Laboratory Examination of Tissues from Badgers and Cattle from the Four-Area Badger Study for Evidence of Tuberculosis

E. Costello¹, F. Quigley¹, D. O'Grady¹, O. Flynn¹, A. Gogarty¹, J. Mc Guirk¹, J. O’Rourke¹ and J. M. Griffin

Introduction
The background, objectives and design of the field study have been described by Griffin (1997) and Hammond (1997). The objectives of laboratory examination were:

(a) to determine the extent of *Mycobacterium bovis* infection in badgers in the four areas,

(b) to study the relationship between strains of *M. bovis* isolated from badgers and cattle in these areas, and

(c) to evaluate the effectiveness of histopathology and various culture methods for the diagnosis of *M. bovis* infection in badgers.

This paper describes the initial results of laboratory examination of badger and reactor cattle tissues undertaken as part of this study.

Materials and Methods
All badgers were examined *post-mortem* at one of the Department of Agriculture and Food’s Regional Veterinary Laboratories or at the Irish Equine Centre, Johnstown, Naas. Where gross lesions were detected a portion of the lesion was fixed in 10% formal saline for histopathological examination. A portion of the lesion was also removed for culture. Where lesions were not detected the retropharyngeal, bronchial, mediastinal and mesenteric lymph nodes along with 1-2 grams both of kidney and lung tissue were removed for culture. Culture and histopathological examination were carried out at the Central Veterinary Research Laboratory, Abbotstown.

Samples were cultured on four tubes of Stonebrinks medium after decontamination with 5% oxalic acid. For comparative purposes 30% of the samples were also cultured on the Bactec 460 system. Isolates were initially typed based on growth rate, pigmentation and cording characteristics pending definitive identification by spoligotyping.

A histopathological diagnosis of tuberculosis was based on the presence of a granulomatous lymphadenitis surrounding areas of caseous necrosis, indicating a positive result on histopathological examination. Where the lesion contained some but not all of the histopathological features associated with tuberculosis it was classified as a granuloma indicating an inconclusive result on histopathological examination.

Samples from badgers were classified as positive based on the isolation of *M. bovis* or on a positive result on histopathological examination.

Lymph node lesions from reactor cattle from the four areas were cultured on Stonebrink’s medium and typed as described above.

¹ Department of Agriculture and Food, Central Veterinary Research Laboratory, Abbotstown, Dublin 15.
M. bovis isolates were strain typed by RFLP analysis using three different probes (IS6110, PGRS and DR) (Skuce et al; 1996) and also by spoligotyping (Kamerbeek et al., 1997). A letter and a number were used to describe each strain identified by spoligotyping. Similarly a letter and a number was used to describe strains identified by IS6110 and PGRS probes. A letter was used to describe strains identified by DR probes. The nomenclature for each strain was then written in the order: spoligotype, IS6110, PGRS, DR.

Results

Culture and histopathological examination of badger tissues

Laboratory examination was completed on tissue samples from 754 badgers. Tissue samples from 219 (29%) badgers contained lesions.

Histopathological examination was carried out on lesions from 210 badgers. A total of 54 of the lesions were classified as tuberculous (positive). A further 61 lesions which had some features consistent with tuberculosis were classified as granulomas (inconclusive) (Table 1) M. bovis was isolated from 82 (71%) of the 115 lesions which were either positive or inconclusive on histopathological examination. M. bovis was isolated from nine of the lesions in which evidence of tuberculosis was not found on histopathological examination and from two of the nine lesions which were not examined histopathologically.

Lesions were not detected in 535 (71%) of the badgers. M. bovis was isolated from 59 (11%) of these NVL tissues (Table 2). In total M. bovis was isolated from 152 (20%) of the 754 badgers.

<table>
<thead>
<tr>
<th>Histopathology findings</th>
<th>N° (% of badgers)</th>
<th>M. bovis isolated N° (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculous granuloma</td>
<td>54 (24)</td>
<td>47 (87)</td>
</tr>
<tr>
<td>Granuloma (inconclusive)</td>
<td>61 (28)</td>
<td>35 (57)</td>
</tr>
<tr>
<td>No evidence of tuberculosis</td>
<td>95 (43)</td>
<td>9 (9)</td>
</tr>
<tr>
<td>Histology not done</td>
<td>9 (4)</td>
<td>2 (22)</td>
</tr>
<tr>
<td>Total</td>
<td>219</td>
<td>93 (42)</td>
</tr>
</tbody>
</table>

Table 2. Gross post-mortem findings and culture of tissues from 754 badgers.

