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Development of a novel nanovaccine against bovine parainfluenza-3 virus for use in calves

Key external stakeholders:

Beef and Dairy farmers; Department of Agriculture, Food and the Marine (DAFM).

Practical implications for stakeholders:

- Bovine respiratory disease (BRD) is one of the most economically important diseases in the cattle industry causing significant annual economic losses.
- A novel nanovaccine containing parainfluenza-3 (PI-3) virus was more effective against bovine respiratory disease in suckled beef calves when administered 4 week versus 1 week pre-weaning.

Main results:

- The mucosal and systemic immune responses to the candidate nanovaccine were compared with those induced by a live attenuated commercially available bovine (B) PI-3V vaccine in dairy calves with pre-existing serum antibodies and then challenged with a 'field' strain of BPI-3V.
- There was an enhanced, more sustained mucosal-based immunological response to the candidate nanoparticles (NP) vaccine in the face of pre-existing systemic BPI-3V-specific IgG in dairy calves.
- Immunising beef calves four weeks before weaning results in greater mucus IgA, serum IgG and IFN-γ responses. In contrast, beef calves immunised one week before weaning failed to show a significant serum IgG and IFN-γ response and showed a weak mucosal IgA response. Weaning affected both the humoral and cell mediated immunity for at least 7 days. Additionally, of the calves weaned one week post-immunisation treatment, only the BPI-3V-NP-treated animals exhibited increased mucus IgA concentrations by the end of the study period, but failed to show significant serum IgG titres or IFN-γ responses.

Opportunity / Benefit:

The results of this research have

- Demonstrated that nanoparticle (NP) stability, measured in terms of size, zeta potential and protein content was highest at 4°C compared with storage conditions at 37^{o}C, 21^{o}C and -20^{o}C.
- Demonstrated that calves given the BPI-3V nanovaccine intra-nasally had significantly greater mucus IgA responses, suggesting an enhanced, more sustained mucosal-based immunological response in the face of pre-existing systemic BPI-3V-specific IgG.
- Demonstrated that immunising calves intra-nasally 4 weeks prior to weaning resulted in greater mucus IgA, serum IgG and IFN-γ responses than calves immunised 1 week before weaning.
- Mucosally delivered nanovaccines also hold out the possibility of inducing a protective immune response in the face of pre-existing maternally-derived antibodies in young calves.

Contact Bernadette Earley Email: bernadette.earley@teagasc.ie.

Teagasc project team: Dr. Bernadette Earley (PI), Dr. Fawad Mansoor.
External collaborators: AFBI (N. Ireland); Dr. Michael Welsh, Mr. Simon Doherty VRO.
UCD; Dr. Joseph Cassidy, Dr. Bryan Markey.

1. Project background:

Bovine respiratory disease (BRD) in cattle results from an interaction between the infectious agents (i.e. bacteria and/or viruses), the environment, genetic factors and host immunity. The important viral causes of respiratory disease are bovine herpes virus type 1 (BHV-1), bovine respiratory syncytial virus (BRSV), bovine parainfluenza-3 virus (BPI-3V), and bovine viral diarrhoea virus (BVDV). Predisposing causes of BRD in pre-weaned calves are typically concurrent, and often synergistic, including stress, immunological background, and changes in nutrition.
Bovine PI-3V plays a vital role in the development of BRD and paves the way for other viral and bacterial pathogens to become involved, resulting in more severe disease. Antigen-loaded microspheres represent an exciting approach to control the release of protein antigens in vaccine formulations by reducing the number of immunisation doses required and optimising the desired immune response via selective targeting of antigen presenting cells. Poly (lactic co glycolic acid) has been commonly used for this purpose because of its proven safety record and their established use in products for controlled delivery of peptide drugs (Kavanagh *et al*., 2013). The objective of this study was to develop a novel, slow-antigen releasing, intranasally delivered, nanoparticle vaccine (nanovaccine) against bovine parainfluenza type 3 virus (BPI-3V), an important pathogen associated with BRD.

2. Questions addressed by the project:

- What is the optimal nanocarrier storage and stability conditions, and protein load, for a novel nanovaccine encapsulating PI-3V?
- What are the immune responses to intranasal administration of the nanovaccine containing PI-3V in mice, and in dairy calves in the presence of maternally derived PI-3 antibodies?
- How does the nanovaccine compare with a commercially available vaccine against BPI-3V in dairy calves?
- What is the optimum immune response time to administer the nanovaccine to suckled beef calves pre-weaning when compared with a live attenuated commercially available BPI-3V vaccine?

3. The experimental studies:

The **first study** characterised and optimised the dose of PLGA-NPs incorporating BPI-3V proteins in terms of their size, zeta potential and entrapped protein load for intranasal delivery.
The **second study** assessed the stability of such NPs when stored at different temperatures.
The **third study** designed a pilot, ‘proof of principle’ study to examine the immune responses to PLGA-NPs incorporating BPI-3V antigens in a murine model before moving to assessing their equivalent effects in experimental models in calves.
The **fourth study** assessed the systemic humoral and cellular, as well as the nasal mucosal immune responses to a candidate PLGA-BPI-3V ‘nanovaccine’ in colostrum-fed calves with pre-existing antibodies and to compare these responses with those induced by a conventional live attenuated BPI-3V vaccine.
The **fifth study** investigated the effect of weaning stress on the systemic and nasal mucosal immune responses to this putative BPI-3V nanovaccine and a conventional live attenuated BPI-3V vaccine.

