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TUBERCULOSIS IN EUROPEAN BADGERS (*MELES MELES*) AND THE CONTROL OF INFECTION WITH BACILLE CALMETTE-GUÉRIN VACCINATION

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ABSTRACT: The eradication of tuberculosis (*Mycobacterium bovis* infection) from cattle herds may be compromised if infected wildlife species, such as European badgers (*Meles meles*), share the same environment and contribute to transfer of infection. Options for dealing with tuberculosis in this wild reservoir host are limited by conservation and social concerns, despite a clear implication that infected badgers are involved with the initiation of tuberculosis in cattle herds. Vaccination of badgers against *M. bovis*, if successfully employed, would directly facilitate the completion of bovine tuberculosis eradication in affected areas. Vaccine trials in captive badgers have established that the *M. bovis* bacille Calmette-Guérin (BCG) vaccine can induce a protective response that limits the distribution and severity of tuberculosis disease following experimental challenge. The protective effect of the vaccine has been demonstrated when the vaccine was delivered by subcutaneous injection, deposited on mucous membranes, and given orally in a lipid formulation. A large-scale field trial of oral BCG vaccine has been designed to measure the protection generated in wild badgers subjected to natural transmission of infection and to estimate vaccine efficacy. These parameters will be estimated by comparing the prevalence of *M. bovis* infection in vaccinated and nonvaccinated badgers. The results will provide a framework for the development and implementation of a national strategy to eliminate the disease in badger populations and if successful will remove this major impediment to bovine tuberculosis eradication.

Key words: Badger, *Meles meles*, tuberculosis, *Mycobacterium bovis*, BCG vaccine.

INTRODUCTION

Mycobacterium bovis is the causative agent of bovine tuberculosis in livestock and in a wide range of wild animals. Where wildlife species are infected with *M. bovis* they may act as a source of infection for domestic and wild animals (Corner, 2006). Tuberculosis in domestic livestock causes economic losses both directly from lost production and from the costs associated with eradication programs, and indirectly through the risk of zoonotic infection. *Mycobacterium bovis* infection is endemic in a number of wild animal species: brushtail possum (*Trichosurus vulpecula*) in New Zealand, white-tailed deer (*Odocoileus virginianus*) in North America, and European badger (*Meles meles*) in Ireland (Gormley and Collins, 2000) and Great Britain (Clifton-Hadley et al., 1993). Tuberculosis in the Irish cattle population has remained at a stable level over many years despite the application of testing programs and disease eradication procedures that have proven successful in the control of bovine tuberculosis in other countries.

That *M. bovis* infection in the badger population constitutes a significant reservoir of infection for cattle has been demonstrated in Ireland via the East Offaly (O'Mairtin et al., 1998a, b) and Four Area (Griffin et al., 2005) projects, and also in the UK (Woodroffe et al., 2005). Based on these studies, the Irish government has instigated a strategy of focused (reactive) culling of infected badgers to lower the density of infection in the badger population. This serves to limit the opportunity for transmission of infection to cattle and to decrease the economic impact of the disease in cattle. The decision to cull is made following an epidemiologic investigation of the possible causes of a breakdown in a cattle herd. If

badgers are identified as the probable source of infection, then the badgers in the immediate area (within 1 km of the farm) are culled. This is an interim strategy while research on alternative control strategies, including vaccination of badgers, is undertaken. Vaccination is an attractive control option for badgers because, if shown to be effective, it would reduce the burden of infection in this species and break the transmission cycle to cattle without provoking conservation and social concerns.

Many studies have shown that tuberculosis in badgers, as in most animal species, is primarily a respiratory disease involving the lung and associated thoracic lymph nodes (Gallagher et al., 1976; Gallagher and Clifton-Hadley, 2000; Gavier-Widen et al., 2001). Transmission between badgers is principally by inhalation of infectious aerosols (Nolan and Wilesmith, 1994). Following inhalation of *M. bovis*, infection becomes established in the lung and, by hematogenous dissemination, spreads to infect distal lymph nodes and visceral organs (Gallagher et al., 1998; Gallagher and Clifton-Hadley, 2000). In infected populations, less than 5% of infected badgers have generalized disease (Dolan, 1993; Gallagher, 1998; O'Boyle, 2003). In the majority of cases examined, infected badgers have no visible lesions (Clifton-Hadley et al., 1993; Corner, unpubl. data). This suggests that infection in the badger progresses slowly, or that it is contained and persists in a latent state. Tuberculosis does not appear to have a significant effect on the size or structure of badger populations (Wilkinson et al., 2000), and infected badgers may survive for several years (Little et al., 1982; Clifton-Hadley et al., 1993).

