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Effect of suckler cow vaccination against glycoprotein E (gE)-negative bovine herpesvirus type 1 (BoHV-1) on passive immunity and physiological response to subsequent bovine respiratory disease vaccination of their progeny

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ABSTRACT

The study objectives were: 1) to characterise the development of immunocompetence in beef suckler calves from birth to three months of age, and 2) to trace glycoprotein E (gE)-negative bovine herpesvirus type 1 (BoHV-1) antibodies from dam to calf and subsequent vaccination against pneumonia. Thirty multiparous beef suckler, spring-calving cows, consisting of two genotypes were involved; Limousin×Friesian (LF) and Charolais×Limousin (CL). Cows were immunised against the inactivated antigen strain of BoHV-1 (gE- (IBR marker vaccine) at day -84 and received a booster at day -56 relative to the expected calving date (d 0). Calves were immunized at 14 and 42 days of age against PI-3 virus, BRSV and *Mannheimia (Pasteurella) haemolytica* serotype A1 using a commercial vaccine administered subcutaneously. Additionally, calves were immunized against BoHV-1 at 42 days of age, using 1 dose of a live commercial vaccine administered intranasally. Blood samples were collected from all calves (n=30) via jugular venipuncture at birth, prior to colostrum feeding (0 h), at 12 hour (h), 24 h, 72 h and 168 h after the initial feeding of colostrum, and at d 7, 14, 28, 42, 56 and 84 post birth. The mean ratio of gE negative antibodies circulating in the blood of LF and CL dams pre-partum scored negative to gE ab (S/N = > 0.70). Antibody levels of BoHV-1 (*wt*) peaked at 12 hours post-birth in calves and declined thereafter, as the maternal antibodies decayed. There was no difference in BoHV-1 and BRSV antibody levels in calves post vaccination.

Keywords

1.   Introduction

The positive association between passive immune status, neonatal health and mortality in calves is well established (Earley *et al.*, 2000; Waldner and Rosengren, 2009; Stelwagen *et al.*, 2009; Furman-Fratczak *et al.*, 2011; Niewiesk, 2014; Raboisson *et al.*, 2016). In the suckled beef calf, there are large differences in passive immunity between cow breed types (McGee *et al.*, 2005a, 2005b; Murphy *et al.*, 2005). Peak incidence of bovine respiratory disease (BRD) occurs in young calves, less than 6 months of age, often in the face of pre-existing maternally derived antibodies (MDA) (Patel and Didlick, 2004). Infectious causes of BRD include viral (bovine herpesvirus-1 virus ((BoHV-1/infectious bovine rhinotracheitis (IBR) virus), bovine coronavirus (BoCV), bovine respiratory syncytial virus (BRSV), bovine viral diarrhoea virus (BVDV), parainfluenza-3 virus (PI3V), bovine adenoviruses, bovine enterovirus-1, -2 and -3), bacteriological, (*Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*)   and mycoplasmal agents *(Mycoplasma bovis)*, many of which are indigenous residents of the bovine respiratory tract (Taylor *et al.*, 2010a; 2010b; 2015; Brooks *et al.*, 2011).

Marker vaccines are commercially available, inactivated or live-attenuated (Kaashoek *et al.* 1994; Kaashoek *et al.,* 1995) and for BoHV-1 the gene coding for the unnecessary glycoprotein E (gE) has been deleted. This allows for serological differentiation between artificial vaccination and cattle that were naturally infected (Van Oirschot *et al.* 1997). This is a useful method to detect infected animals and eliminate them from the herd to prevent the possibility of reactivation of latent virus (Ackermann et al. 1990; Kaashoek *et al.,* 1995).

Many vaccine protocols have been developed to vaccinate young calves at frequencies as often as weekly during the first and second months of age. Finding any experimental studies that support this timing is difficult as limited research is available to indicate the true

effectiveness of vaccine timing or ideal protocols for use in young calves (Chase *et al.,* 2008). Viruses including BoHV-1, BRSV and PI-3V have an important role in BRD, not only in the pathogenesis of the disease but also in the development of immunosuppression (Murray *et al.,* 2017). Furthermore, calf immunisation against BRD causing pathogens may be adversely affected by interference from maternally derived antibodies (Chase *et al.,* 2008), neonatal hormonal factors (Morein *et al.,* 2002) or unfavourable environmental conditions. Infectious bovine rhinotracheitis (IBR) is a highly contagious respiratory disease that is caused by bovine herpesvirus 1 (BoHV-1), in the subfamily *Alphaherpesvirinae* of the *Herpesviridae* family. Vaccines currently used in BoHV-1 control programs in certain EU countries utilize highly attenuated BoHV-1 strains marked by a deletion of the glcoprotein E 9gE gene (Muylkens *et al.,* 2006) also known as the Glycoprotein E (gE)-deleted marker vaccine. Glycoprotein E is the established virulence factor of all known members of the subfamily *Alphaherpesvirinae* (van Engelenburg *et al.,* 1994; Rebordosa *et al.,* 1996).

The objectives of this study were 1), to characterise the development of immunocompetence in beef suckler calves from birth to three months of age, and 2), to trace glycoprotein E (gE)-negative BoHV-1 antibodies from vaccinated dams to calf sera and investigate how passive transfer of immunity affects response in calves to live BoHV-1 vaccine at 14 days of age, and to Bovilis®Bovipast RSP vaccine at 14 and 42 days of age.

2. Materials and Methods

2.1 Animal ethics approval

All animal procedures performed in this study were conducted under experimental licence from the health products regulation authority (HPRA), Dublin, Ireland (individual authorisation number, AE 19132/I073; project licence number, AE 19132/P006).

2.2   Study area

This study was conducted on the beef suckler herd, at Teagasc Grange, located in Co. Meath, Ireland (latitude 53.52187, longitude -6.65247).

