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Influence of antibodies transferred by colostrum in the immune responses of calves to current foot-and-mouth disease vaccines

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ABSTRACT

Immunity to currently used oil-adjuvanted inactivated vaccines against foot-and-mouth disease virus (FMDV) has been studied in detail in adult animals; however, the influence of maternally derived antibodies transferred through colostrum (Mat-Abs) in the immune responses of vaccinated calves is less clear. Here, we report the anti-FMDV humoral responses elicited in calves with or without Mat-Abs that received one or two doses of the current tetravalent oil-adjuvanted commercial vaccine used in Argentina. Anti-FMDV (O1/Campos strain) antibodies (Abs) were evaluated by Liquid Phase Blocking ELISA (LPB-ELISA), virus neutralization test (VNT), isotype ELISA (IgG1, IgG2 and IgM) and avidity ELISA, to allow for the first time a more detailed description of the humoral responses elicited. Our results show that primary IgM responses to FMDV vaccination only became evident as Mat-Abs titers decreased. Likewise, prime and boost vaccination schedules, applied 35 days apart to groups of calves with high or low levels of Mat-Abs, showed that the levels of preexisting neutralizing Mat-Abs prevented the loss of total Abs measured by LPB-ELISA but negatively interfered with the induction of virus neutralizing responses. Altogether, these findings indicate that comprehensive serological characterization of immune responses generated after vaccination in calves may reveal important information on the actual effectiveness of vaccination strategies for young animals, particularly in endemic settings.

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1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious acute vesicular viral disease that affects cloven-hoofed animals. FMD virus (FMDV) belongs to the genus *Aphthovirus* in the *Picornaviridae* family and includes seven serotypes, O, A, Asia, C, and SAT-1, -2, and -3 [1,2]. The circulation of FMDV in susceptible livestock imposes severe restrictions on the movement and trade of animals and derived products, inflicting serious economic damages to the affected countries and regions [3–5].

FMD is endemic in many parts of Asia, Africa, and South America and vaccination of susceptible populations is a widely used method to prevent incursions of the virus in FMDV-free regions. Since immune responses generated by FMD vaccines are strain-specific, many commercial formulations contain more than one virus strain [6]. The current vaccine in Argentina is oil-adjuvanted and contains four FMDV strains of latest regional circulation: O1/Campos/Brazil/58 (O1/Campos), A24 Cruzeiro/Brazil/55 (A24/Cruzeiro), A/Argentina/2001 (A/Arg/01) and C3/Indaial/Brazil/71 (C3/Indaial) [7,8]. Animals older than 2 years are vaccinated once a year, while immunizations are performed every six months to calves aged up to 2 years old [8]. Consequently, calves may be vaccinated when they still have high titers of maternally derived antibodies transferred through colostrum (Mat-Abs).

Vaccine efficacy as well as surveillance of vaccine-induced immunity can be performed by serology [1]. Strain-specific antibody (Ab) titers obtained with Liquid Phase Blocking ELISA (LPB-ELISA) and virus neutralization test (VNT) have been statistically correlated to *in vivo* protection to assess vaccine potency and herd immunity through a percentage of expected protection (EPP)[9–11].

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Vaccines based on inactivated viral antigen are usually capable of inducing T-cell responses but not humoral responses in competent young individuals in the presence of Mat-Abs [12]. This can be disadvantageous for those diseases, like FMD, in which protection correlates to the presence of specific neutralizing Abs [13–15].

Published data from field application of FMDV commercial vaccines and other experimental immunization studies in calves were mostly performed during 80s and 90s [16–19]. These reports utilized different serological assays to measure anti-FMDV humoral responses induced by vaccines formulated with virus strains and adjuvants no longer used, thus hindering comparison and limiting their current application. Some of these authors found that the vaccine effectiveness was not affected by the presence of Mat-Abs when oil-based vaccines were used [17,19,20].

Here, we studied the magnitude and kinetics of the Ab immunity induced by primary FMD-vaccination or by a prime-boost schedule in calves with different levels of Mat-Abs. We analyzed Ab responses in terms of neutralizing capacity, isotypes induced (IgG1, IgG2 and IgM), avidity of FMDV-specific Abs and total Ab titers calculated by LPB-ELISA. This study provides new insights on the immunogenicity of current vaccines when they are applied in the presence of Mat-Abs. Our results demonstrate that the presence of Mat-Abs mainly interferes with the induction of FMDV-neutralizing Abs and IgM-driven primary responses, without greatly affecting total anti-FMDV Ab titers.

