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BIODEGRADABLE MICROPARTICLES AS NON-LIVE VIRAL VECTORS FOR RESPIRATORY TRACT VACCINATION

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SUMMARY

The potential of a microparticulate vaccine delivery system in eliciting a specific humoral response in the upper respiratory tract of calves was evaluated. Microparticles composed of poly(lactide-co-glycolide) containing ovalbumin, a model immunogen, were prepared by a solvent evaporation technique. The microparticles were under 10µm in diameter as determined by fluorescence activated cell sorter (FACS) analysis.

Following immunisation, the microparticles induced production of specific secretory IgA (sIgA) in nasal samples. The sIgA was detected after only one week and persisted throughout the length of the study. Additionally, the effects of microencapsulated synthetic peptides (F peptide (0.5mg) and G peptide (0.5mg), representing known protective epitopes against bovine respiratory syncytial virus (BRSV), on the cellular and humoral immune responses of calves were investigated.

* No significant change in the cellular immune response was detected.

* The secretory IgA response was significantly more prolonged following administration of the SF ($F_{111-148}$) peptide when compared with the SG ($G_{174-187}$) peptide.

* It is concluded that microparticles incorporating antigens show potential in the quest for generating complete protection in the young bovine against respiratory tract pathogens.

* Vaccination of calves with the SF ($F_{111-148}$) and SG ($G_{174-187}$) peptide resulted in a significant reduction in the requirement to treat with antibiotics for respiratory disease in the post-vaccination period.

INTRODUCTION

Respiratory disease in calves and growing cattle has long been recognised as a major problem for the cattle industry throughout the world. The underlying cause of bovine respiratory disease is extremely complex with the involvement of viruses and bacteria. The incidence of infection (morbidity) is usually high, but the mortality rate is variable. Viruses that have been predominantly isolated from outbreaks of calf pneumonia are infective bovine rhinotracheitis (IBR), respiratory syncytial virus (RSV), parainfluenza-3 virus (PI-3 virus), and bovine virus diarrhoea/mucosal disease (BVD/MD virus). Predisposing factors affecting immunocompetence (ability to fight infection) are stress, overcrowding, inadequate ventilation, draughts, fluctuating temperatures, poor nutrition and/or concurrent disease. In most cases it would appear that the primary infective agent is viral, producing respiratory tract damage that is subsequently extended by *Mycoplasmas* and secondary bacterial infections. In an Irish survey (Healy *et al*., 1993) in a slatted unit containing 6399 beef cattle over a six month period respiratory disease was the most frequently recorded case of morbidity and mortality, and this observation is in agreement with reports from large feedlots in America (Jensen *et al*., 1976). In a Northern Ireland bovine mortality study, involving 38% of the total cattle population, respiratory disease was the most common cause of mortality in animals from 1 to 24 months of age, with pneumonia of unknown aetiology being the most common syndrome associated with it (Reul, 1977).

Estimates of economic losses vary but are considered to be substantial as demonstrated by a study in Scotland, in which, the loss incurred was calculated at £21 per animal at risk for 152 outbreaks. For the most severe outbreak the cost was estimated at £114 per calf at risk (Gunn, 1998). The prevalent respiratory disease condition in housed calves is enzootic pneumonia or sudden-onset acute pneumonia. The importance of sound animal management cannot be overlooked and factors such as adequate colostrum intake in early life, prevention of co-mingling of different age groups and avoidance

of stressful procedures.(e.g. weaning, castration, dehorning) during high risk periods for disease cannot be overemphasised. Most pathogens enter the host via a mucosal surface and so one of the best ways to protect against infection would be to stimulate strong specific immunity at these mucosal sites. Most vaccines are administered systemically and so, whilst generating strong systemic immune responses, in general they stimulate only poor mucosal immunity. In the last ten years or so, attention has focused on methods of delivery of vaccines which stimulate strong mucosal immunity, and polymeric microparticles composed of poly DL-lactide-co-glycolide have been widely studied particularly in rodents (O'Hagan *et al.*, 1989;Yan, *et al.*, 1996; Challacombe *et al.*, 1997;Alpar *et al.*, 1998).There is evidence that uptake of orally-administered particulate systems is by the microfold (M) cells of the gut- associated lymphoid tissue (GALT) (Eldridge *et al.*, 1990) and it has been suggested that uptake of particulates by the nasal route is effected by a similar process involving nasal associated ymphoid tissue (Alpar *et al.*, 1994; Heritage *et al.*, 1998). Mucosal application of an antigen can lead to induction of both systemic and mucosal immune responses (Maloy *et al.*, 1994; Eyles *et al.*, 1998) and the use of polymeric particulate systems to deliver antigens to respiratory mucosae could provide a useful system for vaccination of the respiratory tract.

Non-live sub-unit vaccines encapsulated in biodegradable microparticles is a novel approach to achieving a protective immune response against specific pathogens in the host's respiratory tract. The main objective of the present study was to investigate the potential of microparticles composed of poly(lactide-co-glycolide) containing a model antigen, ovalbumin (OVA) in calves. Long experience with this polymer has shown that it is completely biodegradable and non-toxic. The immunogenicity of the microspheres administered intranasally is demonstrated by the presence of an ovalbumin-specific humoral immune response in the upper respiratory tract of the inoculated animal. The appropriate dose of encapsulated OVA was also optimised. Chicken egg albumin (ovalbumin) was chosen as the subunit protein for preliminary work because it is a well charac-

terised model immunogen (Nakaoka *et al.*, 1995; Rafati *et al.*, 1997) and is foreign to the animal species used in this project.