2.3   Animals, feeding and general management

The study consisted of thirty multiparous (MP) beef suckler, spring-calving cows. Two cow breeds were examined: Limousin × Friesian (LF) (n = 17) and Charolais × Limousin (CL) (n = 13). Mean parity (SD) for LF and CL cows was 5.5 (0.94) and 5.3 (1.03), respectively. The mean calving date was 28th February 2015 (26th January to 27th April). The cows were bred using an Aberdeen Angus (AA) and Charolais (CH) sires (natural mating). The beef suckler cows were housed in pens in a concrete slatted-floor shed in November 2014 and offered second-cut grass silage *ad libitum (in vitro* dry matter digestibility (DMD) (615 g/kg). All cows received a mineral and vitamin supplement daily (60g/head) while housed. The cows that calved prior to April were turned out to pasture in early April. Cows that calved from early April onwards were turned out within 2 to 7 days of calving. Prior to parturition (approximately 1 to 7 days) the cows were moved from the slatted-floor shed to straw bedded calving pens. Births were supervised by farm personnel to ensure that calves did not suckle the cow prior to implementing the experimental protocol for collection of the first blood sample from the calf. Immediately post-partum a tincture of 5% iodine was applied to the umbilical cord of each calf, and calf birth weight was recorded. All calves were closely observed to ensure suckling of the dam and any calf not suckling within one hour of birth or showing signs of weakness was assisted to suckle the cow. After parturition, animals remained in the straw pens for a minimum of 4 days with the calf having free access to the dam. The calves had free access to their dams for suckling. Following parturition all cows received first cut grass silage *ad libitum* until turnout to pasture from the 20th March to the 10th April, 2015. Meteorological conditions during the spring calving season (January to June

2015) recorded a mean ambient temperature of $7°C$ (temp. range $-2.7°C$ to $20.6°C$), mean dry bulb temperature of $7.2°C$, and a mean wet bulb temperature of $5.9°C$.

2.4 Cow vaccinations

All cows were immunised against the inactivated antigen strain of BoHV-1 (gE-) with a primary vaccine (day -84) and a secondary vaccine (day -56) relative to the expected calving date (d 0), with 2 ml (S/C) of a commercial vaccine, Bovilis BoHV-1 (IBR) marker inactivated (MSD Animal Health, Buckinghamshire, UK). All cows received a combined rotavirus, coronavirus and *E. coli* F5 (K99) vaccine (inactivated) (Rotavec Corona) by intra-muscular injection between 84 and 28 days prior to calving date.

2.5 Calf vaccinations

All calves were immunised at 14 days of age using 2 ml of a live commercial vaccine, Bovilis IBR marker (MSD Animal Health, Buckinghamshire, UK) delivered intra-nasally (I/N). Additionally, all calves were immunised with a primary vaccination of Bovilis®Bovipast RSP inactivated vaccine at 14 days of age and received a secondary (booster) vaccination at 42 days of age using 2 ml subcutaneously (S/C) and 5 ml (S/C) of a commercial vaccine called Bovilis®Bovipast RSP, for the primary and booster vaccination, respectively (MSD Animal Health, Buckinghamshire, UK). Bovilis®Bovipast RSP vaccine has three components based on inactivated strains of serotype 1 and biotype A of *M. haemolytica* and two viruses, para-influenza -3 virus (PI-3V) and bovine respiratory syncytial virus (BRSV).

2.6 Live weights and body composition

Calf live weights were recorded at birth, and every 28 days thereafter throughout the study.. Cow live weights were recorded using calibrated scales, Tru-Test XR3000, load bars XHD 10000, Auckland, New Zealand.

2.7 Calf records and health

Calf sex, birthweight, and calving difficulty were recorded. Calves were weighed again at turnout to grass and every 28 days thereafter until cessation of blood sampling. Calves were categorised as healthy if they had no incidence of calf disease. If a calf presented with an elevated rectal temperature ($>39.5^{\circ}$C), showed clinical signs of scour and or pneumonia using the Wisconsin Madison chart (McGuirk, 2008), it was considered unhealthy. A total of six calves were treated for navel illness, three calves were treated for scour (*Cryptosporidium parvum*), four calves were treated for pneumonia by day 7 post-birth, and one calf was treated for lameness and infection after disbudding, respectively.

2.8 *Colostrum / milk collection and sampling*

Three $\times$ 50 ml samples of colostrum/milk were collected at calving, prior to the initial feed (d 0), and thereafter at d 2 and d 3 post-calving. Samples were collected from the left quarter of the dams' udders. The udder was cleaned before sample collection to avoid any debris entering the sample. One $\times$ 50 ml colostrum samples (whole) was frozen at -20°C for milk chemical analysis and submitted to the Newforge laboratory, Agri-Food and Biosciences Institute (AFBI), Hillsborough, Co. Down. Colostrum samples were analysed for total IgG. One $\times$ 50 ml colostrum samples were centrifuged at 2000 $\times$ *g* at 7°C for 10 minutes. Following centrifugation, the fat-layer was removed with a sterile spatula. The supernatant (fat-free) was frozen at -20°C for IgG analysis.

2.9 *Cow blood samples*

Cows (n = 30) were blood sampled by jugular venipuncture at d -84, d -56, d-28 pre-calving, at calving (d 0) and at d 14 post-calving. Two serum collection tubes (2 $\times$ 8.5 ml) (BD Vacutainer, Ireland: Serum Separator Tube II Advance Tubes) were collected on all sampling days. Blood samples were refrigerated for 24 hours at 4°C before sera were harvested by centrifugation (2000 $\times$ g at 4°C for 10 min). Sera were frozen at -20°C until assayed for specific antibody analysis (IBR gE$^-$ and BoHV-1 (*wt*)) and maternal passive transfer analysis.

2.10   Calf blood samples

Blood samples were collected from calves (n=30) via jugular venipuncture into 2×8.5 ml BD Serum Separator Tube II Advance tubes (BD Vacutainer; Unitech, Ireland), and 1×6.0 ml tube containing K_3EDTA as anti-coagulant (Vacuette; Cruinn Diagnostics, Ireland). The blood samples were collected immediately at birth, prior to colostrum feeding (0 h), at 12 h, 24 h, 72 h and 168 h after the initial feed of colostrum, and at d 7, 14, 28, 42, 56 and 84 post birth. Blood samples were refrigerated for 24 h before the sera were harvested by centrifugation at 4°C. Sera were frozen at -20 °C until assayed for specific antibody analysis (IBR gE⁻, BRSV, BoHV-1), and passive transfer analyses (serum ZST units and IgG concentrations).