2. Materials and methods

2.1. Animals and sampling

Calves used in this study were a crossbred Jersey/Holstein, born to multi-vaccinated cows or heifers (last vaccination ~6 month before calving) raised in a dairy farm located in Tandil, Buenos Aires, Argentina. They were fed according to a frequent practice in dairy farms of this region. Newborn calves were separated from their dams and fed with a total volume of 4 l of colostrum divided into two administrations (of 2 l each) given at 6 and 12 h after birth. Colostrum used in both rations was a pool of colostrum obtained from dams that had calved during the course of the same day. Colostrum donor dams had received mandatory FMDV-vaccination at least 3 times and the last vaccination was applied 6 months before calving. Serum samples were obtained from the jugular vein using Vacutainer® (BD, Franklin Lakes, NJ) tubes and needles (21G).

2.2. Experimental design

2.2.1. Experiment I

Fifteen colostred non-vaccinated calves, aged 18–49 days-old at the beginning of the experiment, were randomized in 4 experimental groups (Table 1). Calves in group 1A were left unvaccinated while groups 1B, 1C and 1D were vaccinated once at about 1, 3 and 5 months of age, respectively [21].

2.2.2. Experiment II

Twenty-five colostred non-vaccinated calves were selected based on their FMDV-O1/Campos serum LPB-ELISA titers. Group 2A consisted of animals with undetectable titers (<1.3) while group 2B comprised animals with titers >2.9 (EPP = 96%) [10]. Calves in both groups were vaccinated twice, 35 days apart (Table 1). Mat-Abs extinction curves were estimated as described in Section 2. Half-lives of Mat-Abs ranged between 17.10 and 18.36 days, depending on the serological assay (Supplementary File).

2.3. Vaccines

We used officially approved commercial tetravalent single-oil emulsion vaccines containing inactivated FMDV strains A24/Cruzeiro, A/Arg/2001, O1/Campos and C3/Indaial (Bioaftogen®, manufactured by Biogenesis-Bagó). The vaccine was applied subcutaneously in the neck (2 ml/dose) according to the manufacturer's recommendations and official regulations [7].

2.4. Liquid-phase blocking ELISA

Total anti-FMDV O1/Campos Ab responses were assessed by LPB-ELISA performed as stated by the OIE Manual [1], using a rabbit antiserum to capture inactivated whole FMDV O1/Campos particles, and a guinea-pig antiserum as detector Ab, both of them specific for FMDV O1 Campos. Antibody titers were expressed as the reciprocal $\log_{10}$ of serum dilutions giving the 50% of the absorbance recorded in the virus control wells without serum [22].

2.5. Neutralization assay

Serum neutralizing Abs were titrated by a conventional virus neutralization test (VNT) using infective culture-adapted FMDV O1/Campos strain (10^7 $TCID_{50}$/ml) on BHK cell monolayers [1]. Neutralizing Ab titers were expressed as the $\log_{10}$ serum dilution neutralizing 50% of the virus inoculums (100 $TCID_{50}$) [23].

2.6. Single dilution avidity ELISA

Avidity assessment of specific Abs was performed as described by Lavoria et al. [24]. The avidity index (AI) was calculated as the percentage of residual activity of the serum sample after urea washing, relative to that of untreated sample: AI% = (OD sample with urea/OD sample without urea) × 100.

2.7. Isotype ELISAs

Isotype ELISAs were performed as reported by Lavoria et al. [24], using HRP-conjugated Abs anti IgG1 (1:750), IgG2 (1:750) and 1gM (1:500) (AbD Serotec). Serum samples were run in two-fold serial dilutions starting at 1:50. Titers were expressed as Log_2 dilution^{-1} reaching the cut-off value (OD = 0.2; Lavoria et al. [24]).

2.8. Data analysis

The "expected protection percentage" (EPP) was used to estimate and quantify protective vaccine-induced responses. The EPP represents the correlation between Ab titers at 60 days post-vaccination (dpv), measured by LPB-ELISA or VNT, and the percentages of protection achieved for the same groups of animals after *in vivo* challenge experiments, performed at 90 dpv following the "protection against generalized foot infection" (PGP) test [1,9,10]. Titers corresponding to EPP values ≥75% (EPP-75%) for the FMDV O1/Campos strain are 1.66 for VNT [9] and 2.11 for LPB-ELISA [10].

To estimate extinction curves of Mat-Abs measured by each technique, serum samples from calves from group 1A and calves from group 1D sampled up to 150 days of age were used (Table 1). Time-course titers obtained by VNT, LPB-ELISA, and IgG-subtype ELISAs were plotted and each curve was subjected to linear regression analysis. Analysis of covariance (ANCOVA) showed that individual slopes were not statistically different from each other; therefore it was possible to calculate an average slope for the values obtained by the different assays. Average half-life (days) was calculated as Log_{10} (1/2)/slope (data in Supplementary File).

