

# Vaccination of Badgers with *Mycobacterium bovis* BCG: A Preliminary Study to Investigate Blood Lymphocyte Responses associated with Protective Immunity

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## Introduction

The presence of bovine tuberculosis in Irish cattle poses a serious threat to the livestock industry. Although comprehensive test and slaughter policies have proved highly effective in reducing the prevalence of bovine tuberculosis, the transmission of the causative agent (*Mycobacterium bovis*) from badgers to cattle is believed to be a primary reason for the inability to eradicate the disease. Given that the most recent studies indicate a high prevalence of tuberculosis in badgers, there exists a significant potential for continuous re-infection of cattle from wild life reservoirs (Dolan, 1993). Strategies based on the development of novel vaccines against bovine tuberculosis offer an alternative approach to combat the disease by boosting the host immune system to kill the infecting organism. In the 1994 report of a joint UK/Ireland steering committee, it was concluded that development of a vaccine against tuberculosis for use in the badger was a feasible option (Report, 1994).

The sole existing vaccine for tuberculosis, *M. bovis* BCG, is the most widely used vaccine world-wide and millions of doses are given annually, mostly to children in under-developed countries, as part of WHO disease control programs. As such, it is reasonably effective, inexpensive,

with a proven safety track record. Studies have been carried out to test the effectiveness of using BCG in cattle (Buddle *et al.*, 1995), deer (Griffin *et al.*, 1999) and possums (Aldwell *et al.*, 1995) with encouraging results, demonstrating that the vaccine can protect animals from contracting tuberculosis in an experimental setting. However, no rigorously controlled BCG trials have been carried out using badgers and much information is needed to establish the basic principles required to develop an effective vaccination strategy. Immune protection against tuberculosis requires the development of a specific T cell – mediated immune response (CMI) in which the co-operative action of antigen specific T cells and macrophages ultimately controls infection by inhibiting growth of the tuberculous mycobacteria (Orme *et al.*, 1993). The proliferation of peripheral blood lymphocytes (T cells) in the presence of antigens such as bovine-PPD are regarded as indicators of a CMI response against tuberculosis infection (Carpenter *et al.*, 1997).

The ultimate aim of vaccination against tuberculosis is to direct an appropriate CMI response that will afford subsequent protective immunity following challenge with a virulent strain. However, preliminary studies are required to find out how the

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immune system of badgers may respond to vaccination with BCG. The present study has set out to evaluate these responses through defining a number of key objectives:

1. To evaluate immune responses of badgers vaccinated subcutaneously with *M. bovis* BCG.
2. To compare the immune responses of vaccinated badgers with non-vaccinated controls over one year.
3. To measure and compare the immune responses of vaccinated badgers against a panel of known T cell antigens.
4. To assess the heterogeneity of immune responses among individual badgers to BCG vaccination.

## Materials and Methods

### Little Island

Situated off Co. Waterford, Little Island contains a resident isolated population of badgers. The badgers are distributed among 4-6 main active setts. No livestock are present on the island and access to the public is limited. Prior to commencement of the vaccination trial observation studies indicated a healthy population. Cage traps have been deployed at the main setts and are monitored regularly.

### Trapping

Prior to trapping, cages were pre-baited with peanut. Trap mechanisms were set on the evening before trapping commenced. The trapped badgers were anaesthetised with Ketamine<sup>®</sup>/Domitor<sup>®</sup> (0.2 ml/kg), administered by intra-muscular injection. The animals were weighed, tattooed, tagged with microchips (and ear tags), checked for parasites (fleas,

ticks, lice) and other signs of disease or injury (bite wounds, trap wounds etc.). The sex, approximate age and body length, were also recorded. A tracheal aspirate was taken for subsequent culturing to test for presence of *Mycobacterium* spp.

### Analysis of blood samples

Prior to vaccination, a blood sample (20 ml, from the jugular vein) was drawn from each badger, into heparinised Vacutainer tubes. A blood sample (2 ml) was also be drawn into non-heparinised tubes for use in ELISA assays. These samples were sent to Veterinary laboratories Agency, Weybridge, UK (VLA) and analysed by ELISA for the presence of antibodies to the *M. bovis* antigen MPB83. The heparinised samples were transported to the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Dublin, for lymphocyte proliferation assays. Antigens used in these studies included PPD-bovine, PPD-avian, and the mitogen, ConA. Blood samples were also routinely examined for the presence of trypanosomes.