2.11   Colostrum and serum IgG analysis

Colostrum (fat free) and serum (dam and calf) samples were thawed at $4^{o}C$ overnight. IgG concentration was then determined using an enzyme-linked immunosorbent assay (ELISA) kit for bovine IgG from Bio-X Diagnostics (Jemelle, Belgium) (as described by Dunn *et al.* 2017). All components of the kit were brought to room temperature ($21^{\circ}C$) before use. The wash buffer was diluted 20 fold with distilled water. A calibration curve was constructed and all samples were diluted at 1:225 in phosphate buffer saline (PBS), as per manufacturer's instructions. All samples (100 µl) were added to the test plate and tested in duplicate, along with standards using a 96-well dilution plate. Equal volumes of horse radish peroxidase (HRP) conjugate was added to each well of the dilution plate and then 100 µl of this was transferred to the test plate. Each test plate was incubated at room temperature ($21^{\circ}C$) for 1 hour. The plate was washed three times using wash solution. Chromogen solution (tetramethylbenzidine) was added to each well (100µl/well) and the plate was protected from light and incubated for 10 minutes at room temperature. Stop solution was added to each well (50 µl/well) and the absorbance (optical densities) of the samples was read at a wavelength of

450nm using a microplate spectrophotometer (Tecan Magellan, Männedorf, Switzerland).

Negative controls were included on the test plate. An inter-assay CV of < 0.15 was observed.

The mean absorbance at 450nm of each standard were plotted against known concentration to

produce a standard curve. The mean absorbance at 450nm of samples was used to interpret

the IgG concentration from the standard curve. The coefficient of determination value for

each assay had to exceed 0.9 on the standard curve for inclusion within these results.

2.12   *Zinc sulphate turbidity (ZST) test*

Serum samples were analysed for ZST units, a control (100 μL of serum and 6 ml of distilled

water) and test samples (100 μl of serum and 6 ml of zinc sulphate (0.208 g/l) were prepared

and incubated at room temperature for 1 hour; samples were transferred into cuvettes and

read using a spectrophotometer with a 520 nm filter, as described by McEwan *et al.* (1970).

2.13   *Haematology analysis*

Fresh whole K_3EDTA blood samples (1×6 ml) (Vacuette; Cruinn Diagnostics, Ireland) were

analysed within 1-2 h after collection using an ADVIA 2120 analyser (AV ADVIA 2120,

Bayer Healthcare, Siemens, UK), which contained bovine software for haematological

analysis of bovine blood. Red blood cell (RBC) number, differential white blood cell (WBC),

neutrophil and platelet number were analysed.

2.14   *BoHV-1 (gE-) antibody*

Bovine herpesvirsus 1 (BoHV-1/BoHV1) gE- antibody was measured using an enzyme

linked immunosorbent assay (ELISA) for BoHV-1 gE antibody from IDEXX (Hoofddrop,

The Netherlands). The assay is performed using BoHV-1 antigen-coated microtitre plates.

Test samples are applied to the plate and BoHV-1 antibodies, including gE-specific

antibodies in the samples, bind to the viral antigen in the well. Following washing, an anti-

BoHV-1-gE specific monoclonal antibody conjugate is added to the microwells. The amount

of conjugate that reacts to the BoHV-1 gE antigen in the well is inversely proportional to the amount of gE specific antibody in the test sample.

All kit reagents were brought to room temperature before use, by gently swirling and inverting reagents. The test protocol for antibody detection was followed according to the manufacturer's instructions on 96 well plates, each well was coated with gE antigen. The optical densities (OD) of the samples were read at an absorbance of 650 nm using a micro plate spectrophotometer.

The inter-assay CV was less than 15%. In the gE ELISA, values were expressed as a sample-to-negative (S/N) ratio (samples were considered positive at an S/N $\leq$ 0.6).

The S/N ratio of BoHV-1 (gE) antibody was calculated by dividing the absorbance of the sample by the mean absorbance of the negative control. If the S/N ratio was less than or equal to 0.6 then the sample is interpreted as BoHV-1 gE seropositive. An S/N ratio greater than or equal to 0.7 is interpreted as negative for the presence of circulating specific BoHV-1 gE antibodies.

2.15   Infectious Bovine Rhinotracheitis (IBR) antibody determination

BoHV-1 (Infectious Bovine Rhinotracheitis virus ((IBR-Ab)) *wt* and BRSV were analysed using indirect ELISA kits from (Svanova Biotech AB, Uppsala, Sweden). The test wells were coated with a mixture of viral and cellular proteins from virus-infected cells whereas control wells were coated with material from non-infected cells of identical type. All kit reagents were brought to room temperature before use, by gently swirling and inverting. The test protocol was followed according to manufacturer's instructions. The optical density (OD) values were recorded using a microplate spectrophotometer with a 450 nm filter. The corrected OD value for controls and serum samples were calculated by subtracting the OD values of the corresponding wells containing the control antigen. The percentage positivity values were also calculated by dividing the corrected optical density (OD) value (OD_{corr}) of the

sample or negative control by the corrected OD value of the positive control and multiplying by 100.

2.16 Bovine Respiratory Syncytial antibody (BRSV) determinations

BRSV-specific antibodies were detected using a commercially available SVANOVIR[®] BRSV-Ab test kit. Indirect ELISA Samples were analysed in accordance with the manufacturer's instructions. Briefly, serum samples (4μl) were added to wells coated with BRSV viral antigen and to corresponding wells coated with control antigen containing 100μl of sample dilution buffer. Milk samples were added directly to the wells (100μl/well). Positive and negative control sera were added. The plate was shaken thoroughly and incubated at 37°C for one hour. The plate was washed three times with PBS-Tween buffer, HRP conjugate was added to each well (100μl/well) and the plate was incubated for one hour at 37°C and then washed three times using PBS-Tween buffer. Substrate solution was added to each well (100μl/well) and the plate was protected from light and incubated for 10 minutes at room temperature. Stop solution was added (50μl/well) and the optical density of the samples and controls was read at a wavelength of 450nm using a microplate spectrophotometer (Tecan Magellan, Männedorf, Switzerland). Corrected OD (OD$_{corr}$) values for samples were calculated by substracting the optical density of the wells containing the control antigen from that of the corresponding wells coated in BRSV viral antigen. The mean OD$_{corr}$ value for each of the controls was also calculated. The percentage positivity of each sample and negative control relative to the positive control was determined.

