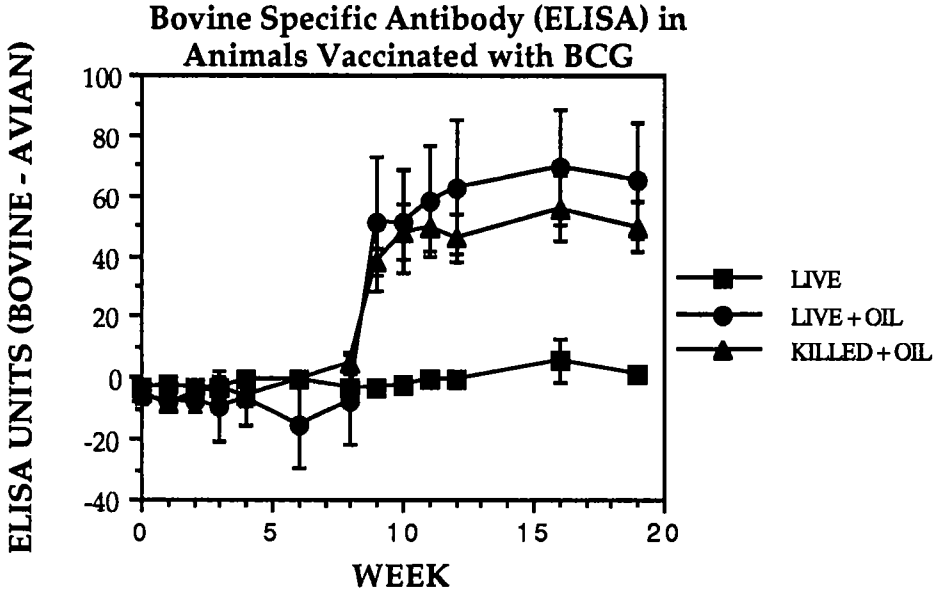
Figure 1:

Bovine Specific Lymphocyte Transformation (LT) in Animals Vaccinated with BCG



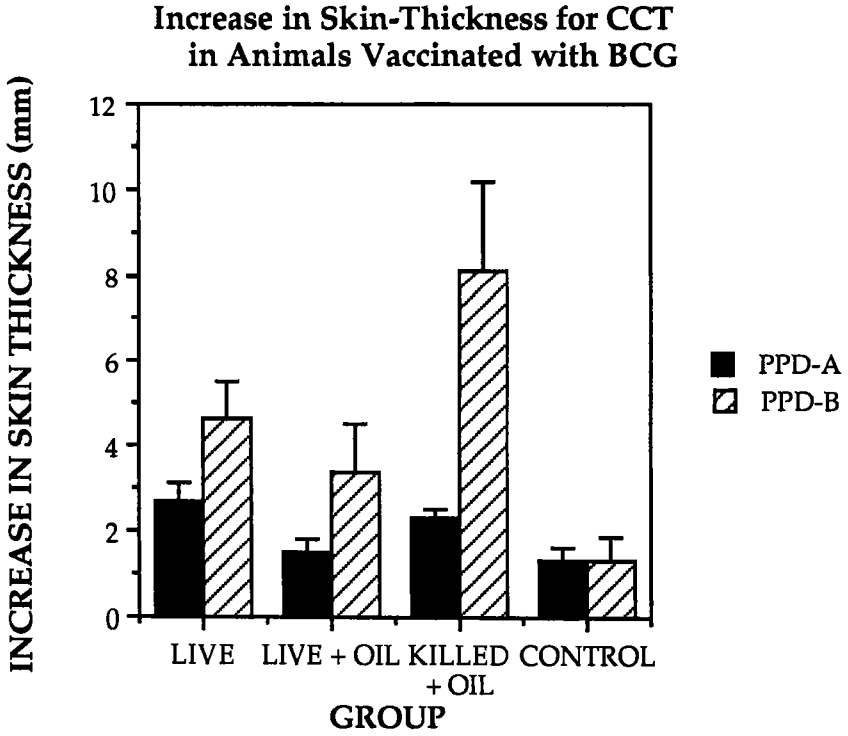
The antibody (ELISA) levels specific for M.bovis (B-A) are given in Figure 2. This shows that the addition of oil to live or killed BCG vaccines generated significant levels of antibody following boosting. The level of antibody specific for M.bovis was similar with killed BCG to that found with live BCG in oil. No antibody was produced in negative (saline) controls or in animals given live BCG alone.

Figure 2:



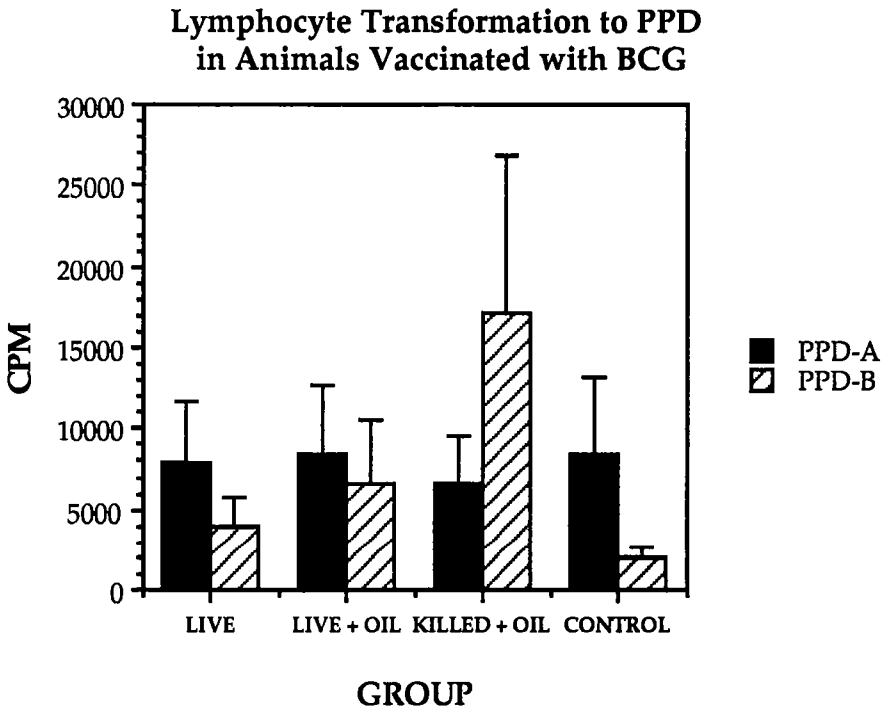
Comparative skin cervical tests (CCT) and lymphocyte transformation (LT) tests were carried out at week 19; 11 weeks post boosting with vaccine. The results obtained from CCT are given in Figure 3, and can be compared with T-cell reactivity to bovine and avian PPD (Figure 4) in parallel LT assays.