Table 1
Experimental designs I and II.

Experimental design	Group	*n*	Vaccinations	Age-range at 0 dpv (days)	LPB-ELISA titer at 0 dpv (Log_{10})	Bleeding schedule (dpv)
I	1A	3	0	–	–	0, 56, 112, 168
	1B	4	1	20–44	3.46 ± 0.25	0, 7, 14, 28, 56, 112, 168
	1C	4	1	74–88	2.54 ± 0.15	0, 7, 14, 28, 56, 112
	1D	4	1	130–148	2 ± 0.29	0, 7, 14, 28, 56
II	2[a]	13	2 (0, 35 dpv)	165–215	<1.3	0, 7, 21, 35, 55, 120
	2B	12	2 (0, 35 dpv)	14–42	3.38 ± 0.01	0, 7, 21, 35, 55, 120

LPB-ELISA titers are expressed as geometric mean ± SEM.

In experiment I, results between different experimental groups were compared by ANOVA 2-factor repeated measures followed by Bonferroni multiple comparisons test. In experiment II, Mann-Whitney test was used. The confidence interval was 95%. Statistical analyses were carried out using GraphPad Prism v5.0 (GraphPad Software).

3. Results

3.1. Experiment I: FMD-colostred calves of different ages that received a single vaccination

Calves from groups 1B, 1C and 1D were vaccinated at about 1, 3 and 5 months of age (Table 1) as stated above, and followed over time to determine humoral responses against O1/Campos. LPB-ELISA results from group 1B revealed that after a single vaccination (30 days old, arrow-white circle; Fig. 1A) Ab titers slightly fell bellow the EPP75% threshold at 60 dpv (∼90 days old) and from then on, LPB-ELISA titers did not decay and were maintained around similar levels throughout the study, close to 2.11 (EPP75%). At 165 dpv (198 days old), LPB-ELISA titers were significantly higher ($p < 0.05$) from those measured in non-vaccinated calves (group 1A). Antibody titers were induced by vaccination in animals from groups 1C and 1D (Fig. 1A). Calves in group 1C had LPB-ELISA titers above the EPP75% (2.54) at the time of vaccination (86 days old) which were maintained within similar levels until the end of the experiment (significantly higher than group 1A from 142 to 198 days old, $p < 0.05$). In group1D (mean LPB-ELISA titers = 2 at 0 dpv, 142 days old) LPB-ELISA titers increased until 14 dpv and were maintained within similar levels until the end of the experiment (titers higher than group 1A from156 to198 days of age, $p < 0.05$).

Antibody avidity followed similar kinetics curves in groups 1A and 1B, even though calves from group 1B were vaccinated (Fig. 1B). In this group, avidity became higher than group 1A only at the end of the experiment (198 days old). Group 1C accompanied Mat-Abs avidity decay from the day of vaccination and up to 56 days later (142 days old), when Ab avidity improved to approximately 50% at 198 days old (112 dpv). Avidity remained higher than that of non-vaccinated calves (1A) after 7 dpv (149 days old) in group 1D (Fig. 1B).

IgG1 and IgG2 titers and kinetics in group 1B were comparable to that of group 1A (Fig. 1C and D). IgG1 and IgG2 mean titers in animals from group 1C were higher than those of group 1A at 112 dpv (196 days old) ($p < 0.05$). Group 1C IgG1 titers (Fig. 1C) remained above 640 ($Log_2 = 9.3$) throughout the experiment, while IgG2 titers (Fig. 1D), which at the time of vaccination were at least 10 times lower than IgG1 titers, increased after vaccination. In group 1D, IgG1 titers were already significantly higher than those in group 1A at 28 dpv (170 days old) while this difference was first observed for IgG2 only at 56 dpv (196 days old; $p < 0.05$).

Neutralizing activity of anti-FMDV Abs was negatively influenced by vaccination in the presence of high levels of Mat-Abs (Fig. 1E, group 1B). VNT titers decreased after vaccination with a slope matching that of non-immunized animals until 58 days of age (28 dpv) and then fell sharply to undetectable levels when calves where about 86 days old (56 dpv). Calves in group 1C elicited a slight increase in the neutralizing titers soon after vaccination. Despite this, neutralizing Ab titers in this group followed a continuous decay, remaining within undetectable or low levels (∼1.3) from 142 days of age and up to the end of the experiment, when titers were not statistically different from those measured in group 1A ($p < 0.05$) (Fig. 1E, group 1C). Animals from group 1D elicited neutralizing Abs shortly after vaccination. Mean neutralizing titers surpassed 1.66 (EPP75%) at 7 dpv and remained constant thereafter (Fig. 1E).

