FOOT-AND-MOUTH DISEASE (FMD) VACCINATION STRATEGIES IN SHEEP AND LAMBS BY USING COMMERCIAL OIL VACCINE

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ABSTRACT

In most countries in South America, FMD routine control and eradication programs require compulsory vaccination of the entire cattle population to be vaccinated compulsorily. However, in an emergency situation or when a buffer zone is being established, the vaccination of all susceptible species is essential. In this context, appropriate vaccination strategies in sheep and lambs with maternal derived antibodies (MDA) are needed in order to reach and maintain a satisfactory protection level in the herd.

A commercial oil adjuvanted FMD vaccine (Biogénesis-Bagó, Argentina) was administered to seronegative adult sheep during breeding season. Three groups were revaccinated at 10, 20 and 30 days after primary vaccination (dpv), respectively.

After lambing, lambs born from vaccinated sheep were grouped according to age and vaccinated at 30, 60 or 90 days of age using the same FMD vaccine. FMDV specific antibodies were examined up to one year after vaccination in adults and up to five months of age in lambs.

One dose induced satisfactory antibody levels in adult sheep, which persisted up to 330 dpv. However, revaccinated groups had significantly higher antibody titres (P<0.05) than the singly vaccinated group from 40 to 120 dpv, regardless of the time the second dose was administered. In the absence of MDA, lambs responded as adults to FMD vaccination. In spite of the MDA interference, vaccination at young age induced a good immune response in the three vaccinated groups and protective antibody levels remained above log_{10} 2.5, until the end of the study.

Taken together these results indicate that a vaccination scheme consisting of one or two doses to adult sheep and a single dose to lambs at any age (with or without MDA) should be encouraged in order to achieve and maintain sufficient herd protection levels and prevent further spread of the disease.

1. INTRODUCTION

In most countries in South America, FMD routine control and eradication programs require compulsory vaccination of the entire cattle population. However, in an emergency situation or when a buffer zone is being established, the vaccination of all susceptible species is mandatory. In this context, and in regions with high density of sheep, appropriate vaccination strategies are necessary in order to reach and maintain a satisfactory protection level in the herd. In addition, there is a need to demonstrate the efficacy and safety of vaccination in newborn ruminants in the presence of maternally derived antibodies (MDA) as they interfere to varying degrees with active immunization (Kitching and Salt, 1995, EMEA, 2007). Previous work had demonstrated that lambs are immunologically competent at an early age and no evidence of a blocking effect of MDA on vaccinal response was found (Cunliffe and Graves, 1970, Terpstra and Dekker, 1996).

The efficacy of mineral oil adjuvanted vaccines in inducing protective immune responses in sheep has been well documented (Cox et al., 1999, Parida et. al, 2008, Selman et. al., 2006). Most of the experiments previously reported used either few animals, experimental vaccines specifically prepared and/or high potency vaccines (Patil et al., 2002, Nair & Sen, 1993, Cox et al., 2003).

The objective of this study was to provide information to allow determination of the optimum vaccination strategy for sheep and lambs that are required in different risk situations and using an oil vaccine commercially available for systematic vaccination in cattle.

2. MATERIALS AND METHODS
2.1. Vaccines

Two industrial batches of commercial polyvalent inactivated water-in-oil single emulsion vaccine containing FMD virus (FMDV) strains O1 Campos, A Arg 2000, A Arg 2001 and A24 Cruzeiro manufactured by Biogénesis Bagó S.A., Argentina (each batch size was 2500 000 cattle doses, Bioaftogen®) were used. In trial 1, a vaccine batch manufactured in 2004 was used, and in trial 2, one manufactured in 2005.

The vaccine batches were controlled by the Argentine National Animal Health Authorities and complied with requirements for efficacy, safety, purity related to non structural protein (NSP) antibodies and stability before released to the market (SENASA, Resolution 351/06).

