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Effect of FMD vaccination schedule of dams on the level and duration of maternally derived antibodies

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ABSTRACT

Vaccination against Foot and Mouth Disease (FMD) in pregnant cows is crucial to produce greater immunity in new born calves, especially in late gestation, as this directly affects neonatal immunity. Therefore, we aimed to investigate how late gestation FMD vaccination of pregnant cows affects the maternally derived antibodies in their offspring. Pregnant cows were vaccinated with and without booster vaccination during the 3rd months (early gestation vaccination, EGV) or the 6.5th months (late gestation vaccination, LGV). Their offspring were investigated for passive immunity transfer, maternal antibody duration, and the first vaccination age of calves (when the maternal antibody has waned sufficiently to allow the first vaccination). Antibody titers were analyzed by a virus neutralization test (VNT). A digital Brix refractometer (% Brix) was used to estimate passive antibody transfer efficiency measuring total protein (TP) content of calf blood sera and also colostrum IgG content. Two linear mixed effects models were fitted: one for the antibody titer values of the dams, and the other for the antibody titer values of calves before the vaccination. A marginal fixed effects model was also fitted to explore the effects of the dam titers on the antibody titers of the calves after their vaccinations. As a result, the average neutralizing antibody titers did not differ between the EGV and LGV groups nor were any differences detected between dams that received a booster and those that were not boosted. However, the LGV calves' mean maternally derived antibody titers were significantly higher (p-values = 0.0001 for both groups) and the duration was longer than that of the EGV calves (120 days in LGV, 60 days in EGV, $p < 0.05$). Since no statistical difference was found between the titers of either group of dams at the beginning of the experiment and parturition, it does not appear that the higher VN titers in LGV calves compared to titers in EGV are directly related to the circulating antibody levels in the dams. Furthermore, the TP value (% Brix) of calf blood sera was higher than > 8.4% in both calf groups (9.3 ± 0.33 in LGV and 8.6 ± 0.40 in EGV, $p > 0.05$) indicating that passive immunity transfer had occurred for both groups. In addition, we found that the % Brix mean colostrum IgG content of the LGV (25.8 ± 1.30) was higher than the EGV (21.8 ± 0.58) dams ($p < 0.01$) and a significant positive correlation found between the colostrum density of LGV dams and TP (% Brix) value of their offspring ($r = 0.73$, $p < 0.01$). Our results show that vaccination during the late gestation period increased the colostrum IgG content of dams of LGV in addition to the maternally derived antibody duration and potentially provided greater protection of the offspring.

1. Introduction

Foot-and-mouth disease (FMD) is a highly infectious, rapidly-spreading viral disease in many farm animals and the containment and control of disease mostly rely on vaccination (Grubman and Baxt, 2004). In Turkey, campaign vaccinations have been used to control the disease, the policy of which consists of two doses of vaccinations of adult cattle in 6 months intervals and booster application in calves

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which receive the first dose of the vaccine at 2 months of age if they consumed adequate colostrum and their mothers were vaccinated regularly. Calves are then boosted dose one month later (3 months of age) and further boosting at 6 months intervals. Thus calves receive 4 vaccinations in their first year. This vaccination scheme is described in FMD Turkey vaccine (Turvac oil) prospectus (https://vetkontrol.tarimorman.gov.tr/sap/Link/3/Asi-Prospektusu).

Newborns which are not or inadequately vaccinated result in an immunity deficit and thus the virus can circulate in the population (Morein et al., 2002; Dekker et al., 2014; Elnekave et al., 2016). This inadequate herd immunity mainly affects newborns, the most sensitive group in the population. Because of syndesmochorial placenta structure in ruminants, newborns are dependent on passively acquired maternally derived immunoglobulins, immune cells, and other substances from colostrum for protection. Immune cell content of the colostrum is mainly T–lymphocytes, which are believed to transfer immune functions and secrete cytokines. Most of the major cytokines have been identified in colostrum and milk, however, their biological effects on the neonate have yet to be determined (Barrington and Parish, 2001). The IgG concentration of colostrum is affected by many factors such as the breed of animal, lactation number, cow's age, calving season or vaccination date of dams, etc (Morin et al., 1997; Jaster, 2005; Wooulms, 2014). Therefore, it is of primary importance to reveal mother's vaccination routine for pregnancy period since it affects colostrum quality of newborn (Morin et al., 1997; Jaster, 2005; Wooulms, 2014; Dekker et al., 2016). Despite this knowledge, there are not adequate studies on how FMD vaccination schedule during pregnancy affects the quality of maternally derived antibodies (Francis and Black, 1984; Panjevic and Valcic, 1989; Dekker et al., 2016). It is generally known that vaccine administration before calving increases maternally derived antibodies (Saif et al., 1983, Murakami et al., 1985). This has been studied extensively for vaccines against neonatal diarrhea pathogens, yet data on the effects of FMD vaccination before calving is scarce (Francis and Black, 1984; Panjevic and Valcic, 1989). Although maternally derived antibodies protect newborns against diseases, they also can result in maternally derived interferences by blocking the response to the vaccine antigen administered to the offspring (Graves, 1963; Nicholls et al., 1985; Chase et al., 2008; Niewesk et al., 2014, Bucafusco et al., 2014). Thus, the optimal vaccination age for young animals is a critical question that needs to be answered.

