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Vaccination against Johne's disease

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ABSTRACT

Vaccines are important tools to aid in the control of Johne's disease. However, currently available live and killed whole organism vaccines have a number of drawbacks including hypersensitivity reactions at the injection site and sensitization to *Mycobacterium bovis* antigens. These problems lead to carcase downgrading and interference with TB control programmes. Alkaline phosphatase fusion methodology is being used to search for immunogenic cell surface associated and secreted proteins of a New Zealand field isolate of *Mycobacterium avium* subspecies *paratuberculosis*. The identification of immunogenic proteins of the bacterium will facilitate the development of a subunit vaccine. This type of vaccine contains only selected components of the organism and therefore it is likely to have fewer adverse side effects. In addition, it may be possible to design a vaccine that does not interfere with TB testing programmes.

Keywords: Johne's disease, immunogenic proteins, subunit vaccines.

Johne's disease (paratuberculosis) is a chronic wasting disease of ruminant animals caused by the bacterium *Mycobacterium avium* subspecies *paratuberculosis*. Animals become infected early in life following ingestion of contaminated colostrum, milk or pasture but clinical disease does not usually develop for a number of years. Infected animals become less productive which results in significant economic losses for farmers (Harris & Barletta 2001). The disease has a global distribution with the cost to New Zealand farmers estimated to be close to 60 million dollars per annum (Brett, 1998).

Good management practices, herd testing and culling of infected animals are important tools for controlling paratuberculosis. However, this strategy alone is unlikely to completely control the problem because current diagnostic tests frequently fail to identify infected animals in the early (preclinical) stages of disease (Whittington & Sergeant 2001). As a consequence more animals are put at risk of infection because preclinically infected animals intermittently shed the bacterium in the faeces, which results in the insidious spread of the organism. Therefore, improvements in diagnostic test sensitivity and/or increased immunity of uninfected animals would be beneficial.

In the long term, genomic studies may lead to the breeding of disease resistant production animals, but in the short to medium term, vaccination is the most realistic option for increasing herd immunity.

Commercial vaccines are available for the control of Johne's disease and these contain whole organisms (either dead or alive), which are mixed with an oily adjuvant and injected subcutaneously into the animal. These vaccines reduce the number of animals that progress to clinical disease and the excretion of organisms in the faeces (Harris & Barletta, 2001). Unfortunately these whole cell vaccines have a number of drawbacks. Firstly, the organism is made up of a complex mixture of proteins, lipids and sugars, that when mixed with the adjuvant, induce a severe hypersensitivity reaction at the injection site. This can cause persistent nodule (granuloma) formation that can occasionally rupture. This causes suffering to the animal and potential downgrading of the carcase at slaughter with diminished returns for the farmer. Histological examination of these nodules and or regional lymph nodes can reveal the presence of granulomatous lesions and acid-fast organisms that can be confused with tuberculosis (Collett & West, 2001). In addition, the current Johne's vaccines can generate cross-reactive responses to *Mycobacterium bovis* skin test antigens which can interfere with TB control programmes (Kohler *et al.,* 2001).

Infection by *Mycobacterium avium* subspecies *paratuberculosis* is particularly challenging for the immune system. The organism targets intracellular compartments of subepithelial macrophages, which shield the pathogen from antibodies. These cells are key players involved in protective cell-mediated immune responses in the gut and survival and replication within these cells is pivotal to the establishment and progression of disease (see review by Valentin-Weigand & Goethe, 1999). Whilst it is not clear if an infected animal can completely eradicate the organism, there is experimental evidence to indicate that activated macrophages can restrict mycobacterial replication (Zurbrick, Follett … Czuprynski 1988). This type of immune response is termed a T helper 1 (Th1) cellular immune response and a key cytokine involved in this response is interferon-gamma (IFN-γ). Therefore, a desirable feature of any new vaccine against Johne's disease is that it can stimulate a Th1 response and not generate any of the side effects seen with the current vaccines.

Recent advances in mycobacterial genetics is creating new opportunities for the development of novel vaccines. Research on *Mycobacterium tuberculosis* suggests that subunit vaccines may offer an alternative approach to traditional whole cell vaccines. This type of vaccine contains only part of the organism (either DNA or protein) and offers the prospect of including only the components that generate the protective immunity and eliminating those components that cause the hypersensitivity at the vaccine site and the cross reactive antigens that interfere with TB skin tests. In addition, because a subunit vaccine

does not contain whole cells, the draining lymph nodes and the vaccine site of immunized animals will not contain organisms that can be confused with *M. bovis*.

