Mycobacterium paratuberculosis infection in sheep: aspects of diagnosis and immunity

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A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy of Veterinary Science. Massey University, 1999.

> ...the essential part of scientific research is dedicated to struggling, not against the evils of Nature, but against the evils begotten by our so-called progress...
>
> *Henri-Frederic Blanc*

Abstract

Paratuberculosis is a chronic, wasting disease of ruminants caused by *Mycobacterium paratuberculosis*. Programmes aimed at controlling paratuberculosis are based on either vaccination or detection and culling of infected animals. Because of its chronic nature, and the lack of suitable tests for early diagnosis, control of the disease using the latter approach is difficult.

Standard procedures for the isolation of *M. paratuberculosis* are time-consuming and some strains are difficult or impossible to grow. Using the published sequence data of IS*900*, an insertion sequence considered unique for *M paratuberculosis*, a polymerase chain reaction (PCR) assay was developed. With purified extracts of bacterial DNA the PCR assay was found to be highly sensitive and specific. Among 30 bacterial species tested, the assay showed cross-reactivity only with DNA from *M. scrofulaceum*. The possibility of *M. scrofulaceum* causing false positive results in clinical samples from sheep was considered remote, and the assay was subsequently applied to clinical samples. In a study involving 20 sheep suspected of having clinical paratuberculosis, *M. paratuberculosis* DNA was detected in 90% of liver samples and 66% of blood samples from sheep with advanced clinical paratuberculosis. However, in a longitudinal study involving 14 sheep infected experimentally with *M. paratuberculosis*, the PCR failed to consistently detect the target DNA in liver biopsy specimens and blood samples of subclinically infected and clinically affected sheep with mild or moderate extraintestinal infection. Furthermore, the sensitivity of the PCR on samples of ileum and ileocaecal lymph node was similar to that achieved by histological examination.

An experimental model of ovine paratuberculosis, which was developed primarily to validate the PCR assay, created an opportunity to evaluate the diagnostic performance of three commercially available antibody assays for paratuberculosis: complement fixation test (CFT), agar gel immunodiffusion test (AGID), and enzyme-linked immunosorbent assay (ELISA). Two experimental trials demonstrated a limited value of serology for the control of ovine paratuberculosis, as none of the antibody assays was able to detect all sheep with histologically confirmed paratuberculosis. In comparison, the whole-blood interferon-γ (IFN-γ) assay, which

was assessed only during the second trial, detected significantly more experimentally infected sheep and over shorter period of time than any of the serological tests. Furthermore, in a pilot study involving 19 sheep infected experimentally with *M. paratuberculosis*, 18 of the 19 sheep gave positive reactions in the IFN-γ assay on samples of prescapular lymph node (PLN). The PLN-based IFN-γ assay detected significantly more experimentally infected sheep than the CFT, AGID, ELISA or the blood-based IFN-γ assay. Since the specificities of the blood- and PLN-based IFN-γ assay were similar to that of the serological tests, these data indicate the potential utility of this assay, using blood or samples of peripheral lymph nodes, for the detection of sheep exposed to *M. paratuberculosis*. Interestingly, among the 18 sheep tested positive by PLN-based IFN-γ assay, 13 had no histological evidence of paratuberculosis at the time of collection of the PLN samples. In addition, results obtained in a study involving 14 sheep infected experimentally with *M. paratuberculosis* suggest a positive relationship between the magnitude of antigen-induced IFN-γ response in blood and animal's ability to control the infection. Thus, attempts to use this assay in control programmes that are based on testing and culling of positive reactors could result in the removal of animals that have successfully mounted an immune response to the infection.

Vaccination provides an alternative method to test-and-cull programmes of controlling paratuberculosis. Results of a study involving 28 lambs infected experimentally with *M. paratuberculosis*, 14 of which were vaccinated against paratuberculosis with a live-attenuated vaccine 2 weeks postinfection, indicate that vaccination of lambs already exposed to the organism triggered early and strong humoral and cell-mediated immune responses and led to a reduced mycobacterial burden.

But remember,
nothing is actually happening,
and nothing will occur
till the end.

M. Zablocki

Acknowledgements

There is only one author listed on the cover of this manuscript. This is more than misleading. It would not have been possible to carry out the work presented here without the help of many people inside the former Department of Veterinary Pathology and Public Health, which after restructuring is currently a part of the Institute of Veterinary, Animal and Biomedical Sciences. I am very grateful to my supervisors Keith Thompson, Bill Manktelow, Alan Murray and Dave West for the opportunity of undertaking the study, their support and intellectual contribution to this thesis. Special thanks go to Colin Wilks for his assistance throughout. Others who have provided me with help, advice and, sometimes, with enlightenment include Frazer Allan, Maurice Alley, Suzanne Borich, Liz Carpenter, Julie Collins-Emerson, Mark Collett, James Dickson, Magda Dunowska, Linley Fray, Stan Fenwick, Eamonn Gormley, Bob Jolly, Richard Johnson, Joanne Meers, Jane Oliaro, Matthew Perrott, Dirk Pfeiffer, Laurie Sandall and Caroline Twentyman. I also wish to express my appreciation of invaluable technical expertise and assistance I have received from Barbara Adlington, Sheryl Bayliss, Shirley Calder, Pat Davey, Jan Schrama, Farris Sharpe, Pam Slack and Peter Wildbore.

Help and support have not been limited to the "old" Department of Veterinary Pathology and Public Health. I would like to thank Dr. G. de Lisle and Dr. D Collins, both of the AgResearch, Wallaceville Animal Research Centre, for their advice and assistance. Staff at the Central Animal Health Laboratory, Wallaceville, performed a considerable part of immunological testing, and I wish to acknowledge contribution made to this thesis by Michael Reichel. Invaluable assistance on statistical analysis was provided by Dr. S. Ganesh of the Department of Statistics, Massey University. I am also grateful to Barry Parlane for his technical assistance during experimental trials.

This study was funded in part by Wools of New Zealand, the Massey University Research Fund and the Veterinary Research Fund. Without the support of these funding agencies much of this research would not have been performed.

But money is not everything, and much of the work presented in this thesis would not have been even contemplated without the encouragement and ongoing support of my wife, Magda, and my children, Karolina and Mateusz. They shared my seldom "ups" and frequent "downs", always showing a lot of patience and understanding. Finally, what would I have done without my friends: Jasiu, Kylie, Magda D, Matthew and Stan? I would probably have done more than I did, but without their delightful company and distraction from work I would certainly have become a "mad scientist". Considering the importance of an individual, this may not have been perceived as a great loss to the mankind. The fundamental question, which I have no intention to provide an answer to, is whether the mankind is more important than an individual.

At the end, I wish to acknowledge the involuntary contribution made by animals used in the experiments.

This thesis is dedicated to my parents.

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