IgM responses followed similar kinetics than VNT titers (Fig. 1F). In the presence of high level of Mat-Abs, IgM titers were indistinguishable from those of colostred non-vaccinated animals. As Mat-Abs decayed, IgM peak increased revealing the induction of genuine primary responses in the vaccinated calves.

3.2. Experiment II: calves with high or undetectable levels of maternal antibodies that received two vaccinations

Efficacy of single vaccination seemed to be interfered by the presence of Mat-Abs. In order to study if this inhibition could be counteracted by a second vaccine dose, a group of 25 calves FMD-colostred calves were selected based on their FMDV-O1/Campos serum LPB-ELISA titers (Table 1). Animals in group 2A responded with a marked increase in total Ab titers and only one vaccination was enough for all the animals to surpass the EPP75% threshold at 21 dpv (mean titer 2.5). In fact, Ab kinetics continued rising almost 10 times after the second vaccination (Fig. 2, upper panel, 55 dpv). Although the Ab titers decreased at 120 dpv, they were above the EPP75%.

Total Abs in group 2B followed a decreasing kinetics after the first vaccination, concordant with the estimated Mat-Abs extinction curve (dotted curves). Mean LPB-ELISA titer slightly changed after the boost, becoming significantly different from its expected decay only at 120 dpv ($p < 0.05$). At that time point, mean LPB-ELISA titer was 2.43, meaning that calves with Mat-Abs vaccinated twice maintained Ab levels above the EPP75% threshold [10]. However, LPB-ELISA titers in group 2B were significantly lower than those in group 2A after the booster ($p < 0.05$).

Titers obtained by VNT had similar kinetics compared to those of LPB-ELISA for group 2A. However, VNT titers in group 2B declined regardless of vaccination following similar kinetics to that of the expected extinction curve for Mat-Abs (Fig. 2, lower panel). From 55 dpv and up to the end of the experiment, neutralizing Ab titers in group 2A significantly outperformed those from group 2B ($p < 0.5$).

Calves from group 2A responded to both vaccinations with an increasing kinetics of IgG1 and IgG2 titers (Fig. 3A and B). While IgG2 titers were maintained over time, IgG1 titers decayed from 55 to 120 dpv. In group 2B, IgG1 titers were higher than IgG2 at 0 dpv. After vaccination, IgG1 and IgG2 curves (group 2B) had similar kinetics to that expected for the decay of Mat-Abs (Fig. 3A, dotted line). However, isotype levels detected after the second dose were

