Ovine Paratuberculosis Control

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Ovine paratuberculosis due to *Mycobacterium avium* subsp. *paratuberculosis (MAP)* is no longer the serious agripolitical animal health issue that it was for many Australian rural communities a decade and a half ago. However, the disease continues to spread, with both OJD extension and funded research programs required to address remaining knowledge gaps, the challenging issues of promulgated misinformation, and the need for continual improvement of diagnostic and disease control tools, respectively. Improved regional and on-farm biosecurity, including the introduction of a risk-based trading system, may have contributed to changing attitudes to OJD control, although decreasing on-farm OJD prevalence is almost certainly attributable mostly to the uptake of persistent vaccination programs with Gudair. Although encouraging the ongoing use of vaccination and improved biosecurity when OJD mortalities disappear remains challenging, vaccination has provided a robust strategy for managing OJD on-farm and contributed significantly to both the health of Australian sheep and the lives of producers with affected properties. As vaccination offers a pathway to reduce the risk of MAP infection entering the human food chain from ruminant products, it should be more widely adopted globally.

Keywords: ovine ; paratuberculosis, ; Gudair ; disease control ; persistent vaccination ; misinformation ; pathogenesis

1. Introduction

Paratuberculosis or Johne's Disease (JD), caused by several strains of *Mycobacterium avium* subsp. *paratuberculosis (MAP),* is an insidious, chronic disease of the global ruminant industries, causing mortalities and reduced production; interference in trading; and, in Australia, an extended period of profound negative social impact on rural communities involved in wool sheep production [1]. Johne's disease was first described in cattle in 1895 as an intestinal condition that partially resembled tuberculosis. With increasing prevalence, geographic distribution and host range over the past 120 years, it has created serious concerns for animal health authorities due to the prolonged periods of incubation prior to clinical expression, plus faecal shedding of the causative bacterial agent *Mycobacterium avium* subsp. *paratuberculosis* (*MAP*) in faeces in subclinical phases. As diagnostic tests are of limited efficiency in the pre-clinical period, control of the disease is problematic due to difficulties in identification and the removal of infected animals [1].

Although bovine JD (BJD) has been recognized in Australia for about a century, Ovine Johne's Disease (OJD) was not described in Australian sheep until the 1980's [2]. OJD then slowly emerged until it was recognised by the turn of this century as the most serious of concerns for the sheep industry, particularly in NSW [3][4]. That OJD caused severe mortalities in many flocks in Australia is widely known, although initially there was considerable resistance to accepting that this was an industry-wide national issue, particularly until the range of economic losses was demonstrated [5][6]. These losses seriously compromised the financial stability of numerous wool sheep producers during a period when OJD caused depressed land values in southern NSW in the late 1990's and early millennium, occasionally resulting in psychological stress and suicide risk [1].

Controversial food safety concerns have continued to "confound" research in relation to *MAP* and Crohn's disease in humans. This is despite identification that people with Crohn's disease had a seven-fold greater odds of *MAP* infection than non-Crohn's sufferers, the increasing evidence of an association of Crohn's disease and paratuberculosis, and acceptance by "experts" that *MAP* is a zoonosis [7][8]. Whilst a meta-analysis on Crohn's and *MAP* concluded that there is an association, public health control measures beyond those provided by animal health control programs that reduce *MAP* in the food chain have been suggested as unjustified at present due to knowledge gaps in the role of *MAP* in human disease [9]. Nevertheless, some countries, including Norway, with a "stamping out" program, and Japan, with a program for removal of *MAP*-infected livestock from the food chain, have instituted stringent regulatory controls on paratuberculosis, and others have advocated for industry-led control programs for Johne's disease. In a recent survey of 48 countries, paratuberculosis was confirmed to be very common in livestock, with more than 20% of herds and flocks infected with *MAP* in half of these countries, but formal control programs were present in only 22 countries [10]. Control programs were justified most commonly on animal health grounds, with protection of market access and public health also of relevance. Government funding was involved in about two thirds of countries, but operations tended to be funded by

