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ORIGINAL ARTICLE

Heterogeneity in the Antibody Response to Foot-and-Mouth Disease Primo-vaccinated Calves

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Summary

Foot-and-mouth disease (FMD) vaccines are routinely used as effective control tools in large regions worldwide and to limit outbreaks during epidemics. Vaccine-induced protection in cattle has been largely correlated with the FMD virus (FMDV)-specific antibodies. Genetic control of cattle immune adaptive responses has been demonstrated only for peptide antigens derived from FMDV structural proteins. Here, we quantify the heterogeneity in the antibody response of cattle primo-vaccinated against FMD and study its association with the genetic background in Holstein and Jersey sires. A total of 377 FMDV-seronegative calves (122 and 255 calves from 16 and 15 Holstein and Jersey sires, respectively) were included in the study. Samples were taken the day prior to primo-vaccination and 45 days post-vaccination (dpv). Animals received commercial tetravalent FMD single emulsion oil vaccines formulated with inactivated FMDV. Total FMDV-specific antibody responses were studied against three viral strains included in the vaccine, and antibody titres were determined by liquid-phase blocking ELISA. Three linear hierarchical mixed regression models, one for each strain, were formulated to assess the heterogeneity in the immune responses to vaccination. The dependent variables were the antibody titres induced against each FMDV strain at 45 dpv, whereas sire's 'breed' was included as a fixed effect, 'sire' was included as a random effect, and 'farm' was considered as a hierarchical factor to account for lack of independence of within herd measurements. A significant association was found between anti-FMDV antibody responses and sire's breed, with lower immune responses found in the Jersey sires' offspring compared with those from Holstein sires. No significant intrabreed variation was detected. In addition, farm management practices were similar in this study, and results of the serological assays were shown to be repeatable. It therefore seems plausible that differences in the immune response may be expected in the event of a mass vaccination campaigns.

Introduction

Foot-and-mouth disease (FMD) is a highly contagious and acute viral disease affecting all ruminants and cloven-footed animals. Domestic species typically infected by the FMD virus (FMDV) include cattle, swine, sheep and goats; numerous susceptible wildlife species may act as reservoirs for FMDV under certain ecological conditions (Alexandersen et al., 2003). Fatal cases are usually restricted to young animals and certain FMDV strains; however, the high

morbidity rate and indirect losses associated with FMD outbreaks result in severe and far-reaching economic losses to the livestock industry (Yang et al., 1999; Thompson et al., 2002).

Disease outbreaks in FMD-free regions are controlled using a combination of mitigation strategies, such as control of animal movements and, in some cases, killing of infected and exposed animals and/or vaccination. In recent decades, social, economic and environmental concerns have increasingly favoured the use of vaccination to control FMD outbreaks (Mackay et al., 2004; Poulin and Christianson, 2006). Moreover, vaccination is routinely used in countries and large regions recognized as free from the disease by the World Organization for Animal Health (OIE), to prevent FMDV incursions (Saraiva and Darsie, 2004).

Current commercial vaccines are based on chemically inactivated whole virus particles formulated in oil or hydroxide–saponine adjuvanted formulations (Doel, 2003). Good quality vaccines prevent the development and transmission of the disease and decrease the incidence of persistently infected animals (Anderson et al., 1974; Orsel et al., 2005; Cox et al., 2006). Protection provided by FMD vaccines is serotype (and in many cases strain specific) specific and closely related to the induction of specific antibody responses (Pay and Hingley, 1987). Moreover, *in vitro* assays such as liquid-phase blocking ELISA (LPB-ELISA) or virus neutralization tests (VNT) may be used to assess vaccine potency based on statistical correlations between antibody titres and OIE-recognized *in vivo* protection assays, such as the 'protection against podal generalization'(PPG) test (Maradei et al., 2008).