Vaccines

Classical vaccines generally consist of either whole inactivated or live attenuated micro-organisms, which are often sufficiently immunogenic to induce potent immune responses without theaddition of an adjuvant. However advances in biotechnology and chemical synthesis have resulted in new approaches to vaccine development involving the synthesis of protein or peptide sequences that are homologous with epitopes capable of inducing protective immunity against infectious organisms. This newer approach can be termed the subunit' approach. Subunit vaccines prepared by chemical synthesis or biotechnology have several advantages over traditional vaccines; they are chemically well defined, can be reproducibly prepared and readily assayed and are inexpensive to manufacture. However a general drawback is poor immunogenicity resulting in the need for multiple administrations thus few effective subunit vaccines have been produced to date.

Encapsulation of subunit antigens into polymeric microparticles may overcome some of the problems with this approach. The polymer particles can be either monolithic with the antigen interspersed throughout the matrix, or consist of true capsule wall structures with a core antigen load within (reservoir microcapsules). The size of particles can range from 300µm to less than 1µm depending on the manufacturing procedure.

The polymer of choice for vaccine delivery is poly DL lactide-co-glycolide (PLG) a biocompatible and biodegradable compound. This copolymer consists of lactic acid and glycolic acid, both by-products of normal mammalian energy metabolism. Polylactide-co-glycolide formation results in compounds of approximately 40kDa in molecular weight and degrades over time by random, non-enzymatic hydrolysis. The rate of biodegradability is influenced by several physicochemical factors so controlled release of antigen may be achieved at pre-determined timepoints to maximise the immune

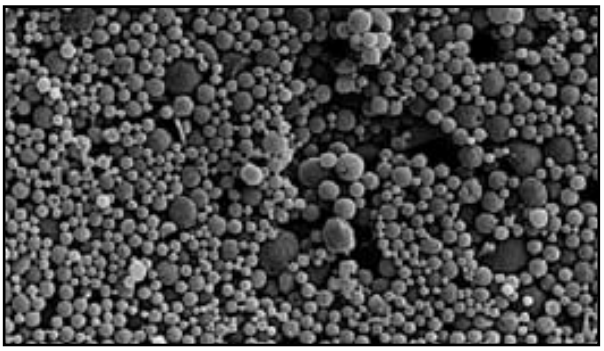
response generated.

Materials and Methods

Microparticle Preparation

Microparticles containing ovalbumin (OVA) were prepared using a poly(lactide-co-glycolide) polymer with a molecular weight of 40-75 kDa and a lactide/glycolide ratio of 50:50. The microspheres were prepared by a solvent evaporation technique from an water-oil-in-water (w/o/w) emulsion as described by o'Hagan. Briefly, 1ml of OVA in double-distilled water (60mg/ml) was mixed with 5ml of 60mg/ml polymer dissolved in dichloromethane using a Silverson homogeniser. This emulsion was then homogenised into 10ml of the emulsion stabiliser, polyvinyl alcohol (PVA)(50mg/ml double-distilled water) to form the final stable w/o/w solution. The emulsion was stirred overnight at room temperature to allow the evaporation of the solvent and resultant microsphere hardening. Microparticles were washed three times in 0.1% PVA, collected by centrifugation, freeze dried and stored at -20ºC.

Scanning Electron Micrograph of microparticles



Microsphere Characterisation

The content of encapsulated ovalbumin was determined using a bicinchoninic acid (BCA) protein determination assay after dissolution of approximately 10mg of microparticles in 600µl 0.1M NaOH containing 5% sodium dodecyl sulphate. The extraction was

stopped after 4 hours at 37°C in an orbital shaker with 500µl 0.1M HCl, the solution was centrifuged and the supernatant removed for analysis. The surface morphology and mean size of the microparticles was determined by scanning electron microscopy. Variations in particle size distribution was observed by flow cytometry using 5µm and 10µm fluorescent beads as size markers.

In vitro protein release kinetics

Duplicate samples of the microsphere preparation (approximately 20mg) were suspended in 2ml sterile PBS (pH 7.2) in 2ml glass universals. The solution was agitated continuously at 200rpm in an orbital shaker at 37°C. After 24hr, 48hr, 72hr and weekly thereafter the samples were centrifuged at 3000rpm for 5 minutes and 1.9ml of the supernatant was removed and replaced by resuspension in fresh sterile PBS. The supernatant was analysed for protein content using the BCA protein assay. The release studies were continued over a 10 week period.

Micro-Bicinchoninic acid protein assay

Bicinchoninic acid (BCA) working reagent was prepared by combining 50 parts of BCA stock solution and 1 part of 4% CuSO4.5H2O. A set of ovalbumin standards in PBS was prepared using sterile PBS for the *in vitro* release study (1000mg/ml-3.125mg/ml) and a 1:1 ratio of 0.1M HCl:0.1M NaOH containing 5% SDS for the evaluation of total protein content (2000mg/ml-25mg/ml). 10ml of each standard, unknown sample and blank was added to a 96-well Nunc plate and 200ml of working BCA solution was added. The microtitre plate was covered with a lid and incubated for 20 minutes at 37°C with gentle shaking. Absorbance of water-soluble purple product was measured at 570nm using a microplate reader.