3.   Statistical analysis

Data were checked for normality and homogeneity of variance by histograms, QQ-plots, and formal statistical tests as part of the UNIVARIATE procedure of SAS (version 9.3; SAS Institute, 2006). Data that were not normally distributed were transformed by raising the

variable to the power of lambda. The appropriate lambda value was obtained by conducting a Box-Cox transformation analysis using the TRANSREG procedure of SAS. The transformed data were used to calculate P-values. The corresponding least squares means (Lsmeans) and SEM of the non-transformed data are presented in the results for clarity. Haematological, physiological, serological data for both the dam and calf were analysed using repeated measures mixed model ANOVA (PROC MIXED, SAS v 9.3) with animal ID, breed (cow breed), sampling time and their interactions included as a fixed effect. REML linear mixed models, was performed to detect significant differences between experimental breeds. The statistical model consisted of the individual animal as the experimental unit in all analyses. Breed was the fixed effect within the model. Animal was included in each model as a random effect. For repeated measures, sampling time was included as the repeated measure. Non-statistically significant interactions were removed from all the models. The type of variance-covariance structure used was chosen depending on the magnitude of the Akaike information criterion (AIC) for models run under compound symmetry, unstructured, autoregressive, heterogeneous 1st order autoregressive, or Toeplitz variance-covariance structures. The model with the least AIC value was selected. The differences between mean values were determined by F-tests using Type III sums of squares. The PDIFF was applied as appropriate to evaluate pairwise comparisons between means. A probability of $P < 0.05$ was selected as the level of significance and statistically tendencies were reported when $P < 0.10$. Average daily gain (ADG) was determined by linear regression analysis. Differences between the means were examined using the PDIFF (*difftype*) option within the MIXED procedure of SAS. Means were considered statistically significant at a probability level of $P < 0.05$.

4.   Results

4.1   *Cow live weight and body composition changes*

The mean (SD) live weight at calving was greater (P<0.01) for CL cows (765 (71.7) kg) than for LF (677 (65.8) kg). There was no difference in calf birth weight, vigour or calving difficulty score between genotypes (Table 1). Cow docility score was greater (P < 0.001) for CL compared to LF cows (Table 1). The mean (SD) BCS for CL and LF cows at calving was 3.0 (0.15) and 2.6 (0.14), respectively.

4.2   Calf performance

Mean (SD) calf birth weight was similar (P>0.05) for the progeny of CL (46.4 (4.74) kg) and LF (46.6 (6.27) kg) cows. The live weight gain of calves from LF cows was greater from birth to weaning (P<0.05) compared to the calves from CL cows. Male calves from LF cows had greater ADG (P<0.05) compared to male progeny from CL cows (Table 1), with no difference among female calves.

4.3   Colostrum IgG profile

There was no effect (P>0.05) of parity on colostrum or milk IgG concentrations. There were no significant breed × time interactions, or breed effect (P>0.05) observed for dam colostrum and transitional milk total IgG concentration. However, time had a significant effect (P ≤ 0.05) on colostrum and milk IgG concentrations (Fig 1). Total IgG concentrations decreased at d 2 and d 3 for CL cows, whereas IgG concentrations decreased at d 3 for LF cows, relative to calving (d 0).

4.4   Cow serum IgG concentrations

Cow serum IgG concentrations pre and post-partum are presented in Table 3 and the effects of breed, time and time by breed interaction both pre and post-partum are summarised. There was no effect (P>0.05) of parity on cow serum IgG concentrations. There was a significant breed × time interaction (P ≤ 0.05) and time effect (P < 0.05) for serum IgG concentrations in CL and LF cows. Pre-calving at d -84, LF cows had greater (P<0.05) serum IgG

concentrations than CL cows (Table 2). LF and CL cow serum IgG concentrations decreased significantly at calving compared to d -84. At calving, serum IgG concentration had decreased (P<0.05) for both genotypes. Post-partum, serum IgG concentrations increased (P<0.05) in LF cows at d 14 with no change (P>0.05) in CL cows.

4.5 Calf immunological profiles

Calf serum was analysed for passive transfer immunological variables (Fig. 2). There were no breed × time interactions, or breed effect (P ≥ 0.05) observed for serum ZST units and IgG concentration. However, an effect of time (P ≤ 0.05) was observed for serum ZST units and IgG concentration (Fig. 2).

4.6 Vaccine response of the dam

There was no effect (P>0.05) of parity on vaccine antibody responses. There was no significant breed × time interaction (P> 0.05), breed effect (P > 0.05) or time (P>0.05) observed for dam vaccine antibody responses for IBR gE⁻, IBR S/N ratio and BoHV-1 (*wt*). (Table 3).

4.7 Vaccine responses of the calves

There was no breed × time interaction (P> 0.05), or breed effect (P>0.05) observed in calves for maternal antibody levels for BRSV %, BoHV-1 gE⁻ S/N ratio, or BoHV-1 % (*wt*) (total antibody) values (Table 4). Time had a significant effect (P < 0.05) on maternal antibody responses. The BRSV%, BRSV S/N ratio, and BoHV-1 % (*wt*) responses increased (P<0.05) at all time-points for the progeny of CL and LF cows. The IBR gE S/N ratios decreased at all time-points, relative to d 0 for the progeny of LF cows, whereas antibody titre levels decreased at 12 h, 24 h, 48 h, 72 h, and d 7, for the progeny of CL cows (Table 4). There was no increase in antibody responses in sera of calves to the agent circulating in the vaccines that were administered at d 14 and at d 42.

