Production and Immune Response Characterisation of *S. equi* Antigens Encapsulated in Surface Modified Polymeric Nanoparticles

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INTRODUCTION: Strangles is a bacterial infection of Equidae family that affects the nasopharynx and draining lymph nodes, caused by *Streptococcus equi* subspecies *equi*, a Lancefield group C streptococcus [1]. It causes suppurative nasopharyngitis and lymphadenopathy, with a long convalescent period [1, 2]. Although *S. equi* is sensitive to some antibiotics, most of the treatments are ineffective and prevention is the key. Since the 1980s new vaccines against strangles have been developed but limited efficacy has been attested [2]. As biodegradable nanoparticles have enormous potential as antigen carriers for the induction of systemic and local immunity, the aims of the present study were to produce surface modified polycaprolactone (PCL) and polylactic acid (PLA) nanoparticles, produced with cationic (glycochitosan) or anionic (sodium alginate or polyvinyl alcohol) polymers, and absorption enhancers (spermine and oleic acid), as *S. equi* carriers for the induction of both systemic and local protective immunity in a mice model.

EXPERIMENTAL: PCL (42.5kDa) and PLA (2kDa) nanoparticles were prepared by the double emulsion (w/o/w) solvent evaporation method described elsewhere [3]. Particle size and surface charge were determined (laser scattering) and morphology was observed using scanning electron microscopy (SEM). The amount of protein encapsulated per unit weight of nanoparticles was measured using the BCA protein assay (Pierce). Protein integrity throughout encapsulation was assessed by SDS-PAGE.

In vivo studies involved six groups of female BALB/c mice (25g; n=4/group), which were immunized on day 1 and boosted on day 29, with 50µl of solution containing *S. equi* antigen equivalent to 10µg of SeM, either free or encapsulated in nanoparticles (Table 1).

Table 1. Vaccines and delivery route of immunized mice.

Set	Groups	Route
A	SeM encapsulated PLA particles	i.m.
B	Ext. encapsulated PLA particles	i.m.
C	Ext. encapsulated PLA particles	i.n.
D	Ext. encapsulated PCL particles	i.n.

Blood samples were collected at appropriate times after immunization and, by week 12, all animals were ethically sacrificed, their lungs were washed and spleens aseptically removed. The levels of anti-*S. equi* specific IgG, IgG1, IgG2a and IgA were assessed by indirect ELISA. The spleen cells were co-cultured with soluble antigen and interleukin concentrations (IL-2, IL-4, IL 6 and IFN-γ) were quantified using ELISA.

RESULTS AND DISCUSSION: The nanoparticles presented a spherical and smooth surface under SEM (Fig.1). The encapsulation efficiency varied from 30.4% to 95.4 %. The SDS–PAGE analysis showed that antigen integrity was maintained. Vaccination of sets A and B induced a strong increase of serum IgG levels (Fig. 2). Differences between IgG2a level were statistically significant, except those induced by GCs/set B. Negatively charged formulations induced significantly higher IgG and IgG2a levels. Simultaneously, differences were observed in serum antibody levels of animals vaccinated with bacterial extract encapsulated in PLA and PCL particles, when compared to the free form of the antigen.

Overall, the results from the IgG subclass titers obtained in all four sets of particles indicates generation of Th1/Th2 mixed response. Differences observed in IL2 and IFN-γ induced by formulations A and B were statistically significant. Levels of IL2 were not different when comparing spermine formulations C and D. Formulation C induced IFN-γ levels statistically higher when comparing with D. Interleukin levels induced by formulations C and D were lower than those obtained in groups treated with formulations A and B. Moreover, the efficacy of vaccines to prevent strangles seems to be dependent on the induction of a mucosal immune response. Nasal mucosal antibody response (IgA) was statistically higher in animals immunized with formulations C than with D.

CONCLUSIONS: PCL and PLA nanoparticles are potential vaccine carriers with strong immunoadjuvant properties for the effective delivery of *S. equi* antigens.

References:

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Figures

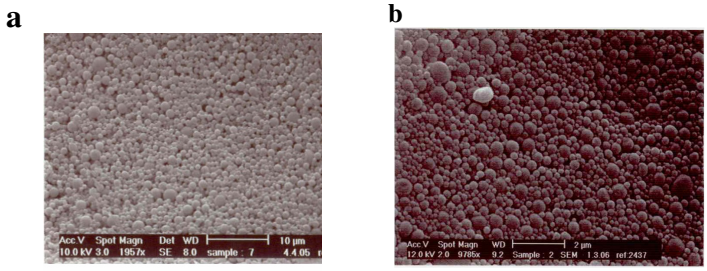

Fig. 1. SEM micrographs of antigen-containing nanoparticles: a) PCL-chitosan; b) PLA-chitosan.

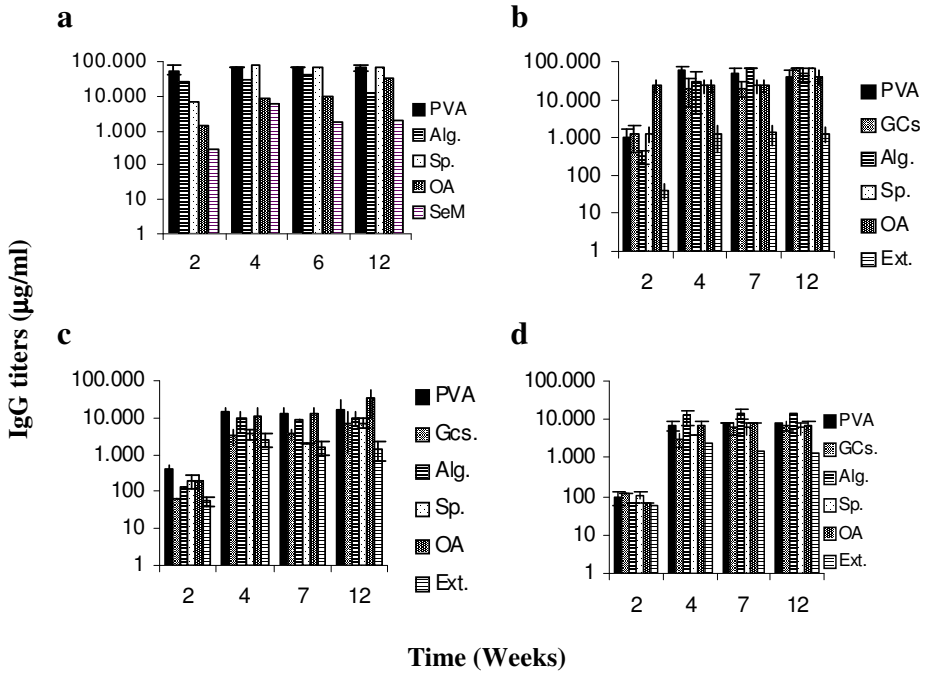

Fig. 2. Systemic immune response to: 1-i.m. administrated a) SeM free or encapsulated, b) Ext. free or encapsulated in PLA nanoparticles; 2-i.n. administrated Ext. free or encapsulated in c) PLA or d) PCL nanoparticles (n=4, mean±sd).

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