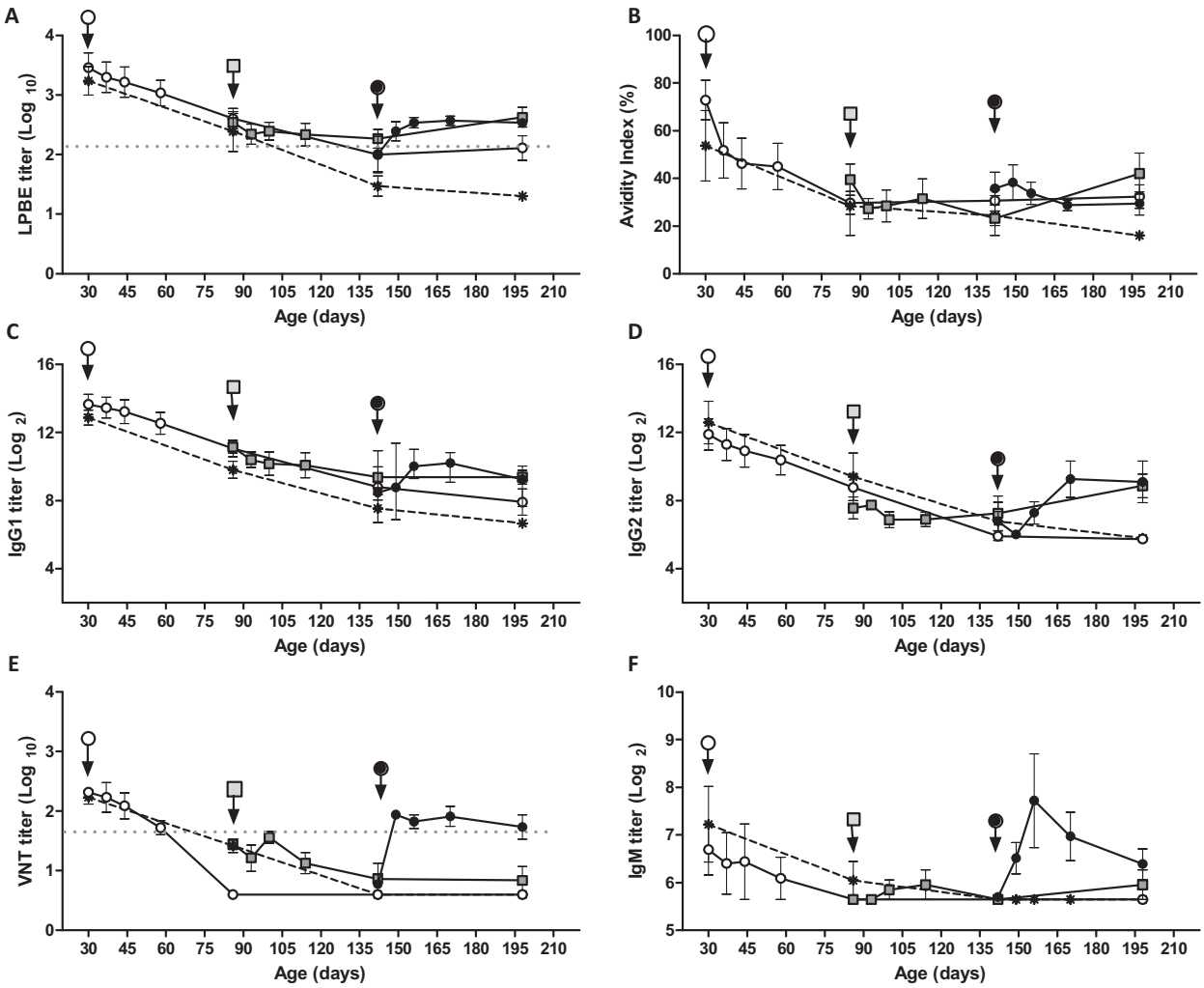

Fig. 1. Serum titers (geometric mean ± SEM) measured by LPB-ELISA ("LPBE", Log_{10} titer (A) avidity index (B), isotype ELISAs (Log_2 titers): IgG1 (C), IgG2 (D), IgM (F) and VNT Log_{10} titers (E), against FMDV O1/Campos in vaccinated and unvaccinated colostred calves of experiment I. Arrows indicate the time of vaccination. Grey dotted lines indicate the EPP75% threshold for LPBE and VNT (O1/Campos strain). (–) Group 1A, unvaccinated calves ($n = 3$). Calves vaccinated once at: 1 months of age (○) group 1B ($n = 4$); 3 months (□) group 1C, $n = 4$; and 5 months (•) group 1D, $n = 4$.

significantly different between groups 2A and 2B at 55 dpv ($p < 0.05$) for both isotypes, and at 120 dpv ($p < 0.05$) for IgG2.

Antibody levels were low in group 2A at 0 and 7 dpv, not allowing the estimation of the avidity indexes. In this group, avidity indexes reached the 24.5% threshold proposed for protection at 21 dpv [25]. These values improved to over 50% at 35 dpv and remained about the same level after the booster. Antibody avidity in group 2B, however, decayed gradually until animals received the second vaccination (35 dpv). From this moment on, the avidity of specific Abs increased and remained above 50% until the end of the experiment (Fig. 3C).

3.3. Immune status of responders and non-responders to booster vaccination

Analyzing individual data from group 2B, we found that there were animals that responded positively to the boost while others did not. For example, only 3 of the 13 animals surpassed the EPP75% level for O1/Campos VNT titers (≥1.66) at 55 dpv. We then categorized the animals in group 2B according to their response to the booster in terms of neutralizing (Fig. 4A) and total Abs (Fig. 4B). A positive response was considered when the slope of the Ab curve between titers at 35 vs. 55 dpv was greater than 0. These animals were called "responders"; while we designated "non-responders" those that either maintained or reduced their titers after the boost.

Four animals (group 2B) that increased their VNT titers after revaccination (responders) had average VNT titers at 0 dpv significantly lower than the other 9 calves that did not raise their VNT titers (non-responders). The same was observed at the time of the booster (35 dpv). Interestingly, these differences between titers at the time of vaccination were not evident when assessing total Abs (LPB-ELISA titers) both at 0 and 35 dpv (Fig. 4B).

Our data shows that calves that increased their titers to booster vaccination always had lower VNT titers than non-responders at the time of vaccination, while these differences could not be appreciated when measuring total Ab levels.