4.8 Calf haematological profiles

There were no breed × time interactions, or breed effect (P ≥ 0.05) observed for any of the haematological profiles. However, time had a significant effect (P ≤ 0.0001) on calf haematological profiles (Table 5). There was no difference in RBC, platelet or neutrophil number at birth for the progeny of CL and LF cows. However, the progeny of LF dams had greater WBC number compared to the progeny of CL. Neutrophil number decreased (P<0.05) at 72 h, d 7, d 28, d 42 in the progeny of CL cows. Likewise, a decrease in neutrophil number was observed across all time-points for the progeny of LF cows, relative to d 0. Red blood cell number decreased at all time-points up to d 14, and increased at d 56 and d 84, in the progeny of CL cows. A similar decrease was observed for red blood cell number in the progeny of LF cows (up to d 14) and numbers increased (P<0.05) thereafter at d 28, d 42, d 56 and d 84. Platelet numbers increased at d 14, d 28, d 42, d 56, d 84 for both progeny of CL and LF cows. White blood cell number decreased (P<0.05) at all time-points in the progeny of LF cows, whereas numbers decreased (P<0.05) at 48 h, 72 h and d7 in the progeny of CL cows (Table 7).

5. Discussion

Our study objectives were to characterise the development of immunocompetence in beef suckler calves from birth to three months of age, and to trace glycoprotein E (gE)-negative BoHV-1 antibodies from vaccinated dams to calf sera and investigate how passive transfer affects response to live BoHV-1 vaccine at 14 days of age, and to Bovilis®Bovipast RSP vaccine at 14 and 42 days of age.

Our results show a greater decrease in cow serum IgG concentration in LF cows than CL cows suggesting that more Ig was transferred into the mammary secretion. There was a subsequent increase in beef cow serum IgG concentrations in LF but not for CL cows, post-partum. McGee *et al.* (2005a; 2006) reported that Charolais cows did not transfer immunoglobulin subclass IgG_1 as efficiently in their bloodstream as beef × Friesian cows and

that Charolais cows produce lower IgG_1 mass. Similarly, Murphy *et al.* (2005) reported a greater decrease in serum IgG_1 concentrations in cows between 90 days pre partum and parturition for LF cows.

In the present study, the progeny of LF cows had a greater pre-weaning average daily gain than the progeny of CL cows. In agreement with these findings, McGee (2005b) and Murphy *et al.* (2008) reported similar pre-weaning live weight gain for the progeny of beef × Friesian cows, compared to the progeny of Charolais cows. This finding of greater ADG from the progeny of LF cows may be associated with a greater milk yield in LF cows compared to purebred continental cow genotypes, such as CL. Furthermore, other studies reported that the progeny of Limousin and Charolais cows have lower pre-weaning weights than SLF cows (Fredeen *et al.*, 1982b; Gregory *et al.*, 1992). This finding concurs with previous studies comparing beef cow genotypes and dairy × beef cow genotypes (Wright *et al.*, 1994; McGee *et al.*, 2005b).

In our study, calf haematological profiles were consistent with the reference range reported (Egli and Bloom, 1998; Knowles *et al.*, 2000; Kramer, 2000). Mean white blood cell number was greater at birth in the progeny of LF cows, however, numbers subsequently decreased and were within the reference range at the other sampling times (Knowles *et al.*, 2000; Zanker *et al.*, 2001a). Neutrophil number was greater than reference values at the 24 h sampling time and then reached normal reference values on subsequent sampling days. It is probable that the increased WBC and neutrophil number (neutrophilia) within 24 h of birth may be attributed to greater cortisol concentration in circulation. Neutrophilia and reductions in the number of monocytes, eosinophils and basophils in circulation have been associated with the stress response to raised cortisol in circulation in bovine animals (Burton *et al.*, 2005; Lynch *et al.*, 2012; O'Loughlin *et al.*, 2014). Moreover, Chase *et al.* (2008) reported that calves produce high levels of cortisol at parturition and serum cortisol concentrations

remain increased throughout the first week post birth. The number of B cells is greatly reduced in the neonate at 4% of the total lymphocytes at a week of age and increase gradually to 20% of total lymphocytes at 6-8 weeks of age. This low number of B cells coupled with the TH2 environment induced by the calves endogenous corticosteroids, maternal and placenta hormones results in a lack of an antibody response until at least three weeks of age.

In the present study, platelet number was not different across sampling times, whereas Knowles *et al.* (2000) and Egli and Blum (1998) reported that the platelet number was within reference range at birth, with an increasing trend, that after six days values reached the adult reference range.

In Ireland, limited information has emerged regarding bovine herpesvirus 1 (BoHV-1) infection, albeit from a subset of Irish beef herds (O'Grady *et al.*, 2008) of which 73.2% were seropositive. BoHV-1 virus causes respiratory infections and abortions in cattle, and is an important component of the bovine respiratory disease complex, which causes a considerable economic loss worldwide (Chowdhury *et al.*, 2016). Pre-partum vaccination programs have been reported to increase colostral antibodies and protect newborn calves from viruses associated with calf pneumonia, for example, *Mannheimia haemolytica*, BRSV, and PI3V (Makoschey *et al.*, 2012; Dudek *et al.*, 2014). *M. haemolytica* is a primary bacterial pathogen associated with BRD (Reddy *et al.*, 2012). Thus, specific pre-partum immunisation of cows provides a significant increase in specific antibody protection against infectious disease via colostrum intake, and effectively stimulates a natural immune response in their offspring (Crouch *et al.*, 2001). Vaccination of the young calf is often practiced in an effort to provide additional protection from common disease causing pathogens during the first few months of life, as the immune system matures and maternal antibodies decay (Chase *et al.*, 2008).

In the present study, CL and LF cows tested negative to the gE antigen of BoHV-1 during the dry period (S/N = > 0.70). Similarly reports from Van Oirschot et al. (1997) demonstrated

that sera from cattle that were vaccinated with a gE- negative marker vaccine scored negative. Van Oirschot et al. (1997) reported that cattle naturally exposed to BoHV-1 tested positive for antibodies to the gE antigen of BoHV-1 in the gE- ELISA. In addition to this, animals that received an initial vaccination and were then challenged with infection, thus having reduced virus replication, also became gE-seropositive.

As a result of vaccinating the dam with a marker vaccine in this current study, the antibodies were then traceable in the calf sera post colostrum intake and calves also scored negative to the gE antibody. This demonstrated the transfer of the *BoHV-1* specific antibodies from vaccination to the calves and consequently it could be verified that this came from the marker vaccine administered to the dam as the calves were also gE negative when tested by ELISA.

