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New Approaches to the Diagnosis and Control of Tuberculosis in Badgers

E. Gormley[1] and E. Costello[2]

Introduction

The principal maintenance host for *Mycobacterium bovis* is the infected bovine animal (O'Reilly and Daborn, 1995). In Ireland, in areas where tuberculosis affects multiple or contiguous herds, the involvement of *M. bovis*-infected wildlife is often suspected as being the source of infection for the cattle. Epidemiological evidence has demonstrated a high prevalence of tuberculosis in badgers in Ireland. As the badger population shares the general environment with the national cattle herd, particularly at grazing, the two species cannot be effectively segregated from each other. The behavioural patterns of cattle and *M. bovis*–infected badgers may influence the probability of transmission of disease. Contamination of farm buildings, pastures and feed by badger excretions (sputum, faeces or urine) containing large numbers of bacilli, or by contaminated bedding material, is a possible route of transmission to cattle (Clifton-Hadley *et al.*, 1993; Hutchings *et al.*, 1999). There is also the possibility of direct transmission of infection between badgers and cattle, as there is anecdotal evidence of cattle investigating dying badgers on pasture and in buildings. Because the process of transmission from badgers to cattle is not fully understood, it is difficult to establish a relationship between the prevalence or density of infected badgers and the rate of transmission of infection to cattle. Vaccination of wildlife species against tuberculosis, if successfully employed, could directly facilitate eradication of bovine tuberculosis in affected areas (Gormley and Collins, 2000). Any strategy to vaccinate badgers would first require detailed knowledge of the prevalence of disease in the badger population as well as the distribution of the infected animals among the general badger population. A vaccine would serve the purpose to reduce the severity of lesions in infected badgers and consequently lower the prevalence of disease in the population. Both outcomes would have the effect of interrupting the chain of infection between infected and susceptible animals. The risk to cattle then posed by infected badgers would be expected to be less than that at present.

Diagnosis of tuberculosis in badgers

The badger appears to be highly susceptible to infection with *M. bovis*. Clinical and *post-mortem* findings suggest that infection commonly spreads between and within social groups *via* the respiratory tract or by bite wounds (Gallagher *et al.*, 1976; Pritchard *et al.*, 1986). Currently, the diagnosis of tuberculosis in badgers is made *post-mortem* by examination for gross lesions followed by histopathology. However, these tests can be insensitive and may not detect animals at the earliest stage of disease. The most sensitive and specific test is the culturing of *M. bovis* from infected tissues. However, the main drawback is that this can take up to three months to obtain a confirmed result. Advances in our understanding of immunological responses to tuberculosis infection have improved prospects for the development of sensitive and specific diagnostic tests for tuberculosis in many species. Immunological tests have been developed that can be carried out *in vitro* on blood samples taken from live badgers. An ELISA system (the Brock Test) measures antibody recognition of MPB83, a glycosylated lipoprotein, that is a major target of the antibody response in *M. bovis* -infected badgers (Goodger *et al.*, 1994). The sensitivity of this test is variable (37% - 62.5%) with the differences attributable to the level of lesion detectability and the sex of the animal (Clifton-Hadley *et al.*, 1995).

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More recently, diagnostic tests based on the cell-mediated immune response have been developed (Dalley *et al.*, 1999; Southey *et al.*, 2002a). A lymphocyte transformation assay (LTA) that uses bovine and avian tuberculin has been shown to detect responses in infected and *M. bovis* BCG vaccinated badgers (Dalley, *et al.*, 1999; Southey *et al.*, 2001a).

In a recent survey carried out to compare conventional diagnostic techniques with immunological analyses, thirty six badgers were removed from two areas each of which had a history of recurrent tuberculosis outbreaks in the associated cattle population. Macroscopic lesions (VL), yielding *M. bovis* on culture, were detected in nine (25%) of these badgers at *post-mortem* examination (Table 1). No macroscopic lesions (NVL) were detected in the twenty seven remaining badgers and tissue samples failed to yield *M. bovis* on culture.

Eight of the nine VL badgers were seropositive by the BrockTest (Table 1). Seven of the twenty seven NVL animals (25.9%) with no visible lesions (NVL) were also positive to the BrockTest. The immune responses of all thirty six badgers were also monitored by measuring proliferation of T lymphocytes cultured in the presence of bovine tuberculin (PPD-bovine) and avian tuberculin (PPD-avian). Only three of the nine VL animals (33.3%) were considered positive by LTA (PPD-bovine). However, in the NVL group, four badgers (14.8%) were positive by this test and of these three were also positive by the BrockTest. The data suggest that reliance upon one particular diagnostic test may result in an under-estimation of the prevalence of *M. bovis* infection in a badger population while a more accurate profile of *M. bovis* exposure may be constructed using a combination of tests (Figure 1).