The parts of the organism that have generated the most interest in mycobacterial vaccine laboratories around the world are the secreted and surface-bound proteins. These proteins are likely to be the first parts of the organism to be exposed to the immune system on infection and many of them have been shown to be highly immunogenic. The challenge for researchers is to identify which of these proteins can be used to produce an efficacious vaccine. Whilst there are no rules for defining a 'protective' protein it is generally accepted that proteins that stimulate IFN-γ production when challenged with the invading organism are potential candidates.

As a first step towards creating a subunit vaccine against Johne's disease we have extracted genetic material from a New Zealand field isolate of *Mycobacterium avium* subspecies *paratuberculosis* and created a secreted protein gene library in the plasmid vector pJEM11 (Dupont & Murray, 2001). This system involves the production of secreted mycobacterial proteins that are fused to alkaline phosphatase (PhoA). When the fast growing lab-adapted strain of *M. smegmatis,* is transformed with the pJEM11 library, the PhoA tag produces a blue colour when it is transported out of the cytoplasm of the cell and comes into contact with a chromogenic substrate (5-bromo-4-chloro-3-indoyl phosphate) present in the media. Individual blue colonies can then be selected and the nucleotide sequence of the mycobacterial DNA determined. A particular advantage of this system is that the *Mycobacterium avium* subspecies *paratuberculosis* proteins are being produced in a mycobacterial host rather than the more commonly used *E. coli* which is more distantly related. Thus, there is an increased likelihood that the gene products will be processed in a manner more similar to the native proteins.

Using IFN-γ secretion in peripheral blood and in-vitro lymphocyte proliferation assays, we have shown that sheep vaccinated with Neoparasec (which contains the live attenuated strain 316F) generate a significant IFN-γ response to *Mycobacterium avium* subspecies *paratuberculosis* secreted proteins. This observation has been extended by vaccinating sheep with a single dose of secreted proteins which also resulted in an IFN-γ response that was measurable for up to 6 months post-vaccination. The injection sites were also monitored by visual inspection and palpation for the first 16 weeks post-vaccination and interestingly, the reactions in sheep vaccinated with the secreted proteins were much smaller than those found in the Neoparasec vaccinated animals even though the adjuvant used in both cases was the same. Preliminary histopathological studies on the injection sites have shown that lesions produced in the Neoparasec vaccinated animals consist of much larger areas of inflammation and more caseous necrosis and mineralization than in the subunit vaccinated group. It is quite conceivable that alternative adjuvants to the oil based preparation currently used, may offer improved immunological responses and reduce further the vaccine site reactions.

Thus, we have provided preliminary evidence that a subunit vaccine for Johne's disease may offer an alternative to the current whole cell vaccines. The nature of subunit vaccines is that it may be possible to differentiate between a vaccinated and an infected animal and because the vaccine is non infectious it is safer for the farmer to use, it cannot spread and contaminate the environment and would be less likely to provoke market access restrictions.

REFERENCES

Brett, E. 1998: Johne's disease: An economic evaluation of control options for New Zealand livestock industries. Agriculture New Zealand report

Collett, M.G. and West, DM.2001: A comparison of the injection site and draining lymph node pathology induced by an attenuated live and a killed Johne's disease vaccine in sheep. *Proceedings of the 5th international sheep veterinary congress*, Stellenbosch, South Africa, pp 28-29.

Dupont, C. and Murray, A. 2001: Identification, cloning and expression of sodC from an alkaline phosphatase gene fusion library of Mycobacterium avium subspecies paratuberculosis *Microbios 51:* 7-19.

Harris, NB. and Barletta, RG. 2001: Mycobacterium avium subspecies paratuberculosis in Veterinary medicine. *Clinical microbiology reviews 14:* 489-512.

Kohler H.; Gyra H.; Zimmer K.; Drager K.G.; Burkert B.; Lemser B.; Hausleithner D.; Cubler K.; Klawonn W.; Hess RG. 2001: Immune reactions in cattle after immunization with a Mycobacterium paratuberculosis vaccine and implications for the diagnosis of M. paratuberculosis and M. bovis infections. *Journal of veterinary medicine B Infect Dis Vet Public Health 48:* 185-195.

Valentin-Weigand, P. and Goethe, R. 1999: Pathogenesis of Mycobacterium avium subspecies paratuberculosis infections in ruminants: still more questions than answers. *Microbes and Infection 1:* 1121-1127.

Whittington, R.J. and Sergeant, E.S.G. 2001: Progress towards understanding the spread, detection and control of Mycobacterium avium subsp paratuberculosis in animal populations. *Australian veterinary journal 79:* 267-278.

Zurbrick, B.G.; Follett, D.M. and Czuprynski, C.J. 1988: Cytokine regulation of the intracellular growth of Mycobacterium paratuberculosis in bovine monocytes. *Infection & Immunity 56:* 1692-1697.