Mycobacterium bovis vaccination and challenge trial in red deer : preliminary findings

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Abstract

Six groups of female red deer weaners received two doses 8.5 weeks apart by subcutaneous injection of the following forms of live attenuated *Mycobacterium bovis* BCG vaccine : 5 x 10^7 cfu Connaught strain lyophilised, 5 x 10^7 cfu Pasteur strain lyophilised, 5 x 10^8 cfu Pasteur strain subcultured, 5 x 10^7 cfu Pasteur strain subcultured, 5 x 10^4 cfu Pasteur strain subcultured, 5 x 10^7 cfu Connaught strain subcultured. One group of six unvaccinated weaners acted as controls. Twelve weeks after the second dose of vaccine all seven groups were challenged with 2 x 10^2 live virulent *M. bovis* by intratonsil inoculation. Seven months later they were slaughtered and critically necropsied. Four of the six control animals had lesions typical of tuberculosis whereas only two of the 29 vaccinated animals had gross lesions. There were no obvious differences between any of the vaccination regimes.

Introduction

Tuberculosis (TB), due to *Mycobacterium bovis*, continues to be a problem in some areas of New Zealand where herd "breakdowns" are caused by the sporadic reintroduction of infection from wild animal reservoirs in endemic areas. The possum is considered the most important maintenance host, although recently wild deer, ferrets and feral pigs have come under suspicion. Ultimately the solution to this problem is to eliminate possums and other wildlife vectors from these areas or alternatively to eliminate TB from them. This is a long term goal and there is no available means of achieving it in the forseeable future. Short term measures rely on trapping, poisoning and shooting to reduce wild animal populations and this appears to reduce reactor rates in cattle and deer (Livingstone, 1991).

The National Science Strategy Committee, established by the Ministry of Research, Science & Technology recently recommended that vaccines be investigated as a potential medium term solution for livestock in high risk areas. It is also likely that vaccination may be more acceptible than culling for the control of TB in wild deer in New Zealand. This paper describes the preliminary findings of a trial which is the fourth in a series of studies into the immunological response of deer to vaccines against *Mycobacteria* conducted by the Deer Research Laboratory, Otago University and AgResearch Invermay (Griffin *et al.* 1992; Griffin *et al*, 1993 a,b).

Materials and Methods

Thirty six female red deer weaners received the following treatments with various forms of live attenuated *M. bovis* Bacillus Calmette-Guerin (BCG) vaccine with the dose in colony forming units (cfu):

Group A (n = 5)	2 doses of BCG
Connaught strain* lyophilised	5×10^7 (cfu)
B (n = 5)	2 doses of BCG
Pasteur strain** lyophilised	5×10^7 (cfu)
C (n = 5)	2 doses of BCG
Pasteur strain subcultured	5×10^8 (cfu)
D (n = 5)	2 doses of BCG
Pasteur strain subcultured	5×10^7 (cfu)
E (n = 5)	2 doses of BCG
Pasteur strain subcultured	5×10^4 (cfu)
F (n = 5)	2 doses of BCG
Connaught strain subcultured	5×10^7 (cfu)
G (n = 6)	unvaccinated control

The first dose of vaccine was given by subcutaneous injection in the right side of the neck on April 15, 1993, and the second dose on the right side of the neck on June 21, 1993 (8.5 weeks after the first dose). The animals were blood sampled every 1 to 2 weeks until August 20. They were skin tested on August 20 and transported to the Infected Deer Farm (IDF) at Milton on September 2. They were allowed to settle for three weeks then challenged with 2×10^2 cfu live virulent *M. bovis*, by the intra-tonsil route while anaesthetised with xylazine/fentanyl citrate/azaperone (Fentazin)*** (i.e. 12 weeks after the second dose of vaccine). They were blood sampled every two to four weeks until March 22, when they were skin-tested and then slaughtered three weeks later (April 15) (i.e. seven months after challenge). At slaughter all major lymph nodes were removed and sliced finely. All lesions were measured, described and sections taken fresh for culture and fixed for histopathology. The tonsils, retropharyngeal lymph nodes (LNs), a pool of the rest of the head LNs, and a pool of thoracic LNs were cultured from animals with no gross lesions at slaughter.