Immune responses elicited by commercial FMD vaccines vary between different hosts (Doel et al., 1994; Patil et al., 2002; Barnett et al., 2004; Parida et al., 2007), and thus, formulations should be tailored to affected animal species. Variations are also observed depending on the age of the animal (Spath et al., 1995; Samina et al., 1998) and the pre-existence of vaccine-induced passive maternal immunity (Nicholls et al., 1984; Sadir et al., 1988). Genetic factors involved have also been investigated in laboratory animal models and natural hosts, mainly through the study of specific immune responses elicited by discrete FMDV-derived peptide sequences(Francis et al., 1987; Glass et al., 1991, 2000; Glass and Millar, 1994; Van Lierop et al., 1995; Garcia-Briones et al., 2000; Baxter et al., 2009; Leach et al., 2010). However, to the authors' knowledge, only one early report has included some observations on the association between genetic factors and immune responses against whole inactivated FMDV particles within vaccines (Samina et al., 1998).

The hypothesis assessed in the study here was that the antibody response to FMD primo-vaccination in cattle varies in association with sire's breed, which is suggestive of genetic-borne variation in the response. This was tested using data from total anti-FMDV serum antibody titres induced 45 days post-vaccination (dpv) against three FMDV vaccine strains. A significant association ($P < 0.05$) was found between humoral responses and sire's breed, with offspring from Jersey sires exhibiting less of an immune response than Holstein sires. No significant intra-breed variation was identified. Results presented here provide supporting evidence of genetic-borne influence on the immune response to FMD vaccination.

Materials and Methods

Animals

Four to seven months old naïve calves ($n = 606$) raised in four dairy farms in Buenos Aires Province, Argentina, were screened during a twelve-month period. As all the animals were born to FMD-vaccinated dams, only calves without detectable anti-FMDV colostral antibodies at the time of vaccination (62.2%, $n = 377$) were included in the study. Calves included in the study were from Holstein (16 sires, 122 calves) and Jersey (15 sires, 255 calves) breeds, and all sires analysed presented at least three calves in their progeny. Two of the farms (referred to as farms No. 1 and 2) included only Holstein calves and another farm included only Jersey calves (farm No. 4), whereas the remaining farm included both Holstein and Jersey calves (farm No. 3).

Samples and vaccination

Paired whole blood samples were obtained from the 377 calves. Samples were taken the day prior to primary vaccination and 45 days later. Vaccinations were performed in the frame of the national FMD campaigns in Argentina using officially approved commercial tetravalent FMD single oil emulsion vaccines from same manufacturer. Formulations contained inactivated FMDV from A24 Cruzeiro/Brazil/55 (A24 Cruzeiro), A/Argentina/2001 (A/Arg/01), O1/Campos/Brazil/58 (O1 Campos) and C3/Indaial/Brazil/71 (C3 Indaial) strains. Vaccines were provided, handled and applied by trained professionals authorized by the national sanitary authority (SENASA) following current regulations (SENASA, 2002, 2006, 2011). Whole blood samples taken at 0 and 45 dpv were centrifuged to separate plasma from cells, and both fractions were stored at −20°C until used.

Serological assays

Total FMDV-specific antibody responses were determined against three vaccine strains (A24/Cruzeiro, A/Arg/01 and O1/Campos) belonging to serotypes with recent circulation in South America. Anti-FMDV antibody titres

were studied by means of a liquid-phase blocking ELISA (LPB-ELISA) performed under ISO standards and utilized by the regional OIE FMD Reference Laboratory in Argentina to assess herd immunity and vaccine efficacy (Periolo et al., 1993; Maradei et al., 2008). Briefly, serial dilutions of plasma samples were incubated with inactivated whole FMDV particles corresponding to these three vaccine strains. Plasma–virus mixtures were transferred to ELISA plates previously coated with strain-specific rabbit polyclonal sera to capture non-associated virus. Captured virus was finally detected using panels of MAb specific for each of these three strains and anti-mouse Ig sera HRP-conjugated. Antibody titres were expressed as the reciprocal $\log_{10}$ of serum dilutions giving the 50% of the absorbance recorded in the virus control wells without plasma. Calves carrying FMDV-specific colostral antibodies prior to primary vaccination were also identified using this test and removed from the study. Thirty-five randomly selected samples were retested to evaluate repeatability of the assay by computing the concordance correlation coefficient (ρc) (Lin, 1989), using an online application (National Institute of Water and Atmospheric Research, 2011).