4. Discussion

In this report, we evaluated how the presence of Mat-Abs influenced the development of humoral responses in FMD-vaccinated calves in terms of total and neutralizing Abs, currently used as correlates of protection [10,13,14]. Antibody isotype and avidity were also determined to further characterize the immunity developed. We worked with two experimental groups to get an overview of immune responses elicited after one or two vaccinations in the presence or absence of Mat-Abs.

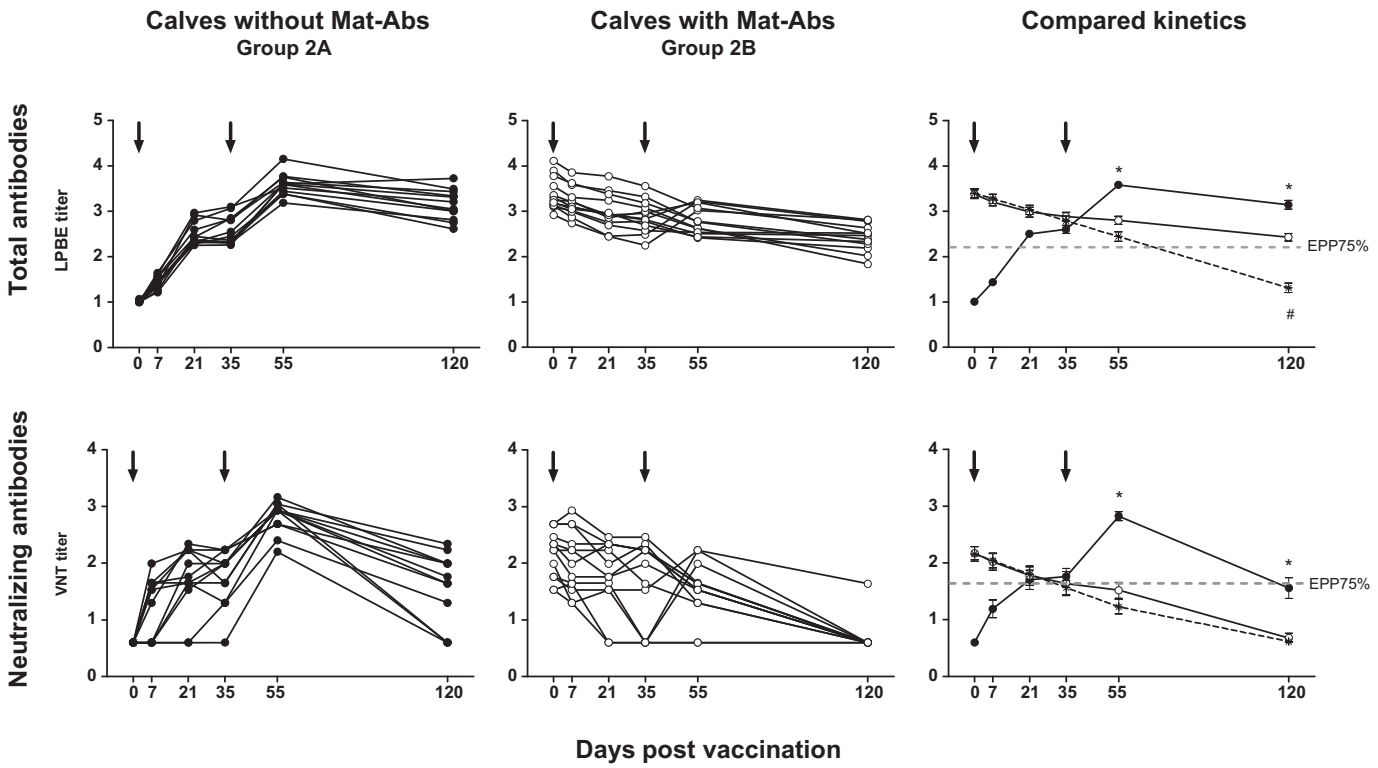

Fig. 2. Kinetics of LPB-ELISA serum titers ("LPBE", upper panel) and VNT titers (lower panel) against FMDV O1/Campos in calves with (◯) or without (•) Mat-Abs that were immunized twice with FMDV tetravalent vaccine. Arrows indicate the time of vaccination (days 0 and 35 post vaccination). Data are shown as individual curves (left and central panels) and also as compared kinetics (geometric mean $\log_{10}$ titers ± SEM, right panel) where titers for LPBE and VNT related to an EPP = 75% are shown. *Significant differences between groups 2A and 2B, $p < 0.05$. #Significant differences between groups 2B and the expected Mat-Abs decay titer, $p < 0.05$.