Immunisation protocol (1)

Holstein/Friesian calves (approximately 8 months old) were randomly divided into 3 groups of 4 and administered either (1) 0.5mg, (2) 1.0mg, or (3) 5.0mg of encapsulated OVA. Microspheres were suspended in 2ml sterile PBS immediately before immunisation and administered using an intranasal applicator attached to a 5ml syringe. The animal's heads were held upright for 30 seconds to prevent rejection of the inoculum. All animals received the appropriate booster dose of encapsulated OVA intranasally 5 weeks after the primary inoculation.

Collection of Biological Fluids

Pre-immune sera and nasal mucous samples were collected the morning before primary immunisation and weekly thereafter for 10 weeks. Blood samples were collected by jugular venipuncture and left overnight at 37°C to separate the serum from plasma. The serum was obtained by centrifugation at 3000RPM for 5 minutes and stored at -20°C. Nasal mucosa washings were collected by inserting a sponge into the animals' nostril for 10 minutes. Nasal secretions were centrifuged at 3000 RPM for 5 minutes at 4°C to remove aggregated mucus and stored at -20°C.

Antibody Detection.

The presence of OVA-specific IgA and IgG antibody content in nasal washings and sera respectively, was detected using an ELISA procedure. Briefly, ninety-six-well ELISA plates (Dynatech) were coated overnight with 10µg/ml OVA in 0.05 M sodium carbonate-bicarbonate buffer (pH 9.6) at 4°C. Plates were washed thoroughly with PBS containing 0.05% Tween 20 (PBS-T). Nasal wash samples and sera samples were diluted 1/10 and 1/20 respectively, in 5% nonfat dried milk (Marvel) in PBS-T and incubated for 1 hour at 37°C. Plates were washed thoroughly and mouse anti-bovine IgA or rabbit anti-bovine IgG horse radish peroxidase conjugate at a dilution of 1/100 and 1/20,000 respectively in PBS-T was added to the wells for 1 hour at 37°C. Plates were washed and goat anti-mouse IgG horse radish per-

oxidase conjugate diluted 1/500 in PBS-T was added to the nasal IgA plates for 1 hour at 37°C. The plates were washed and 3,3'5,5'-tetramethlbenzidine (TMB) substrate was added to each well and the colorigenic reaction was stopped after 5 minutes at ambient temperature with 0.1M HCl. Absorbance values were read at 450 nm by an ELISA reader. The results for the IgA assay were expressed as antibody units calculated from a standard curve of a pooled nasal wash sample diluted between 1/2 and 1/32. Furthermore, to allow for differences in nasal wash protein content, these results were normalised to total protein concentration for each sample, as determined by the BCA assay. For the IgG ELISA, themean absorbance values were standardised using sera from a calfhyperimmunised to OVA.

Immunization protocol (2)

Seventy-eight mart purchased Holstein/Friesian calves were approximately 18 days of age on arrival at the research centre. The calves were allocated randomly and received one of the following 6 treatments (13 per treatment); 1). Soluble F-peptide (SF) and soluble G-peptide (SG) in phosphate buffer saline (PBS); 2). Microencapsulated Ppoly(lactide-co-glycolide (PLG)-peptide-F and PLG-peptide-G); 3). Microencapsulated coupled to hexapeptiide (PLG Hexa-peptide-G and PLG hexa-peptide-F); 4). Microencapsulated coupled to ovalbumin (OVA) (PLG OVA-peptide-G and PLG OVA-peptide-F); 5). Ovalalbumin + 20mg microencapsulated cholera toxin 6). Phosphate buffered saline control. The sequence of the BRSV fusion protein from the RB94 (Rispoval vaccine) representing the amino acid region was 111-PELIHYTRNSTKRFYGLMGKKRKR-RFLGFLLG IGSAI-148 ($_{F111-148}$) (SF). The amino acid sequence 174-STCEGNLACLSLSK-187 with a Cys-Ser substitution at position 184 ($_{G174-187}$) (SG) represents the corresponding region of the attachment protein of the BRSV strains. Free or conjugated peptide was encapsulated in microparticles consisting of poly(lactide-co-glycolide) polymer with a molecular weight of 40-75kDa and a lactide/glycolide ratio of 50/50 (Sigma-Aldrich Co. Ltd, Poole, UK). There is preliminary evidence that the G protein is incorporated into the envelope of the BHV-1 virions and it may, therefore, facilitate

virus attachment and replication in the lower respiratory tract.

On day 38 after arrival and immediately before nasal administration the freeze-dried microspheres containing the encapsulated peptides (F and G) were suspended in 2ml of sterile physiological PBS and administered to individual calves in a syringe fitted with an intranasal applicator. Nasal mucosa washings were collected on the day of inoculation and on days 28 and 77 post-primary inoculation for the determination of secretory IgA antibody levels in response to inoculation. Gamma interferon production in response to concanavalin-A (Con-A) was determined in cultured lymphocytes on days 28 and 142 post-primary inoculation. Ten ml of blood wascollected into evacuated blood collecting tubes containing heparin (Vacutainer Systems, Becton Dickinson Systems Europe, Meylan Cedex, France) at predetermined timepoints. A commercially available sandwich ELISA kit (Bovigamä, CSL Veterinary Ltd.,Victoria,Australia) was used for the quantification of bovine interferon-γ in blood samples. Serum immunoglobulins (IgG_1) were measured

Laminar flow unit for cell culture procedures

quantitatively by single radial immunodiffusion on day 28 post-inoculation.The calves had *ad libitum* access to a concentrate ration and roughage (straw) throughout the experimental period. Clean fresh water was available at all times. Individual disease episodes were determined by the requirement to treat for either enteric disease or respiratory disease.The data were analysed using analysis of variance procedure and if a significant difference was observed, Duncan's multiple range test was applied to determine statistical differences between treatments.