As mentioned before, newborn calves depend on the efficient passive transfer of maternally derived IgGs from colostrum. Measurement of colostrum IgG content is crucial to determine passive transfer efficiency. The radial immunodiffusion, density measurement using a colostrometer, zinc sodium sulphate turbidity tests and the Brix refractometer are known methods to measure the colostrum IgG content. Similar to the colostrum density, calf serum IgG content is also a crucial parameter to determine passive transfer efficiency. The radial immunodiffusion assay is the gold standard method to determine passive transfer by measuring IgG in the serum (Mancini et al., 1977). Serum-refractometers are also used to measure serum IgG concentration. However, all these methods require trained persons and at least one day to determine results. Brix refractometer provides a reliant estimate of calf serum IgG concentration in recent years (Morill et al., 2013). A study (Deelen et al., 2014) reported that passive transfer efficiency can be estimated by evaluating the IgG content of calf blood serum by digital Brix refractometer. In another study (Quigley et al., 2012) digital Brix refractometer was used to estimate the IgG content of colostrum.

Vaccination of pregnant cows during late pregnancy is still controversial in Turkey due to animal owners' concern that FMD vaccination at late gestation causes abortion. FMD Turkey vaccine prospectus is indicated that vaccine can be administrated safely to pregnant cows if stress factors are eliminated, but unfortunately there is no data about the timing of vaccination during pregnancy. Hence, the goal of the present study was to measure the effects of timing FMD vaccination in dams on the level and duration of maternally derived antibodies.

2. Materials and methods

2.1. Vaccines

A current circulating (A Nep 84, Genotype VII) commercial Turkish vaccine (Turvac oil) produced by the SAP Institute, Turkey was used in this study. The potency of the vaccines was previously determined by the vaccination-animal challenge experiments described in the World Organisation for Animal Health (OIE). Montanide ISA 206 (SEPPIC, France) oil adjuvant was used to prepare double oil emulsions. The antibody cut off values were set with a provision for complete protection against 10,000 homologous infectious virus particles. A cut off titer of 1.2 (log 10) was considered as the lower threshold.

Animals

A total of 34 Holstein-Friesian pregnant cows which were 2–3 lactation ages (average ages 2.5 years) owned by a state farm in Ankara, Turkey were used in the study. All dams were vaccinated against FMD at least 6 times before the beginning of the experiment. A total of 24 of their offspring were used in the study because 10 calves were sold according to state farm rules, were kept separate in a calf paddock on the farm under regular management practices. All calves received their colostrum from their own dams during the first two days. FMD outbreaks were not reported at the farm and its surroundings. The farm has also been certified FMD-free for the last 7 years. The study was undertaken in accordance with the International Harmonization of Animal Care and Use guidelines. The study protocol was approved by the ethics committee of the FMD Institute (Ankara, Turkey) (Approval no. 2016-04). Fig. 1 shows the animal groups and number (n).

2.2. Immunization and sampling

Dams were initially assigned to two groups. EGV (early gestation vaccination), as a control group, was vaccinated during the early gestation period (three months) in accordance with Turkish field routines and consisted of 16 pregnant cows. LGV (late gestation vaccination), group was vaccinated during a later gestation (six and a half months) and consisted of 18 pregnant cows. Each main group was divided into subgroups – with and without booster vaccinations, respectively (Fig.1). Blood samples were taken every month after vaccinations from each group. Thus, there was six blood sampling time periods for EGV dams, and this period was three for LGV.

Two groups of calves were maintained to define the duration of maternally derived antibody and passive transfer efficiency. One had their mothers vaccinated in the early period of gestation–EGV-calf group (n = 12) served as the control group and the other LGV-calf group (n = 12) had their mothers vaccinated in the late period of gestation. The mother of each calf group consisted of both subgroups of dams (booster and without booster) (Fig.1), (A total of 12 LGV calves was born from LGV dams: 6 of 12 calves were from the booster and other 6 of 12 calves were from without booster LGV dams. A total of 12 EGV calves was born from EGV dams: 6 of 12 calves were from the booster and other 6 of 12 calves were from without booster EGV dams). Blood samples were collected on days 4, 14, 21, 45, and 60 after parturition in the EGV-calf group and on days 4, 14, 21, 45, 60, 90, and 120 after parturition in LGV-calf group. Sera were simultaneously tested by VNT to define the first optimal vaccination time for both groups; calf groups were vaccinated at the time when the maternally derived antibody titer was lower than the 1.2 $\log_{10}$. Hence, the LGV-calf group was vaccinated on day 120 and the EGV-calf group on day 60 after birth. Then each calf group (LGV and EGV) received a booster dose one month after the first vaccination. Blood samples were taken one month after the booster then sera were stored at −20 °C until the time of analysis.

2.3. Virus neutralization test (VNT)

Two-fold serial dilutions of heat-inactivated 50 µl serum samples

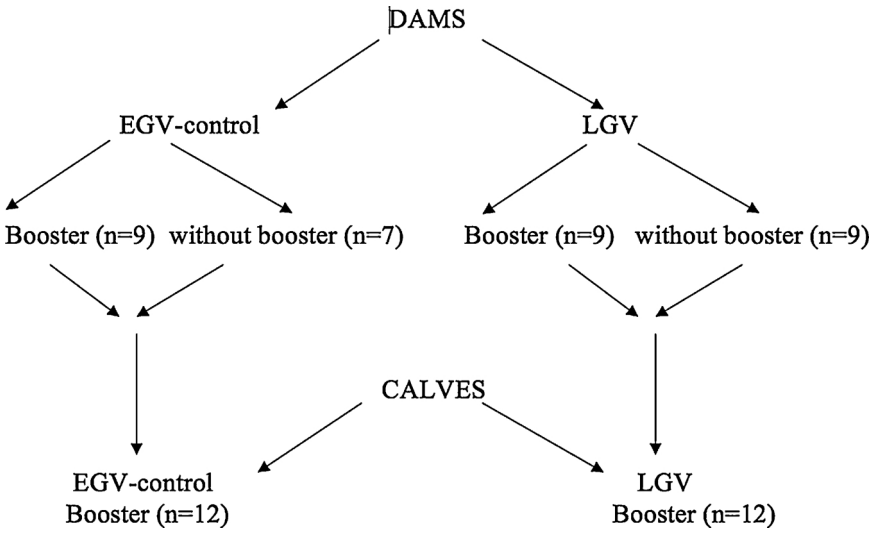

Fig. 1. Experimental design of dams and calves.