All calves in the present study had elevated maternally derived antibodies to BRSV and BoHV-1 (*wt*) by 12 hours post-birth. Cowley *et al.* (2009) reported herd-level seroprevalence of BoHV-1 in Ireland at a rate of 74.9%, which is in agreement with previous findings for bulls (O'Grady *et al* 2008), and with the results of the present study. Our findings showed that the cows had total BoHV-1 (*wt*) antibodies circulating in their blood; this indicated that these animals had been naturally exposed to BoHV-1 at some stage in their lifetime. Consequently we found calves to have a considerable level of BoHV-1 (*wt*) as well as BRSV antibodies circulating in their blood post colostral consumption as a result of MDAs. This finding supports a study that was carried out in Ireland during 2009 to estimate dairy and beef herd seroprevalence of BoHV-1; findings showed a high seroprevalence of 74.9% (95% C.I. 69.9 to 79.8%), where only 2% of herds within the study vaccinated the dams, which indicates that infection in widespread (Cowley *et al.*, 2011). This is similar to reports from the UK where 83.2% of unvaccinated herds had a minimum of one BoHV-1 seropositive animal, and the mean cattle and herd sero-prevalence of BoHV-1 was 42.5% and 43.1%, respectively (Woodbine *et al.*, 2009).

A study by Munoz-Zanzi *et al.* (2002) reported that the duration of passively transferred immunity to respiratory diseases was associated with the concentration of antibodies during the first 3 days of life. Therefore the greater the antibody concentration, the longer time it takes for MDAs to decay. This may be due to the high quality and timely manner in which calves suckled their dams post-birth. It is likely therefore that at 14 days post birth not all of these MDAs have decayed. Furthermore, Chamorro *et al.* (2014) reported that calves with adequate IgG concentration can have highly variable responses to specific respiratory viruses such as BRSV and BoHV-1. It has been suggested that this may be due to the differences in maternal colostrum sources, dam vaccination history, natural exposure to infection and levels of specific viral antibodies in the colostrum.

At the time of the primary BRSV vaccination of the calves at 14 days of age we observed that there was no increase in antibody levels to the agent circulating in the vaccine in the calves' blood. This may be due to the presence and interference of maternal antibody from colostrum and the immaturity of the immune system at this young age (Tyler *et al.*, 1989). Although post primary or secondary vaccination did not result in an increase in antibody levels in the blood, it is likely that a cell mediated response may have occurred post vaccination (Fulton *et al.*, 2004). BRSV is a challenging issue in calves; maternal antibodies are often present in 1 to 6 month old calves, this can have a knock on effect on vaccination programs because these antibodies do not decay until approximately 40 days of age (van der Poel *et al.*, 1999; Chase *et al.*, 2008). Our results are similar with no response to vaccination.

Reports from Lemaire *et al.* (2001) showed the ability of a BoHV-1 gE-negative vaccine strain to establish latency in both seronegative and calves that received passive immunity subsequent to a single intranasal immunization. However the efficacy of the gE negative vaccine was reduced in calves which received maternal antibodies. Passively acquired antibodies to BRD viruses affect the development of active immunity to these viruses in

calves. The presence of potentially 'inhibitory' maternally derived antibodies, in combination with the bias of the immature neonatal immune system towards Th2 responsiveness, are clear obstacles to the effectiveness of vaccines delivered in the neonatal period (Morein *et al*., 2002).

In conclusion, the mean level of antibodies to the gE antigen of BoHV-1 during the dry period were negative due to the presence of artificial infection via marker vaccine. The results demonstrated that repeated vaccination of the dams with the gE marker vaccine produced a gE negative colostrum with very high levels of BoHV-1 antibody and that calves efficiently consumed the colostrum to acquire adequate transfer of passive immunity. As expected the maternally derived antibody titres for the specific respiratory viruses were at their peak levels post colostrum ingestion. Antibodies to the gE antigen of BoHV-1 tested negative within both treatment groups as a result of pre-parturient immunisation. There were no differences between the progeny of CL and LF cows in the level of BRSV antibodies post vaccination at 2 weeks of age and post booster vaccination 4 weeks later. Further research relating to BRD complex infections and the processes involved in the acquisition of immunity via vaccination are required to both improve the effectiveness of current preventative immunisation strategies and to aid development of new and more effective vaccines that could be used in the presence of MDAs.

Conflict of interest statement

There were no conflicts of interest in the preparation of this work. None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Table 1: Cow and calf live weight, characteristics of cows at calving (d 0), and calf performance. The vales are expressed as mean (SEM).

	Cow genotype		
	Charolais × Limousin (CL)	Limousin × Friesian (LF)	Significance
Cow live weight at d 0	765 (71.7)	677 (65.8)	P<0.01
Calf birth weight	46.4 (4.74)	46.6 (6.27)	ns
[1] Calf vigour	4 (0.82)	3.76 (0.66)	ns
[2] Calving difficulty score	1 (0)	1.29 (0.59)	ns
[3] Cow docility	3.4 (1.26)	2.1 (0.66)	P<0.001
Calf average daily gain (ADG) from birth to weaning	1.12 (0.14)	1.3 (0.16)	P<0.01
Male calf ADG from birth to weaning	1.16 (0.13)	1.4 (0.13)	P<0.01
Female calf ADG from birth to weaning	1.08 (0.15)	1.15 (0.07)	ns

[1]Calf vigour [1= normal, vigorous calf; 2= weak calf that sucked without assistance; 3= weak calf needed assistance to suck]; [2]Calving difficulty score (0 to 5 scale), [1 = no assistance; 2 = slight assistance; 3 = moderate mechanical assistance (calving jack needed); 4 = 'hard' mechanical assistance / veterinary assistance, 5 = caesarean]; [3]Cow docility score after calving (1 to 6 scale) with 1 being very docile to 6 being very aggressive); ns = not significant.

