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<tr>
<td>Publication date</td>
<td>2000-10</td>
</tr>
<tr>
<td>Series</td>
<td>Selected Papers, 1999</td>
</tr>
<tr>
<td>Publisher</td>
<td>University College Dublin. Centre for Veterinary Epidemiology and Risk Analysis</td>
</tr>
<tr>
<td>Item record/more information</td>
<td><a href="http://hdl.handle.net/10197/8846">http://hdl.handle.net/10197/8846</a></td>
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Spatial Distribution of RFLP Types Identified in Mycobacterium bovis Isolates from Badgers and Cattle in a Study Area in the Republic of Ireland

E. Costello, O. Flynn, J. Griffin, F. Quigley, D. O’Grady, G. McGrath and R. Hammond

Introduction

A programme to eradicate bovine tuberculosis commenced in the Republic of Ireland in 1954. The prevalence of disease decreased rapidly in the first ten years of the programme. However, since then a low level of infection has persisted within the cattle population, despite annual tuberculin testing of herds and removal of reactor cattle. Mycobacterium bovis infection has been identified in some populations of feral deer and is endemic in badger populations. Badgers are widely distributed throughout the country: the prevalence of infection has been estimated to be 17% and infected badgers have been identified in every county (Dolan and Lynch, 1992). Badgers forage for food on pastures grazed by cattle; therefore, there is a possible risk of transmission of infection between the two species.

Differentiation of M. bovis strains by DNA fingerprinting is a potentially useful method of investigating possible transmission of infection between cattle and badgers. A survey of strain types occurring in cattle and badger isolates in the Republic of Ireland has recently been completed (Costello et al., 1999). Restriction fragment length polymorphism (RFLP) analysis was performed on a small number of cattle and badger isolates from each county, using IS6110, PGRS and DR probes. A total of 85 RFLP types were identified in 452 isolates examined; however, approximately 70% of the isolates were represented by nine RFLP types. All of these prevalent types were identified in both cattle and badger isolates and there was a similar geographic distribution of the RFLP types identified in both species. A further study is presently in progress which will complement this overview of the distribution of RFLP types with a more detailed examination of the spatial distribution of RFLP types in badgers and cattle herds in four geographic areas. Interim results from one of these study areas are presented in this paper.

Materials and methods

The study area selected was situated in county Cork, and had an area of approximately 300 km². This was one of four areas where badgers are being culled in order to study the effect of badger removal on the incidence of tuberculosis in cattle. The area was surveyed in detail and the location of all badger sets was recorded on a Geographic Information System (GIS) database. The current intensive badger removal programme has been in progress over the past three years. Tissues from all badgers removed were cultured. RFLP analysis was performed on M. bovis isolates using IS6110, PGRS and DR probes. RFLP types were named by assignment of an alphanumeric designation to each probe in the order,

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IS6110, PGRS, DR. There are approximately 700 cattle herds with approximately 60,000 cattle in the study area. The location and boundaries of all farms were entered on the GIS. Macroscopic lesions detected in cattle were cultured and RFLP analysis was performed on isolates.

Results and Discussion
A total of 202 main sets and 905 other sets were identified in the study area. Over the study period 623 badgers were removed. *M. bovis* was isolated from 177 (28%) of the badgers. One or more infected badgers was found at 75 (37%) of the main sets and at 45 (5%) of the other sets. To date RFLP analysis has been performed on isolates from 121 (68%) of the 177 infected badgers and on 86 isolates from 59 cattle herds.

Using a combination of the three probes 13 RFLP types were identified in the badger isolates and 12 RFLP types were identified in the cattle isolates. The most common RFLP type was C1 H1 J, which was identified in isolates from badgers in 43 sets and from cattle in 27 herds. The second most common RFLP type was A1 A3 A which was identified in isolates from badgers in 33 sets and from cattle in 14 herds. These two prevalent types were identified in isolates from 76 (79%) of the 96 badger sets, and in isolates from 41 (69%) of the 59 cattle herds for which results of RFLP analysis are available. A further five RFLP types were identified in isolates from both badgers and cattle. Six RFLP types were identified only in isolates from badgers. One of these was identified in badgers from four sets, one was identified in badgers from two sets and the other four were only identified in badgers from a single set. Five RFLP types were identified only in isolates from cattle herds, each of which was confined to a single herd.