Haematological variable

Calves were blood sampled by jugular venipuncture on arrival (and on days 35 and 140 after arrival at Grange Research Centre. The physiological parameters measured were: red blood cell number (RBC), haemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV), total white cell (TWC) count, platelet number, % lymphocytes, blood copper (Cu^{2+}), glutathione peroxidase (GPX). Haematology parameters were determined for unclotted (K3-EDTA) whole blood samples using an electronic particle analyser (Celltac MEK-610K). (Tables 2 - 7) (Appendix 1). Specific changes in haematological parameters are referenced to in the Tables (2-7).

Immunoglobulin determination

Serum immunoglobulins (IgG_1) were measured quantitatively by single radial immunodiffusion (sRID) (Mancini *et al.*, 1965) andcalculated via an internal Ig standard (BINDARID, NANORID kits.The Binding site Ltd., R&D, Birmingham, UK.). The zinc sulphate turbidity (ZST) test was performed on all serum samples at $20^{o}C$ with turbidity readings carried out at 520nm using a spectrophotometer (McEwan *et al.*, 1970).

Results and discussion

In this series of experiments we set out to determine the suitability of a novel micro particulate vaccine delivery system to elicit a mucosal antigen-specific immune response in the upper respiratory tract of young calves. In all inoculated calves significant levels of ovalbumin-specific secretory IgA was detected as early as one week post-administration and levels persisted throughout the duration of the study (Figures 1 and 2). Peak levels of IgA were detected in all calves at week 3.The lowest dose of encapsulated antigen elicited the greatest

Figure.1 Immunogenicity of different doses of OVA encapsulated in poly(lactide-co-glycolide)(PLG-OVA): Detection of ovalbumin-specific IgA in nasal mucosa washes 8-month old calves.

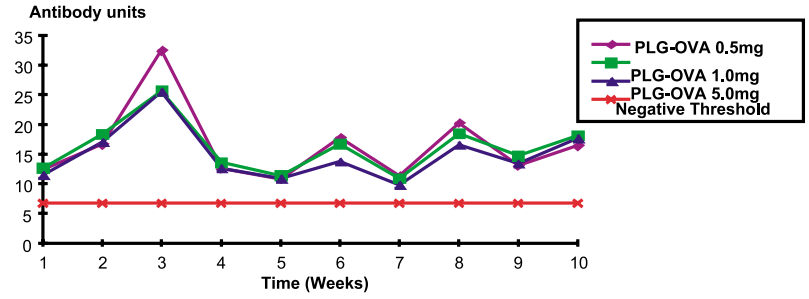
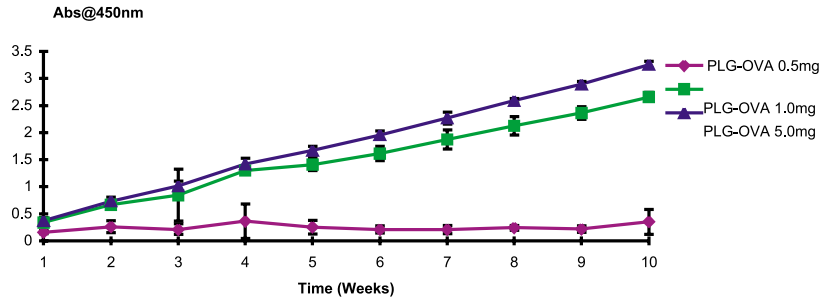


Figure 2. Detection of specific IgG in calf sera intranasally inoculated with either 0.5mg, 1.0mg or 5.0mg of microencapsulated ovalbumin.



immune response. Conversely this same dose resulted in the weakest systemic humoral response.The baseline Ig antibody response to maternal RSV antigens are presented for all treatments at 5, 8 and 10 weeks of age in Table 1. It was important to quantify the RSV antibody titers in calves prior to vaccination in order to rule out any possible neutralization of the novel vaccine when intra-nasally administered to calves. The titres wre significantly lower at 10 weeks of age when values were compared with the data obtained at 5 weeks of age. There was no difference ($P>0.05$) between treatments in response to concanavalin-A (Con-A) induced interferon (IFN) and production from lymphocytes cultured on days 28 or 142 indicating that the vaccination had no effect on the cellular immune response (Table 2). There was no significant difference in serum Ig concentrations (Table 3) or ZST units across treatments ($P>0.05$) (Table 4) indicating that there was no changes in the Ig status of calves throughout the experimental period.