from 1:4 to 1:512 were prepared by using the Glasgow cell culture medium (Biochrome; Merck, Germany) in a microplate. Subsequently, 50 µl of a 100 $TCID_{50}$ A Nep 84 (Genotype VII) working virus suspension (same genotype with the vaccine) was added to each well. After an hour of incubation at 37 °C, 50 µl of a suspension of 6×10^5 BHK-21 cells/ml was added into each well. Then, incubation at 37 °C for 72 h, the wells were observed under a microscope to observe the cytopathic effects. The neutralizing antibody titer was expressed as the $\log_{10}$ serum dilution that neutralized 50% of the virus (OIE Manual).

2.4. Estimation of passive transfer efficiency of calves with digital Brix refractometer

The passive transfer efficiency was estimated with a digital Brix refractometer (Misco, Cleveland, OH) and the following the manufacturer's instructions (Deelen et al., 2014). The Brix results state that passive transfer did not occur if the TP value of calf blood sera on the fourth day after birth is under 8.4% Brix.

2.5. Estimation of colostrum IgG concentration with digital Brix refractometer

As above, Brix refractometry was used, and 21% Brix is to be considered to be the cut off point for high-quality colostrum (> 50 g/l of IgG) (Quigley et al., 2012).

2.6. Kinetics of maternal antibody decay

The natural logarithm values of the average maternally derived antibody titers of the unvaccinated –LGV calves were plotted against time (days) to calculate maternal antibody decay. The decay reaction was depicted to conform 1 st order kinetics. Therefore, the average half life of maternal antibodies was calculated from the slope of the linear graphic (Çokçalışkan et al., 2017).

2.7. Statistical analysis

Statistical analyses were performed in R (3.5.1) (R Development Core Team, 2018). Linear mixed models were fitted by using the lme4 package of R (Bates et al., 2015) and the p-values were calculated by using the Imer Test package (Kuznetsova et al., 2017; Luke, 2017). Two linear mixed effects models were fitted: one for the antibody titer values of the dams, and the other for the antibody titer values of calves before the vaccination. In these models, antibody titer values measured at repeated time points were taken as a response. The group, time, at which the measurement was taken (e.g. the gestation time of the dam), and the quadratic effect of the time were considered as possible fixed explanatory variables. On the other hand, each animal was taken as the random variable to allow different starting titer values for each animal. Time was also included as a random variable to allow for different slopes for each animal, but it was dropped since no significant individual differences were estimated.

A marginal fixed effects model was also fitted for explaining the antibody titer values of the calves after the vaccination. In this model, the antibody titer values were the dependent variable, while the group and time were taken as independent variables. The gee package (Carey et al., 2015) of R was used for parameter estimation of the marginal model.

Pearson correlations were used to reveal whether there was any correlation amongst the antibody titer of the dam (end of gestation), colostrum density, the antibody titer of calves and TP (on the fourth day after parturition). Student's *t*-test was used to compare % Brix TP of calves between the calf groups and % Brix colostrum IgG content between the dams group.

3. Results

3.1. Neutralizing antibody response of the dam-groups (LGV and EGV)

As shown in the Figs. 2–4, there was no significant VN antibody titer difference across time until parturition between the dams group and subgroup and no significant increase VN antibody titers after vaccinations. The output for fixed effects of the linear mixed model fitted for dams is given in Table 1. Estimate column in this table provides the point estimates of fixed effect parameters. For instance, the average titer values of dams in LGV without booster group were expected to be 0.073 unit lower than that of dams in LGV booster group, while keeping other variables in the model same. As it is clear from this table, there was no significant difference between the antibody titer values for different treatment groups (i.e., p-values for groups are < 0.05). However, time and squared time variables were significant (p-values 0.022 and 0.007, respectively). There seems to be an increase and then a decrease in the average antibody titers as the gestation time passes by. The expected titer values at a specific time point can be estimated from this linear mixed effects model for the four subgroups. For instance, for an average dam (i.e. when the random coefficient is set to 0), the expected titer values were estimated to be 2.52, 2.447, 2.5277 and 2.5137 for LGV booster, LGV without booster, EGV booster and EGV without