Table 2: Least square means (SEM) for the effect of cow breed on dam serum IgG concentration, at d-84, d-56, d-28, calving (d 0) and d 14, relative to calving

Variable	Cow genotype	Time in days (d)					Significance		
		d-84	d-56	d-28	d 0	d 14	Breed	Day	Breed × Day
Dam serum IgG (mg/ml)		18.3	19.0	18.5	20.8 [a]	20.5			
	CL	(1.46)	(1.46)	(1.46)	(1.46)	(1.46)	0.26	0.01	<0.0001
		23.8[b]	22.4	21.3[a]	17.4[a]	22.91[b]			
	LF	(1.46)	(1.46)	(1.46)	(1.46)	(1.48)			

Cow genotype CL = Charolais × Limousin (n=13); LF = Limousin × Friesian (n=17); [a] Within rows, Lsmeans differ from d 0 by P < 0.05; [b]Lsmeans differ between CL and LF at d-84;

Table 3: Least square means (SEM) for antibody response to vaccination in cows at d-84, d-56, d-28, calving (d 0) and d 14, relative to calving

Variable	Cow genotype	Time in days (d)					Significance		
		d -84	d -56	d -28	d 0	d 14	Breed	Day	Breed × Day
BoHV-1 gE S/N Ratio	CL	0.85 (0.09)	0.86 (0.09)	0.89 (0.09)	0.85 (0.09)	0.86 (0.09)	0.25	0.71	0.94
	LF	0.73 (0.08)	0.72 (0.08)	0.77 (0.08)	0.73 (0.08)	0.70 (0.08)			
Total antibody to BoHV-1 (*wt*)	CL	1.17 (0.05)	1.24 (0.05)	1.21 (0.05)	1.15 (0.05)	1.13 (0.05)	0.87	0.09	0.61
	LF	1.12 (0.04)	1.18 (0.04)	1.21 (0.04)	1.19 (0.04)	1.13[a] (0.04)			

Cow genotype CL = Charolais × Limousin (n=13); LF = Limousin × Friesian (n=17); [a]Within rows, Lsmeans differ from d 0 by $P < 0.05$.

Table 4: Least square means (SEM) for maternally derived antibodies, and responses to vaccination in calves.

Variable	Cow genotype	0 h	12	24	48 h	72 h	d 7	d 14	d 28	d 42	d 56	d 84	Breed	Time	Breed × Time
BRSV (%)	CL	1.4	55.	70.	82.4	87.0	88.8	88.7	84.0	76.3	84.5	66.7	0.2	<0.00	0.90
		(5.80)	(6.02)	(5.45)	(5.45)	(5.45)	(5.45)	(5.45)	(5.45)	(5.80)	(5.61)	(5.45)			
	LF	2.7	58.	83.	90.7	92.1	93.1	95.3	89.2	82.5	83.3	67.3			
		(5.93)	(5.20)	(5.07)	(5.07)	(5.07)	(5.07)	(5.07)	(5.07)	(5.20)	(5.07)	(5.07)			
BoHV-1 gE S/N Ratio	CL	0.9	0.8	0.8	0.84	0.85	0.85	0.97	0.88	0.89	0.87	0.89	0.1	0.000	0.43
		(0.10)	(0.10)	(0.09)	(0.09)	(0.09)	(0.09)	(0.09)	(0.09)	(0.10)	(0.10)	(0.09)			
	LF	0.8	0.6	0.6	0.68	0.65	0.65	0.61	0.66	0.68	0.64	0.73			
		(0.09)	(0.09)	(0.09)	(0.09)	(0.09)	(0.09)	(0.09)	(0.09)	(0.09)	(0.09)	(0.09)			
Total antibody to BoHV-1 (wt)	CL	0.1	1.0	1.0	1.00	1.00	0.97	1.05	1.07	1.02	1.04	0.91	0.4	<0.00	0.89
		(0.0	(0.09)	(0.08)	(0.08)	(0.08)	(0.08)	(0.08)	(0.08)	(0.08)	(0.08)	(0.08)			
	LF	0.2	1.1	1.2	1.17	1.05	1.09	1.01	1.11	1.00	1.05	0.95			
		(0.08)			(0.07)	(0.07)	(0.07)	(0.07)	(0.07)	(0.07)	(0.07)	(0.07)			

Time in days (d) — Significance

(0.07)   (0.07)

Cow genotype; CL = Charolais × Limousin (n=13); LF= Limousin × Friesian (n=15); [a]=Within rows, Lsmeans differ from d 0 by $P < 0.05$.

Wild type = (*wt*)

Table 5: Least square means (SEM) for neutrophil, red blood cell, platelet and white blood cell numbers from birth (d 0) to d 84 for the progeny of CL and LF cows

Variable	Cow genotype	Time in days (d)										Breed	Time	Breed × Time
		0 h	24 h	48 h	72 h	d 7	d 14	d 28	d 42	d 56	d 84			
Neutrophils ($\times 10^3$ cells/µl)	CL	5.4 (0.73)	6.5 (0.85)	4.1 (0.48)	3.7[a] (0.45)	3.4[a] (0.41)	4.9 (0.71)	3.4[a] (0.49)	3.2[a] (0.76)	4.2[a] (0.40)	2.6[a] (0.45)	0.23	<0.0001	0.11
	LF	6.2 (0.69)	7.2[a] (0.76)	5.0[a] (0.43)	4.0[a] (0.40)	3.8[a] (0.37)	4.4[a] (0.64)	4.2[a] (0.45)	4.4[a] (0.69)	3.3[a] (0.37)	3.4[a] (0.42)			
RBC ($\times 10^6$ cells/µl)	CL	9.8 (0.34)	8.1[a] (0.32)	8.1[a] (0.32)	8.0[a] (0.32)	7.9[a] (0.32)	8.5[a] (0.32)	9.4 (0.32)	10.2 (0.33)	11.2[a] (0.32)	13.1[a] (0.32)	0.26	<0.0001	0.14
	LF	9.1 (0.34)	8.3[a] (0.29)	7.9[a] (0.29)	7.6[a] (0.29)	7.7[a] (0.29)	8.7[a] (0.29)	9.3 (0.30)	9.7 (0.30)	10.5 (0.30)	11.8 (0.30)			
Platelets ($\times 10^3$ cells/µl)	CL	623.9 (52.67)	468.2[a] (39.80)	498.2 (46.91)	550.9 (58.87)	966.5[a] (75.75)	963.8[a] (57.00)	815.6[a] (55.26)	825.6[a] (59.58)	806.3[a] (65.06)	703.9[a] (59.88)	0.06	<0.0001	0.69
	LF	539.2 (51.95)	445.9 (35.88)	465.3 (42.28)	445.3 (53.06)	895.0[a] (68.28)	768.6[a] (51.38)	815.4[a] (50.98)	692.9[a] (53.78)	740.6[a] (60.23)	631.6[a] (55.23)			
WBC ($\times 10^3$ cells/µl)	CL	12.3 (1.44)	11.4 (1.34)	7.7[a] (1.34)	7.7[a] (1.34)	8.3[a] (1.34)	10.8 (1.34)	9.5 (1.34)	10.0 (1.38)	11.9 (1.34)	11.4 (1.34)	0.46	<0.0001	0.08
	LF	20.3[b] (1.49)	11.3[a] (1.21)	8.0[a] (1.21)	7.3[a] (1.21)	8.6[a] (1.21)	10.2[a] (1.21)	10.6[a] (1.24)	10.9[a] (1.24)	10.6[a] (1.25)	12.0[a] (1.25)			