The results presented in Table 5 and Table 6 demonstrated that intranasal administration of ovalbumin in biodegradable poly(lactide-co-glycolide) particles less than 10µm in diameter generated an antigen-specific systemic and mucosal antibody response in calves. A sustained antigen-specific IgA response was induced in the calves nasal mucosa and lungs by all treatments ($P < 0.05$) when compared with PBS control calves on day 28. On Day 77 post-primary inoculation the antigen-specific IgA response to the F peptide subunit was significantly higher (Table 5) and more sustained compared with the response of the G peptide subunit ($P< 0.01$) (Table 6). Haematological parameters were determined using an electronic particle analyser (Nihon Kohdon). On day 35, prior to vaccination, all calves were blood sampled. The total white blood cell counts were significantly higher ($P < 0.05$) in the SF+SG treatment (11.35 ± 0.90) and PLG-F+PLG-G treatment (11.02 ± 1.10) versus PLG OVA-F+PLG OVA-G treatment (8.33 ± 0.39). Similarly, calves in the PBS control group had significantly lower white blood cell counts (8.63 ± 0.49) than calves in the SF+SG treatment (11.35 ± 0.90) and PLG-F+PLG-F treatment (11.02 ± 1.10).

By 140 days after arrival at Grange, the total white blood cell counts were significantly higher ($P < 0.05$) in the SF+SG treatment (13.35 ± 0.63) and PLG-F+PLG-G treatment (13.52 ± 0.79) compared with the PBS control (10.75 ± 1.97). The total white cell counts were significantly higher ($P<0.05$) in the PLG-F+PLG-G treatment (13.52 ± 0.79) compared with the PBS control treatment (10.75 ± 1.97) and PLG-OVA G+PLG-OVA F treatment (11.33 ± 0.65). The incidences of respiratory disease prior to (Table 7a) and in the period after vaccination were recorded in calves. Following vaccination there was a significant reduction in the requirement to treat calves with antibiotics for respiratory disease (Table 7b). Non-vaccinated calves had significantly higher incidences of respiratory disease compared with calves that had been treated with different formulations of the soluble F and soluble G peptides. There was no significant difference in incidences of enteric disease across treatments (Table 7c). Liveweight gain from day 0 to day 140 of the study (Table 8) was not affected ($P > 0.05$) by vaccine administration.

Immunisation Protocol (3)

18 Calves (Holstein x Fresian) were randomly assigned to treatments. All animals were intranasally inoculated with the microparticle preparation containing 1mg of protein antigen. Nasal mucosal wash samples were collected to measure the levels of antigen-specific IgA in the bovine upper respiratory tract. Samples were collected fortnightly for 16 weeks and monthly thereafter.

Antibody Detection

The presence of OVA-specific IgA antibody content in nasal washings was detected using an ELISA developed by the authors. The results for the assay were recorded as a fraction of a positive control and normalised to the total protein concentration expressed as IgA antibody units.

Results

Following intranasal (in) inoculation the microparticles generated a persistent antigen-specific immune response in the nasal washings which lasted for the duration of the 4 month study (Figure 3 and 4). Furthermore, nasal antigen-specific IgA levels for animals not given a secondary booster immunisation were equivalent to those animals which were boosted due to the controlled release of antigen (Figure 4).

It is concluded that intranasally administered antigen encapsulated in biodegradable microparticles generates specific immunity at the site of pathogen infection in the upper respiratory tract of calves and the young bovine. The persistence of antigen-specific IgA antibody in the nasal washings of immunised animals is a very encouraging feature of

Figure 3. Persistence of antigen-specific immunity in the bovine upper respiratory tract: Measurement of ovalbumin -specific IgA levels in nasal washings of 5 week old calves intranasally administered 1mg of microencapsulated antgen.

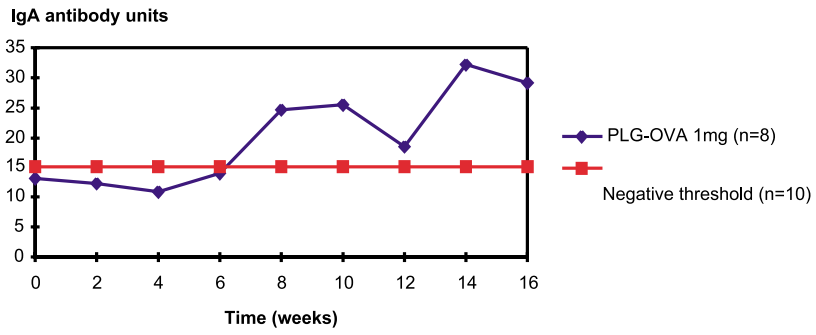
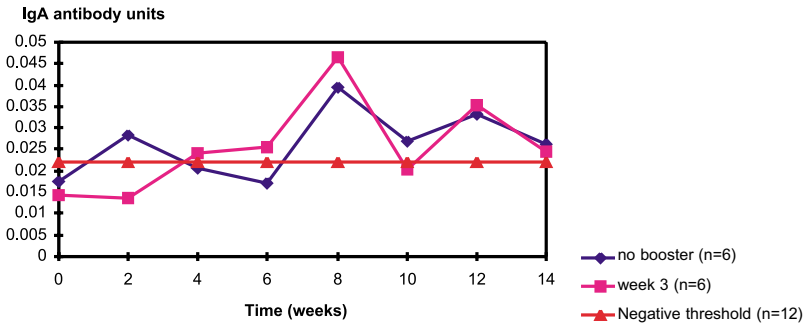


Figure 4. Efficacy of a single shot vaccine delivery system in the young bovine: Measurement of ovalbumin-specific IgA levels in nasal washings generated by intra nasal (I.N.) administration of one or two 1mg doses of encapsulated antigen.



this novel vaccine delivery system and may generate long lasting levels of local anti-viral protection. Furthermore, nasal OVA-specific IgA levels for animals not given a secondary booster immunisation were equivalent to those of animals which were boosted due to the sustained release of antigen from particles over time. These results suggest this novel vaccine delivery system may not only be more effective in neutralising pathogens which reside in the respiratory tract but may also be effective as economical single shot vaccines. It is concluded that this protein delivery system may possess several advantages over conventional methods of respiratory tract vaccination in large animals.