### Vaccine Preparation and Delivery

The BCG (Pasteur) strain was provided as frozen stock of predetermined titre of colony forming units (cfu), by VLA (Weybridge). The stock was thawed on site and diluted appropriately in phosphate-buffered saline (PBS) to the desired concentration. Animals were vaccinated subcutaneously with 0.5 ml ( $10^4$  cfu/ml) of *M. bovis* BCG (Pasteur). The vaccination site, approx. 1.0 cm above the right ear, was lightly shaved prior to injection. Non-vaccinated control badgers were injected subcutaneously with 0.5 ml of PBS.

## Results

Sixty cage traps were deployed on the island in 1998. Trapping has since taken place on five occasions, November, January, March and April and June. To date, thirty three adult badgers and seven cubs have been captured.

### General badger health

Gross examination of badgers throughout the course of the study indicated that the population remained healthy. Average weight changes fluctuated through the seasons. In addition, several lactating females were identified in March. Recorded injuries were highest in January, with severe lacerations found in many animals. Such injuries are generally associated with the breeding season. Trap injuries were minimal, the majority being skin grazes on the head or paws. During visits to the island, it had been observed that some individual badgers appeared to show gross signs of ocular cataract formation. Dr. Terry Grimes (Faculty of Veterinary Medicine, UCD) carried out ophthalmic examination of anaesthetised badgers and obtained data on the ocular abnormality. Eighteen badgers were submitted to ocular examination. Five of these showed evidence of cataract development. The cause of cataract formation was not established; however, changes seen in two animals were consistent with a developmental abnormality. Three animals showed unilateral cortical cataract and, although trauma as a cause of cataract is possible, no associated abnormality of the lids and eye was observed.

Trypanosomes (likely to be *T. pestanai*) were present in blood samples of many of the badgers. The trypanosomes co-precipitated with lymphocytes during blood

fractionation. When 23 adults were examined, trypanosome numbers ranged from  $1 \times 10^3$  –  $2.3 \times 10^5$  /ml blood. No trypanosomes were observed in the remaining six adult badgers. Of the two cubs tested, one was free of trypanosomes, while the second harboured  $1.5 \times 10^4$  /ml blood. The consequences of trypanosome infection in badgers is, at present, unknown.

### Vaccination and lymphocyte responses

During the first visit to the island in November 1998, blood was taken from eighteen badgers and analysed for responses against *M. bovis* antigens. The preliminary results of the lymphocyte proliferations, ELISA, and culture of tracheal aspirates confirmed that the badgers on the island were free of tuberculosis. Thirteen received a BCG vaccination and five received a saline placebo.

Throughout the course of our studies, the anti-mycobacterial immune responses of the vaccinated badgers were routinely monitored by measuring lymphocyte proliferation by T lymphocytes cultured with PPD-bovine, and PPD-avian. The mitogen, ConA, was also included as a positive control to monitor for cell survival. Lymphocytes were considered to be responding to the ConA when the proliferative stimulation index was greater than 5 (SI > 5). Although the individual responses of all animals varied over time, T lymphocytes incubated with ConA consistently proliferated with a stimulation index ranging from SI= 5 – 85.

The proliferation responses from vaccinated and control animals were monitored throughout the course of the study. A representative analysis of SI ratios of bovine-PPD / avian-PPD responses is shown in Figures 1 & 1a .