farmers and their organizations and not by government alone. The majority of countries (60%) had voluntary control programs. Generally, programs were supported by incentives for joining, financial compensation and/or penalties for non-participation, although security of funding for long-term control activities was a widespread problem. Control programs were reported to be successful in 16 (73%) of the 22 countries. Recommendations for future control programs include a primary goal of establishing an international code for paratuberculosis, leading to universal acknowledgment of the principles and methods of control in relation to endemic and transboundary disease. A holistic approach across all ruminant livestock industries is required for control of paratuberculosis because *MAP* is exchanged between species and there must be long-term commitment [10].

Control of paratuberculosis in sheep in Australia has largely depended on persistent use of an “old technology” vaccine (killed whole bacteria) (Gudair™, Zoetis, Australia) that appears to provoke generalized up-regulation of cell-mediated immunity and humoral immunity [11][12][13]. However, addressing concerns and knowledge gaps in the control of *MAP*, particularly in other species, requires improved understanding of the immunopathogenesis of paratuberculosis [9].

2. Pathogenesis of Paratuberculosis

The appearance of Johne’s disease in a sheep flock is a complex interplay between events at population, whole animal, tissue and cellular levels, as reviewed [14].

Population level events involve exposure to *MAP* with infection of a proportion of subjects at risk, with only some (5–15%, although rarely up to 25% in some flocks) developing clinical disease and dying. However, clinical cases are the “tip of the iceberg” due to high rates of subclinical infection. It has been shown that the presumed pathway from silent infection with no faecal shedding of *MAP*, through subclinical infection with light faecal shedding, to heavy faecal shedding and clinical disease, is incorrect. Surgical biopsies of infected animals examined over 3 years confirmed that a proportion of individuals avoided infection or resisted colonization by *MAP*, with some infected then recovering, whilst others progressed to clinical disease [15]. Understanding the pathways that determine these outcomes has progressed with work in an infection model in sheep [16][17][18]. Host dependency (age, genotype) and environment (stress, nutrition and dose of *MAP*) are considered factors of relevance, with Merinos and other breeds developing clinical disease, although some breeds may show earlier clinical presentation than others. This is accompanied by changes in early immunological pathways, including lymphocyte proliferation, apoptosis and cytokine activity, that are detected in blood cells of sheep following *MAP* exposure and lead to eventual disease expression in paratuberculosis [16][17][18].

Whole animal events involve colonisation of host tissues by *MAP*, and this occurs before inflammatory reactions are visible. The silent period of infection lasts for variable periods until granulomatous inflammatory foci arise that may become generalized, with shedding of *MAP.* There is a systemic cellular immune response that can be detected by the intradermal skin test, lymphocyte proliferation assay or whole blood IFN-gamma tests, indicating exposure to *MAP* and offering potential early detection of exposed individuals and farms, although they are still unable to distinguish between active infection and exposure. A switch from a cell mediated (Th1-like) response to a humoral antibody (Th2-like) response associated with progression of the infection to more severe forms has been proposed for paratuberculosis, although switches from Th1 to Th2 vary between sheep and are not observed at all in some animals [19][20].

Tissue events arise due to an interaction between *MAP* antigens and the antigen-specific CD4+ and CD8+ T cells in gut lymph nodes that are required for an effective immune response. Although B cells are activated to release specific antibody, this humoral response is ineffective as *MAP* is intracellular, although recently, gut antibodies have been suggested to have a role in protecting against recolonisation by *MAP* in sheep previously exposed to infection. It is the activated T cells returning to the intestine and interacting with infected phagocytes that produces the typical granulomatous inflammatory responses. Growing CD4+ cells and gamma-delta T cells are found in the Peyer’s patches in young sheep a few weeks after exposure to *MAP*, with granulomas eventually occupying the lamina propria. These enteric lesions appear to progress from paucibacillary to multibacillary, although mild paucibacillary lesions can regress [15]. Factors determining the switch from silent to active clinical disease are uncertain (although a novel regulatory pathway related to this disease switch has been identified in experimental sheep models), as are mechanisms enabling *MAP* to disseminate to other tissues, including within macrophages to the intestinal lumen, and then release to initiate faecal shedding.

