Addition of saponin to double oil emulsion FMD vaccines enhances specific antibody responses in cattle and pigs

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Introduction:
Single oil emulsion (SOE) vaccines have been extensively used in cattle for effective control and eradication programs against foot-and-mouth disease (FMD) in South America (Sutmoller et al., 2003).

Double oil emulsion (DOE) vaccines have proved to induce early protective response in different target species (Doel et al., 1994), particularly in pigs, therefore systematic vaccination with DOE has been limited to this species (Sutmoller et al., 2003). The safety and efficacy of SOE and DOE vaccines have been demonstrated in different studies (Barnett et al., 1996, PIADC-PANAFTOSA, 1975), but little information is available on trials comparing their performance in both cattle and pigs.

We have previously shown the enhancing effects of saponin on the immune response of cattle vaccinated with single oil emulsion (SOE) vaccines (Smitsaart et al., 2000). Saponins extracted from Quillaja saponaria are complex chemical adjuvants and have the capacity to stimulate adaptive immunity, by inducing strong Th1 and Th2 responses and cytotoxic T-lymphocyte (CTL) activity (Marciani, 2003).

In this work, we studied immune responses induced by double oil emulsion (DOE) vaccines containing saponin, in both pigs and cattle. Antibody responses following a single dose of SOE and DOE vaccines were compared in both species under controlled conditions. In addition, the safety of these formulations was assessed.

Materials and Methods:

Vaccines: Vaccines were formulated at laboratory scale with various concentrations of inactivated, polyethylene glycol-concentrated, FMDV antigen derived from the O1 Campos strain. SOE vaccines were prepared by adding 40% v/v of aqueous phase to 60% v/v of oily phase (90% Marcol 52, Exxon, France/10% Montanide 888, Seppic, France). Mixtures were left overnight, at 4ºC on a magnetic stirrer (300 rpm) followed by homogenisation using an Ultraturrax T 25 (IKA, Works, Inc., USA) at 25ºC for 2.5 min at 24000 rpm. DOE vaccines were prepared by adding 50% w/w of aqueous phase to 50% w/w of oily phase (Montanide ISA 206, Seppic, France) and emulsification at 25ºC for 12 min at 2000 rpm with a Eurostar power control-visc emulsifier (IKA Works, Inc USA). Saponin-vaccines contained 3 mg per dose of saponin (Quest, Ireland) which had been added to the aqueous phase (Table 1). Formulated vaccines were tested for sterility, conductivity, viscosity, 140S content (after emulsion disruption), safety (in the mouse and guinea pig) and stability at different temperatures (DOE: 25ºC and 4ºC, SOE: 4ºC, 37ºC and 56ºC); the drop test was also applied. In addition, SOE vaccines had to comply with the centrifuge test and glycerine test (Casas Olascoaga et al., 1990, Dossier Montanide ISA 206, Seppic, France).

Animals and experimental design: Fifty cattle and 50 pigs which were seronegative to FMDV were inoculated intramuscularly with 2 ml/dose of each formulation (Table 1).

Serology: at different intervals after vaccination, specific antibody responses were determined by liquid-phase blocking sandwich ELISA (Robiolo et al., 1995) and by the microserum neutralization test (Rweyemamu et al., 1977) using baby hamster kidney (BHK21 clone 13) cell monolayers (Table 1).

Local reaction detection: the inoculation site was examined at each time that animals were bled. The local tissue reaction was further assessed when cattle were slaughtered in a commercial abattoir at 70 days post vaccination (dpv); at post mortem examination, inflamed tissue at the site of inoculation was dissected away from the surrounding tissue and weighed.

Statistical analysis: one way ANOVA and two tailed unpaired Student´s t- test were used to calculate differences in ELISA antibody titers between groups of animals. For each group, the mean weight of inflamed tissue at the inoculation site was compared with the reference value (30 g) and statistical significance determined by one tailed Student´s t-test. This reference value was obtained from a database constructed by the Pathology Department of the National Sanitary Authorities (SENASA) and based on official vaccine potency tests which were conducted on a total of 5000 cattle for 294 commercial vaccine batches approved during 1990-1996.
Results:

Saponin enhances immune responses of DOE vaccines in cattle and pigs

DOE-vaccinated groups, both cattle and pigs, reached peak antibody levels at 23-24 dpv, regardless of whether or not saponin was included in the vaccine. Similar antibody profiles were apparent by virus neutralization and ELISA tests in both cattle and pigs (Trial 1-2, Fig 1).