With most of the viruses involved in severe respiratory disease in calves the immune response is complicated, and particularly with RSV there is substantial evidence that the immune response to certain viral proteins contributes to the severity of the ensuing disease. Most of the viruses involved are also immunosuppressive and can adversely affect the normal function of macrophages and lymphocytes (Adair and McNulty 1992; Adair *et al.*, 1992a,b). Conventional respiratory virus vaccines largely comprise whole virus preparations administered live or as killed preparations. Fieldexperience tends to

suggest that efficacy declines rapidly with time following vaccination. Current approaches to vaccine development are therefore aimed at improving vaccine performance by eliminating the protein components of pathogens which contribute to adverse reactions or immunosuppression, while retaining immunogenicity. This can only be achieved by improved understanding of immune responses, particularly cellular events, and how protective immunity can be stimulated over long periods at mucosal surfaces where infection is initiated.

CONCLUSIONS

* No significant change in the cellular immune response was detected. It is concluded that at eleven weeks post-inoculation, the IgA response was significantly more prolonged following administration of the SF ($F_{111-148}$) peptide when compared with the SG ($G_{174-187}$) pep tide.

* It is concluded that microparticles incorporating antigens show potential in the quest for generating complete protection in the young bovine against respiratory tract pathogens.

* Vaccination of calves with the SF ($F_{111-148}$) and SG ($G_{174-187}$) peptide resulted in a significant reduction in the requirement to treat with antibiotics for respiratory disease in the post-vaccination period.

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Table 1. RSV antibody titers in calves. The values are expressed as Mean ± s.e.m.

Age of calves	SF+SG	PLG-F + FPLG-G	PLG Hexa G + PLG Hexa F	PLG Ova G + PLG Ova F	Ova + 20 mg Cholera Toxin	PBS Control
5 weeks	0.84 ± 0.054	0.74 ± 0.049	0.7 2 ± 0.077	0.74 ± 0.053	0.61 ± 0.070	0.79 ± 0.059
8 weeks	0.74 ± 0.035	0.67 ± 0.052	0.68 ± 0.229	0.69 ± 0.061	0.59 ± 0.057	0.69 ± 0.050
10 weeks	0.55 ± 0.043	0.46 ± 0.069	0.54 ± 0.061	0.49 ± 0.055	0.46 ± 0.059	0.52 ± 0.056

Calf serum RSV antibody levels in response to maternal levels.

Table 2. Plasma interferon gamma levels in control and vaccinated calves on days 28 and 142 post-vaccination. The values are expressed as Mean levels (OD at 450 nm) ± s.e.m.

	SF+SG	PLG-F + PLG-G	PLG Hexa G + PLG Hexa F	PLG Ova G + PLG Ova F	Ova + 20 mg Cholera Toxin	PBS Control
			Microencapsulated			
Gamma interferon in response to Con-A stimulation. Mean OD ± sem units at 450nm						
Day 28	0.95 ± 0.181	1.05 ± 0.297	0.88 ± 0.194	1.11 ± 0.182	0.73 ± 0.111	0.68 ± 0.083
Day 142	0.67 ± 0.117	0.79 ± 0.198	0.57 ± 0.161	0.58 ± 0.137	0.53 ± 0.122	0.65 0.127

Table 3: Serum IgG1 concentrations in control and vaccinated calves on arrival and at 21, 42 and 63 days post-arrival at Grange Research Centre.

Groups	Serum $IgG1_1$ concentrations			
	Day 0	Week 3	Week 6	Week 9
SF+SG	20.07 ± 3.05	20.24 ± 2.95	25.05 ± 1.65	21.56 ± 1.64
PLG-F + PLG-G	22.87 ± 3.99	22.55 ± 3.00	23.82 ± 2.42	20.35 ± 1.03
PLG Hexa G + PLG Hexa F	22.81 ± 4.16	18.35 ± 1.93	22.77 ± 1.33	21.19 ± 1.13
PLG Ova G + PLG Ova F	25.03 ± 3.39	22.31 ± 2.13	23.38 ± 2.008	20.43 ± 1.28
Ova + 20 mg CT	20.66 ± 2.04	25.08 ± 2.82	25.37 ± 2.24	24.28 ± 1.99
PBS Control	20.81 ± 3.29	20.71 ± 2.50	23.64 ± 2.60	21.83 ± 1.53

Table 4 : ZST Units in control and vaccinated calves. The values are expressed as mean Units ± sem.

