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Efficacy of oral BCG vaccination in protecting free-ranging cattle from natural infection by *Mycobacterium bovis*

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ABSTRACT

Vaccination of cattle against bovine tuberculosis could be a valuable control strategy, particularly in countries faced with intractable ongoing infection from a disease reservoir in wildlife. A field vaccination trial was undertaken in New Zealand. The trial included 1286 effectively free-ranging cattle stocked at low densities in a remote 7600 ha area, with 55% of them vaccinated using *Mycobacterium bovis* BCG (Danish strain 1311). Vaccine was administered orally in all but 34 cases (where it was injected). After inclusion, cattle were exposed to natural sources of *M. bovis* infection in cattle and wildlife, most notably the brushtail possum (*Trichosurus vulpecula*). Cattle were slaughtered at 3–5 years of age and were inspected for tuberculous lesions, with mycobacteriological culture of key tissues from almost all animals. The prevalence of *M. bovis* infection was 4.8% among oral BCG vaccinates, significantly lower than the 11.9% in non-vaccinates. Vaccination appeared to both reduce the incidence of detectable infection, and to slow disease progression. Based on apparent annual incidence, the protective efficacy of oral BCG vaccine was 67.4% for preventing infection, and was higher in cattle slaughtered soon after vaccination. Skin-test reactivity to tuberculin was high in vaccinates re-tested 70 days after vaccination but not in non-vaccinates, although reactor animals had minimal response in gamma-interferon blood tests. In re- tests conducted more than 12 months after vaccination, skin-test reactivity among vaccinates was much lower. These results indicate that oral BCG vaccination could be an effective tool for greatly reducing detectable infection in cattle.

1. Introduction

Bovine tuberculosis (TB), caused by species of the *Mycobacterium tuberculosis* complex, remains a major animal health problem globally, with the main causative pathogen (*M. bovis*) infecting about 50 million cattle worldwide (Waters et al., 2012). Although TB has been eradicated from some developed countries (Cousins, 2001), efforts to eliminate the disease from livestock have been less effective where there are wildlife reservoirs of *M. bovis* infection. Examples of this are the brushtail possum *Trichosurus vulpecula* in New Zealand (Livingstone et al., 2015), badger *Meles meles* in the United Kingdom and Ireland (Delahay et al., 2007), white-tailed deer *Odocoileus virginianus* in Michigan, USA (O'Brien et al., 2011) and wild boar and red deer (*Sus scrofa*, *Cervus elaphus*) in Spain (Vicente et al., 2007). There is therefore interest in vaccines for cattle to help control TB, but there are currently no TB vaccines licensed for routine practical use in cattle.

The human TB vaccine (*M. bovis* bacille Calmette-Guérin (BCG)) protects against childhood tuberculous meningitis and miliary TB, although its efficacy against the major form of adult human disease (pulmonary TB) is highly variable, ranging from 0 to 80% (Colditz et al., 1995). This live, attenuated vaccine has also been trialled on an experimental basis in cattle, with significant protection shown to be conferred by both parenteral- and oral-route BCG administration against experimental challenge with virulent *M. bovis*, and also by parenteral administration against natural exposure via intra-species contact (reviewed in Waters et al., 2012). In the artificial challenge experiments mostly used to identify vaccine-mediated protection, BCG vaccination has been shown to reduce severity of the ensuing disease but not prevent infection, with that form of protection waning after 12 months (Thom et al., 2012). Revaccination when this immunity has waned has been shown to be effective in enhancing protection against TB in cattle (Parlane et al., 2014). It has been unclear whether BCG vaccination would protect cattle against natural exposure to *M. bovis* from a wildlife source for longer than observed in artificial challenge studies, and whether it can also reduce infection rates in that context.

One potential drawback with cattle BCG vaccination is that it can

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sensitise animals to respond in the most commonly-used *ante mortem* TB diagnostic tests. There are modified tests that differentiate infected from vaccinated animals (so-called DIVA tests; Buddle et al., 1999; Whelan et al., 2010) but these are more expensive than the standard low cost tuberculin-based skin-tests. However, the false positive reactivity to standard tests may wane with time, as BCG-induced reactivity to skin testing has been shown to decline rapidly between 6 and 9 months with the single intradermal comparative test (Whelan et al., 2011) and after 12 months in the caudal fold skin test (Parlane et al., 2014). In addition, the false positive reactivity appears to be less severe when the vaccine is delivered orally rather than parenterally, at no cost to the protective effects against experimental *M. bovis* challenge (Buddle et al., 2005; Wedlock et al., 2011) although the BCG doses used for oral vaccination have tended to be very high (doses of 1×10^8 colony- forming units (cfu) or greater orally *vs.* $< 2 \times 10^6$ cfu applied to cattle via injection; Ameni et al., 2010; Lopez-Valencia et al., 2010; Canto Alarcon et al., 2013). Thus oral vaccination with high doses of BCG could be used to protect cattle from TB while also reducing the magnitude, and possibly also the duration, of BCG-induced skin-test reactivity. Although an oral BCG vaccine has been used for many decades in humans (Cosgrove et al., 2006), an oral BCG vaccine has not previously been tested in cattle against natural exposure to *M. bovis*.

This field study was conducted in New Zealand, where transmission of *M. bovis* from infected possums to cattle is the major impediment to eradicating TB from livestock (Livingstone et al., 2015; Crispell et al., 2017). It aimed to determine whether oral BCG vaccination would reduce the rate and severity of *M. bovis* infection and false-positive test reactivity in free-ranging cattle naturally exposed both to other infected cattle and to infected wildlife (possums and other species). A total of 1286 cattle were included in the trial, with 55% of them receiving BCG vaccine. The effect of BCG vaccination was assessed from TB testing outcomes (including abattoir inspection) and vaccine efficacy was determined when the animals were slaughtered at commercial abattoirs.

