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Sustained protection against tuberculosis conferred to a wildlife host by single dose oral vaccination

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ABSTRACT

Background: Vaccination of wildlife against bovine tuberculosis (TB) is being considered by several countries to reduce the transmission of *Mycobacterium bovis* infection to livestock. In New Zealand, where introduced brushtail possums (*Trichosurus vulpecula*) are the major wildlife hosts, we have previously shown that repeat applications of a lipid-encapsulated oral bacille Calmette-Guerin (BCG) vaccine reduce the incidence of naturally acquired TB in wild possums. Here we extend this conceptual demonstration to an operational level, assessing long-term protection against TB conferred to free-living possums by a single oral immunisation.

Methods: Possums in a non-TB area were randomly allocated to receive lipid-formulated BCG vaccine or remained unvaccinated. After initial trials to assess vaccine immunogenicity and establishment of protection within the first year post-vaccination, 13 individuals of each treatment group were relocated to a biosecurity facility and challenged (at 28 months post-vaccination) by subcutaneous injection of virulent *M. bovis*.

Results: Vaccine immunogenicity and short-term protection were confirmed at 2 months and 12 months post-vaccination, respectively. In the long-term assessment, vaccinated possums had significantly reduced bacterial counts in peripheral lymph nodes compared to controls, with 0.6–2.3 $\log_{10}$-fold reductions in *M. bovis* burdens.

Discussion: The magnitude of protective response by possums to experimental challenge at 28 months post-vaccination is known to equate to a high degree of protection against natural infection in this species. With techniques for oral bait delivery well advanced, the longevity of protection demonstrated here shows that an operable wildlife vaccine against TB is feasible.

1. Introduction

Ruminant livestock are highly susceptible to infection with virulent strains of *Mycobacterium bovis*, the causative agent of bovine tuberculosis (TB). Until the mid-1900s, bovine TB in the United States persisted almost entirely due to in-herd transmission; from the 1940s onwards the disease was eradicated from many states by animal testing and infected livestock culling [1]. However, the livestock-TB problem has often proved less tractable to management due to occurrence of the disease in wildlife reservoirs [1–3]. In such cases, spill-over infection from wildlife to livestock [4] confounds efforts to eradicate TB by livestock control alone. Infected white-tailed deer (*Odocoileus virginianus*) in Michigan, for example, are a persistent contributor to livestock TB infection in that state [5].

In New Zealand, the introduced brushtail possum (*Trichosurus vulpecula*) is the major wildlife reservoir of *M. bovis* infection [6]. A sustained programme of possum depopulation by lethal control, coupled with traditional livestock disease control methods, has reduced New Zealand's cattle and deer herd infection rate by 94% in less than two decades [7]. Although successful, however, lethal control of wildlife is often socially contentious even in New Zealand (where the possums are seen as pests), and certainly would be in both the United States (where white-tailed deer are a recreational hunting species) and Western Europe (where a major reservoir – the Eurasian badger *Meles meles* – is a native species protected by conservation legislation). This has prompted research over the last 15 years, in Europe, North America and New Zealand, into non-lethal means of controlling TB in wildlife,

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including prophylactic vaccination with bacille Calmette-Guerin (BCG), an attenuated form of the *M. bovis* bacterium [8–11].

Oral baiting is generally considered the only practical and affordable means of vaccine delivery for large-scale disease management in wildlife populations [11–14]. Successful examples are the oral rabies vaccine (which has largely eradicated enzootic rabies among mesocarnivores in several Western European countries, and halted its westward expansion in the eastern United States [15]) and the classical swine fever oral vaccine (which has eradicated that disease among most free-ranging wild boar [*Sus scofa*] populations in Germany and France [16]). Both these vaccines provide effective protective immunity after a single dose and the immunity established is sufficiently long-lasting that regular vaccine boosting is unnecessary. However, in the comparative case of a BCG vaccine against TB, only relatively short-term protection has been documented to date, as follows.

Buddle et al. [17] demonstrated that protection of captive brushtail possums against experimental challenge with virulent *M. bovis* lasted for at least a year after a single oral BCG vaccination. Such protection involves a slower rate of disease progression and logarithmic-scale reductions in bacterial counts in affected organs, rather than absolute protection against the establishment of infection. Similar protective responses have been recorded in a range of species after artificial challenge following oral BCG vaccination, e.g. [18–21]. Tompkins et al. [9] also showed that such vaccination provides free-living possums with a high degree of protection against natural exposure, with a 95–96% reduction in infection rate in animals vaccinated, on average, 1 year earlier (with repeat 6-monthly vaccination for those animals re-caught). This slowing of disease progression by vaccination against experimental TB challenge, equating to a high degree of protection against infection by natural exposure, has also been demonstrated in cattle [18,22,23].