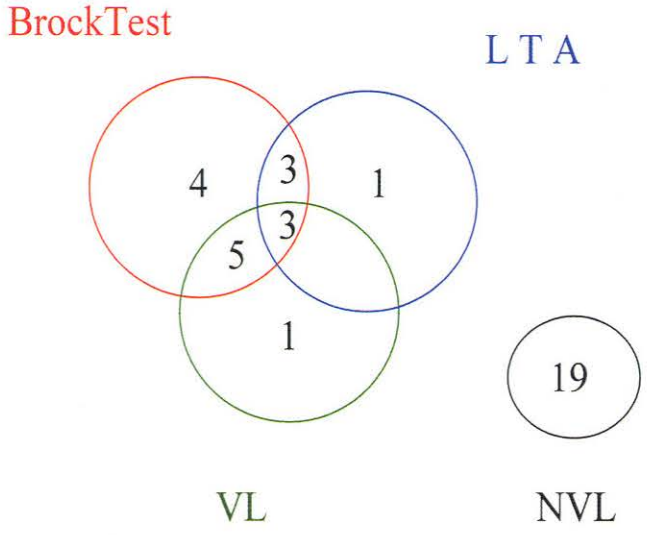

Figure 1. Comparative diagnosis of *M. bovis* infection in thirty six badgers.
VL = Visible Lesions; NVL = No Visible Lesions; LTA = Lymphocyte Transformation Assay

Development of a vaccine against tuberculosis in badgers

The development of a vaccine for badgers is a long term control measure which, it is proposed, will reduce the severity of lesions in infected badgers, thereby interrupting the chain of infection between infected and susceptible animals. A live vaccine such as *M. bovis* BCG is likely to be the vaccine of choice if it can be delivered and can persist in the host so as to continuously prime the protective cellular immune response. In addition, specific diagnostic tests that can distinguish vaccinated from *M. bovis*-infected hosts need to be developed in parallel (Pollock *et al.*, 2000). The ecological impact of

introducing vaccines into the environment must also be considered. Other factors which may influence the effectiveness of a vaccine programme include the underlying prevalence of disease in the target population, the formulation of BCG vaccine used, the delivery route, exposure to environmental non-tuberculous mycobacteria and virulence of challenge strains.

In recent field trials conducted in Ireland, controlled studies were carried out with baits containing biomarkers to measure uptake rates by free–living badgers in a number of areas (Southey *et al.*, 2001b; Southey *et al.*, 2002b). Up to 90% of the badgers studied had ingested baits, with some variation in the percentage of marked badgers attributed to seasonal timing of bait deployment. In parallel with the bait uptake project, a study of the immunological responses of badgers to BCG vaccination was conducted (Southey *et al.*, 2001a). The animals were immunised subcutaneously with *M. bovis* BCG and blood lymphocyte recognition of mycobacterial antigens was compared with that of a population of non-immunised control animals. The specific responses to PPD-bovine in the vaccinated group of animals were significantly greater than in the non-vaccinated group. Given these data it appears there are grounds for optimism that vaccination of badgers is an option worth pursuing.

Conclusions

Although the badger is an ecologically important and protected species, there is considerable circumstantial yet compelling evidence to implicate the tuberculous badger in the introduction of *M. bovis* infection into naïve cattle populations. Any attempt to eradicate the disease from cattle by conventional means without taking due account of the badger reservoir of infection is, therefore, likely to fail. However, in trying to devise novel control programmes, there remains a paucity of information on the dynamics of the disease in badgers and their interactions with cattle that may influence transmission. It remains to be determined, for example, if there is a clear relationship between the prevalence of tuberculosis in badgers and prevalence in cattle herds. Part of the problem is that the current tests may underestimate the true prevalence of *M. bovis* infection in a population of either species, because some infected animals may have undetected lesions, while others may have an immune response that limits the growth of the intracellular pathogen. Our recent studies indicate that a combination of immunological tests may give more reliable results and can detect *M. bovis* infection in badgers that are found to be negative by gross *post-mortem* examination and histopathology.

Vaccination, in spite of the formidable challenges, offers a potential means of eliminating badger-related foci of *M. bovis*. A combination of procedures in which optimal use is made of GIS technology to target areas for the allocation of resources and strategic vaccination of badgers, together with improvements in the diagnostic tests used in cattle, should make a significant contribution to the control and eradication of tuberculosis in the national cattle herd in Ireland.

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Table 1. Lesion status, Lymphocyte Transformation Assay (LTA) and BrockTest analyses of badgers.

Badgers	Lesion	LTA	BrockTest
1	VL	+	+
2	VL	-	+
3	VL	-	-
4	VL	-	+
5	VL	+	+
6	VL	-	+
7	VL	-	+
8	VL	+	+
9	VL	-	+
10	NVL	-	+
11	NVL	-	+
12	NVL	-	+
13	NVL	-	+
14	NVL	+	+
15	NVL	+	+
16	NVL	+	+
17	NVL	+	-
18-36	NVL	-	-

VL = Visible Lesions; NVL = No Visible Lesions; LTA = Lymphocyte Transformation Assay result

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