Cow genotype CL = Charolais × Limousin (n=13); LF = Limousin × Friesian (n=17); RBC = red blood cells; WBC = white blood cell. The values are expressed as least square means (Lsmeans) and SEM; [a] Within rows, Lsmeans differ from d 0 by P < 0.05. [b] Within column different from CL progeny.

Figure legends

Fig 1. The effect of cow breed on colostrum and transitional milks IgG concentrations, at calving (d 0) and at day 2 and 3 relative to calving.

Cow genotype CL = Charolais × Limousin (n=13); LF = Limousin × Friesian (n=17); Fig 2. Least square means (SEM) for serum Zinc Sulphate turbidity (ZST) Units and IgG concentration, from birth (0 h) to d 7 for the progeny of CL and LF cows

Fig 2. Least square means (SEM) for serum Zinc Sulphate turbidity (ZST) Units and IgG concentration, from birth (0 h) to d 7 for the progeny of CL and LF cows
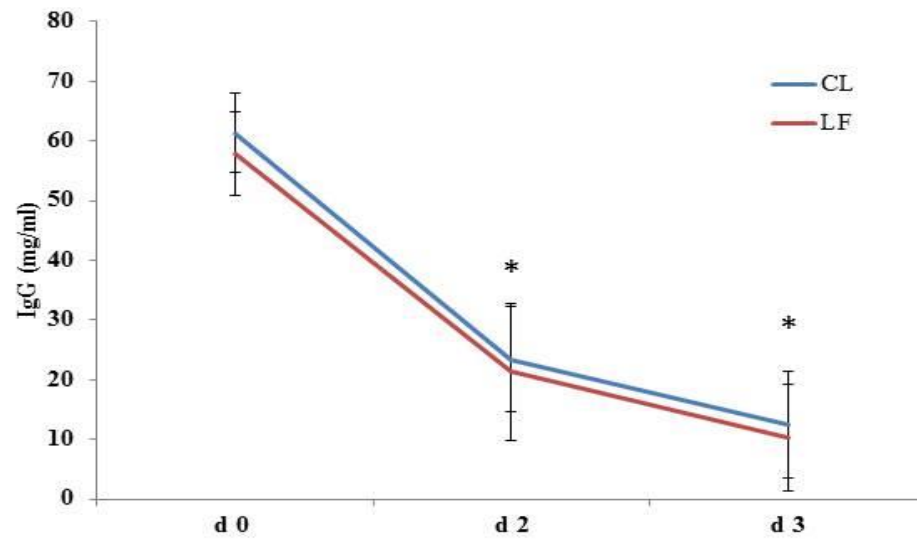
* Lsmeans differ from d 0 by P < 0.05.

Figure 1
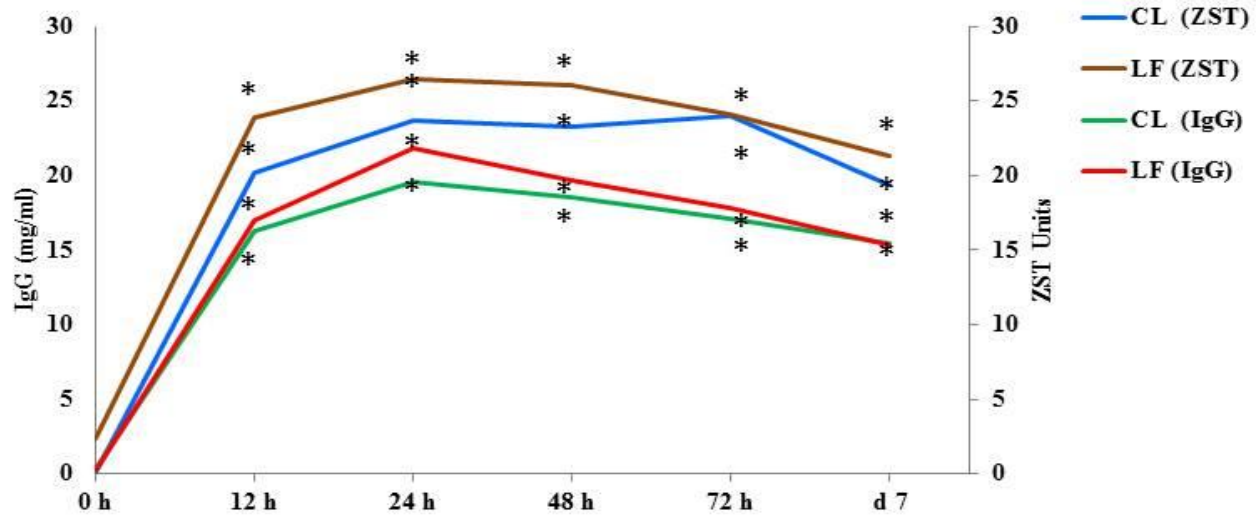
* Lsmeans differ from d 0 by P < 0.05.

ZST (Units) Breed (P = 0.14) Time (P<0.0001) Breed × Time (P = 0.44);
IgG (mg/ml) Breed (P = 0.65) Time (P<0.0001) Breed × Time (P = 0.93)

Figure 2

Highlights

- Greater decrease in cow serum IgG concentration in LF cows than CL cows pre-partum;
- Progeny of LF cows have a greater pre-weaning ADG than the progeny of CL cows;
- Maternally derived antibodies affects response to vaccination in suckled beef calves;