<table>
<thead>
<tr>
<th>Post-mortem findings</th>
<th>N° (% of badgers)</th>
<th>M. bovis isolated N° (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesions present</td>
<td>219 (29)</td>
<td>93 (42)</td>
</tr>
<tr>
<td>No Visible Lesions</td>
<td>535 (71)</td>
<td>59 (11)</td>
</tr>
<tr>
<td>Total</td>
<td>754</td>
<td>152 (20)</td>
</tr>
</tbody>
</table>
For comparative purposes, tissue samples from 227 badgers were cultured in parallel on both Stonebrink’s medium and the Bactec 460 system. *M. bovis* was isolated from 62 samples using the Bactec 460 system and from 50 samples using Stonebrink's medium. The isolation rate from 52 lesions classified as either positive or inconclusive on histopathological examination was 69% with the Bactec 460 system and 53% with Stonebrink’s medium. The isolation rate from the remaining 175 tissue samples in which evidence of tuberculosis was not found on gross or histopathological examination was 15% with the Bactec 460 system and 13% with Stonebrink’s medium.

**Figure 1.** Strain types identified in cattle herds and badgers from the Cork Project Area.

**Figure 2.** Strain types identified in cattle herds and badgers from the Kilkenny Project Area.

**Figure 3.** Strain types identified in cattle herds and badgers from the Donegal Project Area.

**Figure 4.** Strain types identified in cattle herds and badgers from the Monaghan Project Area.

**Strain typing of *M. bovis* isolates from badgers and cattle**

A total of 45 *M. bovis* isolates from badgers and 46 *M. bovis* isolates from cattle were strain typed by RFLP analysis and spoligotyping. One or, in some cases, two predominant strain types were present in both cattle herds and badgers in each of the four project areas. Strain type D1, C1, H1, J was the predominant strain type in both Cork (Figure 1) and Kilkenny (Figure 2). Strain type A1, A1, A5, A was the predominant strain type in Donegal (Figure 3), while A2, B1, C1, C was the predominant strain type in Monaghan (Figure 4).
Discussion

*M. bovis* was isolated from 152 (20%) of the 754 badgers from which tissue samples were cultured. The isolation rate varied according to the histopathological and gross *post-mortem* findings. Lesions which showed all of the histopathological features associated with tuberculosis had the highest isolation rate (87%). Lesions which showed some but not all of the histopathological features of tuberculosis had an isolation rate of 57%, while 11% of tissue samples from badgers in which gross lesions were not detected yielded *M. bovis* on culture. The isolation rate from lesions which did not resemble tuberculosis on histopathological examination was 9%, which was similar to the isolation rate from NVL tissues.

In total, 159 (21%) of the badgers were positive based on culture and histopathological examination. *M. bovis* was isolated from 152 and the remaining seven were positive on histopathological examination only.

The Bactec 460 system utilises a liquid culture medium in which growth of bacilli is detected by the metabolism of a radioactive substrate. An initial evaluation of this system in 227 tissue samples showed that it had a higher sensitivity for the detection of mycobacteria than solid media both in tissues containing confirmed tuberculous lesions and in NVL tissues.

This study reinforces earlier observations that some strain types were associated with certain geographical areas, typically extending over a number of counties (Costello *et al.*, 1997). Strain type D1, C1, H1, J, which was the most common strain isolated from the Cork and Kilkenny project areas, has been identified in cattle and badger isolates from Clare, Limerick, Tipperary and Waterford. This strain has not been identified, to date, in isolates from the northern half of the country. Strain A2, B1, C1, C, which was the most common strain isolated from cattle and badgers in the Monaghan project area, is the strain most frequently identified in cattle and badger isolates from Cavan, Monaghan and Meath. Strain A1, A1, A5, A, from the Donegal project area, has previously been identified in cattle and wildlife isolates from Donegal and from North East Leinster. Both strains A2, B1, C1, C and A1, A1, A5, A have infrequently been identified in isolates from Munster.

The prevalent strain type (A1, A1, A1, A) which is widely distributed throughout the country (Costello *et al.*, 1997) was identified in only two of the four project areas to date, comprising five badger isolates from Monaghan and a single badger isolate from Cork.

Acknowledgements

The authors acknowledge the contribution of staff at Abbotstown, Cork, Kilkenny and Sligo Regional Veterinary Laboratories and the Irish Equine Centre, Naas who carried out *post-mortem* examination of badgers. The authors thank staff at the Histopathology Section, Central Veterinary Research Laboratory, Abbotstown for the preparation of slides.

References