Statistical analysis

Differences in the serotype-specific responses were assessed using a Student's *t*-test, and the correlation of immune responses between individuals was assessed using an *R*-Spearman test (Zar, 1972).

Association of the antibody responses with sire's breed was assessed using three linear hierarchical mixed regression models (Goldstein, 2011), one for each virus strain. The dependent variable was antibody titre induced against FMDV O1/Campos, A24/Cruzeiro and A/Arg/01 at 45 dpv, whereas sire's breed was included as a fixed effect, sire was taken as a random effect, and farm was considered as a hierarchical factor to account for intraherd lack of independence in the observations. The fixed effect (breed) was tested for significance, and the random effect (sire) was evaluated based on their contribution to the model improvement as indicated by the value of Akaike's information criterion (AIC) (Akaike, 1974).

Table 2. Mean and standard deviation of antibody titres[a] in calves at 45 dpv per farm and sire breed

		A24/Cruzeiro		O1/Campos		A/Arg/01	
Sire breed	*n*	Mean	SD	Mean	SD	Mean	SD
Holstein	122	2.782	0.515	2.592	0.573	2.817	0.534
Jersey	255	2.229	0.425	2.122	0.488	2.279	0.469

[a]Mean FMDV-specific antibody responses were measured by LPB-ELISA expressed as described in Materials and Methods.

Results

Antibody titres against three of the vaccine strains (A24/Cruzeiro, O1/Campos and A/Arg/01) were measured by a validated and controlled LPB-ELISA utilized by the regional OIE FMD Reference Laboratory in Argentina. However, because samples were obtained and processed during a 12-month period, we studied the repeatability of the LPB-ELISA by retesting a subset of 35 randomly selected samples after analysing the whole set of plasma. The assay used here demonstrated good repeatability of results, as indicated by the high values of ρc estimated, which were (95% CI) ρc = 0.94 (0.88, 0.97), ρc = 0.92 (0.85, 0.96) and ρc = 0.87 (0.76, 0.94) for O1/Campos, A24/Cruzeiro and A/Arg/01, respectively.

A total of 377 FMDV-seronegative naïve calves from 31 different Holstein or Jersey sires distributed throughout four farms were primary vaccinated with commercial tetravalent FMD vaccines and included in this study. As it is shown in Table 1, 45 days after primary vaccination, titres of antibodies against A/Arg/01 were on average 0.04 log10 higher ($P < 0.05$) than those against A24/Cruzeiro, which in turn were 0.14 log10 higher ($P < 0.05$) than those against the O1/Campos strain (Table 1). Also, correlation between strain-specific antibody responses was high for all strains studied ($R = 0.85$–0.9). Moreover, primary FMD vaccination in all these groups of calves was efficient, inducing mean antibody titres against each of the strains at 45 dpv above the 75% of expected protection, based on the correlation with the *in vivo* potency tests (Maradei et al., 2008). (Table 2).

Table 1. Mean antibody titres[a] at 45 dpv per FMDV strain and mean difference between strains

FMDV strain	Mean Ab response at 45 dpv[a]	Compared to	Mean difference	Paired t-test significance	Pearson's correlation	Pearson's significance
O1/Campos	2.27	A24/Cruzeiro	−0.13	<0.01	0.85	<0.01
A24/Cruzeiro	2.41	A/Arg/01	−0.04	<0.01	0.87	<0.01
A/Arg/01	2.45	O1/Campos	0.18	<0.01	0.9	<0.01

[a]Mean FMDV-specific antibody responses were measured by LPB-ELISA expressed as described in Materials and Methods.

The whole set of mean antibody titres detected for each virus strain, grouping calves according to their sire and breed, is shown in Fig. 1. Intrabreed differences were negligible, as indicated by the similar averages and overlapping intervals estimated for specific sires (Fig. 1).