2.2. Animals and experimental design

A Corriedale sheep flock of the Experimental Station from INTA localized in Balcarce, Buenos Aires province, Argentina was used. The last FMD outbreak reported in this area was 4 years prior the commencement of trial 1 (2000). Animals were identified individually by ear tags. The vaccine was administered by the intramuscular route on the side of the neck, using a 1 ml dose. General and local reactions at the site of vaccination were recorded. Two experiments were carried out, one in adult sheep and the other in lambs (Trials 1 and 2, respectively). Figure 1 shows a scheme of reproductive time and vaccination/bleeding schedule. The flock was kept under grazing conditions, in a 7 hectare paddock with a consociated pasture (about 15 sheep per hectare). No nutritional supplement was given during the winter months. Two rams were introduced in the flock during the two breeding months (April-May). Ewes were pregnancy tested the 14th of June using ultrasonic equipment, and marked according to pregnancy status (empty, single, double). Afterwards, lambing ewes were moved to a second paddock of the same size. All sheep were de-wormed prior to the trial beginning and fecal egg counts were performed routinely to monitor internal parasite burden. Only one treatment was necessary during the trial.

Blood samples were taken before and at regular intervals after vaccination (up to 1 year in adults and 5 months in lambs), and the serum was separated, aliquoted and stored at −20ºC for further serological determinations.

2.2.1. Trial 1

One-hundred and two female sheep aged 1-4 years, (free of structural and NSP antibodies to FMDV) were allotted to 4 vaccinated groups (n=23 each) homogeneously distributed with regard to age, weight and body condition. Ten non-vaccinated sheep served as control animals. FMD vaccination was performed during breeding season. Three groups were revaccinated at 10 (Group 2), 20 (Group 3) and 30 (Group 4) days after primary vaccination (dpv), respectively; Group 1 was not revaccinated and received only a single dose.

2.2.2. Trial 2

After lambing, lambs born from vaccinated sheep were grouped according to age and vaccinated at approximately 35±1 (n=28), 61±3 (n=28) and 94±2 (n=30) days old (abbreviated as G-30, G-60 and G-90, respectively). Non-vaccinated lambs born to vaccinated sheep were sampled to evaluate the decay of levels of MDA (n=13). Four (4) and 2 lambs born to non-vaccinated ewes free from FMDV antibodies (from control group of Trial 1) were vaccinated at 30 and 90 days of age, respectively. Body weight was measured at birth, at 32-49 and at 122-140 days of age, and daily weight gain calculated for each group.

2.3. Serology

Specific antibody responses were determined by liquid-phase blocking sandwich ELISA (LPBE) against FMDV strains O1 Campos and A Arg 2001 (Robiolo et al., 1995). Neutralizing antibodies against O1 Campos were also determined in serum samples of sheep of Trial 1 by the virus neutralization test (VNT, OIE, 2004) using baby hamster kidney (BHK21 clone 13) cell monolayers. Antibodies to the 3ABC NSP were determined by 3ABC ELISA (Robiolo et al., 2006) as a screening test, and the positive reactors determined using the Ceditest FMDV-NS, (Cedi-Diagnostics). The Ceditest FMDV-NS was also used for serum samples of Group 4, Trial 1 (before vaccination, 30 days after the first dose, and again 30 days after the second dose). Serum samples of non-vaccinated sheep were also tested at those intervals.

2.4. Statistical analysis

Analysis of variance was used to compare the means of the LPBE and VNT antibody titres, and mean daily weight gain of the experimental groups. A nonparametric Kruskal-Wallis test was used to compare VNT antibodies at 330 days of age due to lack of homogeneity of variance of data at this bleeding time. Statistical analyses were performed using SAS version 9.1, Mixed and Glimmix procedures were used (SAS Institute, 2002-2005). Differences were considered significant at
P<0.05. Linear Regression was used to analyze the MDA decay according to age to estimate mean half-lives, and also to analyze the interference of MDA to vaccination at 30 days of age. A Student’s t-test was used to compare LPBE antibody titres at 30 days of age between lambs born to sheep receiving one or two doses of vaccine.