VACCINATION AGAINST TUBERCULOSIS

The bacille Calmette-Guérin (BCG) vaccine (a live attenuated strain of *M. bovis*) is widely used in humans and studies have also shown its potential to limit the spread of disease in wild animals. Two studies in wild brushtail possums in New Zealand showed that the BCG vaccine was protective. In the first study, vaccine efficacy was estimated to be 69% (Corner et al., 2002) and in the second, the estimated efficacy was 95–96% (Tompkins et al., 2009). These studies demonstrated that the BCG vaccine was more effective in wild possums than the results in captive studies had suggested. In the captive studies, as a consequence of the infection procedure used, all challenged possums developed lesions whereas in the wild possum studies vaccination prevented possums from becoming infected through natural transmission (Corner et al., 2002).

A cornerstone of developing a vaccine for tuberculosis in badgers is understanding the pathogenesis of the disease and how the infection progresses after an animal is infected. Experimental infections have been used to study pathogenesis in a variety of species and to test the protective efficacy of vaccines (Aldwell et al., 1995; Griffin et al., 1999; Hewinson et al., 2003; Buddle et al., 2005). In vaccine and challenge studies the objective of the experimental infections is to generate tuberculosis in the lungs in a uniform manner while maintaining a profile of lesion development and distribution that is consistent with natural *M. bovis* infection. We have shown that experimental endobronchial inoculation of badgers mimics natural disease (Corner et al., 2007, 2008a). Doses from <10 colony-forming units (cfu) to $>10^3$ cfu lead to the establishment of infection and clearly demonstrated that badgers are very susceptible to *M. bovis* inoculated by this route, with infection consistently established with even with the lowest dose. The highest dose produced a uniform level of pathology when the response to inoculation was assessed over 24 wk.

In 1994, a joint consultative group of the World Health Organization, the Food and Agriculture Organization of the United Nations, and the Office International

des Épizooties recommended that BCG Pasteur strain 1173P2 be used in animal studies (World Health Organization, 1994). This strain has been used successfully in studies in domestic cattle (Buddle et al., 1995), red deer (*Cervus elaphus*) (Griffin et al., 1999), ferrets (*Mustela furo*) (Qureshi et al., 1999), and brushtail possums (Aldwell et al., 1995). The BCG is an appropriate choice for vaccine studies in badgers because it has a long history of safe use in humans and animals (Murphy et al., 2008).

If effective in badgers, vaccination could form an alternative strategy for controlling *M. bovis* infection in badger populations (Gormley and Collins, 2000). The purpose of vaccination would be to decrease the burden of infection in the population and thereby decrease the risk to cattle. It has been shown that vaccination of badgers with BCG is safe when administered by the intramuscular and subcutaneous route (Lesellier et al., 2006), and can induce a protective response when administered intradermally (Stuart et al., 1988).

BACILLE CALMETTE-GUÉRIN VACCINATION IN CAPTIVE BADGERS

The badger vaccine research project in Ireland commenced in 2001. Studies were undertaken to determine if the BCG vaccine could induce a protective immune response in badgers against an endobronchial challenge with a virulent strain of *M. bovis*. The responses of vaccinated and control badgers to challenge were assessed by severity of disease and infection. In these studies, using BCG Pasteur, badgers were vaccinated by subcutaneous injection, by spraying the vaccine into the nasal cavity and instilling a drop of vaccine suspension into the conjunctival sac (mucosal vaccination) (Corner et al., 2008b), or by giving the BCG orally in a lipid formulation (Aldwell et al., 2003) to prevent degradation in the stomach. At 3 mo postchallenge, the badgers were examined postmortem to assess the pathologic and bacteriologic responses to challenge. Gross and histologic lesions of tuberculosis were observed and *M. bovis* was recovered from all challenged badgers. In both studies, infection in the vaccinated badgers was less severe than in the control group, demonstrating that BCG vaccine induced a significant protective effect. A follow-up study was conducted to compare BCG Pasteur and BCG Danish strains when delivered as lipid-formulated oral vaccines, because BCG Danish is the only vaccine strain currently registered for human use in the European Union. Following virulent challenge, both vaccines generated protective immunity that was significantly different from that in the nonvaccinated controls. However there was no difference in the levels of protection achieved by either strain.

