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Vaccination of Badgers against Tuberculosis: the Optimal Dose of *Mycobacterium bovis* Required for Challenge

S. Lesellier¹, L.A.L. Corner¹, D.P. Sleeman², M.A., E. Costello³ and E. Gormley¹

**Introduction**

Wildlife species, such as badgers, act as maintenance hosts for *Mycobacterium bovis* and contribute to the spread and persistence of tuberculosis in the Republic of Ireland and elsewhere (O'Reilly and Daborn, 1995). While continued tuberculin testing will serve to contain tuberculosis in cattle at a low level, there is a growing consensus that the problem of *M. bovis* infection in badgers and, consequently, in cattle, will not be solved without a vaccine (Hughes et al., 1996). The badger vaccine research group have embarked on a program to evaluate the potential of a vaccine to protect badgers against tuberculosis (Southey et al., 2001). It is expected that vaccination will reduce the severity of disease in the infected badger and decrease the levels of excretion of *M. bovis*, thereby breaking the chain of transmission between badgers and cattle (Gormley and Collins, 2000). The vaccine most likely to be used is the live attenuated *M. bovis* strain BCG. There are expectations that this vaccine will be successful, based on the results of vaccination studies carried out in wild brushtail possums in New Zealand (Corner et al., 2002).

The establishment of an experimental infection protocol is an essential prerequisite for the rational development and testing of a vaccine against an infectious disease. An infection is required that induces pathology in a manner that mimics that seen in natural infections. It is only then that vaccine candidates can be fully evaluated for their potential to alter the nature of an experimental infection. Given that tuberculosis in badgers is primarily a respiratory disease, studies were carried out to investigate the optimal dose of *M. bovis* required to establish infection and progression of disease in badgers using the intratracheal route.

**Methods**

- Badgers were held in groups of 2–4 in pens each with an area of 200 m². Each pen contained two wooden setts. For handling the badgers, they were anaesthetised with ketamine hydrochloride, Vetalar® (10mg/kg) and medetomidine, Domitor® (0.1 mg/kg) administered by intra-muscular injection.
- Badgers were experimentally infected using the intratracheal route (Pfeiffer et al., 1994). With the anaesthetised badger held in sternal recumbency, a 2 mm external diameter plastic cannula was passed *per os* down the trachea until the end lay at the tracheal bifurcation or in a bronchus. When in place, 1 ml of the *M. bovis* suspension was instilled into the lungs and the cannula was flushed with 1.5 ml of sterile PBS.
- Three groups (each of three badgers) were inoculated with *M. bovis* and received either a low dose (≤10¹ cfu), medium dose (≤10² cfu) or high dose (≤10³ cfu). A fourth control group (4 badgers) received a placebo (PBS) inoculation.

¹ Department of Large Animal Clinical Studies, Faculty of Veterinary Medicine, UCD, 4, Dublin.
² Department of Zoology and Animal Ecology, National University of Ireland, Cork.
³ Central Veterinary Research Laboratories, Abbotstown, Dublin 15.
• At 3-week intervals starting from the *M. bovis* inoculation date, blood samples were collected from each animal. Samples were drawn from the jugular vein for isolating Peripheral Blood Mononuclear Cells (PBMC), as well as for haematological analysis, serology and haematochemistry. A tracheal aspirate was taken and cultured for the presence of *Mycobacterium* spp.

• The cellular immune responses of each badger were monitored by measuring the proliferative response of PBMC when cultured with PPD-bovine, PPD-avium.

• At 17 weeks after infection the badgers were euthanased and subjected to a detailed necropsy. At the necropsy each badger was examined for the presence of granulomatous lesions characteristic of tuberculosis. The following tissues were examined: lungs and thoracic lymph nodes (LNs), abdominal organs, mesenteric and hepatic LNs, head and body LNs. Sections of all lymph nodes and organs were collected, using a strict aseptic technique, for bacteriological examination for *M. bovis*. Also collected for culture were urine and faeces. Tissues fixed in formalin were collected for histopathological examination.

Results

Immunology

Following infection, a steady increase in lymphocyte proliferative responses to PPD-bovine was observed in the badgers inoculated with medium and high doses of *M. bovis* (Fig. 1). A lower (and delayed) response was observed in the low dose group. In contrast, no specific responses were observed in the control group during the course of the study. Responses to PPD-avium were consistently lower than those measured against PPD-bovine (data not shown). The specific T lymphocyte responses to *M. bovis* antigens provide an immunological indication of the establishment and progression of infection.

![Graph showing lymphocyte responses of badgers infected with different doses of M. bovis](image-url)

**Figure 1.** Lymphocyte responses of badgers (N=3/group) infected with different doses of *M. bovis*. 
**Gross pathology**

At necropsy macroscopic lesions consistent with tuberculosis were observed in all badgers in the high and medium dose groups and 2/3 in the low dose group (Table 1). No macroscopic lesions were seen in the controls. In the low dose group macroscopic lesions in the lungs consisted of a few 1 mm foci scattered throughout the lung. In the medium and high dose groups the lung lesions consisted of 10 – 40 mm diameter spherical sections of the right caudal lobe that contained myriad, sometimes confluent, 1mm foci.