Early studies showed that calves over 30 days of age, or even younger, responded as adults to FMD-vaccination [17,26], however, the influence of the age in the immune responses of calves is still controversial [27]. In our regional epidemiological situation age and Mat-Abs titers are always linked, so considering them together reflects our field situation.

In the first set of experiments, a group of colostred calves born to immune dams received a single vaccination at different ages. FMD-specific total antibody titers were marginally affected by the presence of Mat-Abs at the time of vaccination as demonstrated by their practically constant levels throughout the experiments for all three vaccinated groups. Even in calves vaccinated at ~30 days of age, LPB-ELISA titers were maintained close to the EPP-75% after 60 dpv, as reported by Spath et al. in animals of the same age and equivalent colostral titers [19]. Conversely, neutralizing responses induced after vaccinations were hampered by the amount of circulating Mat-Abs.

Our results show that IgG1, IgG2 and IgM levels increased as Mat-Abs titers decreased (Fig. 1) even though we cannot rule out, by measuring serum Abs, that detection of *de novo* responses may be masked by the presence of high levels of high avidity Mat-Abs. Nevertheless, the observation that neutralizing activity and avidity maturation of anti-FMDV Abs were effectively interfered by Mat-Abs over time supports the idea of impairment on the generation of FMDV-specific humoral responses.

Progression of IgM titers followed that observed for the neutralizing Abs. In line with these results, a previous work from our laboratory demonstrated that at short times after infection, IgM is

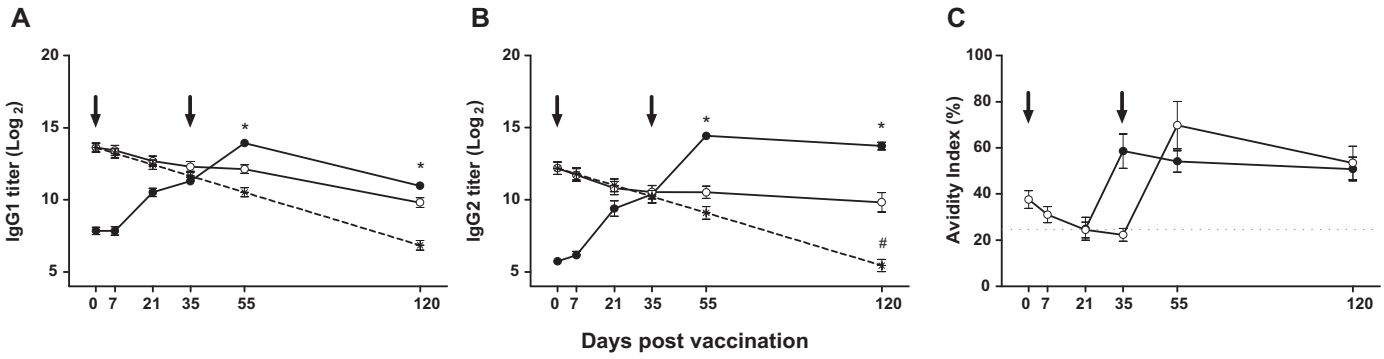

Fig. 3. Compared kinetics of mean IgG1 titers (A), IgG2 titers (B) and avidity indexes (C) of Abs against FMDV O1/Campos in calves with (◯, $n = 12$) or without (•, $n = 13$) Mat-Abs that were vaccinated at 0 and 35 dpv (indicated by arrows). Dotted-lines of (A) and (B) depict the expected decay of IgG1 and IgG2 Mat-Abs. Titers are expressed as geometric mean titers Log_2 ± SEM. Avidity is expressed as mean avidity index (%AI ± SEM). Dotted grey line of (C) indicates the 24.5% avidity threshold. *Significant differences between groups 2A and 2B, $p < 0.05$. #Significant differences between groups 2B and the expected Mat-Abs decay titer, $p < 0.05$.