3. RESULTS

3.1. Tolerance Trial 1 and 2
No general adverse side effects were observed in adult sheep or lambs of any age after vaccination or revaccination. No visible and palpable local reactions were detected at the site of inoculation after vaccination/s in any adult or lamb. Lambs had at birth an average body weight of 4.41 kg (SD 0.83) and was balanced for all groups. At 32-49 days of age the mean body weight reached 15.3 kg (SD 3.15) and at 122-140 days of age it reached 29.17 kg (SD 4.36). The mean for daily weight gain in the vaccinated groups was 0.182 g, whereas in the non-vaccinated group was 0.191 g (P> 0.05) showing no effect of the vaccination on the body weight gain during the study.

3.2. Trial 1. Immune response in adult sheep after single or double vaccination at different intervals
Non antibodies to FMDV were detected in the control group during the whole trial (either by LPBE or 3 ABC ELISA). One dose of vaccine induced satisfactory LPBE antibody levels with regards to O1 Campos and A Arg 2001 in adult sheep, reaching log10 2.80 and log10 2.71, respectively at 20 dpv. These levels persisted up to the end of the trial (330 dpv). However, revaccinated groups had significantly higher LPBE antibody titres (P<0.05) than the single vaccinated group from 40 to 120 dpv, regardless of the time the second dose was administered (Figure 2). Neutralizing antibody kinetics of O1 Campos followed a similar pattern to that of the LPBE antibody profile (Figure 3), and revaccinated groups had higher VNT antibody titres at 60 dpv (P<0.05). At 180 and 330 dpv, the mean VNT antibody titres ranged between log10 1.35 and 1.61, and non significant differences were detected between vaccinated and revaccinated groups.

3.3. Trial 2. Decay of MDA in non vaccinated lambs born to vaccinated ewes
Four out of the 13 lambs did not have detectable antibodies to both FMDV strains in the LPBE presumably due to either inadequate ingestion or very poor absorption of colostrum, and were consequently excluded from this analysis. High MDA titres were observed in the 9 remaining lambs at the first sampling date corresponding with an average age of 17.4 days (SD 1.4, range 15-19): log10 3.47 for O1 Campos and log10 3.41 for A Arg 2001. Figure 4 shows the decay of the MDA antibodies determined by LPBE to both FMDV strains up to 153 days of age. The regression lines calculated with 83 observations were:
O1Campos: y = 3.8061-0.0191x; r²=0.82
A Arg 2001: y = 3.6487-0.0177x; r²=0.78
From these equations, the half-lives of MDA for A Arg 2001 and O1 Campos were estimated in 17 and 15.2 days, respectively.

3.4. Trial 2. Immune response of lambs with MDA vaccinated at 30, 60 and 90 days of age
High MDA titres were recorded in lambs at 30 days of age (log10>3.00 for both FMDV strains). Moreover, lambs born to revaccinated sheep (Group 2, 3 and 4, Trial 1) had significantly higher MDA titres than those born to singly vaccinated sheep (Group 1, Trial 1) (P<0.01). Vaccination at this age induced seroconversion in 39 and 49% of the lambs (G-30 group) at 30 dpv (Figure 5). High titres were also detected before vaccination in G-60 group lambs (mean titre log10 2.42 for O1 Campos and log10 2.48 for A Arg 2001) and seroconversion after vaccination was evident in 27 out of 28 lambs. At 90 days of age, lambs had medium to low levels of MDA (mean titre log10 1.94 for A Arg 2001, and log10 2.08 for O1 Campos) and vaccination at this age induced high immune response with seroconversion in the 100% of the lambs.
High antibody titres were detected at 30 dpv (LPBE titres log10 3.04-3.29) with non significant differences among G-30, G-60 or G-90 lamb groups. In addition, similar antibody kinetics was observed in all three groups after vaccination. Lambs showed high levels of LPBE antibodies (mean titre group log10>2.70) at 5 months of age regardless of age the vaccination was administered (Figure 5).
Individual responses were related to the antibody level at the time of vaccination and a negative postvaccinal antibody increase or decrease at 32 dpv (γ) to the antibody titres at vaccination (x) at 30 days of age (G-30 group)
produced a significant fit for both FMDV strains with a negative slope (Figure 6). Regression equations are: y=2.8208-0.9270x; r²= 0.8515 for A Arg 2001 and y=3.1787-1.0193x; r²=0.8658 for O1 Campos.