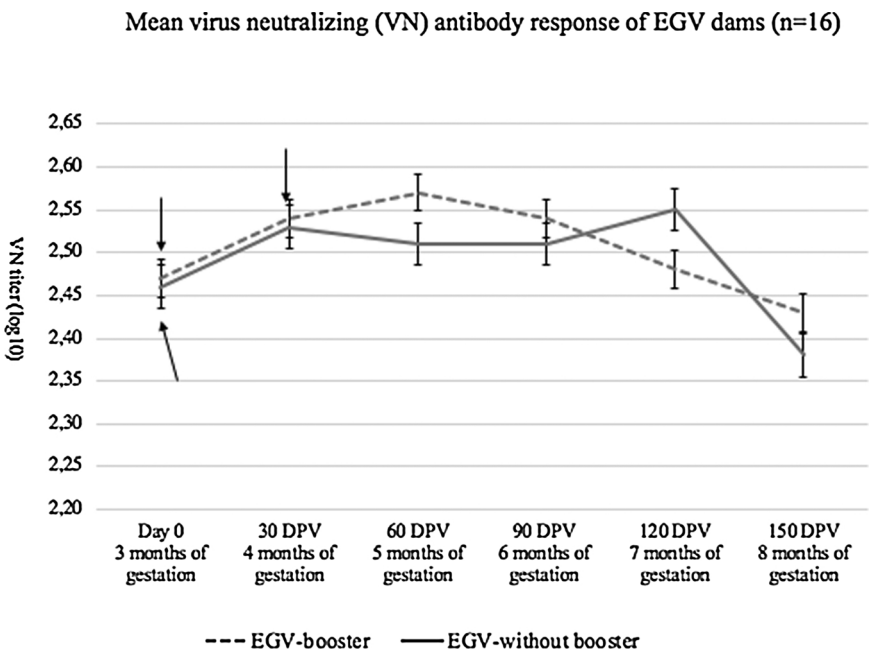

Fig. 2. Mean virus neutralizing (VN) antibody response of EGV- dams.
Data shows the mean VN antibody responses with % 95 confidence intervals ($CI_{95\%}$) during pregnancy of EGV dams (booster and without booster subgroups). Error bars represent the ($CI_{95\%}$). Vaccine administrations are indicated with black arrows. Dotted and solid lines show with and without booster subgroups. DPV (days post vaccination).

booster, respectively, at the 6.5th month of the gestation (i.e. when time = 0 for LGV groups and Time = 3.5 for EGV groups). Similarly, these expected values were 2.59, 2.52, 2.41 and 2.43, respectively, at the 8th month of gestation (i.e. when time = 2.5 for LGV groups and Time = 5 for EGV groups).

At the end of gestation, it was not determined significant VN antibody titers between the EGV and LGV dams and their subgroups. A few of the random intercepts were significant, indicating that although slight, there were only a few individual differences in the expected antibody titer values at the beginning of the experiment in both dams group (data were shown in supplementary Figs. 2 and 3). For instance, three dams (one was in LGV and two were in EGV dam groups) were expected to have lower antibody titer values at the beginning of the experiment compared to the average.

The last blood sampling time during pregnancy was different in both dams group. It was 8.5 months of gestation in LGV dams and 8 months of gestation in EGV dams (The last sampling time in EGV was in 15 days before the LGV).

3.2. Virus neutralizing (VN) antibody response of the EGV and LGV calf groups (maternally derived antibody period, before their primary vaccination)

As shown in Fig. 5 and Table 2, the mean maternally derived antibody titers against A Nep 84 (Genotype VII) were significantly (t-value= -4.66, p-value = 0.0001) higher in the LGV calves across time from day 0 to day 60 following parturition than the values for the EGV calves. The antibody titers decline significantly in both groups (p-value $< 2 \times 10^{-16}$), however the LGV calves maintained a VN antibody titer of greater than 2.0 $\log_{10}$ until at least day 45 and the levels

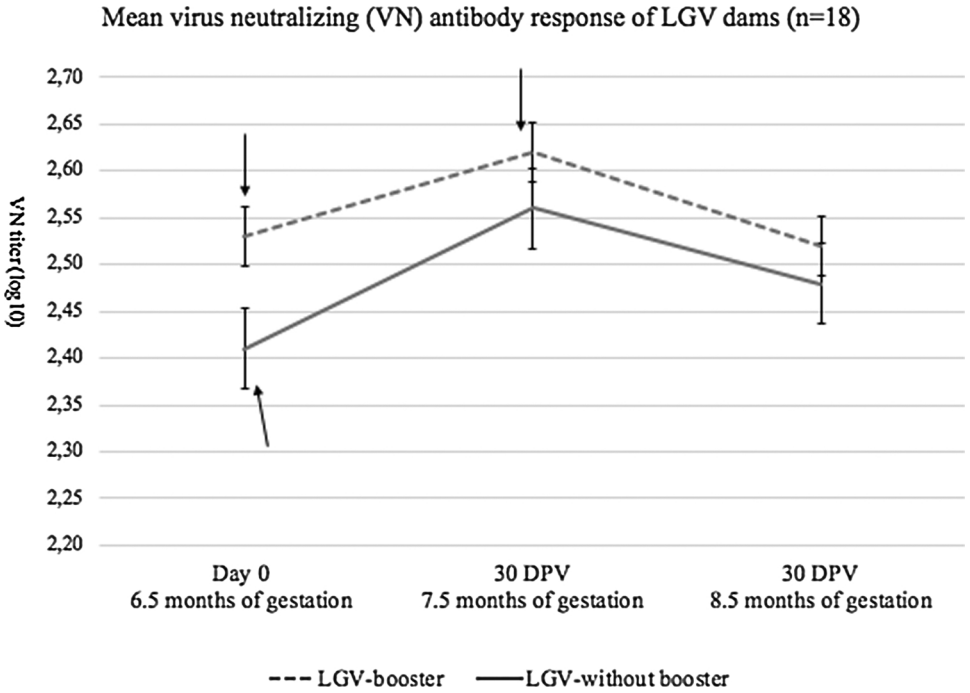

Fig. 3. Mean virus neutralizing (VN) antibody response of LGV- dams.
Data shows the mean VN antibody responses with % 95 confidence intervals ($CI_{95\%}$) during pregnancy of LGV dams (booster and without booster subgroups). Error bars represent the ($CI_{95\%}$). Vaccine administrations are indicated with black arrows. Dotted and solid lines show with and without booster subgroups. DPV (days post vaccination).