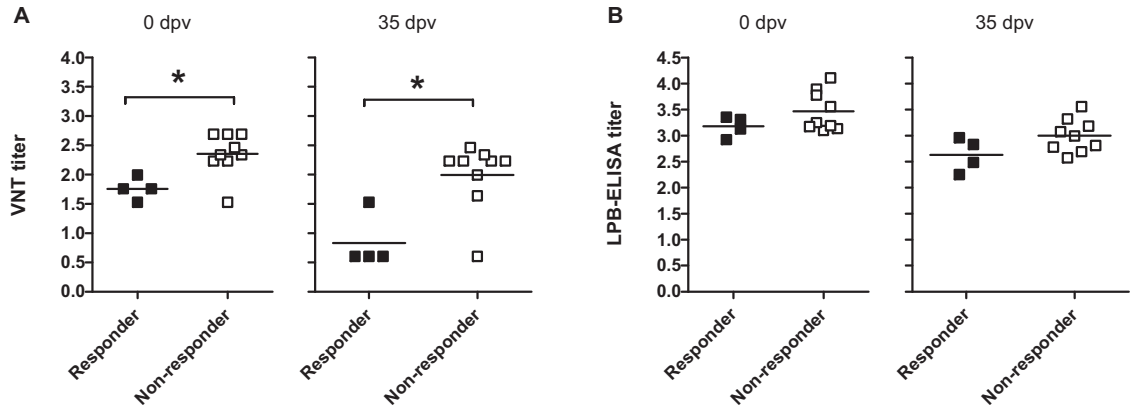

Fig. 4. Calves with Mat-Abs from experiment II (group 2B) were categorized according to their capacity to increase Ab titers after booster vaccination (measured at 55 dpv). Graphs show VNT (A) and LPB-ELISA (B) titers at the time of the first vaccination (0 dpv) and the day of the booster (35 dpv) in both responder and non-responder calves. *Significant differences, $p < 0.05$.

the predominant isotype and accounts for most of the neutralizing activity [28], although differences can occur when comparing responses against an inactivated antigen.

Results from the second experiment, using calves with undetectable (group 2A) or high levels (group 2B) of total anti-FMDV Abs subjected to a vaccination-revaccination schedule reinforce the idea that VNT reveals Mat-Abs interference better than LPB-ELISA. Experimental data supporting such conclusions are: (i) the different outcome after revaccination between groups 2A and 2B, despite the existence of comparable levels of mean Ab titers (measured by all techniques at 35 dpv), and (ii) individual data analyses which showed that booster-responders within group 2B had lower VNT titers than non-responders at the time of vaccination and revaccination, while total Ab levels were irrelevant. However, and probably due to the small number of animals considered, we cannot rule-out the influence that the first vaccination had on the humoral response after the booster.

Publications studying the interference of Mat-Abs in FMD-vaccinated calves only used either LPB-ELISA or VNT separately [16–20]; in this study these aspects of humoral immunity and others (isotypes, avidity) were assessed altogether for the first time. The different conclusions obtained when analyzing data either by VNT or LPB-ELISA, indicate that further adjustment of the different serological techniques might be needed, preferably supported with challenge tests, for this particular age group.

Vaccination in the presence of Mat-Abs creates a particular scenario. We showed that high levels of high avidity IgG (mainly IgG1) FMDV-specific Mat-Abs are passively transferred to calves. Mat-Abs probably diminishes the arrival of sufficient amounts of antigen to the draining lymph-nodes as to activate immune cells; consequently primary responses are poorly elicited [29]. As Mat-Ab levels decay, chances of free antigen to encounter naïve B-cells increase. This is revealed by the capacity of the vaccine to induce IgM-driven responses. Conversely, those animals without Mat-Abs at the time of primo-vaccination are able to induce memory B-cells clones which may be expanded by the revaccination even in the presence of neutralizing Abs, as they can be stimulated by low amounts of free viral antigen. Moreover, those animals receiving the primary vaccination in the presence of Mat Abs but reaching the revaccination time-point with low levels of neutralizing Abs were able to raise their titers after the booster.

An intriguing observation in this study was the fact that particularly neutralizing responses were abrogated when vaccinating calves carrying high levels of Mat Abs. It can be speculated that the presence of high levels of neutralizing Abs may block the availability of neutralizing epitopes that can be recognized by naïve B-cells, thus only those B cells reactive to non-neutralizing epitopes can be activated. However, no direct evidence of such mechanisms has been reported.

Assuming an association between IgG1 and FMDV-neutralizing activity [21,25,30], another explanation can be an isotype-specific colostrum-associated suppression of Ab production, already described in calves [31–34]. Later studies have indicated that colostrum feeding actually reduces the number of immunoglobulin-positive cells in the lymphoid tissues of newborn calves in an isotype-specific manner [35]. Again, further fit-for-purpose experiments are required to substantiate such possibility.

To summarize, our results show that the presence of high levels of Mat-Abs at the time of vaccination interferes with the induction of primary and secondary neutralizing responses in calves immunized with a current inactivated oil-adjuvanted FMD vaccine. This study opens up a number of alternative hypotheses on the mechanisms underlying the immunomodulatory effects of colostrum, and the interaction of Mat-Abs during the priming of immune responses in calves.

Conflict of interest statement

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.06.056.

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