3.5. Trial 2. Immune response of lambs without MDA vaccinated at 30 or 90 days of age

Vaccination of lambs born to non vaccinated ewes either at 30 or 90 days of age induced good immune responses similar to those of G-30 or G-90 in presence of MDA and high antibody levels persisted to 5 months of age (Figure 5). The mean group antibody titres at 60 dpv were equivalent in lambs and sheep (Trial 1) regardless of the presence of MDA in lambs.

3.6. Serology to NSPs

NSP antibody reactors (by 3ABC ELISA-CEVAN) were detected in 7/1684 serum samples (0.41%), with a low percentage of positive reactivity (% reactivity= 22 to 32%) and 17/1684 serum samples (1 %) were classified as suspicious (% reactivity= 15 to 19%). No association was established between positive or suspicious reactors and age group, interval post vaccination, vaccinated group, or to samplings of an individual animal. Results were verified by applying the Ceditest NSP FMDV to all the serum samples of animals that were scored as suspicious or positive at any bleeding time by 3ABC ELISA CEVAN; no reactors were detected. In addition, no reactors were detected by Ceditest NSP FMDV in serum samples 30 dpv of group 4 and in the non vaccinated group.

4. DISCUSSION

FMD control in most South American countries relies on systematic vaccination of cattle -once or twice a year- movement control, differential diagnosis with other vesicular diseases and serosurveillance to monitor antibodies to NSP in the susceptible population. In South America, specific strategies are being implemented in areas with a high level of risk in which vaccination of all susceptible domestic livestock is practiced. This study provides information regarding vaccination strategies in sheep and lambs with MDA gained by using a commercial polyvalent oil adjuvanted vaccine currently used in the vaccination campaign in Argentina (120 million doses per year during the last 5 years).

The safety of the vaccine was demonstrated in vivo in lambs and sheep as no side effects - local or systemic - were observed after vaccination in any animal. With regards to the purity of antigens formulated in the vaccine, previous work has demonstrated a sufficient level of purity based on the lack of seroconversion to NSP in cattle after repetitive vaccinations and under field conditions (Matton et al., 2004, Espinoza et al., 2004). In this study, no detectable NSP antibodies were found in sera from vaccinated and revaccinated sheep. Therefore, the use of this immunogen in sheep in addition to cattle will not interfere with the surveillance programs for the detection on NSP and recognition of FMD free status. Previous challenge studies performed in vaccinated sheep have shown protection against clinical disease and viremia when sheep had an antibody titre ≥ log₁₀ 1.3 (VNT) at 10 dpv (Parida et al., 2008); approximately ≥ log₁₀ 1.3 (VNT) at 14 dpv (Barnett et al., 2004); ≥ log₁₀ 2.45 (LPBE) at 90 dpv (Fondevila et al., 1993) and ≥ log₁₀ 2.3 (LPBE) at 21 dpv (Deghaidy et al., 2002). However, it is difficult to directly compare titres obtained in different locations and this is particularly true for the VNT when different cells are used. In our study, protective levels were observed in adult sheep one year after a single dose (mean group VNT titre of log₁₀ 1.35 and mean group LPBE titre log₁₀ 2.8), suggesting a long lasting duration of immunity which is of particular relevance in areas with difficulties in moving animals for booster doses. However, when a higher protection level is required in high risk situations, a second dose given 10 to 30 days after primary vaccination assures a high flock immune status is achieved within a short period. Further studies are necessary to determine the antibody levels required in sheep to confer protection against clinical signs, reduce viremia and prevent transmission of relevant FMDV strains.

Lambs born to non vaccinated sheep responded as adults against FMD vaccination. This observation was in line with previous reports (Terpstra and Dekker, 1996, Culiffe and Graves, 1970). Half-lives of MDA of 15-17 days found in this study were slightly shorter than the values calculated by other authors. Culiffe and Graves (1970) reported a mean value 22,4 days (range of 12.2 to 29.7 days) for different FMDV strains.