DOE vaccines to which saponin has been added (DOE-saponin) induced higher antibody titres in cattle at 23 dpv (P< 0.05) and in pigs at 50 dpv (P<0.05) when compared with DOE vaccines lacking saponin (Fig 1).

In cattle, the enhancing effects of saponin were only apparent in the group which had received vaccine with a low antigen payload (5 µg of 140S per dose). In the group which received DOE vaccine without saponin, the antibody titre decreased between 23 and 60 dpv (P<0.05). Moreover, a greater proportion of the cattle receiving DOE-saponin vaccines had protective antibody levels when compared with those receiving vaccine without saponin (Fig 2-B).

On the contrary, in pigs, the enhancing effects of saponin were observed with both low and high antigen payloads (5 and 20µg of 140S/dose). As in cattle, the proportion of pigs which had high antibody titres (log 10 >2.5) was greater in the group of animals vaccinated with DOE-saponin vaccine than in those receiving vaccine without saponin (Fig 2-A).

When comparing the variation in the immune responses of cattle and pigs, less variation in antibody titres was found in pigs than in cattle. However, greater variation in antibody titres was observed in the group of pigs which received DOE lacking saponin than in the group of pigs which received DOE-saponin vaccine (Fig 1-A and B).

SOE-saponin vaccine induced higher antibody responses in pigs than DOE-saponin vaccine

SOE-saponin vaccine induced higher levels of antibody in pigs at 10 dpv (P<0.05) and at 60 dpv (P<0.01) than similar DOE-saponin vaccine (Fig 3-B). In cattle, differences in antibody titres between the groups receiving these formulations were not statistically significant at any bleeding time (Fig 3-A).

As observed in trial 1-2, DOE-saponin-vaccinated groups (both pigs and cattle) reached peak antibody titers at 20-30 dpv whereas SOE-saponin-vaccinated groups did not reach peak values until 60 dpv. In this regard, a significant increase in antibody titres was detected in SOE–saponin-vaccinated group at 60 dpv when compared to 30 dpv (P<0.05) (Fig 3-B).

DOE-saponin and SOE-saponin formulations proved to be safe in both pigs and cattle

None of the vaccines produced systemic or visible local reactions. The local tissue reaction at the site of inoculation in vaccinated cattle was quantified and in each group the mean weight of inflamed tissue at this site was less than 20 g, not significantly different from the reference value (Table 2). No correlation was found between the extent of the local tissue reaction and antibody titers after vaccination (data not shown). These vaccine formulations produced a local reaction characterized as either a granuloma (localized nodule) or diffuse granulomatous inflammation. No specific pattern of local reaction (localized or diffuse) was associated with any particular vaccine formulation, with the presence or absence of saponin or with different types of emulsion. However, it should be noted that only one group of cattle vaccinated with a formulation lacking saponin was included in post-mortem studies, reducing the statistical power of this analysis. In this study, vaccinated cattle displayed a diffuse local reaction more often than a localized nodular reaction.

Discussion:

Saponins have been widely used as veterinary adjuvants and are generally included in aluminium hydroxide-adjuvanted vaccines. Saponins induce strong Th1 and Th2 responses and moderate CTL responses to some proteins, probably as a result of forming mixed protein-saponin micelles (Cox and Coulter, 1997). Partially-purified saponins are simple to formulate and generally safe (Cox and Coulter, 1997).

In South America, single-oil emulsion vaccines have been developed and applied for systematic vaccination of cattle population since 1990 (Sutmoller et al., 2003). Water-in-oil, single oil emulsion vaccines based on mineral oil (Marcol 52) and Montanide 888 as the emulsifier resulted in fluid emulsions which were easy to prepare, safe and of adequate stability (Casas Olascoaga, 1990). Double oil emulsion vaccines based on Montanide ISA 206 have low viscosity and have performed well in pigs.