Results of the linear hierarchical mixed regression models are depicted in Table 3. The intercept in the model represents the overall mean antibody response of the reference breed value (Jersey), taking into account the herd correlated data. The estimate of the breed represents the mean increase in antibody titre increase of Holstein breed compared with Jersey, and the standard error (SE) measures data dispersion from the expected (mean) values based on the sampling distribution. A greater standard error indicates less statistical precision of the estimate, which will be reflected in wider confidence intervals. Analyses using the regression models indicated that sire's breed was significantly associated ($P < 0.05$) with the immune response for the three strains (Table 3), resulting in an antibody response to FMD vaccination at 45 dpv significantly lower ($P < 0.01$) for the progeny of Jersey compared with those of Holstein (Table 2). Addition of sire as a random effect did not improve the fit of the model for any of the three virus strains, suggesting that individual factors other than differences in sire breed were negligible.

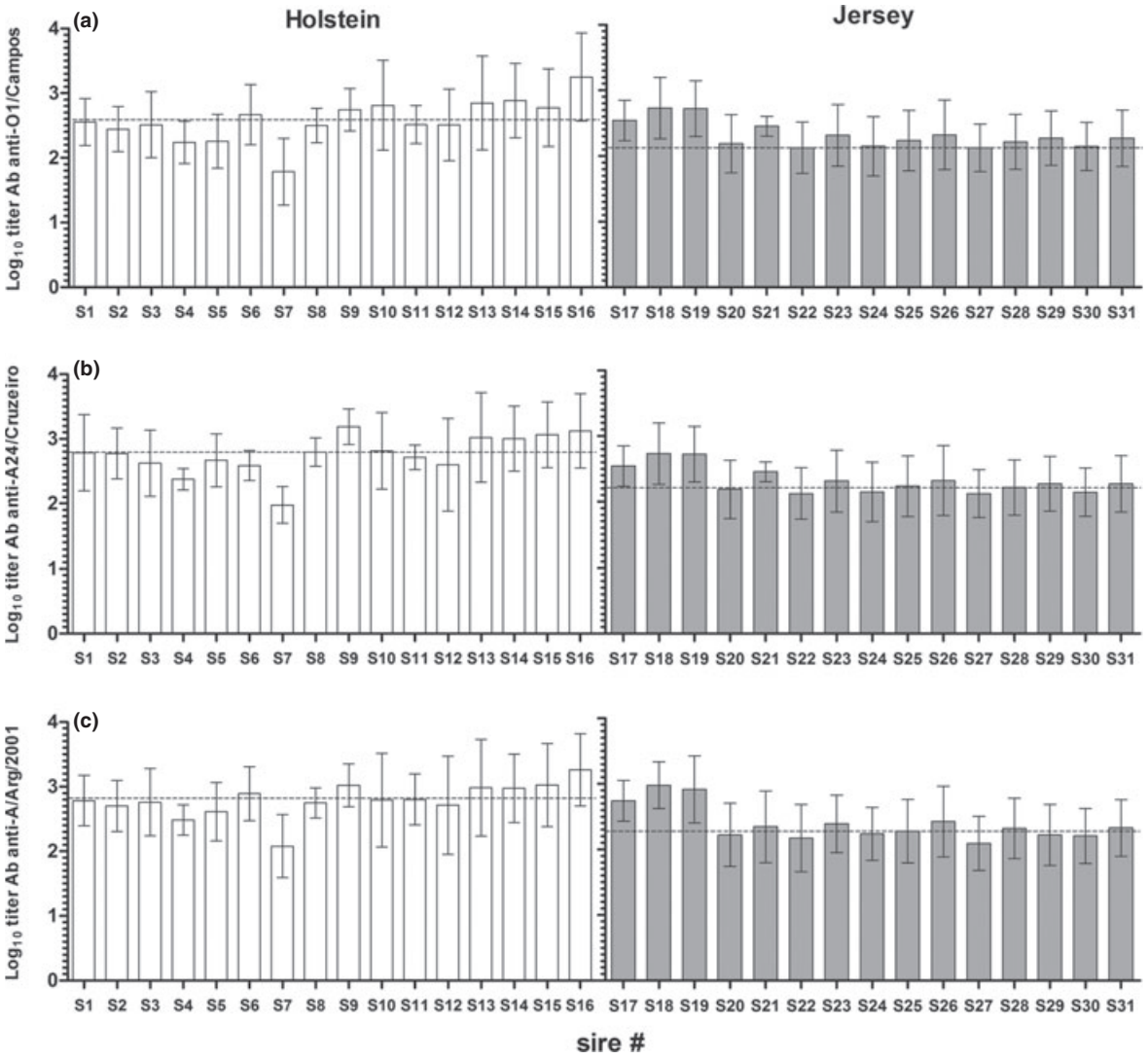

Fig. 1. Mean antibody titres against FMDV O1/Campos (a), A24/Cruzeiro (b) and A/Arg/01 (c) in calves at 45 dpv. Calves were grouped by breed and sire from which they derived. Antibody titres were measured by LPB-ELISA and expressed as the reciprocal $\log_{10}$ of serum dilutions giving the 50% of the absorbance recorded in the virus control wells without serum. Error bars represent the standard deviation of the mean titres, and dotted lines indicate average values for each breed and virus strain. Sires S1-S7 correspond to farm 1; S8–S15 to farm 2; S16–S19 to farm 3; and S20–S31 to farm 4.

Table 3. Hierarchical linear model results for antibody titres[a] at 45 dpv against O1/Campos, A24/Cruzeiro and A/Arg/01

	Estimate	SE	95% CI	Sig.
O1 Campos				
Intercept	2.24	0.21	1.71–2.78	<0.01
Breed[b]	0.45	0.18	0.09–0.81	0.02
A24/Cruzeiro				
Intercept	2.39	0.16	1.98–2.79	<0.01
Breed	0.42	0.16	0.10–0.73	0.01
A/Arg/01				
Intercept	2.49	0.18	2.02–2.96	<0.01
Breed	0.35	0.17	0.01–0.70	0.04