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
SF+SG	9.56 ± 1.35	11.27 ± 0.975	14.20 ± 1.05	10.45 ± 0.57	13.40 ± 0.53
PLG-F + PLG-G	9.57 ± 1.45	11.37 ± 1.45	13.87 ± 1.24	10.76 ± 1.01	13.30 ± 0.77
PLG Hexa G + PLG Hexa F	8.54 ± 0.77	9.94 ± 0.86	13.64 ± 0.81	10.47 ± 0.51	13.47 ± 0.69
PLG Ova G + PLG Ova	10.94 ± 0.96	11.66 ± 0.70	13.88 ± 0.78	10.82 ± 0.57	13.48 ± 0.50
Ova + 20 mg CT	10.58 ± 1.03	11.41 ± 1.06	13.88 ± 0.88	10.84 ± 0.55	14.02 ± 0.49
PBS Control	8.66 ± 0.99	10.71 ± 0.90	14.01 ± 0.93	10.24 ± 0.76	13.33 ± 0.62
	Day 35	**Day 42**	**Day 49**	**Day 56**	**Day 63**
SF+SG	11.59 ± 0.49	10.58 ± 0.52	11.12 ± 0.57	13.01 ± 0.96	16.47 ± 1.54
PLG-F + PLG-G	11.40 ± 0.54	10.27 ± 0.63	10.96 ± 0.48	12.21 ± 1.08	18.02 ± 1.44
PLG Hexa G + PLG Hexa F	11.96 ± 0.43	10.70 ± 0.58	10.70 ± 0.70	12.55 ± 0.85	18.22 ± 1.76
PLG Ova G + PLG Ova F	11.03 ± 0.56	9.86 ± 0.28	10.84 ± 0.53	11.62 ± 0.73	17.78 ± 1.33
Ova + 20 mg CT	12.02 ± 0.42	11.13 ± 0.68	11.97 ± 0.73	13.92 ± 0.99	18.48 ± 1.36
PBS Control	11.20 ± 0.54	10.67 ± 0.78	11.76 ± 0.77	13.46 ± 1.03	20.22 ± 1.60

Table 5. Secretory IgA concentrations in response to F peptide microencapsulation in control and vaccinated calves. The values are expressed as Mean ± s.e.m.

	SF+SG	PLG-F + FPLG-G	PLG Hexa G + PLG Hexa F	PLG Ova G + PLG Ova F	Ova + 20 mg Cholera Toxin	PBS Control
			Microencapsulated			
			G peptide IgA response			
Day 0	0.007 ± 0.001	0.007 ± 0.001	0.006 ± 0.001	0.008 ± 0.001	0.006 ± 0.001	0.008 ± 0.002
Day 28	0.022[a] ± 0.002	0.021[a] ± 0.002	0.017 [a] ± 0.002	0.020 [a] ± 0.003	0.019 [a] ± 0.004	0.008[b] ± 0.002
Day 77	0.012 ± 0.002	0.011 ± 0.002	0.013 ± 0.002	0.012 ± 0.002	0.011 ±0.001	0.007 ± 0.002

[a,b,] means without a common superscript differ (P< 0.05)

Table 6: Secretory IgA concentrations in response to G peptide microencapsulation in control and vaccinated calves. The values are expressed as Mean ± s.e.m.

	SF+SG	PLG-F + FPLG-G	PLG Hexa G + PLG Hexa F	PLG Ova G + PLG Ova F	Ova + 20 mg Cholera Toxin	PBS Control
			Microencapsulated			
			F peptide IgA response			
Day 0	0.023 ± 0.003	0.020 ± 0.002	0.016 ± 0.001	0.020 ± 0.002	0.016 ± 0.001	0.020 ± 0.001
Day 28	0.049 [a] ± 0.004	0.056 [a] ± 0.007	0.045 [a] ± 0.005	0.052 [a] ± 0.067	0.044 [a] ± 0.009	0.019 [b] ± 0.002
Day 77	0.037 [a] ± 0.003	0.037 [a] ± 0.005	0.035 [a] ± 0.003	0.034 [a] ± 0.004	0.034 [a] ± 0.004	0.016 [b] ± 0.002

[a,b,] means without a common superscript differ (P< 0.05)

Table 7a. Incidences of pneumonia in calves prior to vaccination

	SF+SG	PLG-F + FPLG-G	PLG Hexa G + PLG Hexa F	PLG Ova G + PLG Ova F	Ova + 20 mg Cholera Toxin	PBS Control
Treatment	**Pneumonia**					
0	3	5	2	5	4	4
1	7	4	6	4	4	5
2	1	-	3	3	2	3
3	1	3	1	1	2	1
4 or more	1	1	1	-	-	-

Table 7b. Incidences of pneumonia in control and vaccinated calves.

	SF+SG	PLG-F + FPLG-G	PLG Hexa G + PLG Hexa F	PLG Ova G + PLG Ova F	Ova + 20 mg Cholera Toxin	PBS Control
Treatment	**Pneumonia**					
0	9	10	11	11	11	7
1	3	3	1	2	1	4
2	1	-	1	-	-	2
3	-	-	-	-	1	-
4 or more	-	-	-	-	-	-
Significance of post versuspre treatment	P = 0.022	P = 0.026	P = 0.001	P = 0.011	P = 0.012	P = 0.198 ns

Mann-Whitney U test - incidences of calf pneumonia prior to and post vaccine administration.
NS = non significant

Table 7c. Incidences of enteric disease in control and vaccinated calves.

Treatment	Enteric disease					
0	10	12	11	13	13	12
1	2	2	2	0	0	1
2	0	0	0	0	0	0
3	1	0	0	0	0	0
4 or more	0	0	0	0	0	0

Table 8: Liveweight gain (kg/day).