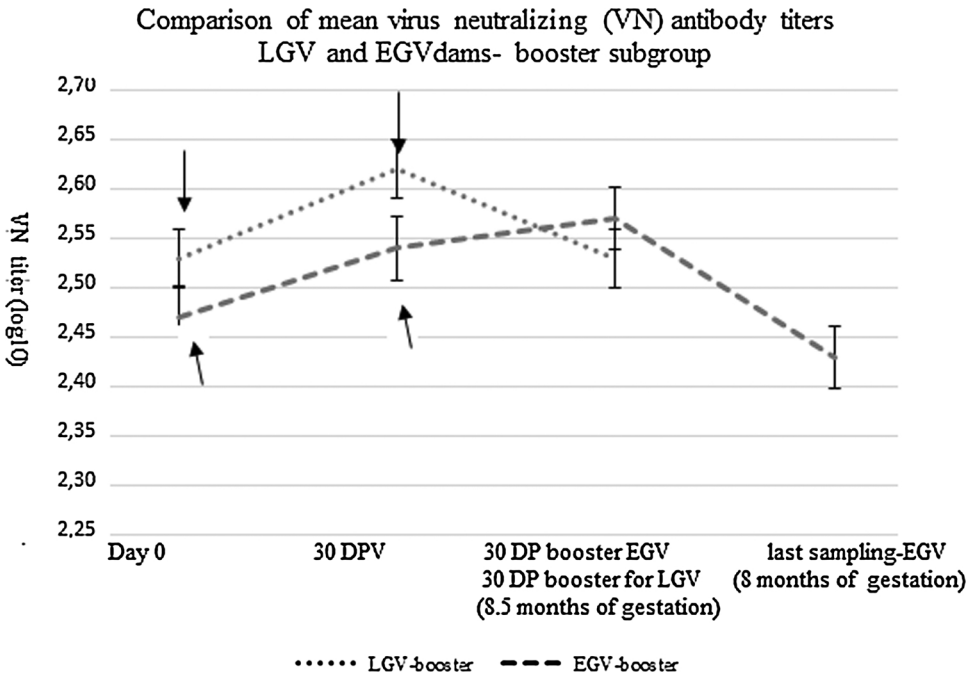

Fig. 4. Comparison of mean serum virus neutralizing antibody titers LGV and EGV dams–booster subgroups.
Data shows the mean VN antibody responses with % 95 confidence intervals ($CI_{95\%}$). Error bars represent the ($CI_{95\%}$). Vaccine administrations are indicated with black arrows. Dotted and solid lines show with and without booster subgroups. DPV (days post vaccination).

Table 1
Estimate, standard error, t-value and p- value of fixed effects for the final model fitted to dams.

	Estimate	Standard error	t-value p-value
Intercept	2.52	0.0548	$45.99 < 2 \times 10^{-16}$
LGV booster	reference		
LGV without booster	−0.073	0.071	−1.03 0.311
EGV booster	−0.032	0.069	−0.46 0.648
EGV without booster	−0.046	0.071	−0.65 0.518
Time	0.067	0.029	2.31 0.022
$Time^2$	−0.0159	0.0058	−2.73 0.007

only dropped significantly (1.0 $\log_{10}$) ($p < 0.05$) by day 90, further decreasing to a level below the cut off VN antibody titer on day 120 (0.8 $\log_{10}$). In the EGV calf group, at day 45, the titers dropped significantly (1.6 $\log_{10}$) decreasing to a level below the cut off VN antibody titer on day 60 (0.9 $\log_{10}$). Thus the duration of maternally derived antibody titers was significantly shorter for the LGV calves compared to the EGV calves (120 days at LGV and 60 days at EGV-calf groups). One calf in both groups was estimated to have smaller starting titer compared to the rest of the calves (data were shown in supplementary Fig. 5).

3.3. VN antibody response of the EGV and LGV calf groups (after the primary and booster vaccinations)

The initial mean of the VN antibody titer was 0.8 $\log_{10}$ for the LGV_calf and group and 0.9 $\log_{10}$ for the EGV_calf group (day 0). By thirty days post vaccination (dpv) these titers had increased to 1.9 $\log_{10}$ for the LGV and 1.7 $\log_{10}$ in the EGV_calf groups (p-values = 5×10^{-7} and 4×10^{-5} for LGV and EGV groups, respectively). On day 30 after primary vaccination, the calves received a booster vaccination and the titers increased further to 2.5 $\log_{10}$ in the LGV and to 2.3 $\log_{10}$ in the EGV_calf groups by day 60 following the first vaccination (p-values = 2.5×10^{-10} and 2.5×10^{-7} for LGV and EGV groups, respectively) (Fig. 6).

Exploratory analyses indicated that there were no clear differences between the individual traces of calves after the vaccination. An attempt to fit a random effects model (even a simple random intercept model) confirmed that observation, since the model resulted in overfitting. Therefore, a marginal fixed effects model was fitted to this data. While fitting a marginal fixed effects model, a correlation structure should be assumed to account for the repeated measurements taken from the same calf. Some possible structures include exchangeable, auto progressive, and unstructured correlation matrices. The choice may depend on an educational guess, based on implied structure from data. However, sandwich estimators have been proposed, which provide results that are insensitive to this correlation assumption (Diggle and Liang, 2002). Sandwich estimators from the marginal fixed effects model are given in Table 3. Moreover, the difference between the groups was smaller (β_{EGV} = -0.468 in Table 2 vs. -0.112 in Table 3), but still significant at a 10% significance level (p- value = 0.099). Hence, the expected antibody titer values were slightly lower for the EGV calf group.

3.4. Estimation of passive transfer efficiency of calves with digital Brix refractometer

Mean % Brix total protein (TP) of blood sera in the calf groups on the fourth day after parturition were higher than > 8.4% Brix (9.3 ± 0.33 in LGV and 8.6 ± 0.40 in EGV) ($p > 0.05$). Therefore, according to the study result of Deelen et al. (2014) it was proposed that passive transfer failure was not determined in both groups (Table 4).

3.5. Estimation of colostrum IgG concentration with digital Brix refractometer

% Brix mean colostrum IgG of the LGV (25.8 ± 1.30) was higher than the EGV (21.8 ± 0.58) group ($p < 0.01$) (Table 4).