For effective vaccination, it is desirable that the immune response conferred by vaccines is strong and homogeneous, inducing high levels of protective antibodies and a long duration of immunity in most vaccinated individuals. In this study (trial 1) the ability of saponin-adjuvanted vaccines to achieve uniformly high antibody titres in pigs and a longer duration of immunity than vaccines which lack saponin, has been demonstrated. We found that animals receiving DOE-saponin vaccine with a low antigenic payload reached maximum antibody levels at 30 dpv and that this antibody level was
maintained at least until 60 dpv in both species. Conversely, when the equivalent vaccine without saponin was administered to cattle, antibody levels decreased after 30 dpv, as previously reported for DOE–Montanide ISA 206 vaccines (Barnett et al., 1996). In another report, Hunter (1996) showed that cattle vaccinated with either DOE or SOE emulsions reached peak antibody levels at 30 dpv and maintained high antibody titres for 6 months, although he did not specify which type of oil was used in the vaccine formulations.

The adjuvant capacity of saponin in DOE and SOE formulations was evaluated in cattle and pigs after single vaccination with O1 Campos antigen. The results in pigs showed that SOE-saponin vaccine elicited significantly higher antibody levels than DOE-saponin vaccine. Similarly, Barnett et al. (1996) have reported single oil-in-water emulsion vaccine to be more effective than DOE at stimulating a response against A24 Cruzeiro virus.

In our study, antibody levels to O1 Campos virus as determined by ELISA were lower in cattle than in pigs, whereas neutralizing antibodies were similar in both species or higher in cattle than in pigs. Although these results do not agree with an earlier report (Barnett et al. 1996), the difference might be explained by differences in the virus strain, antigen payload and serological assay which were used in both studies.

Additionally, we observed greater variation in the antibody response among individual cattle than in individual pigs. This may simply be a species difference or may be due to a combination of factors: vaccine formulation, FMDV strain and animal species. This finding would suggest that more than five individual cattle would be required in each experimental group to that statistical tests have adequate power to explore the significance of differences between groups.

One possible problem associated with the use of saponin is its possible adverse effect on vaccinated. Our results demonstrated the safety of vaccines containing saponin in cattle and pigs and a negligible local tissue reaction at the site of vaccine inoculation. This was confirmed following post mortem studies in cattle, in which the extent of tissue inflammation at this site was quantified. From previous studies recorded at SENASA, it was established that where the inflamed tissue associated with local reaction to vaccine exceeded 150 grams weight, this had a detrimental effect on muscle function, diminished the quality of the carcass and was more likely to lead to rejection of the beef at the slaughterhouse. In our study, none of the formulations assayed developed localized nodules which exceeded 37 g weight (data not shown).

In Argentina, SOE-saponin-adjuvanted vaccine has been used for many years. In the last four years more than 400 million doses of vaccine have been administered to cattle and no adverse effects have been reported associated with the use of saponin.

Conclusions:
- Addition of saponin to double oil emulsion vaccines based on Montanide ISA 206 significantly enhanced the immune response in vaccinated pigs and cattle.
- Saponin-SOE vaccine induced higher antibody responses in pigs than saponin-DOE vaccine
- No adverse side effects were observed after administration of single or double oil emulsion vaccines containing saponin.
- Oil-saponin-vaccines can be used for rapid and effective immunization of susceptible animal population against the disease

Recommendations:
- Research should continue to investigate the protective capacity of these vaccine formulations.
- Further studies are encouraged to characterize specific immune response associated with the adjuvant effect of saponin components.

