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Bovine Tuberculosis in Brushtail Possums
(*Trichosurus vulpecula*):
Studies on Vaccination, Experimental Infection, and
Disease Transmission.

A thesis presented
in partial fulfilment of the requirements for the degree of
Doctor of Philosophy
at
Massey University

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2001

Abstract:

The objectives of the research program were to obtain a better understanding of BCG as a tuberculosis vaccine in possums, and assess its potential as a tool for controlling tuberculosis in wild possum populations. A series of vaccination and challenge experiments were conducted, as well as studies on alternative experimental infection procedures. The program included two field studies, one on the epidemiology of tuberculosis in a population of possums regenerating after localised possum eradication, and the other examined the efficacy of BCG vaccine in a wild population in which tuberculosis was endemic.

The first experiments confirmed the earlier published findings that BCG delivered as an intranasal aerosol induced a protective response. The protective response was found to be present 12 months after vaccination and therefore of sufficient longevity to make vaccination a practical control tool. A second study demonstrated that revaccination of possums enhanced protection and a third showed that conjunctival vaccination was as effective as intranasal aerosol. These findings supported the development of a possum activated self-vaccinator that would deliver vaccine as an aerosol. In delivering the spray to both the external nares and the eyes a simple and cheap device could be designed to efficiently vaccinate wild possums.

The intratracheal experimental infection procedure used in the vaccination and challenge experiments was not entirely suitable for our purposes. Although it provided an assured level of exposure and repeatable results, all infected possums developed fulminant, rapidly progressive disease, irrespective of the vaccination regime used. Two alternative methods of challenge were examined; the conjunctival route of infection, and natural transmission between experimentally infected possums and susceptible in-contact possums. Conjunctival infection was shown to be a reliable procedure for infecting possums, with the disease that resulted from infection having many of the cardinal features of natural tuberculosis in wild possums. Infection following conjunctival inoculation progressed slowly and may be suitable for studying pseudo-vertical transmission and the efficacy of post-infection vaccination.

In studies with captive possums there was little or no transmission of infection between experimentally infected possums and susceptible in-contact possums in the same pen when

the experimentally infected animals were selected at random. However, when possums with high levels of social interaction were experimentally infected there was a significant increase in transmission rates. In addition, the possums that became infected by transmission were more socially active than those that remained free of infection.

Two aspects of the pathogenesis of tuberculosis in possums were clarified during the experimental infection and natural transmission studies. The duration of preclinical infection, impossible to determine accurately in longitudinal studies on wild possums, was found to range from 6 - 20 weeks. Secondly, the pre-eminence of the aerosol route in naturally transmitted tuberculosis was confirmed.

After eradication of possums from a 36 ha site, tuberculosis reappeared within four months. Re-emergence of infection on the site was due to immigration of infected possums, not to the survival of *M. bovis* in the environment. Each of the four restriction endonuclease analysis (REA) types of *M. bovis* that caused disease in the possum population showed a different temporal and spatial pattern.

BCG vaccine had high efficacy in a wild possum population. Over 2 years, 300 possums were recruited to a study of BCG vaccination. Approximately 50% of the possums were vaccinated, where each possum was vaccinated using both intranasal aerosol and conjunctival instillation. There were significantly more cases of tuberculosis in unvaccinated possums than in vaccinates, with a relative risk of tuberculosis in unvaccinated possums of 3.21. The vaccine efficacy was 69%. The most important question relating to BCG vaccine that remains to be addressed is the ability of vaccination to control tuberculosis in possum populations.

This research has demonstrated that BCG vaccine provided protection against *M. bovis* infection in both captive and wild possums. Future research should be directed towards developing delivery systems for vaccinating wild possums and strategies for vaccine use in wild tuberculous possum populations.

Acknowledgements

“A Chinese fable tells of a young man discovering a sage at the village well. The old man was lowering a wooden bucket on a rope and pulling the water up slowly, hand over hand. The youth disappeared and returned with a pulley. He approached the old man and showed him how the device worked. “See, you put your rope around the wheel and draw up the water by cranking the handle”. The old man resisted. “If I use a device like this, my mind will think itself clever. With a cunning mind I will no longer put my heart into what I am doing. Soon my wrists alone will do the work. If my heart and whole body are not in my work, my work will become joyless. When my work is joyless, how do you think the water will taste.”

Kornfield, J. 2000, “After the Ecstasy, the Laundry”, Random House, Sydney, p. 189.

For me there are few greater joys than learning. I cannot imagine a more satisfying vocation than research. No greater responsibility could be asked of me than to conduct research openly, honestly, diligently and ethically. However, life is for living, and is to be lived here and now. Living is to be joyful and not to be wasted, life is too valuable to be “put off” until the PhD is finished. The Buddha advised that we live intentionally, live in the moment. That is what I intended to do. There was no greater sadness for me than when my PhD studies became a burden. When that happened my research suffered, the quality became poor because I was not attending to the work with all my mind and all my heart. My research, my learning, my life became joyless. But with the help of my friends and fellow students I rebounded after a short time. During my time as a student I have conducted the best research in my career although it has not been my most productive time.

Many people have helped me during my studies and I am indebted to each and every one. My fellow students have been an inspiration and support, adding their humour, wisdom and friendship. I want to acknowledge especially Carola Sauter-Louis, Solis Norton and my daughter René. Deb McCrae listened to my grumbles, pleas and joys and went beyond her role as the EpiCentre administrator. There were numerous others who helped especially a stream of local and foreign students, and a number of technicians and administration staff. I want to acknowledge the support, help and guidance of the academic staff outside of the EpiCentre, in the “Vet Tower”, especially that of Professors Maurice Alley, WAG (Tony) Charleston, Colin Wilks, and Dr Stan Fenwick. There were a number

of professional colleagues outside of the University who were of great assistance, particularly Dr Geoff de Lisle of AgResearch and Dr Phil Cowan of Landcare Research. Outside funding for the research came from the Animal Health Board, for which I am grateful.

Dr Bryce Buddle, Professor Dirk Pfeiffer and Professor Roger Morris were my supervisors, all contributed significantly and in their own fashion. Bryce engaged very much in the “stand beside and help” style of supervision. Dirk was more inclined to respond to the problem (experimental design and statistical analysis) and we would attempt to solve the problem together. Roger facilitated the whole research and study program, from the initial invitation to join the EpiCentre, the overall plan of the research program, and acquiring the funds. I am greatly indebted to these three gentlemen.

My time at Massey University has been anything but plain sailing. When I arrived I was in the process of divorce and I had resigned from my position at Commonwealth Scientific and Industrial Research Organization (CSIRO), a position that I had held for 23 years. I had left behind two daughters, extended family, friends, my whole support network. Settling down in New Zealand was difficult. At CSIRO I had been a member of several highly productive, multidisciplinary research teams and in the EpiCentre I felt isolated, and at home I was alone.

In November of 1996 I met Laurie Lawler, my wife, and my personal world once again took on some joy. Laurie has a wisdom that is unique in my experience, a wisdom based on intuition and borne of experience. She is a very intelligent person engendered with love, compassion and a level of common-sense that is very uncommon. Being supported, nurtured and loved by Laurie enabled me to continue my search for self-awareness, to understand myself, my reactions, and to continue my academic studies when I was sorely tempted to “pack it all in”. More than to anyone else I am indebted to her and to her I dedicate this work.

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19th August 2001

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