[a]Mean FMDV-specific antibody responses were measured by LPB-ELISA expressed as described in Materials and Methods. SE, standard error.
[b]Indicates the fixed effect analysed in the regression model.

Discussion

The induction of neutralizing antibodies is the mechanism most frequently related to protection against FMDV (Barnett and Carabin, 2002; Golde et al., 2005; Orsel et al., 2005; Cox et al., 2006, 2007; Maradei et al., 2008; Robiolo et al., 2010), and a number of reports have provided in-depth information about the genetic control of humoral responses in cattle (O'Neill et al., 2006; Minozzi et al., 2010; Glass et al., 2011; Leach et al., 2012). Previous work has shown MHC-based restrictions to FMDV-derived antigens in cattle. Early studies focused on the genetic restrictions imposed on T-cell recognition via major histocompatibility complex class II (MHC II) polymorphism (Francis et al., 1987; Glass et al., 1991; Van Lierop et al., 1995). Some alleles of the MHC II system in cattle (Glass et al., 2000) have been associated with both the magnitude (Glass and Millar, 1994) and the quality (Garcia-Briones et al., 2000; Baxter et al., 2009) of humoral responses induced against FMDV peptides. Only recently, whole genome analyses also revealed quantitative trait loci (QTL) which controlled both humoral and cellular immunity against FMDV peptides in regions outside the BoLA genes (Leach et al., 2010).

Complexity of the associations between genetic-related factors and immune responses to FMDV runs in parallel with that of the immunizing antigen. Some important aspects of the anti-FMDV responses observed in cattle, such as T-independent antibody responses (Juleff et al., 2009), are tightly associated with structural features of the intact particle. Consequently, they do not follow the same immune pathways as for peptide antigens and cannot be explained as the addition of independent responses to an array of discrete peptides. Two other factors add difficulties to the FMDV model in cattle: the genetic variability associated with outbreed bovine populations and the fact that FMDV antigens included in vaccines are not antigenically homogenous but rather a mix of multiple variants produced during virus replication (Piatti et al., 1995).