CONSIDERATIONS IN BCG VACCINE FIELD TRIAL DESIGN

Whereas captive badger studies are the most cost effective way of examining the protective response to vaccination, such studies cannot be used to predict whether BCG will be protective in free-ranging badgers or to estimate vaccine efficacy. Estimates of vaccine efficacy are extremely valuable in modelling potential vaccine strategies, but data from field trials are needed to reliably estimate protection and vaccine efficacy parameters. Any field trial will by necessity use an oral vaccine delivery system, because this is the likely method of choice for any broad-scale mass vaccination of free-ranging badger populations.

A BCG vaccine field trial likely will have two principal objectives. These are to validate the results of captive badger studies and show that BCG vaccine is protective in naturally exposed wild badgers, and to estimate vaccine efficacy under field conditions. These objectives will be met by comparing the prevalence

of *M. bovis* infection in vaccinated badgers with that in nonvaccinated controls. A secondary outcome of field trials will be to measure the effect of BCG vaccine in badgers with pre-existing *M. bovis* infection. There is some evidence from laboratory animals that vaccination may exacerbate pre-existing infection (Moreira et al., 2002). However, the effects reported were observed in inbred mice and were marginal with a single dose of vaccine (but more pronounced after repeated vaccination). In addition to providing a measurement of protection and an estimate of vaccine efficacy, field trials will provide a practical basis for understanding the logistics of oral vaccine delivery to wild badger populations.

Three different trial designs have been considered. The first design was to compare disease prevalence in an area where 100% of the population was vaccinated with a matched control area. However, this design was regarded as difficult to implement because it would require replication with the inherent problems of matching study sites. The second candidate design was to compare disease prevalence in vaccinated and control badgers within one area. In this design, 50% of the badgers in the trial area would be vaccinated and the nonvaccinated animals would constitute the control group. Although this design adopts a more pragmatic approach and takes into account geographic clustering, it would only measure the vaccine efficacy in badgers as individuals and not at a population level. In the third candidate design, different proportions of a badger population would be vaccinated, for example, 0, 50, and 100%. The advantage of this latter design is that effects on vaccine efficacy arising from changes in the force of infection as a result of vaccination could be estimated. Consequently, this third design has been chosen for planned field studies. The required proportion of vaccinates will be achieved by systematically trapping the area. When first encountered, indivdual badgers will be allocated to either the vaccination or control group as required for the particular area. To allow for continued exposure to infection, the trial will be conducted over a 3-yr period. It is estimated that an initial population of 300 badgers (100 in each of the treatment areas) will be required to accurately estimate vaccine efficacy, based on an assumed initial tuberculosis prevalence of 20–30% and vaccine efficacy of 50–70%. for an individual badger.

The BCG Danish strain, encapsulated in a lipid formulation for oral administration and containing about 10^8 cfu/ml, will be used in the planned field trial. During the trial, badgers will be individually vaccinated by administration of the lipid vaccine or lipid placebo directly into the pharynx. Vaccine and placebo control samples will be "double-blind" coded. Badgers will be revaccinated annually and the population will be examined three times per year by trapping the entire study site in a continuous process. Throughout the trial, estimates of changing tuberculosis incidence will be made from the measurements of humoral and cellular immune responses.

At the end of the 3-yr study period, each site will be depopulated and all badgers will be examined for tuberculosis by detailed postmortem examination that will include an examination for visible lesions, histologic lesions, and mycobacteriology to demonstrate infection with *M. bovis*. The isolation of *M. bovis* from postmortem or clinical samples (wound exudates or tracheal swabs) will be used to define a case of tuberculosis.

INCORPORATING BCG VACCINE INTO A NATIONAL CONTROL PROGRAM

Prior to implementing a wildlife vaccination strategy at a national level there would be many hurdles to overcome. Among these are issues related to licensing of the vaccine for use in badgers and development of efficient bait delivery

systems to achieve high coverage in targeted populations. The environmental impact and effects of the vaccine on nontarget species also will need to be considered. In addition, the vaccination program will need to be carried out against the background of exhaustive investigation of tuberculosis in cattle, animal husbandry methods, and herd management–related factors that may affect cattle-to-cattle and badger-to-cattle transmission. Following vaccination, the risk to cattle posed by infected badgers would be expected to decrease as tuberculosis prevalence in badgers declines. By removing the influence of the reservoir host, an effective badger vaccination program could improve efficiency of the tuberculin testing program for controlling cattle-to-cattle spread and address a major impediment to the eradication of tuberculosis in Ireland and elsewhere.

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