4. Main results:

Nanoparticles of a biodegradable polymer encapsulating BPI-3V antigens were prepared and optimised for intranasal delivery, initially in mice where they induced a greater serum IgG response at an earlier time point compared to solubilised BPI-3V antigen alone. The mucosal and systemic immune responses to the candidate nanovaccine were then compared with those induced by a live attenuated commercially available BPI-3V vaccine in dairy calves with pre-existing serum antibodies and then challenged with a ‘field’ strain of BPI-3V. The calves given the BPI-3V nanovaccine had significantly greater mucus IgA responses, suggesting an enhanced, more sustained mucosal-based immunological response in the face of pre-existing systemic BPI-3V-specific IgG. The serum IgG responses in these BPI-3V-NP-treated calves were largely similar to those in the animals given the live attenuated vaccine.
The impact of weaning stress on the mucosal and systemic immune responses of beef calves to the BPI-3V nanovaccine, and to a live attenuated commercial vaccine was then investigated.
The findings indicated that immunising calves 4 weeks prior to weaning resulted in greater mucus IgA, serum

Contact Bernadette Earley Email: bernadette.earley@teagasc.ie.

IgG and IFN-γ responses than animals immunised 1 week before weaning for both vaccine treatments (Figure 1). Of the calves weaned one week post-immunisation, only the BPI-3V nanovaccine-treated animals exhibited increased mucus IgA concentrations by the end of the study period, but failed to show significant serum IgG titres or IFN-γ responses. This more sustained mucosal immunity induced by the BPI-3V nanovaccine may have potential if it translates into enhanced protective immunity in the face of virus challenge. Overall, the findings of this 'weaning stress' experiment suggest beef calves under such management conditions should be vaccinated at least 4 weeks prior to weaning in order to minimise the effect of this well-recognised stress on their immune responses.

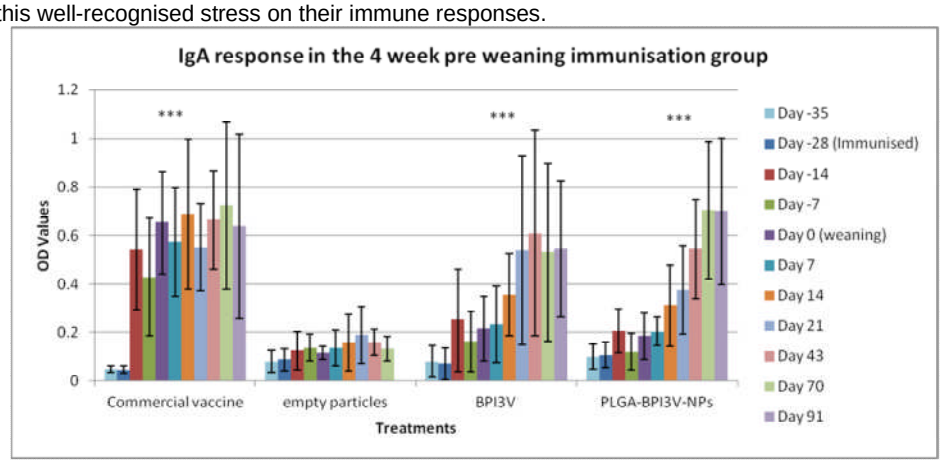

Figure 1: IgA immune response (±s.d) in four weeks pre weaning immunisation group: commercial Rispoval Intranasal vaccine; empty - negative control (empty particles); BPI-3V -positive control (purified solubilised BPI-3V proteins); and PLGA-BPI-3V-NPs vaccine under test. *** = P < 0.001.

5. Opportunity/Benefit:

The results of this research have; 1) Demonstrated that nanovaccine stability, measured in terms of size, zeta potential and protein content, indicate that NPs were most stable at 4°C and do not require low temperature storage; 2), Demonstrated that calves given the BPI-3V nanovaccine had significantly greater mucus IgA responses, suggesting an enhanced, more sustained mucosal-based immunological response in the face of pre-existing systemic BPI-3V-specific IgG; 3), Demonstrated that immunising calves 4 weeks prior to weaning resulted in greater mucus IgA, serum IgG and IFN-γ responses than calves immunised 1 week before weaning; 4), Demonstrated that the more sustained mucosal immunity induced by the BPI-3V nanovaccine may have potential if it translates into enhanced protective immunity in the face of virus challenge; and 5), Demonstrated that mucosally delivered nanovaccines also hold out the possibility of inducing a protective immune response in the face of pre-existing maternally-derived antibodies in young calves. This is the first project of its kind to develop, and assess the mucosal and systemic immune responses to a viral nanovaccine in calves. The use of such novel nanotechnology in the context of vaccine delivery could contribute to state-of-the-art prevention strategies against BRD and other infectious diseases of livestock and potentially make a significant contribution to improving animal health and welfare.

6. Dissemination:

Main publications:

Kavanagh, O., Adair, B.M., Welsh, M.D., Earley, B. (2013). Local and systemic immune responses in mice to intranasal delivery of peptides representing bovine respiratory syncytial virus epitopes encapsulated in poly (DL-lactide-co-glycolide) microparticles. *Research in Veterinary Science*. doi:pii: S0034-5288(12)00353-0. 10.1016/j.rvsc.2012.12.001. PMID 23312498.

Mansoor, F., Earley, B., Cassidy, J.P., Markey, B., Foster, C., Doherty, S. Welsh, M.D. (2011). Immune response to the intranasal delivery of Poly Lactide-co-Glycolide (PLGA) nanoparticles containing Bovine Parainfluenza Type 3 virus (BPI-3V) proteins in mice. *Proceedings of the Agricultural Research Forum meeting*, Tullamore, Ireland, 14 - 15th March 2011.

7. Compiled by: Dr. Bernadette Earley

Contact Bernadette Earley Email: bernadette.earley@teagasc.ie.