Only one early report presented results about the influence of the genetic background on the immune response to FMD vaccines (Samina et al., 1998). In that paper, groups of cattle ($n = 10$) deriving from four different sires were compared. Animals received three vaccinations with a trivalent FMD aqueous vaccine formulated with aluminium hydroxide and saponine and were tested 1 year after the last vaccination. The authors indicate that the sire effect was significant for daughters' antibody responses against the vaccine strains, although there was no association between the bulls' own response and their daughter's responses for any of the serotypes, thus hindering interpretation of the results.

With this background, we decided to use FMDV vaccines as model antigens. Humoral responses raised against three strains used in commercial tetravalent vaccines at 45 days after primo-vaccination were measured by a reliable serological assay and associated with sire and breed effects. Highest mean antibody titres were found for A/Arg/01 strain, followed by A24/Cruzeiro and O1/Campos, 0.04 and 0.18 Log_{10} titre units below A/Arg/01, respectively ($P < 0.01$). As previously described, these differences between strains could be related to different antigenic payloads for each particular strain included in the vaccine (Rweyemamu et al., 1984) and also to the differential capsid stability among FMDV strains (Doel and Baccarini, 1981) which may impact on the immunogenicity of the viral antigens (Doel and Chong, 1982). In any case, it is important to note that all of these average values were above the LPB-ELISA titres determined to confer 75% of expected protection to the viral challenge (Maradei et al., 2008). As a whole, our results demonstrated that antibody responses induced after primo-vaccination were homogenously high for all calves assayed, with a good inter-strain correlation between titres.

Significant differences found between antibody responses against each vaccine strain reinforce the idea of the poor inter-strain cross-reactivity (Alexandersen et al., 2003) and the independence of the pathways involved in the induction of antibodies against each virus. Thus, we decided to study all three humoral immune responses independently.

Associations of the humoral responses elicited in calves with sire or breed effects were analysed by three linear hierarchical mixed regression models. Design of the experiment included animals from different farms. The possible impact of environmental factors was reduced by including dairy farms with identical management and similar environmental characteristics. The potential bias introduced by this

variability was also addressed by including farms as a hierarchy in the statistical model, so the final immune response attributed to the sire characteristics was unbiased. Also, although the number of calves per breed was uneven between both groups (122 Holstein and 255 Jersey), using herd as a hierarchical factor controls for correlated data, in this case, regarding breed and other factors related with within herd similarities. This model deals with the potential for overestimation of the estimate precision when similar (correlated) data are represented with large sample sizes.

We found no significant intrabreed differences in the antibody responses obtained in calves grouped by sire for any of the three strains. Moreover, mean antibody titres grouped by sire followed a similar pattern among strains. This lack of intrabreed differences may be in part explained by the within-farm genetic homogeneity; however, the vigorous humoral responses induced by these vaccines may also have an impact. Good quality vaccines are quite efficient in inducing FMDV-specific antibodies, and thus, they might be hiding or compensating some of the inherent, genetically driven, immunological differences between individuals.

Sire's breed, however, significantly affected the outcome of the antibody responses registered here, as indicated by the results of the regression models. Progeny of Jersey sires developed a post-vaccination immune response significantly lower ($P < 0.05$) than progeny from Holstein sires for the three FMDV strains analysed at 45 dpv. Titre values differed from 2.24- (for A/Arg/01) to 2.82-fold (for O1/Campos) between these two breeds. These observations are also in agreement with the idea that less potent humoral responses, in this case those induced against the O1/Campos strain, may be better than high potency vaccines in revealing small differences in humoral responses related to the genetic background of the animals.

Results presented here provide evidence that the immune response to primo-vaccination in cattle is affected by the genetic background of the calves, as indicated by the association detected between sire's breed and immune response. These results suggest that additional exploration into the limitations and potentials of genetic markers may help to predict immune responses to FMDV immunization and other vaccines in cattle.

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