The most prevalent RFLP type (C1 H1 J) was widely distributed throughout the study area, both in badgers (Figure 1) and cattle. RFLP type A1 A3 A was concentrated in the western half of the study area, again both in badgers (Figure 1) and cattle. The third most prevalent type A2 A1 B was found predominantly in the eastern part of the study area, where it was identified in badgers from seven sets and in cattle from three herds. Three other RFLP types were identified in isolates from cattle herds and badger sets which were in close proximity (i.e. less than 2 km apart). One RFLP type was identified in cattle from two herds and badgers from two sets that were not in close proximity. Frequently, more than one RFLP type was present in a locality; usually, between two and four RFLP types were identified in cattle and badger isolates in a 10 km² area. In almost all instances where the same RFLP type was present in badgers and cattle in a locality, the set from which the infected badger was culled was not on the infected farm but was located some distance away (Figures 2 and 3). This distance typically varied from 200 metres to 2 kilometres.

The results of this study show that there was some correlation between the spatial distribution of RFLP types in badgers and cattle, and suggests that transmission of infection occurs between these species. Further analysis of these data will include mapping the extent of badger social group territories and determining the proximity of the territories of infected social groups and infected farms, which have the same RFLP type. This may provide further evidence regarding the modes of spread of *M. bovis*. Additional RFLP analysis will be carried out on all isolates using the probe pUCD (O’Brien et al., 2000). As the study progresses, changes in the distribution of RFLP types over time will be assessed.
Acknowledgments
The authors thank Mr. Michael Sheridan, Mr. Ian O’Boyle and Ms. Margaret Good, Department of Agriculture Food and Rural Development for their contribution to the organisation of this study. We also thank Dr Paddy Sleeman, University College Cork, who supervised the survey of badger setts and Mr. Kieran Towey, Veterinary Epidemiology and Tuberculosis Investigation Unit for data analysis. We also thank Mr. Arthur O’Grady and staff at Cork District Veterinary Office and staff at Cork Regional Veterinary Laboratory. We also thank staff at the CVRL, Abbotstown for culture of samples.

References


**Figure 1.** Spatial distribution of prevalent RFLP types in *M. bovis* isolates from badgers in the Cork Study Area. Each coloured square shows the location of setts at which badgers, infected with an *M. bovis* strain of the RFLP type represented by the colour shown, were captured.

**RFLP types**
- C1 HI J
- A1 A3 A
- A2 A1 B
- A2 A2 B
- A1 A1 A
- A2 C1 D
- B2 A3 Y
- A1 E3 A
- C1 H7 J

5 kilometres

**Figure 2.** Spatial distribution of RFLP types in *M. bovis* isolates from cattle and badgers in southwest part of the Cork Study Area. The irregular coloured areas show the location of farms from which *M. bovis* isolates from cattle were the RFLP type represented by the colour shown. Similarly, the coloured squares show the location of setts at which badgers, infected with an *M. bovis* strain of the RFLP type represented by the colour shown, were captured.

**RFLP types**
- C1 HI J
- A1 A3 A
- A1 A1 A
- A1 H7 J
- B2 A3 Y

1 kilometre
Figure 3. Spatial distribution of RFLP types in *M. bovis* isolates from cattle and badgers in the northeast part of the Cork Study Area. The irregular coloured areas show the location of farms from which *M. bovis* isolates from cattle were the RFLP type represented by the colour shown. Similarly, the coloured squares show the location of setts at which badgers, infected with an *M. bovis* strain of the RFLP type represented by the colour shown, were captured.

**RFLP types**
- C1 H I J
- A1 A3 A
- A2 A1 B
- A2 A2 B
- A1 A1 A
- B1 C1 C

1 kilometre