**Mycobacterium bovis** surveillance in European badgers (*Meles meles*) killed by vehicles in Northern Ireland: an epidemiological evaluation.

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Keywords: Bovine tuberculosis, badgers, surveillance, roadkills

**Summary**

A survey of bovine tuberculosis (TB) in badgers killed by vehicles has been ongoing since 1999, with an annual mean of 78 badgers submitted for laboratory examination. The sample prevalence was 20.0% and temporal trends were similar to those of bovine herd prevalence. TB in badgers and surrounding cattle herds was spatially associated at distances up to five kilometres. The survey provides valuable epidemiological information and is a useful monitoring tool, but is constrained by several inherent weaknesses. Validation, through comparison with badgers captured at setts, is therefore required before the survey can be fully utilized.

**Introduction**

Bovine tuberculosis (TB) remains endemic in parts of the British Isles despite nationwide eradication programmes being in place for more than 50 years and significant progress in the early stages. Final eradication has proved elusive with lack of success attributed to a range of cattle-related factors and the presence of wildlife reservoirs, most notably the European badger (*Meles meles*).

Northern Ireland has an estimated population of 34 000 badgers with a mean sett density of 0.56 social groups per square kilometre (7). Culling is not permitted and monitoring of TB has therefore been conducted through informal surveys of badgers killed by vehicles. In this paper, results of the current survey are presented and its value and limitations discussed.

**Materials and methods**

Reports of badgers killed by vehicles and collection of carcases (“road traffic accident” badgers or “RTAs”) are managed by a Wildlife Officer within the Veterinary Epidemiology Unit of the Department of Agriculture and Rural Development (DARD). Reporting was initially limited to government employees to restrict reporting bias but later extended to farmers and members of the public. When collecting the RTA, the Officer confirms the map reference and identifies the two nearest bovine herds. Carcases are submitted to one of two laboratories of the AgriFood and Biosciences Institute (AFBI), a non-departmental government body, who undertake all laboratory procedures. Following a standardized *post mortem* examination, a range of tissue samples are routinely collected from lymph nodes (a “cranial” pool comprising parotid, submaxillary and retropharyngeal lymph nodes, prescapular glands pooled with popliteal, and the mesenteric lymph nodes), as well as faeces and urine. Samples from other lymph nodes or organs are taken when gross pathological changes are detected. Bacteriological culture is undertaken on all samples using standard methods and genotyping (spoligotyping, VNTR) performed where positive growth is observed, to confirm *M. bovis* to strain level.

Summary measures are reported for the period January 1999 to September 2010. Bovine TB test data were obtained from the central animal health database of DARD. Stepwise logistic regression was used to explore associations between TB-infected carcases and regional distribution. For this, locations were classified by county (one of six) and by veterinary administrative area (one of ten). Thereafter, spatial associations between TB in badgers and surrounding bovine herds was assessed at three levels. First, the risk of TB in the two herds nearest to infected badgers, for one year either side of the RTA collection date, was compared to those nearest TB-negative badgers. Second, the cumulative herd prevalence of TB in five concentric rings of one kilometre was compared in herds around infected with those around TB-negative badgers. Third, the ratios of the nearest neighbour distance from a TB positive and a TB negative herd were calculated for each badger, based on Woodroffe *et al* (6), and the ratios compared between infected and uninfected badgers using Wilcoxon rank sum tests. For the analyses, a positive bovine herd was defined as a herd having one or more skin test reactors or a culture-positive abattoir case. An infected badger was one confirmed as *M. bovis* by molecular means. Prior to 2006 however, confirmation was not possible for a minority (27%) of badgers, which were allocated a TB status based on the annual proportion of samples confirmed. These data were included in the cumulative and annual prevalence estimates but excluded from the spatial analyses. For the latter two spatial analyses, data were restricted to the period 2006 to 2009 to limit temporal variation. Mapping was undertaken in ArcMap version 9.3.1 (ESRI), logistic regression in SPSS 17.0 and nearest-neighbour distances in R version 2.12.0 using the spatstat package.

