PROJECT SUMMARY

RURAL INDUSTRIES
Research & Development Corporation

Rhodococcus equi – Development of new vaccine candidates

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Background

In this project we developed a new vaccine candidate for the foal respiratory pathogen *Rhodococcus equi*. This vaccine which has been tested in the mouse model shows great promise for further development. The next stage would be evaluation of safety and efficacy in horses before moving into clinical trials.

Issue

Rhodococcus equi is a bacterial respiratory pathogen of horses, primarily foals less than 6 months of age. *R. equi* causes supperative bronchopneumonia and pyogranulomatous lesions on the lungs, and is often known as 'Rattles' due to the wheezing and rattling sound heard from the foal (Giguère et al. 2011a; Prescott 1991). *R. equi* has been isolated from the soil in endemic farms and affects 1 out of every 3 on these farms and has a mortality rate of 5-100% and a morbidity rate of greater than 20% (Muscatello 2012a). This bacteria is found worldwide and is endemic in parts of the eastern states of Australia, particularly the Hunter Valley and Northern NSW. A high incidence of *R. equi* is associated with dry and short pastured pens and laneways and foals are infected by inhaling or ingesting the bacteria (Muscatello 2012b). The foal immune system is not yet developed or competent enough to combat this pathogen, leading to a fulminant infection in this host (Dawson et al. 2010).

Current treatment for *R. equi* pneumonia is a long term course of dual antimicrobial therapy, often a Macrolide and rifampicin for approximately 3 months. The only prevention that is currently available is *R. equi* hyperimmune plasma, which is expensive and does not offer consistent, adequate or complete protection to the foals (Giguère et al. 2011b; Sanz et al. 2014). Vaccination is the only mechanism to adequately protect the foals and support the foals' immune system against *R. equi* infections. *R. equi* has proven to be a very difficult bacteria to vaccinate against due to its intracellular nature and specific virulence factors. Currently there is no *R. equi* vaccine commercially available despite years of research. Researchers have tried various approaches including the traditional vaccine candidates (live, killed, attenuated), more recently newer vaccine technologies have been tested (DNA, genetically attenuated and subunit vaccines) (Giles et al. 2014; Phumoonna et al. 2008; Vanniasinkam et al. 2005). More recently some progress has been made in using new vaccine vector techniques, whereby a non-pathogenic bacteria or virus is programmed to deliver an immune stimulating antigen to induce the target immune system to protect against a pathogen, e.g. the ALVAC vaccines available for horses. However, the use of a viral vector vaccine technique had not been tested for *R. equi*, with success in the development of an adenoviral vector vaccine for the *R. equi* relative Mycobacterium tuberculosis.

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Aims/Objectives

The aim of this project was to develop an adenoviral vector vaccine against *Rhodococcus equi* for foals and test the developed vaccine candidate in mice for safety, viability, efficacy and immunogenicity.

Methods Used

In this study, an adenoviral vector based vaccine carrying specific *R. equi* antigens was developed. The Adenovirus used is a known non-pathogenic, non-virulent virus that is not capable of causing disease in horses and deemed safe in humans and livestock. It was not practical to work with foals for the initial studies so the vaccine was administered to mice intramuscularly in two doses; a prime and a boost 2 weeks apart. Two weeks after vaccination the mice were challenged with aerosolised virulent *R. equi* ($1x10^9$ cfu/ml), in order to establish the efficacy and viability of the vaccine. Following challenge the mice were monitored to see how much bacteria they received (baseline) and the clearance of the *R. equi* from their system. The immune response to the vaccine was also monitored by measuring antibody levels (IgG, IgG1, IgG2a, IgG2b, and IgA) and cytokine production.

Results/Key findings

The vaccine developed elicited a strong antibody response. The prime/boost vaccination regime produced the strongest antibody response; a strong total IgG and a significant IgG1, IgG2b and IgG2a antibody response was produced. The antibody profile and cytokines secreted in vaccinated mice suggests that a mixed Th2/Th1 antibody response was elicited by the vaccine which based on previous research is considered a protective immune response against *R. equi*. A supportive mixed cytokine response was also found indicating a strong immune response against *R. equi* is present.

The efficacy of the vaccine was demonstrated by the clearance of the bacteria from the murine system following challenge. The results obtained showed that the vaccine developed in this study was equivalent to that of the 'gold standard' positive control of live *R. equi* which is the most immunogenic vaccine candidate developed for *R. equi* to date. The vaccine had no adverse effects in mice and is considered to have good efficacy and immunogenicity and appears to be an outstanding vaccine candidate for *R. equi*. This vaccine has the potential to be an effective vaccine in foals and could aid in reducing mortality and morbidity rates, improve foal health and reduce disease in the foals.

Implications for Relevant Stakeholders

The studies with this new vaccine candidate have to date been conducted in the mouse model. The positive results obtained so far show this vaccine to be a strong candidate for further development. It should hearten the horse industries world-wide that there is a potential *R. equi* vaccine candidate. For the global thoroughbred and standardbred horse breeding industries this vaccine has the potential to reduce morbidity and mortality of foals to *R. equi* infections, provide a healthier foal for the producers, improve the welfare of the mares and foals on farms and improve the economic return of the foal for producers. The wider horse community will benefit from this vaccine as the pleasure and performance horse breeding industries will also benefit with improved foal welfare and reduction in disease.

Recommendations

More research is required to confirm safety and efficacy in horses and once this is demonstrated commercial vaccine producers should be interested in taking the development of a vaccine further.

Information about the project is available from Professor Mary Barton, **mary.barton@unisa.edu.au**.

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