Table 1. Distribution of macroscopic lesions in badgers experimentally infected with *Mycobacterium bovis* by intratracheal inoculation.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Low dose &lt;10 cfu</th>
<th>Medium dose ~100 cfu</th>
<th>High dose ~3000 cfu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left bronchial LN</td>
<td>1/3</td>
<td>1/3</td>
<td>2/3</td>
</tr>
<tr>
<td>Right bronchial LN</td>
<td>2/3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Anterior mediastinal LN</td>
<td>0/3</td>
<td>0/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Posterior mediastinal LN</td>
<td>1/3</td>
<td>2/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Lung</td>
<td>2/3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Liver</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Mesenteric LN</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Hepatic LN</td>
<td>0/3</td>
<td>2/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

*LN – lymph node.*

Table 2. Distribution of *Mycobacterium bovis* infection in badgers experimentally infected with *M. bovis* by intratracheal inoculation.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Low dose &lt;10 cfu</th>
<th>Medium dose ~100 cfu</th>
<th>High dose ~3000 cfu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left bronchial</td>
<td>0/3</td>
<td>1/3 (10³)</td>
<td>2/3 (10⁵)</td>
</tr>
<tr>
<td>Right bronchial</td>
<td>1/3 (&gt;10⁴)*</td>
<td>2/3 (10³ – &gt;10⁴)</td>
<td>3/3 (10¹ – &gt;10⁴)</td>
</tr>
<tr>
<td>Anterior</td>
<td>0/3</td>
<td>0/3</td>
<td>2/3 (10³ – &gt;10⁴)</td>
</tr>
<tr>
<td>Posterior</td>
<td>3/3 (10² – 10³)</td>
<td>2/3 (&gt;10⁴)</td>
<td>3/3 (10³ – &gt;10⁴)</td>
</tr>
<tr>
<td>Lung</td>
<td>2/3 (10² – &gt;10⁴)</td>
<td>3/3 (10³ – 10⁵)</td>
<td>3/3 (10³ – &gt;10⁴)</td>
</tr>
<tr>
<td>Liver</td>
<td>0/3</td>
<td>0/3</td>
<td>2/3 (10)</td>
</tr>
<tr>
<td>Spleen</td>
<td>0/3</td>
<td>0/3</td>
<td>1/3 (2)</td>
</tr>
<tr>
<td>Mesenteric LN</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Hepatic LN</td>
<td>1/3 (+)</td>
<td>1/3 (25)</td>
<td>0/3</td>
</tr>
<tr>
<td>Right</td>
<td>0/3</td>
<td>0/3</td>
<td>1/3 (+)</td>
</tr>
<tr>
<td>Right</td>
<td>0/3</td>
<td>0/3</td>
<td>2/3 (10¹)</td>
</tr>
<tr>
<td>Left Prescapular</td>
<td>0/3</td>
<td>0/3</td>
<td>1/3 (10⁵)</td>
</tr>
<tr>
<td>Right</td>
<td>0/3</td>
<td>1/3 (30)</td>
<td>0/3</td>
</tr>
<tr>
<td>Mesenteric LN</td>
<td>0/3</td>
<td>0/3</td>
<td>2/3 (10² – 10³)</td>
</tr>
</tbody>
</table>

*LN – lymph node.*

* number in parentheses is the range of counts of *M. bovis* in the tissues (colony forming units per gram); (+) indicates isolation by BACTEC® only.
Bacteriology

*M. bovis* was recovered from all badgers exposed to experimental infection but not from the control badgers (Table 2). There were no extra-thoracic sites of infection in the low dose group. One badger in the medium dose group had infection in a prescapular LN. There were extra-thoracic sites of infection in all of the high dose group: a mesenteric LN in one; mandibular and retropharyngeal LNs and liver in the second; and retropharyngeal, prescapular and mesenteric LNs, the liver and spleen in the third. Tracheal lavage was positive for only one badger, a member of the high dose group.

Conclusions

In these studies we successfully established infection of badgers with *M. bovis* by intratracheal inoculation including three animals which received fewer than 10 cfu. The immunological assay showed a progressive increase in responsiveness over the first 9 weeks after infection. At 17 weeks after infection gross pathological lesions were present in all except one of the inoculated badgers. However, bacteriological examination showed that infection had been established in all the inoculated animals and that in the high dose group, infection was much more widely disseminated than was indicated by gross pathology. The severity of the lesions in the inoculated badgers appeared to be related to the inoculum dose, being most severe in the high dose group. This dose provided the most reproducible pattern of infection and disease.

The benefit of this study is that it has provided the necessary basic information to test the protective efficacy of candidate vaccines. This will increase the likelihood of developing a vaccine to eliminate tuberculosis in targeted badger populations. This will not only serve to protect this ecologically important native mammal from tuberculosis infection in the future, it will also help break the chain of infection to associated cattle.

References