**Result**

935 badgers were tested between 1999 and Sept 2010, an annual mean of 78 (range: 20 in 2001 to 134 in 1999). The sample prevalence was 0.20 with annual prevalence varying from 0.07 to 0.49 (2009 and 2002 respectively, Figure 1). The trend over the period was similar to that observed in the bovine population.

The prevalence by county varied from 0.10 (Co. Londonderry; 95% exact binomial confidence interval =
0.05, 0.15) to 0.29 (Co. Fermanagh; 0.16, 0.42) and, between 2006 and 2009, from 0.06 (Co. Londonderry and Co. Antrim; 0.01 to 0.16) to 0.25 (Co. Fermanagh; 0.03 to 0.65). No significant association (p>0.05) was detected between the infection status of badgers and location, at either county or administrative area level.

Figure 1: Annual herd prevalence and RTA survey prevalence (solid line = adjusted for samples not confirmed by genotyping, with 95% confidence intervals; broken line = samples confirmed by genotyping).

The risk of TB in the two herds closest to each RTA was significantly higher in those located near infected badgers than uninfected animals (Odds Ratio = 1.67; 95% confidence limits = 1.27 and 2.21; $\chi^2$ test, p<0.001). Herds located in each of five zones surrounding infected carcases had a significantly increased risk of TB between 2006 and 2009 compared to those around TB-negative badgers (Table 1).

The ratio of distances between herds (TB-positive, TB-negative) and RTAs (TB-positive, TB-negative) was significantly lower for infected than for uninfected badgers (p = 0.019), suggesting infected badgers were closer to infected TB herds than uninfected badgers.

Figure 2: Distribution of samples by county.

The cranial pool of lymph nodes was the most commonly affected of the routinely sampled carcase sites (9% of sites, 60% of infected badgers, Figure 3). Of 141 badgers sampled at three or more carcase sites and confirmed as infected by spoligotyping, infection was disclosed at a single site in 77 (54.7%). Conversely, three or more sites were affected in 33 (23.4%).

Figure 3: Prevalence of infected sites in all badgers (left) and in infected badgers (right). Cranial = pool comprising parotid, submaxillary and retropharyngeal lymph nodes, Prepop = pool comprising prescapular and popliteal lymph nodes, Mesent = mesenteric lymph nodes.

Discussion

Surveys of RTA badgers are a relatively inexpensive and ecologically friendly means of monitoring TB. They have been used previously in Great Britain, where prevalence estimates ranged from 0.12 to 0.21, with a mean of 0.15 (2), (3) and in Ireland where prevalences of between 0.10 and 0.14 were reported (5). In Northern Ireland, a survey in the 1980s recorded a prevalence of 0.12 with TB-infected badgers more likely to be within 2km of an infected bovine herd than TB-negative badgers (n = 240; Odds Ratio = 5.52; 95% C.I. = 1.59 to 23.38; p <0.005; O. Denny, unpublished data). In an early analysis of data from the current survey (1999 to 2001; n = 254), the sample prevalence was 0.18 and no association was detected between TB in the badgers and that of the nearest two herds (Odds Ratio = 1.56; 95% C.I. = 0.76, 3.11; $\chi^2$; p = 0.193). However, the risk of TB in the previous four years in herds within three kilometres of a positive carcase was significantly higher than those around a TB-negative carcase (0.26 and 0.20 resp. $\chi^2$ test; p<0.001); (1).