Recent modelling of the management of TB in possums in New Zealand, accounting for their approximately 10 year maximum lifespan and other life-history details, predicts that BCG vaccination every three years will provide a cost-effective adjunct to lethal control [24]. Hence, demonstrating that vaccine efficacy is maintained into a third year in individual animals is a priority in developing oral BCG vaccination as an operational tool. We address this here by assessing the extent of protection against artificial challenge with *M. bovis* in free-living wild possums 28 months after a single oral immunisation with lipid-formulated BCG vaccine. This work was conducted in tandem with methods development for TB challenge and assessment protocols, necessitating some complexity in trial design.

2. Materials and methods

2.1. Possum vaccination

The experimental animals comprised a free-living possum population inhabiting a 200 ha trapping site in the Kaimai mountain range (upper North Island, New Zealand [lat. −37.695, long. 175.886]). The site comprises mixed native broadleaf/exotic conifer forest with a grid of 400 wire mesh cage traps at 50 m × 100 m spacing, in a region from which TB has not been previously recorded in wildlife.

Traps were baited with apple coated with flour and aniseed, and set for four consecutive nights in May 2009. All captured adult possums were sedated with ketamine hydrochloride at 25 mg/kg body weight, marked with an individually numbered metal ear tag in each ear, and assessed as free of clinically identifiable TB by palpation of the major peripheral lymph node sites [20,25]. Animals were designated vaccinate or control by coin-toss, with each vaccinate receiving 10^7 colony-forming units (cfu) of live *M. bovis* BCG (strain Danish 1331) in 1 ml of edible lipid matrix [26], delivered via syringe to the rear of the oral cavity (which, under ketamine sedation, causes possums to ingest the vaccine by reflex swallowing [9]). Possums were monitored for recovery from sedation and then released at the site of capture.

2.2. Post-vaccination immune responsiveness

BCG-based vaccines are expected to invoke strong effector cell-mediated immune responses within 6–8 weeks post-vaccination [27]. One quarter of the trapping grid was thus set for a single night in July 2009, and sterile blood samples taken from captured possums to assess this response. Approximately 2 ml of heparinised blood was drawn under sedation from either the tail or jugular vein of each trial animal caught; possums were again monitored for recovery from sedation and then re-released at the site of capture. A standard lymphocyte proliferation assay was conducted on each sample the following day [17,28]. Briefly, blood was depleted of erythrocytes, and mononuclear cells were cultured for 4 days with or without bovine PPD. Proliferation of lymphocytes was measured by the uptake of tritiated thymidine added 18 h prior to the end of the incubation.

2.3. Short-term protection

A new experimental challenge model for TB in possums that emulates natural disease, involving inoculation of virulent *M. bovis* into subcutaneous connective tissue via the paws, was developed in parallel with this study [29,30]. This approach leads to an incidence and pattern of lesion development more closely resembling that in naturally infected wild possums [25,31] than that of existing respiratory tract inoculation models [17,20]. Since the new model had not been employed previously in vaccination trials, we first sought to confirm that protection against such inoculation was evident in a subset of the free-living possums at 1-year post-vaccination, the longest length of protection demonstrated to date in other possum vaccination/artificial infection studies [17].

Half of the trapping grid was set for a single night in July 2010, and 24 vaccinates and 24 control animals sedated and transported to a second study site in a TB-endemic part of the lower North Island (Orongorongo Valley [9]). Once there, each animal was sedated for a second time and received two subcutaneous challenges of 100 cfu of virulent *M. bovis* (strain 83/6235 [20]) injected through the skin webbing between the fourth and fifth digits of the left front and right rear paws using a 26 gauge needle [29]. All possums were fitted with radio-tracking collars (Sirtrack Ltd., Lincoln, New Zealand), monitored for recovery and released. Eight weeks post-challenge, the day-time den site of each radio-collared possum was located, and an array of traps placed around them. Thirty-nine possums (20 vaccinates and 19 control animals) were recaptured, euthanised, and subject to necropsy; the other nine were not caught at this time-point.

2.4. Long-term protection

To assess whether any short-term protective response observed was sustained into the third year post-vaccination, a second subset of the free-living possums was challenged in a similar manner at 28 months post-vaccination.

The other half of the trapping grid was set for a single night in September 2011, and 13 vaccinates and 13 control animals sedated and transported to a PC3 biocontainment facility [32] where they could be more closely monitored than in the short-term trial. Animals were housed in individual wire-mesh cages and allowed to acclimate to captivity for 3 weeks. Each animal was sedated and received two subcutaneous challenge injections of 10 cfu of