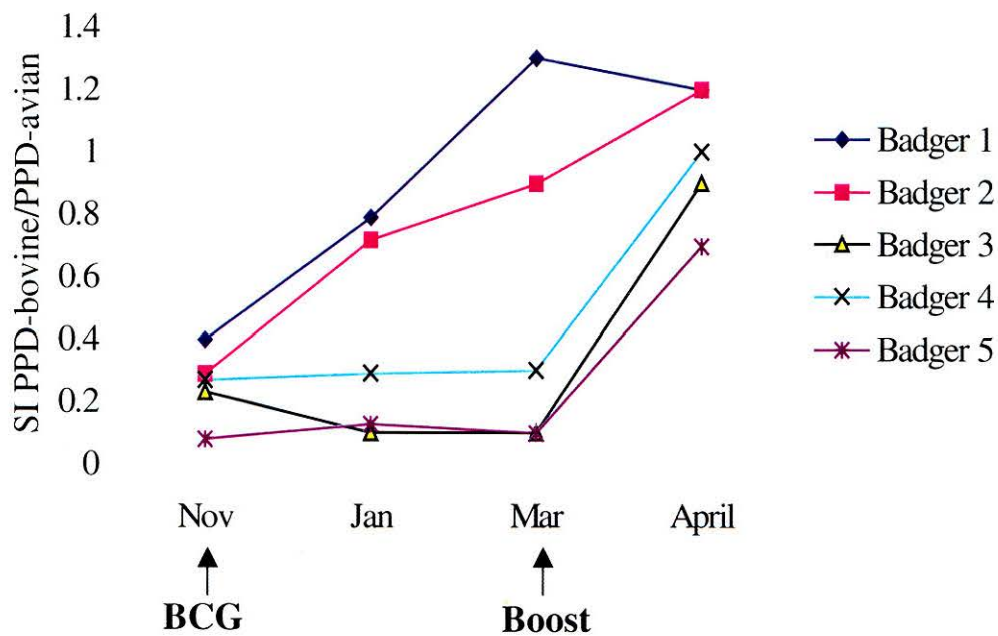


Figure 1 Immune responses of *M. bovis* BCG vaccinated badgers.

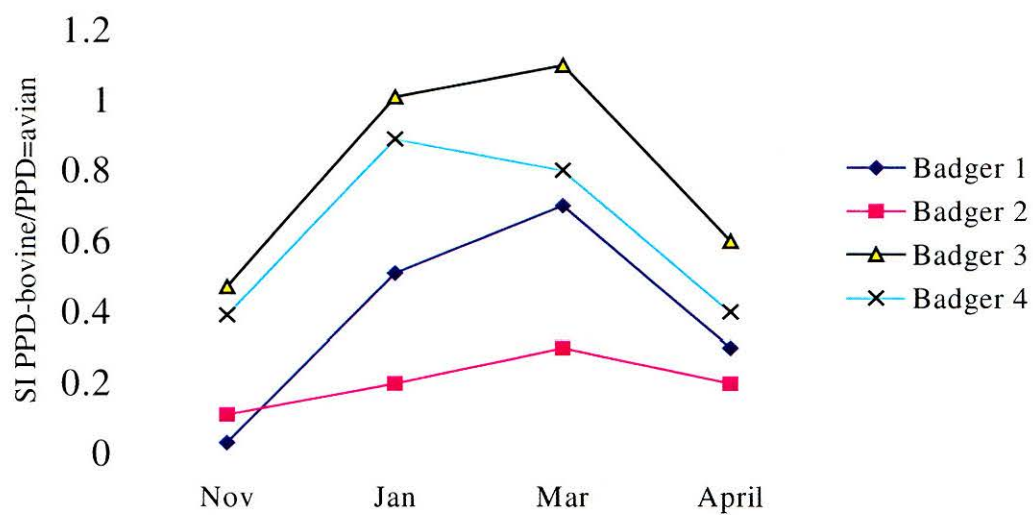


Figure 1a. Immune responses of control non-vaccinated badgers.

In January, the lymphocyte SI ratios of the BCG vaccinated animals were not found to be significantly different from those of the control animals. However, both had increased from the base line results obtained in November. This increase in the lymphocyte activity from both groups of animals in the first three months of study may reflect seasonal variation in responses. During the March trapping a subcutaneous 'booster' dose of BCG ( $10^5$  cfu) was delivered to the primary vaccinated badgers. Following delivery of booster BCG, it was observed that the PPD-bovine/ PPD-avian T cell SI ratio increased among the vaccinates when compared to the control non-vaccinates. These results suggest that vaccination had enhanced and switched the responses of badgers from an *M. avium* type response to an *M. bovis* BCG type response. Such a response is indicative of the generation of a specific immune response directed at *M. bovis* antigens.

## Discussion

Because vaccination of cattle with a low dose of *M. bovis* BCG has been shown to confer significant protection against challenge with virulent *M. bovis* (Buddle *et al.*, 1995) we used a similar vaccination regime as the basis for generating a source of anti-mycobacterial specific T lymphocytes. A group of badgers were vaccinated subcutaneously with *M. bovis* BCG and their lymphocyte responses to bovine-PPD and avian-PPD was compared during a period of six months. We could readily measure proliferation in T lymphocytes cultured with ConA, the variation observed in the responses probably reflecting the heterogeneous nature of responses in relatively outbred animals. When lymphocytes were cultured in the presence of bovine-PPD and avian-

PPD, it was found that the ratio of responses to both sets of antigens changed throughout the course of study. The delivery of a booster injection appeared to enhance the responses to bovine-PPD. Although proliferation of lymphocytes to PPD does not strictly correlate with the generation of a protective immune response, it is indicative of an appropriate antigen specific response. Future studies will be carried out to investigate protocols designed to enhance this response and evaluate how differences in dosage and/or routes of administration influence the generation of specific responses.

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