The results of the survey are consistent with a close association between TB in badgers and cattle. Notwithstanding the small sample sizes in some years, the temporal trend in sample prevalence is similar to that of

<table>
<thead>
<tr>
<th>Zone</th>
<th>Prevalence</th>
<th>Z-test</th>
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<tr>
<td>&lt;1km</td>
<td>0.382</td>
<td>0.303</td>
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<tr>
<td>1to2km</td>
<td>0.331</td>
<td>0.264</td>
</tr>
<tr>
<td>2to3km</td>
<td>0.310</td>
<td>0.273</td>
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<td>3to4km</td>
<td>0.278</td>
<td>0.235</td>
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<tr>
<td>4to5km</td>
<td>0.274</td>
<td>0.224</td>
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Table 1. Cumulative herd prevalence, 2006 to 2009, around infected and uninfected badgers and Z-test for difference in proportions.
the bovine herd prevalence. Furthermore, a consistent, significant spatial association existed between infected badger carcases and TB in surrounding herds, from those closest to where badger carcases were collected, to the five kilometre limit of the analysis. The lack of association between RTA positivity and region may arise from a limited sample size and short-term variation but may also indicate that alteration of prevalence within one species may affect the other, but on a localized scale.

As these results do not indicate direction of transmission, they provide no information on the relative roles of badgers and cattle in the persistence of TB in Northern Ireland. Nevertheless, work elsewhere (for example, the Four Areas Trial in Ireland and the Randomised Badger Culling Trial in England) indicates a prominent role for the badger and risk mitigation measures have been implemented (e.g. badger culling and improving on-farm biosecurity) or are currently under trial (e.g. vaccination). RTA surveys prove a useful role in monitoring trends and providing epidemiological information. They may also assist in identifying potential areas for intervention or those with increasing infection to allow early remedial action or enhancement of measures already in place. However, surveys of this nature contain a number of inherent weaknesses. As a passive means of surveillance, they are highly dependent on the cooperation of the public. Even with quotas in the current survey, reporting varied greatly within the country and it is not clear if sufficient RTAs will be reported within a sufficiently short period to allow sufficient spatial precision. RTA badger surveys are also subject to bias; they are not a random sample of the population and may not therefore be typical of all badgers with respect to age, sex or severity of TB infection. Heavily infected badgers may be less likely to roam, resulting in an under-estimate of infection, which will also occur if younger badgers are more likely to range away from setts. The sample population is biased to areas surrounding roads and thus regions with a lower road density or with roads less used by cars will be under-represented. Conversely, high-speed roads provide few RTAs as carcases are often too damaged for retrieval.

A more serious bias results from the passive nature of the surveillance and consequent reporting bias whereby carcase sightings are more likely to be reported by farmers with infected herds than those from TB-free herds. This risk was initially addressed by limiting reports to government employees but was not maintained due to a need to increase the sample size and to farmers reporting RTAs through local government offices. In a field survey of setts in Northern Ireland, Menzies et al (4) found that farmers with herds recently infected with TB were more likely to know the location of setts on their farm than those with no infection. If such farmers are more likely to report RTAs, the bias will lead to an over-estimate of prevalence and the spatial associations reported above. The decay in risk with distance from TB-infected carcases (Table 1) may point to causality but a similar trend with TB-negative badgers is more consistent with reporting bias.

These weaknesses have been long recognized (3) and may be partly addressed through strict controls on reporting, setting of regional quotas and proactive searching for badger carcases. However, where the purpose is to provide information on the geographic distribution of infection in badgers as a prelude to intervention (e.g. vaccination), the survey method needs to be first validated before the results can be applied (8). This can be achieved through a series of comparisons with badgers captured at the sett, to ensure the RTA badgers are representative of the population.

Acknowledgement
The assistance of staff from DARD and AFBI in all aspects of the survey is gratefully acknowledged, in particular, Kathryn McBride and the staff of the disease surveillance branch of AFBI who carry out the pathological examinations of badgers.

References
(2) ISG (2004) Fourth Report, DEFRA
(5) O’Boyle I. Tuberculosis Investigation Unit, University College, Dublin. Selected Papers from 1997 to 2002
(8) ISG (2003), DEFRA