



Mycobacterium paratuberculosis: **pain in the gut and challenge for science**

Introduction

Mycobacterium paratuberculosis causes Johne's disease in sheep, cattle, goats and other ruminants worldwide. It is a fatal, untreatable, chronic, granulomatous infection of the intestine.

According to some researchers, the organism may be responsible for Crohn's disease in man and, although not proven, this has led to a higher public profile for the disease. For this reason and because of animal welfare concerns and economic loss, animal health authorities in most developed countries aim to reduce the prevalence of infection.

Australian researchers have already delivered cost effective microbiological and molecular diagnostic tests for all species, proven the efficacy of a killed whole cell vaccine in sheep and developed management strategies to control this disease. Nevertheless, gaps in our knowledge of the disease and shortcomings in the effectiveness of reagents and tests for the disease mean that it will continue to spread and long-term technical solutions must be devised.

The scale of the problem

The disease occurs in almost all countries. Typically, 25% of dairy herds in developed countries are infected. In Victoria, there are currently over 1100 infected dairy herds and the disease is also seen in another 200 cattle herds in NSW, South Australia and Tasmania. In NSW, more than 40% of sheep in some districts are infected, with the infection seen in many flocks in South Australia, Tasmania and Victoria.

Zoning of regions of Australia according to the prevalence of the disease and a

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voluntary national market assurance programme is used to control the spread of bovine Johne's disease, but a more vigorous approach has been tried with ovine Johne's disease. A \$40M national control programme from 1998-2004 failed to halt the spread of ovine Johne's disease, which has recently been confirmed in eight flocks in Western Australia, leaving the NT and QLD as the only States free of the infection in sheep.

When established on a sheep farm, there is considerable economic loss from emaciation, reduced productivity and the eventual death of affected livestock. Annual mortality rates of 2-18% have been recorded under extensive grazing conditions, and up to 25% under intensive grazing when the disease is well-established. Vaccines are now available for sheep and cattle which prevent clinical signs but not necessarily infection or shedding of bacilli.

In the face of strong sheep industry concerns about the high costs and lack of

progress in control of the disease, Australian animal health authorities have recently opted to discontinue harsh and restrictive quarantine controls because of the low probability of success, and instead now encourage farmers to manage the problem themselves by providing advice on risk assessment when trading livestock.

Due to the long incubation period of the disease and the impact of vaccination on suitable test methods, the risk will be difficult to objectively and accurately assess. For these reasons, bovine and ovine Johne's disease are likely to continue to spread within Australia.

This much is known

The organism has a tropism for the lamina propria of the intestine, survives within macrophages and is transmitted mostly by the faecal-oral route. It survives for 12 months in the environment and may be dormant during this period. Some strains can be very difficult to grow in the laboratory.

Researchers in Australia have developed specific media based on Middlebrook 7H9 using the BACTEC 460 format to culture the 'non-cultivable' strain responsible for most disease in sheep, and can now routinely isolate the organism from both sheep and cattle.

Rapid molecular typing tests based on IS1311 PCR-REA have also been developed in Australia and have confirmed that the diseases in sheep and cattle are quite distinct and can be controlled separately if necessary.

There is an unusual pattern of inflammation with some similarities to the human disease leprosy. Some



infected animals develop a multibacillary form of disease in which macrophages laden with acid fast bacilli dominate every high power field of view and Ziehl Neelsen-stained histological glass slides held up to the light are visibly red, while other animals have a paucibacillary form in which acid fast bacilli can be almost impossible to find. In these cases how do so few bacteria cause so much tissue damage?

It is also known that within a flock or herd the disease will affect only a proportion of individuals. What factors influence the outcome for a given individual? Level or timing of exposure, genetic factors, environmental factors or stressors may be involved. There is much still to learn.

Solutions are awaiting discovery

The single most useful technological advance will be a diagnostic test for early infection, so that animals can be removed from a flock or herd prior to transmission of the infection. In favour of this approach is the extreme incubation period after exposure in the post-weaning period. It ranges from 1-7 years in sheep and 3-14 years in cattle.

Studies have begun to identify the principal immunopathologic processes at animal, tissue, cell, genome and protein level using laser capture microdissection, gene expression technologies, proteome analysis, FACS, ELISPOT and other tools for immunology, cell and molecular biology.

The recent publication of the genome sequence of *M. paratuberculosis* provides a great resource to facilitate and underpin our understanding of molecular pathogenesis from the point of view of the bacterium. This is complemented by on-going bovine and ovine genome sequencing projects which are beginning to assist in understanding the host response at the molecular level.

However, to gain full value from this wealth of information, appropriate experimental models are necessary. These models have now been developed and characterised to study the disease both *in vivo* and *in vitro*.

Australia is participating in a global research effort with innovative research projects investigating microbiological, molecular and immunological aspects of the disease.

A large research programme involving collaboration between NSW Agriculture Elizabeth Macarthur Agriculture Institute, the University of Sydney Faculty of Veterinary Science and CSIRO Livestock Industries at AAHL Geelong is a key development in the pursuit of a better understanding of this disease.

Further information

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PhD scholarships

Research opportunity in *Mycobacterium paratuberculosis* programme

Johne's disease, caused by *Mycobacterium paratuberculosis*, is a serious current issue for the livestock industry in Australia (see Whittington's article in this issue).

A new microbiological, immunological and molecular research programme will provide young scientists with opportunities to contribute solutions and add to Australia's biotechnology capacity. A research team with around 20 staff and students is established at Camden to reinforce collaboration between The Faculty of Veterinary Science and Elizabeth Macarthur Agricultural Institute, and CSIRO Livestock Industries at Geelong.

Three PhD scholarships provided by Meat & Livestock Australia will be available at the University of Sydney to commence in February 2005. Prospective candidates with an honours level degree should contact:

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