Cellular events in *MAP* infection follow entry via epithelial cells, presumably involving numerous receptors on cell membranes of the intestinal mucosa. Infection occurs within 30 mins of *MAP* contact with M cells and goblet cells, with mycobacteria passed rapidly to macrophages to which they attach and enter, mediated by the fibronectin receptor and components of the *MAP* cell wall. The host responds when pathogen-associated molecular patterns (PAMP) such as cell wall lipoproteins engage the host’s pathogen recognition receptors (PRR) such as the toll-like receptors (TLR), leading to

the induction of cytokines and activation of innate immune mechanisms. TLR2/TLR6 heterodimers are involved in the recognition of mycobacterial antigens. The fact that pathogenic mycobacteria survive phagocytosis, acquire nutrients for growth within the phagolysosome, spread between macrophages, disseminate within them and finally escape the confines of the macrophage to infect additional animals is fundamental to *MAP* pathogenesis. Mycobacteria are passed from infected macrophages to uninfected macrophages, presumably by apoptosis, and *MAP* alters its gene expression within macrophages, using numerous metabolic pathways and dozens of altered proteins to withstand stresses such as hypoxia, nitrosative and oxidative conditions, and nutrient depletion compared to optimal growth conditions. Gene expression studies in paratuberculosis have identified that *MAP* may utilise lipids as an energy source once within the macrophage, with several gene families differentially regulated, including S100 calcium binding, lysozyme function, MHC class I and class II, T cell receptor and transcription factors [21]. These differentially regulated genes are considered as putative biomarkers of *MAP* exposure, or of the specified disease or resilience outcomes.

3. Ovine Paratuberculosis Control Strategies

Control strategies for paratuberculosis include the following:

- Test and cull programs;
- Management interventions to reduce faecal–oral transmission;
- Vaccination to limit and suppress infection [1].

In extensive sheep flocks in Australia, it is the latter strategy that has proven to be most successful. However, further research solutions may provide more effective JD control programs, a priority being the development of tests that can detect late subclinical infections to enable removal of future sources of infectious material from flocks/herds and the food chain, plus predict the likely outcomes of animals exposed to the organism at an early age. The recent developments in OJD control include an improved understanding of the immune and cellular profiles of sheep with varying paratuberculosis outcomes (as above), offering potential improved testing regimes, plus increasing recognition of the importance of ongoing annual lamb vaccination and improved biosecurity to reduce losses and risks of disease [1][4]. However, improving national paratuberculosis control programs should also be a priority to manage disease risk from trade. Strong leadership and communication from livestock health authorities is required to build trust within rural communities confused by the difficulties in managing this insidious disease.

Change management considerations provide a framework for reflection on animal health strategies, potentially enhancing our understanding of the strengths and weaknesses of national programs that are driven by livestock industry stakeholders keen to enhance the welfare of animals and quality of ruminant-derived products [1][4]. However, control of paratuberculosis in Australia by government regulation, de-stocking and other approaches generally failed to prevent or control the disease or build the trust required for producer compliance with these approaches [1][4]. The OJD situation in NSW in particular, the state with the largest sheep numbers where the disease was first established and recognized, precipitated into a rural community crisis until the introduction of Gudair™ vaccination in 2002. Vaccination was then supplemented by the introduction of the “sheep health statement”, a vendor declaration system to facilitate disease risk awareness during the trading of sheep, plus eventually a more effective program of general biosecurity risk awareness, supported by legislation.

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