Results

At the time of writing the culture results are incomplete and so only the gross pathology will be reported. The immunological results have also not been analysed completely and they will be reported elsewhere.

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* Connaught lyophilised BCG (Lot 1348-12) supplied by Connaught Laboratories Ltd, Ontario, Canada

** Pasteur lyophilised and Culture BCG (1173 P2) supplied by Pasteur Institute, Davis, France

*** Parnell Laboratories NZ Ltd, PO Box 141, Takanini

At slaughter, two of the 29 remaining vaccinated animals and four of the six unvaccinated animals had lesions typical of TB. Of the two vaccinated animals with lesions, one was from a high dose (5×10^8) Pasteur subcultured Group C (n = 4) and one was from a medium dose (5×10^7) Pasteur subcultured Group D (n = 5). There was no difference between Connaught and Pasteur strains (0/10 v 2/19) or between lyophilised and subcultured(0/10 v 2/19) vaccines. Therefore, if the results are grouped by vaccine dose, lesions were seen in 0/5 low dose, 1/20 medium dose and 1/4 high dose animals. The high dose animal was the most severely affected in the trial, with head, thorax and abdominal lesions. The medium dose animal had a single retropharyngeal LN lesion. Of the four unvaccinated animals, two had retropharyngeal LN lesions, one had a parotid LN lesion and one had mesenteric and ileocecal LN lesions.

Early in the trial an outbreak of pneumonia caused by *Pasteurella haemolytica* killed two deer and they were replaced with spare animals which received their first vaccination two-three weeks after the others. All animals received their second doses at the same time. One month after moving to the IDF and two weeks after challenge one of the Group C (High dose Pasteur Live) animals broke its leg and was euthanased. *M. bovis* (challenge strain) was isolated from the left medial retropharyngeal LN but not the left tonsil. BCG strain *M. bovis* was isolated from a small (5 x 5 mm) granuloma at the vaccination site in the neck.

Discussion

These results show that two doses of live BCG vaccine gave significant protection against TB lesions using this intra-tonsil *M. bovis* challenge model. The results did not show any significant differences between Connaught versus Pasteur strains of BCG, reconstituted lyophilised versus freshly subcultured vaccine or between 5×10^4, 10^7 and 10^8. However, it clearly demonstrates that the low dose (5×10^4) is at least as effective as higher doses. There is still some suspicion that the high dose (5×10^8) may be too high, and could drive the immune reaction away from a protective cell mediated response to a non-protective humoral response (Griffin *et al*, 1993a). The high dose group had one out of four with TB lesions and this case was the most severely affected of all the animals in the trial. The other vaccinated lesion animal was in a medium dose (5×10^7) group.

In three other trials the intratonsil challenge model has resulted in typical TB lesions in 50-80% of weaners and adult stags challenged with 10^1 to 10^4 cfu (Mackintosh *et al*, 1993; Mackintosh unpub). In this trial where all the animals received 2×10^2 organisms, 4/6 (67%) of the unvaccinated control weaners developed typical TB lesions (two with retropharyngeal, one with parotid and one with mesenteric and ileocaecal LN lesions). Thus, it appears that the vaccinated animals received a very realistic challenge and the vaccine should give similar protection under natural challenge conditions.

This trial examined the protection given by two doses of vaccine. It could be argued that one dose of a live attenuated vaccine should give adequate protection. However, the decision to give two doses was based on the principle that this was essential to clearly show protection in this initial trial. Any poor or non-responders to a single dose, should be immunised with a second dose, thereby increasing the chance of demonstrating significant protection. Future trials will examine the optimal size and number of doses, as well as the optimal route of administration.

Acknowledgements

We would like to thank the Animal Health Board and FRST for funding this work. Thanks also to the workers in the Deer Research Laboratory, especially Bridget McMillan and AgResearch Invermay, especially R Labes, I Woodhouse and K Waldrup.

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