Although vaccination of lambs with high MDA titres produced a high antibody response on average, individual differences were observed. In lambs with high MDA titres (>log₁₀ 3.00), there was a slightly impaired response at 30 dpv; however, the biological impact of this suppression is negligible as protective levels of antibodies persisted during the whole trial.

MDA in lambs stayed at or above protective levels (log₁₀2.30) for up to 80 days of age. Vaccination of lambs at 30, 60 or 90 days of age in the presence of MDA induced a protective response that persisted at least up to 150 days of age.
In this regard, an increase of mean titre was observed in all lambs with MDA after vaccination. In contrast, a previous study in calves with MDA vaccinated at 20, 30 or 40 days of age showed a decline in the mean group titres up to 90 dpv, even though half of calves had protective antibody levels (Späth et al., 1994). Differences between immune responses of calves and lambs with MDA could provide insights in the role of colostrum in the regulation of the immune system and the mechanisms of active immunisation in young ruminants.

Interestingly, both lambs (with or without MDA) and adult sheep reached high antibody levels at 60 dpv. Moreover, these levels were higher against both FMDV strains than those detected in cattle at the time of the official batch release potency test of the two vaccine batches used in this study (differences ranged from log_{10} 0.45 to 0.89; data not shown).

Other correlates of protection such as gamma interferon, IgA in probang samples or saliva need to be examined in further vaccination/challenge studies to characterize the protective immune response induced after vaccination of sheep.

In conclusion, these results indicate that a vaccination scheme consisting of one or two doses for adult sheep and a single dose to lambs at any age (with or without MDA) should be encouraged in order to achieve and maintain sufficient herd protection levels and prevent further spread of the disease.

5. CONCLUSIONS

One dose of oil adjuvanted vaccine induces a duration of immunity in adult sheep of at least one year.

A booster effect is observed shortly after revaccination at 10, 20 or 30 days post primo vaccination. Lambs 30-90 days old with MDA respond to FMD oil vaccine and produce high levels of antibodies that persist at least for 4 months.

6. RECOMMENDATIONS

One or two doses annually in adult sheep is recommended for systematic vaccination programs.

Two doses of oil vaccine in adult sheep, 10-30 days apart, is encouraged in order to enhance herd protection in a high risk situation.

Vaccination of young lambs with high levels of MDA is beneficial as this induces an effective immune response.

7. ACKNOWLEDGEMENTS

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8. REFERENCES


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**Figure 1:** Breeding cycle and vaccination/bleeding schedule in adult sheep and lambs
Figure 2: Mean group LPBE antibody titres (log 10) following vaccination (Group 1-1 dose) and revaccination at 10 days (Group 2-rv 10), 20 days (Group 3-rv 20) or 30 days (Group 4-rv 30) after primary vaccination. Open circles indicate non vaccinated control group.
Figure 3: Neutralizing antibody titres (log 10) following vaccination (Group 1-1 dose) and revaccination at 10 days (Group 2-rv 10), 20 days (Group 3-rv 20) or 30 days (Group 4-rv 30) after primary vaccination. Open circles indicate non vaccinated control group.

Figure 4: Decay of maternally derived antibodies in lambs born to vaccinated ewes, as measured by LPBE to A Arg 2001 (open circles) and O1 Campos (solid squares) FMDV strains.
Figure 5: Immune response in lambs following vaccination as measured by LPBE (mean group titres log 10+SD). Arrows indicate vaccination. Open circles indicate naïve control sheep. Open squares indicate decay of colostral antibodies. A. Lambs born to vaccinated sheep (G-30) and non vaccinated sheep (w/o MDA vacc 30) vaccinated at 30 days of age. B. Lambs born to vaccinated sheep vaccinated at 60 days of age (G-60) C. Lambs born to vaccinated sheep (G-90) and non vaccinated sheep (w/o MDA vacc 90) vaccinated at 90 days of age.
**Figure 6:** Effect of maternally derived antibodies (MDA) on primary response. Y axis: Difference between LPBE titres (log$_{10}$, A Arg 2001) at 30 dpv and day 0 (30 days of age, G-30 group). X axis: MDA titres at day 0