Figure 3:



The results given in Figure 3 show that animals exposed to BCG vaccine produce M.bovis specific (CCT+) reactivity when tested 11 weeks post boosting with BCG. While the CCT response in animals vaccinated with killed BCG in oil is particularly high, all other groups of vaccinated animals are CCT(+) by standard NZ interpretation of CCT.

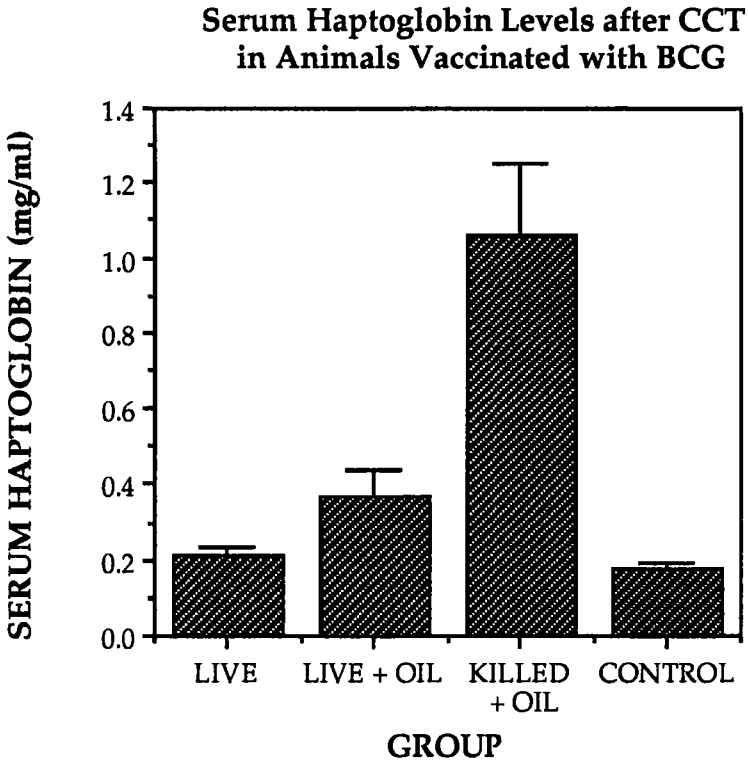
Figure 4:



By contrast with CCT, *in vitro* culture tests (LT) for mycobacterial reactivity (Figure 4), carried at just before CCT was applied, show significantly different patterns of specificity (Bovine vs Avian PPD), in BCG vaccinated animals. The only vaccine which produced LT specific reactivity to bovine PPD was the group vaccinated with killed BCG + oil.

The final parameter used in the BTB assay measured levels of plasma inflammatory cofactors (ICF) following skin testing. The data given in Figure 5 show that a significant incrrease in ICF was found only in animals vaccinated with killed BCG in oil. No significant increase was seen in negative control (CCT(-)) animals, or other groups exposed to live BCG (alone or in oil), even though both of these were CCT(+), though LT(-).

Figure 5:



Conclusions

The data discussed in this paper has focussed on a preliminary BCG vaccine study, which has evaluated patterns of immune reactivity in animals exposed to live BCG and BCG; live or killed, in an oil adjuvant. The conclusions which can be drawn from this study are:

1. BCG stimulates low grade primary immune reactivity which is increased significantly following boosting.
2. Unless an oil adjuvant is used, no antibody is produced.
3. BCG in oil induces lymphocyte transformation which is highly specific for M.bovis. Live BCG alone stimulates cross reactive cellular responses to common antigens found in both M.bovis and M.avium.
4. The overall response to killed BCG in oil; LT, ELISA and ICF, found in deer is identical to patterns of immune reactivity found in naturally diseased animals with active tuberculosis. It appears to induce immune reactivity (**Hypersensitivity**) typical of tuberculous disease caused by virulent M.bovis. This is quantitatively and qualitatively different from the reaction to live BCG vaccine, which may reflect protective immune reactivity (**Immunity**).

5. The response to live BCG; Non-specific LT, ELISA(-) and ICF(-) post skin testing is similar to the reaction found in non-diseased, (Immune?) animals exposed to M.bovis infection.
6. Small groups of 5 animals gave highly consistent results. This infers that valid studies in immunity to Tb vaccines can be carried out on relatively small groups.
7. Data not shown in this paper affirm that the patterns of immune reactivity found in yearling animals is almost identical to those found in 2-4 month old deer. This suggests that BCG will evoke reasonable levels of immunity in young animals; the group which will be the primary target for exposure to vaccines.

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