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References:


Table 1: Vaccine composition and experimental design

<table>
<thead>
<tr>
<th>Trial (species)</th>
<th>Vaccine</th>
<th>Number animals/group</th>
<th>Saponin content (µg/dose)</th>
<th>Adjuvant</th>
<th>Bleedings (dpv*)</th>
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<tr>
<td><strong>1 (Pig)</strong></td>
<td>DOE/20/sap 5</td>
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<td>DOE/5/sap 5</td>
<td>Yes</td>
<td>5</td>
<td>ISA 206</td>
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<td>No</td>
<td>20</td>
<td>ISA 206</td>
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<td>ISA 206</td>
<td>0,24,50</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>SOE/20/sap** 5</td>
<td>Yes</td>
<td>20</td>
<td>Marcol 52-Montanide 888</td>
<td>70 dpv</td>
<td>ND</td>
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<td></td>
<td>SOE/5/sap** 5</td>
<td>Yes</td>
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<td><strong>4 (Cattle)</strong></td>
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<td>10</td>
<td>ISA 206</td>
<td>0,15,30,60</td>
<td>70 dpv</td>
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</table>

* dpv: days post vaccination. ND: not done. **82% of protection in cattle by protection to podal generalization test (PGP) at 30 dpv.
Table 2: Local reaction and post-mortem results of cattle vaccinated with DOE and SOE formulations containing or lacking saponin.

<table>
<thead>
<tr>
<th>Vaccine*</th>
<th>Trial</th>
<th>Deep palpation 15 dpv</th>
<th>Post mortem results-70 dpv</th>
<th>Media weight (g)</th>
<th>SD</th>
<th>Type of reaction**</th>
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<tbody>
<tr>
<td>DOE/20/sap</td>
<td>2</td>
<td>0/5</td>
<td>12.86</td>
<td>3.76</td>
<td>80%</td>
<td>20%</td>
</tr>
<tr>
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<td>0/5</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
</tr>
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<td>DOE/20</td>
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<td>5.06</td>
<td>60%</td>
<td>40%</td>
</tr>
<tr>
<td>DOE/5</td>
<td>2</td>
<td>0/5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SOE/20/sap**</td>
<td>2</td>
<td>0/5</td>
<td>10.22</td>
<td>2.92</td>
<td>40%</td>
<td>60%</td>
</tr>
<tr>
<td>* SOE/5/sap</td>
<td>2</td>
<td>0/5</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>DOE/10/sap</td>
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<td>0/10</td>
<td>13.14</td>
<td>3.9</td>
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<tr>
<td>SOE/10/sap</td>
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<td>0/10</td>
<td>15.66</td>
<td>8.49</td>
<td>20%</td>
<td>80%</td>
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</table>

* see composition in Table 1 (DOE/SOE denotes type of emulsion, number indicates µg of 140S/dose, sap denotes addition of saponin). dpv: days post-vaccination. ND: not done. SD: standard deviation.
** Percentage of cattle with localized or diffuse local reaction. ***82% of protection in cattle by protection to podal generalization test (PGP) at 30 dpv.
Figure 1: Mean group antibody responses of pigs of trial 1 (Panels A and B) and cattle of trial 2 (Panels C and D) after vaccination with DOE formulations of O1 Campos strain. Panels A and C: ELISA antibody titres. Panels B and D: neutralizing antibody titers. References x axis: see table 1. * : significant difference compared to its equivalent in antigen content without saponin (P<0.05). Δ : significant difference compared to 62 dpv (P<0.05). T-bars represent the standard deviation.
Figure 2: Frequency of pigs of trial 1 with ELISA titres above log_{10} 2.5 (Panel A), and: frequency of cattle of trial 2 with ELISA levels compatible with protection (>log_{10} 1.85) (Panel B) after vaccination with DOE or SOE vaccines containing or lacking saponin.

A

Frequency of pigs with high antibody titers. Effect of saponin in DOE and SOE vaccines

B

Frequency of protected cattle after vaccination with DOE and SOE vaccines. Effect of saponin
Figure 3: Mean group ELISA antibody responses of pigs of trial 3 (Panel A) and cattle of trial 4 (Panel B) vaccinated with DOE and SOE vaccines containing saponin. * significant difference vs DOE vaccine (P<0.05). ** very significant difference vs DOE vaccine. Δ : significant difference to 60 dpv (P<0.05). T-bars represent the standard deviation.