Correlation amongst the VN antibody titer of dam groups (end of gestation), % Brix colostrum IgG content of dam groups, the % Brix Total Protein and the VN antibody titers of calf groups just after 4 day parturition

A positive correlation was determinate % Brix colostrum IgG content of LGV dams and TP (% Brix) value of LGV calves ($r = 0.73$; $p < 0.01$), (Fig. 7). There was no correlation between the EGV dams' colostrum IgG content and the TP value of EGV calves.

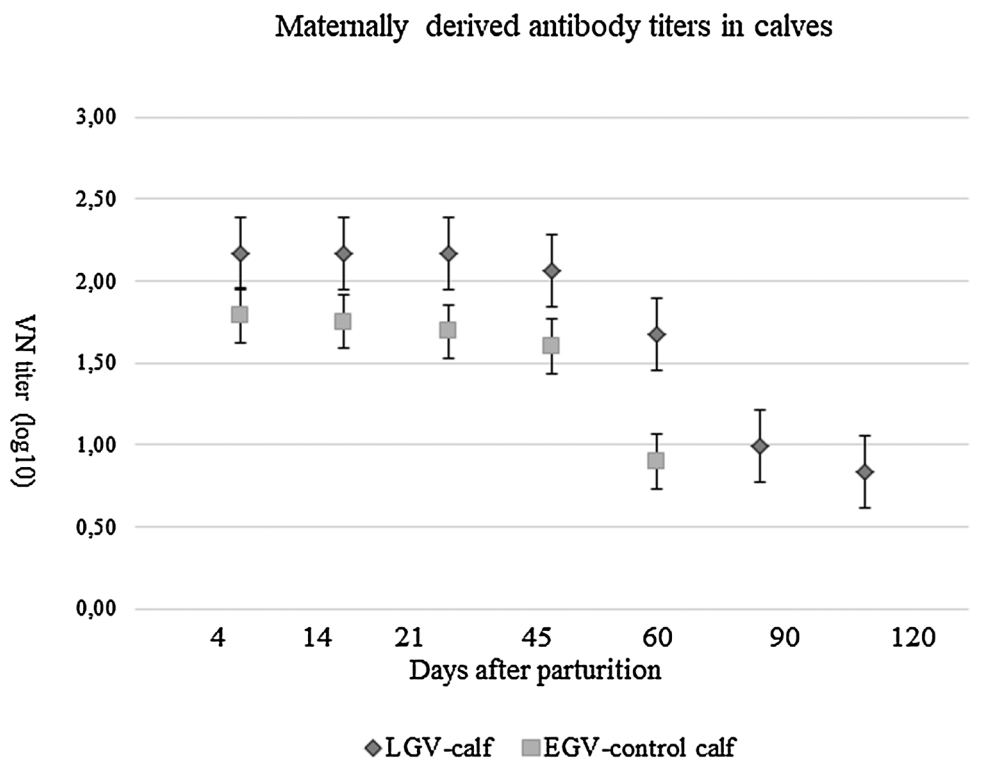

Fig. 5. Maternally derived neutralizing antibody titers in calf groups.
Error bars represent the % 95 confidence intervals ($CI_{95\%}$).

Table 2
Estimate, standard error, t-value and the p-value of fixed effects for the final model fitted to calves before the vaccination.

	Estimate	Standard error	t-value p-value
Intercept	2.41	0.1	$30.25 < 2 \times 10^{-16}$
LGV	reference		
EGV	−0.468	0.092	−4.66 0.0001
Time	−0.01334	0.00117	$-16.7 < 2 \times 10^{-16}$

Table 3
Estimate, standard error, t-value and p-value of fixed effects for the final model fitted to calves after the vaccination.

	Estimate	Standard error	t-value p-value
Intercept	0.99	0.066	$15 < 2 \times 10^{-16}$
LGV	reference		
EGV	−0.112	0.0678	−1.65 0.099
Time	0.77	0.038	$20.2 < 2 \times 10^{-16}$

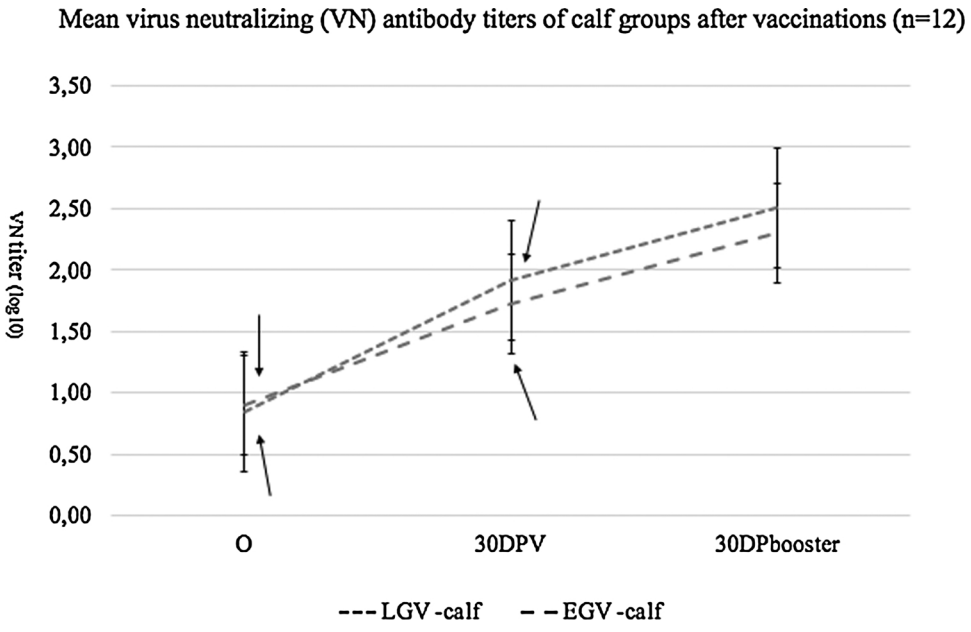

Fig. 6. Mean virus neutralizing (VN) antibody titers of calves after first and booster vaccinations.
Data shows the mean VN antibody responses with % 95 confidence intervals ($CI_{95\%}$). Error bars represent the ($CI_{95\%}$). Vaccine administrations are indicated with black arrows. Both calf group were shown as dotted blot since all calves received booster. DPV (days post vaccination).