	SF+SG	PLG-F + FPLG-G	PLG Hexa G + PLG Hexa F	PLG Ova G + PLG Ova F	Ova + 20 mg Cholera Toxin	PBS Control
Liveweight gain (kg/day)						
Day 0 - day 35	0.56 ± 0.032	0.55 ± 0.036	0.58 ± 0.042	0.56 ± 0.034	0.52 ± 0.055	0.59 ± 0.042
Day 35 – day 63	0.99 ± 0.044	0.97 ± 0.020	1.03 ± 0.054	1.01 ± 0.039	0.88 ± 0.078	0.93 ± 0.070
Day 35 – Day 77	1.094 ± 0.054	1.052 ± 0.032	1.107 ± 0.064	1.086 ± 0.045	0.98 ± 0.080	0.98 ± 0.064
Day 35 – Day 140	0.93 ± 0.051	0.92 ± 0.041	0.94 ± 0.045	0.97 ± 0.048	0.83 ± 0.073	0.85 ± 0.053

Animals vaccinated on day 35 after arrival at Grange Research Centre.

REFERENCES

Adair, B.M. and McNulty, M.S. (1992) Effect of 'in vitro' exposure of bovine alveolar macrophages to different strains of bovine respiratory syncytial virus. Veterinary Immunology and Immunopathology 30: 193-206.

Adair, B.M., Bradford, H.E.L., Mackie, D.P. and McNulty, M.S. (1992a) Effect of macrophages, and 'in vitro' infection with parainfluenza type 3 and respiratory syncytial viruses on the mitogenic response of bovine leukocytes. American Journal of Veterinary Research 53: 225-229.

Adair, B.M., McNulty, M.S. and Foster, J.C. (1992b) The effect of two adenoviruses (type 1 and type 8) on the functional properties of bovine alveolar macrophages 'in vitro' American Journal of Veterinary Research 53: 1010-1014.

Alpar, HO; Almeida, AJ and Brown, MRW. (1994). Microsphere absorption by nasal mucosae of the rat. Journal of Drug Targeting. 2: 147-149.

Alpar, HO; Eyles, JE and Williamson, ED. (1998). Oral and intranasal immunization with microencapsulated clinically relevant proteins. STP Pharmaceutical Sciences. 8 (1): 31-39.

Challacombe, SJ; Rahman, D and O'Hagan, DT. (1997). Salivary, gut, vaginal and nasal antibody responses after oral administration with biodegradable microparticles. Vaccine. 15 (2): 169-175.

Eldridge, JH; Hammond, CJ Meulbroek JA; Staas, JK; Gilley, RM and Rice, TR. (1990). Controlled vaccine release in the gut-associated lymphoid tissues. I. Orally administered biodegradable microspheres target the Peyer's patches. Journal of Controlled Release. 11: 205-214.

Eyles, JE; Sharp, GJE; Williamson, ED; Spiers, ID and Alpar, HO. (1998) Intranasal administration of poly-lactic acid microsphere co-encapsulated Yersinia pestis subunits confers protection from pneumonic plague in the mouse. Vaccine. 16 (7):698-707.

Gunn, GJ and Stott, AW. (1998). Economic losses associated with calf pneumonia. Cattle Practice. 6 (1): 259.

Healy, AM; Monaghan, MI; Bassett, HF; Gunn, HM; Markey, BK and Collins, JD. (1993). Morbidity and mortality in a large Irish feedlot; microbiological and serological findings in cattle with acute respiratory disease. British Veterinary Journal. 149: 549-560.

Heritage, PL; Brook, MA; Underdown, BJ and McDermott, MR. (1998). Intranasal immunization with polymer-grafted microparticles activates the nasal-associated lymphoid tissue and draining lymph nodes. Immunology. 93: 249-256.

Jensen, R; Pierson, RE and Braddy, PM. (1976). Shipping fever pneumonia in yearling feedlot cattle. Journal of the American Veterinary Medical Association. 169: 500-506.

Maloy, KJ; Donachie, AM; O'Hagan, DT and Mowat, AM. (1994). Induction of mucosal and systemic immune responses by immunization with ovalbumin entrapped in poly(lactide-co-glycolide) microparticles. Immunology. 81: 661-667.

Mancini, G., I. Carbonara, and Heremans, J.F. 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry, 2: 235-241.

McEwan, A.D., E.W. Fisher, I.E. Selman, and Penhale, W.J. 1970. A turbidity test for the estimation of immune globulin levels in neonatal calf serum. Clinica. Chemica. Acta. 27; 155-163.

Nakaoka, R; Tabata, Y; Ikado, Y. (1995). Enhanced antibody production through sustained antigen release from biodegradable granules. Journal of Controlled Release. 37: 215-224.

Rafati, H; Lavelle, EC; Coombes, AGA; Stolnik, S; Holland, J and Davis, SS. (1997). The immune response to a model antigen associated with PLG microparticles prepared using different surfactants. Vaccine. 15: 1882-1897.

Reul, GJ. (1977). Use of Vicryl (polyglactin 910) in general surgery and cardiothoracic procedures. American Journal of Surgery. 134: 297.

O'Hagan, DT; Palin, K; Davis, SS; Artursson, P and Sjoholm, I. (1989). Microparticles as potentially orally active immunological adjuvants. Vaccine. 7: 421-424.

Yan, C; Rill, WL; Malli, R; Hewetson, J; Naseem, H; Tammariello, R and Kende, M. (1996). Intranasal stimulation of long-lasting immunity against aerosol ricin challenge with ricin toxoid vaccine encapsulated in polymeric microspheres. Vaccine. 14 (11): 1031-1038.

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