2. Materials and methods

2.1. Study site and TB history

The study was conducted between 2009 and 2014 in a 7600 ha part of Muzzle Station in the Clarence Valley, New Zealand (42°15′ S, 173°05′ E). The study area is described in detail in previous publications by our group (Yockney et al., 2013 and Nugent et al., 2016). Cattle were stocked at low densities (< 0.05/ha), and were essentially free ranging. Tuberculosis has been present in the area for several decades among both livestock and in wildlife (Nugent et al., 2014). The annual reactor rate from caudal-fold tuberculin (CFT) skin-testing of the Muzzle Station breeding herd averaged 2.3% (range 1.1 − 4.2%) for the 2009–2013 study period, although it fell to 0.5% in the 2014–2015 period (S. Loeffler and M. Neill; OSPRI New Zealand cattle surveillance statistics, Wellington, New Zealand). Among wildlife, culture-confirmed tuberculous lesions were detected in 5.1% of possums surveyed on Muzzle Station between 2009 and 2011 (Nugent et al., 2016), and in 2.7% of possums surveyed in 2014 (Yockney et al., 2016). Tuberculosis has also been recorded in feral ferrets (*Mustela furo*) and red deer in the area, and occurs at a particularly high prevalence in feral pigs (*S. scrofa*) (Nugent et al., 2011 & 2014, and see Supplementary Fig. 1).

2.2. Approvals and consents

Requisite consents to experimentally vaccinate cattle with BCG/lipid vaccine were obtained from (i) Ministry of Agriculture and Fisheries Biosecurity; (ii) Agriculture Compounds and Veterinary Medicines group, New Zealand Food Safety Authority; (iii) Department of Conservation; (iv) Kaikoura Runanga of Nga Tahu; and (v) Landcare Research Animal Ethics Committee (AEC#08/11/02).

2.3. Vaccine formulation and delivery

For oral vaccination, BCG bacilli (strain Danish 1331) were grown to mid-log phase in Tween/albumin supplemented Middlebrook 7H9 broth, and subsequently harvested, sedimented and formulated into an edible lipid matrix (Liporale™; University of Otago, Dunedin, New Zealand) as described previously (Buddle et al., 2005; Wedlock et al., 2011). Each 10 mL vaccine dose contained 1×10^8 cfu of BCG (or a reduced dose of 2×10^7, for one trial group) as determined by retrospective mycobacteriological culture, and was held in a sealed syringe at 4 °C until use. For vaccination, the vaccine was warmed to 15–20 °C, and then squirted over the back of the tongue of the animal while its head was tilted upwards.

A small number of cattle were vaccinated subcutaneously using a commercial source of human *M. bovis* BCG Danish 1331 vaccine (Statens Serum Institute, Copenhagen, Denmark). Each lyophilised vial of this vaccine contained an estimated 2 to 8×10^6 cfu of BCG. The contents of a vial were reconstituted with 1 mL of Sauton medium (Statens Serum Institute) and this was further diluted 1:4 in PBS before administration; 0.5 mL of the reconstituted diluted vaccine was administered per dose to each animal. Retrospective mycobacteriological culturing indicated that the final delivery dose of injected vaccine was 3×10^5 cfu of BCG per animal.

2.4. Experimental design

The overall design involved vaccination of five cohorts of cattle nominally born in 2006–2010 followed, ultimately, by inspection and testing (including mycobacterial culture) of the animals for *M. bovis* infection at slaughter up to four years after vaccination. The cattle were raised to a target weight and then sent to slaughter at commercial abattoirs.

The study began with vaccination in 2009 of cattle nominally born in spring 2006 (2006 cohort), 2007 (2007 cohort) and 2008 (2008 cohort). Cattle born in 2009 (2009 cohort) and 2010 (2010 cohort) were subsequently vaccinated in 2010 and 2011, respectively. Cattle were recruited as they became available over several musters, with the timing (and completeness) of those depending heavily on the prevailing weather.

Candidate cattle were first subject to a standard CFT, with 65 skin test-positive reactor animals excluded from the trial but remaining in the herd (Table 1). Non-reactors were allocated randomly to vaccinated and unvaccinated groups and fitted with an electronic ear tag. The numbers of animals vaccinated (and the vaccination method and dosages) are shown for each cohort in Table 1, along with numbers used as non-treatment controls. For the 2006 cohort, the non-vaccinated animals were sham-vaccinated with 10 mL of plain lipid matrix (no BCG), but from then onwards the other four cohorts remained as non-treated controls (Table 1).

For the 2006 cohort, short-term immune responses were compared for the oral and sub-cutaneous BCG vaccinates; the cattle close to the cattle yards were mustered for the CFT and gamma-interferon (IFN-γ) blood tests at about 70 days after vaccination. The four later-born cohorts were tested with the CFT and IFN-γ tests between 10 and 18 months after vaccination. The widespread distribution of the cattle over rugged terrain meant it was never possible to muster all trial cattle at any single point in time, so testing was always incomplete at a cohort level.

The trial cattle were grazed alongside others from the same cohorts that were not formally entered into the trial ('non-trial cattle'), some of which were sent to slaughter with the trial animals and were subject the same inspection processes (see below). The prevalence of TB in these non-trial cattle was also determined. To confirm the on-going presence of a risk of infection from wildlife, surveys of the age class-specific prevalence of infection in feral pigs in the study area were conducted in most years, using the methods described in Nugent et al. (2014) (with results presented as Supplementary material).