Table 4
% Brix dam's colostrum IgG and % Brix TP of calf blood sera.

	Dams	Calves
Groups	Colostrum IgG	TP, on day 4 after parturition
LGV	25.8 ± 1.30	9.3 ± 0.33
EGV (control)	21.8 ± 0.58	8.6 ± 0.40

3.6. Maternal antibody decay

Fig. 8 illustrates the relationship between the log10 (1/2)/slope titer value and time. The calculated average half-life (t ½) was 23 ± 3 days (%95 CI) of maternally derived VN antibody titers of LGV calf group.

4. Discussion

It is crucial to determine the FMD vaccination schedule for dams during pregnancy to improve immunity in newborns. However, there are no definitive scientific reports on this topic, and detailed information was not found in the Turkish vaccine prospectus about the vaccination of pregnant animals. In Turkey, although there has been an increase of FMD vaccination coverage of calves and booster dose implementation in recent years, newborns are always under risk due to endemic nature of FMD in Turkey and surrounding countries. In addition, there are risks of incursion from exotic strains because of inadequate animal movement control. Moreover, the animal owners tend not to vaccinate their cows when the dams are in the late period of pregnancy due to their concern that the late gestation vaccination causes abortion. In the present study, we aimed to investigate the effects of FMD vaccination timing of dams during pregnancy on the level and duration of maternally derived antibodies.

When evaluating VN antibody titer results in mother groups, the VN antibody titers remained above the antibody cut off value until parturition in both groups of dams although there was an increase and then decrease in the average VN antibody titers as the gestation time passes by. This may be as a result of pregnancy-induced immunosuppression which has been shown previously to continue until parturition (Hansen, 2011). In addition production of colostrum in the udder starts 4-6weeks prior to calving transfer of maternally derived antibodies from the blood to the colostrum occurs, potentially leading to lower blood VN titers in the dams (Mauncell, 2014). It was also concluded that the booster dose during pregnancy might not be necessary if the dams were vaccinated regularly until the time of pregnancy, since no statistical difference in the mean VN antibody titers was determined in any of the dam subgroups, with or without booster vaccinations prior to calving. It may be related to immunosuppression during pregnancy or genetic variations among the individuals in response to the vaccination (Davies et al., 2009; Hansen, 2011; Glass et al., 2012; Wankhade et al., 2017).

There has been insufficient research on the timing of FMD vaccination of cattle and the effect on their calf antibody levels. In an earlier study on pigs (Francis and Black, 1984), when the dams received FMD vaccine before farrowing, this positively affected the colostrum IgG content and immunological response of the newborns. In another study (Panjevic and Valcic, 1989) high levels of maternal antibody titers in the offspring were obtained when one vaccine was given to the pigs before pregnancy and another (booster) during the pregnancy. As suggested in the above two studies, (Dekker et al., 2016), reported that the duration of the maternally derived antibody titer in piglets mainly depends on the titer at birth, which, in turn, depends on the titer in the sows. However, our results contradicted this result of Dekker et al. (2016). There was no significant statistical difference in the antibody titers in the dams, yet the LGV calves had significant higher neutralizing antibody titer. One explanation of our results could occur from the difference between the last blood sampling time of dam groups during pregnancy (In EGV dams, this was 15 days before the LGV, at 8 months of gestation). If we had taken blood samples also at 8.5 months in EGV there might have been the difference in dam titer as suggested by Mauncell (Mauncell, 2014). Future studies are warranted to clarify this result. Another explanation may be related to the VN titer in LGV dams at the start of the experiment, although there was no detectable between the titers in the LGV and EGV dams. Third explanation, as reported previously in another study (Reber et al., 2017), could be related to the level and/or characteristic of immune cells transfer via colostrum in such a way that a recent immunization of LGV dams affects the colostrum immune cell content, which might lead to higher maternally derived antibody response in LGV calves than EGV. Their results underline the complexity of pregnancy immunology and gaps still exist in our knowledge and understanding about pregnancy immunity as suggested previously (Oliverira et al., 2012; Fair, 2016). For example, many factors are directly affecting the amount of leucocyte expression

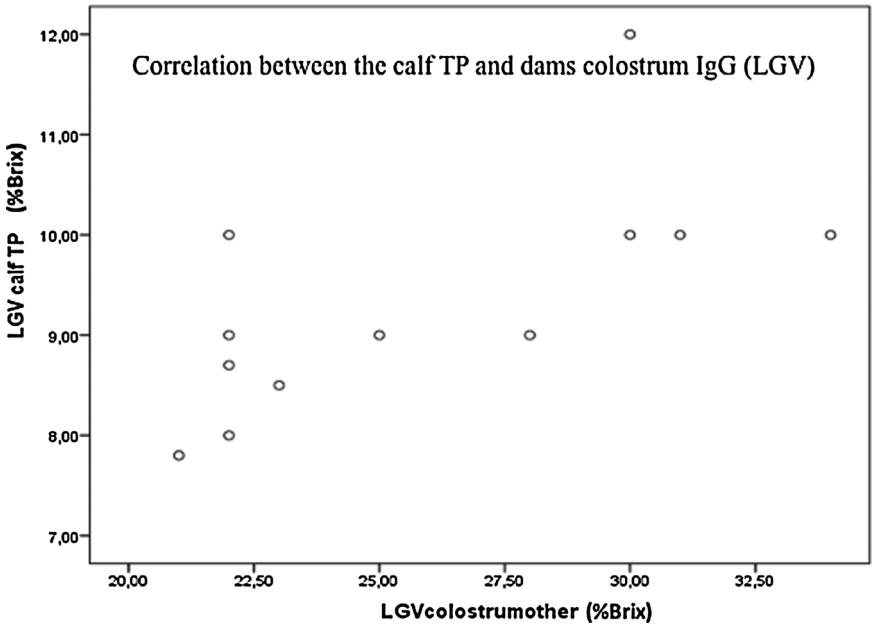

Fig. 7. The relation between dams (LGV) % Brix colostrum density and the % Brix Total Protein in the LGV calves just after 4 days parturition.

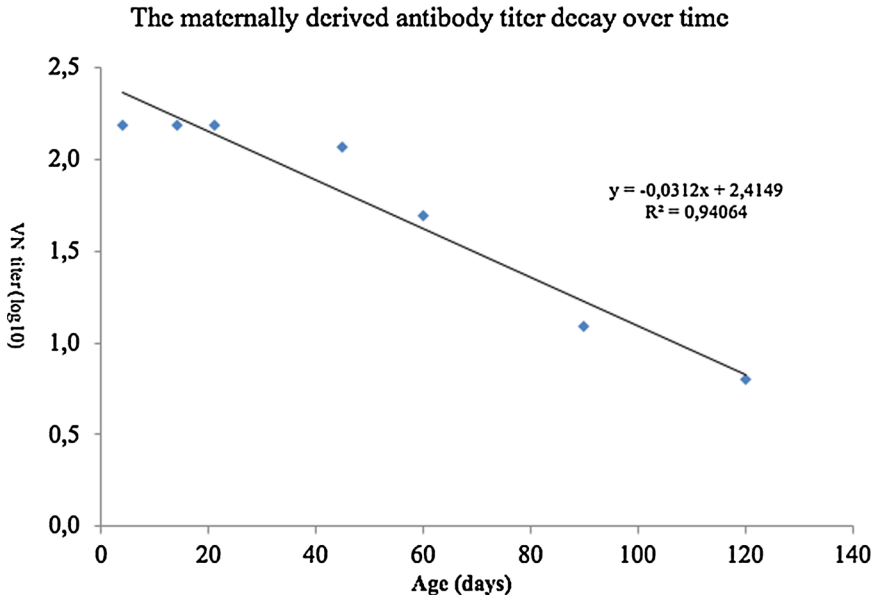

Fig. 8. The maternally derived antibody titer decay over time.
The linear association between the calves VN titer and time periods. The regression line and the R square are indicated.

levels in the dam placenta including dams' breed, age, health status, stress, physiological conditions, the quarter of pregnancy and surrounding temperature (Chucri et al., 2017).

There was a positive time effect in the calf groups with VN antibody titers increasing after vaccination. Moreover, there was a significant difference between the groups (albeit at a 10% significance level) with a slightly lower level observed in the EGV calves. Similarly to previous reports (Patil et al., 2014; Elnekave et al., 2016; Çokçalışkan et al., 2017), our study also confirms that the booster vaccination of the calves has critical importance in protecting the newborn calves since VN antibody titers of calves were increased after the booster dose. Additionally, the colostrum IgG content of LGV_dams was significantly higher than the EGV ($p < 0.01$) and there was a positive correlation between the colostrum IgG content of LGV dams and TP of their calves ($r = 0.73$, $p < 0.01$). These results provide further support that vaccination later in pregnancy has a beneficial effect on colostrum density of the LGV dams which in turn improves the TP and VN titers in their offspring. Here we also determined the half life of maternally derived antibodies in LGV group.

Since antibody half life determination helps to clear the interference period and first vaccination age of calves (Murphy et al., 2014). Previous studies (Nicholls et al., 1985; Spath et al., 1995; Dekker et al., 2014; Murphy et al., 2014; Çokçalışkan et al., 2017) have revealed that the average half-life of maternally derived antibodies is around 21 to 28 days. Similar to these previous reports, in this study, it was defined as 23 ± 3 days. There is varying information available about the maternal immune interference period. In general, this period varies from two to six months in ruminants (Gaglierdi and Andzoletto, 1972; Van Bekkum, 1974; Shankar and Uppal, 1982; Madhanmohan et al., 2009; Niewesk, 2014; Elnekave et al., 2016). In the present study, maternally derived antibody titers in the LGV group were both higher and longer (120 days) duration than EGV group titers. A previous study (Çokçalışkan et al., 2017), like as our result, showed that the first vaccination of calves should be optimal around four months if the dams received the recent Turkish FMD vaccine.

5. Conclusions

If dams were regularly vaccinated before their pregnancy, transfer of high-quality maternally derived antibodies to their offspring with a single vaccination after six and a half months of gestation appears possible and thus their calves could be vaccinated after four months of their birth instead of 2 months, thus reducing the number of FMD vaccines given to calves in their first year to three times instead of four. This would reduce both vaccine production (raw material, labor) and implementation (logistic, labor, and transfer) costs within a year.

To our knowledge, this is the first study that has investigated how the FMD vaccination time of pregnant cows affect their offsprings' maternally derived antibody titers in Turkey. This study was conducted with a small number of animals in a regular FMD vaccinated state farm. Therefore, further studies should be carried out with a larger number of animals in the field. Additionally, it would be advisable for further research in countries using vaccination as a control strategy in cattle populations to determine the optimum vaccination time for pregnant dams, and periodic measurement of this would be required for every new FMD vaccine introduced. Moreover, last but not least, detailed research about immunological changes during pregnancy period with FMD vaccination awaited future studies.

Declaration of Competing Interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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[1]See: https://www.tarim.gov.tr/TAGEM

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetimm.2019.109881.

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