Effect of passive transfer status on response to a glycoprotein E (gE)-negative bovine herpesvirus type 1 (BoHV-1) and bovine respiratory syncytial virus (BRSV) vaccine and weaning stress in pre-weaned dairy calves

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ABSTRACT
The study objectives were to: 1) examine how calves of divergent immune status respond to BRSV vaccination at 3 weeks of age; 2) trace glycoprotein E negative BoHV-1 antibodies from vaccinated dams to calf sera and to investigate how passive transfer affects response to live BoHV-1 vaccine at 6 weeks of age; 3) explore the impact of passive transfer status on blood metabolites around weaning. Thirty seven Holstein cows and their calves were included in the study. All cows were immunised with a commercial marker vaccine against BoHV-1 (gE-) administered intra-muscularly at 4 month prior to the start of calving. Calves were assigned to 1 of 2 colostrum treatment groups: 1) 5% of BW in colostrum fed at birth, or 2) 10% of BW in colostrum fed at birth. Calves were also immunised at 3 weeks of age with a respiratory commercial vaccine, and a booster administered 4 weeks later. Calves were also immunised against BoHV-1 at 6 weeks of age, using one dose of a live commercial vaccine. The results demonstrated that level of passive immunity had no effect on immune response to vaccination and the importance of feeding colostrum from vaccinated BoHV-1 gE- dams to provide calves with passive protection against IBRV.

Introduction
Bovine respiratory disease (BRD) is a common illness in young calves and it has been documented that calves which have suffered this illness also experience reduced growth, and the mortality rate is increased (Wendeyer et al., 2015). A Canadian study carried out across 17 dairy herds reported the risk of pneumonia to be as high as 39%, with a reoccurrence risk of 56% and the median age of this first occurring was at 27 days of age (Van Donkersgoed et al. 1993). Mortality cases as a result of pneumonia are most common during the pre-weaning period and shortly after weaning. It is thought that weaning, painful procedures, housing and ventilation can all be factors associated with an increase in disease at this stage of a calf’s life (Lorenz, Earley, et al. 2011).

Calves primarily depend on maternal colostrum after birth as a source of protection against disease. It is the maternal derived antibodies (MDA) from their dam which are transferred in the colostrum that activates and regulates the innate response to combat infection (Chase et al. 2008). However, although colostrum is required to protect the calf in the early stages of life before they develop their own immunity, there may be interference from the MDA inhibiting the calf’s ability to mount an immune response to antigens from vaccination or natural infection (Morein et al. 2002). Fulton et al. (2004) reported that most MDA have a decay half-life of 16–28 days, although the duration of this immunity is dependent on the mass of antibodies ingested and absorbed by the calf in the first 24 hours of life. There are reports in the literature that the prolonged survival of passively acquired immunity in calves that receive maternal colostrum at birth is extremely inconsistent (Menanteau-Horta et al. 1985, van der Poel et al. 1999, Kirkpatrick et al. 2008). It is during the phase of decaying MDA that the calf is presented with a window of susceptibility to pathogens, the calf is vulnerable to infectious diseases mainly because the MDA or endogenous antibodies are not at a significant level to prevent the infection (Morein et al., 2002; Niewiesk, 2014). It has been confirmed by several researchers that vaccinating calves when MDA are still present can activate T-cells (Endsley et al. 2003, Niewiesk 2014) which are specific to certain disease-causing pathogens, and this can occur without seroconversion post vaccination. Consequently, when the calf receives a secondary vaccination after the MDA have declined, production of high levels of antibody consistent with a memory response occurs.

Marker vaccines are commercially available, inactivated or live-attenuated (Kaashoek et al. 1994, 1995) and for bovine herpesvirus type 1 (BoHV-1) the gene coding for the unnecessary glycoprotein E (gE) has been deleted. This allows for serological differentiation between vaccinated animals and animals that have had natural contact with the microorganism (seropositivity...
does not necessarily imply infection) (Van Olirschot 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).

Weaning is often a very stressful time for calves due to the alteration of several environmental and social husbandry practices, such as changing their diet in which they receive no milk, and usually calves are re-housed with different animals at weaning which they are unfamiliar with, and this can lead to social stress. The separation from the dam and termination of milk feeding do not occur simultaneously in the dairy calf (Flower and Weary 2003). Dairy calves are normally removed from their dams at birth and fed milk and concentrate by artificial means thereafter. Therefore, weaning of dairy calves solely involves the removal of milk from their diets (Flower and Weary 2003). Artificially reared dairy calves are typically weaned from milk either abruptly, or gradually. Gradual weaning is preferable to abrupt weaning (Khan et al. 2007, Jasper et al. 2008, Nielsen et al. 2008, Sweeney et al. 2010, Lorenz, Mee, et al. 2011, Hill et al. 2012, De Passillé and Rushen 2016).

Abrupt weaning of artificially reared dairy calves has led to increased vocalization, activity, cross-suckling and unrewarded occupancy at a milk feeder (Budzynska and Weary 2008, Nielsen et al. 2008), whereas gradual weaning has increased both starter consumption and feed efficiency and improved rumen function and average daily gain (ADG) (Khan et al. 2007, Sweeney et al. 2010, Hill et al. 2012, De Passillé and Rushen 2016). Acute phase proteins (APPs) are often used as an indicator of infection, inflammation, surgical trauma or stress-related illness. Haptoglobin is an important APP in the bovine species. It reduces pro-oxidative and pro-inflammatory stress by means of binding haemoglobin and removing it from the animal’s circulation (Murata et al. 2004).

The objectives of this study were (1) to examine the effect of divergent immune status on the response to bovine respiratory syncytial virus (BRSV) vaccination at 3 weeks of age; (2) to trace the transfer of e-negative BoHV-1 antibodies from vaccinated dams to calf sera and investigate how passive immunity affects response to live infectious bovine rhinotracheitis (IBRV) vaccine at 6 weeks of age; and (3) to explore the effect of passive transfer status on blood metabolites pre and post weaning. The hypothesis of the study was that calves receiving a lower volume (5% body weight (BW)) of colostrum, thus possessing less MDA than calves fed 10% BW would respond better to BRSV and IBRV vaccination, and that there would be no effect of passive transfer immune status on blood metabolite concentration around weaning.

Methods and materials

All procedures and treatments within this study were conducted under the UK Animal (Scientific Procedures) Act (Office, 1986). This study was conducted at AFBI research dairy farm in Hillsborough, located in County Down, in Northern Ireland (latitude 52°27’; longitude 6°14’). The study population consisted of 37 multiparous Holstein cows, which received their annual vaccinations in October and calved between 9 February and 17 April 2014 and 37 dairy bred calves.

Dam vaccination

All cows involved in this study were immunized against BoHV-1 (gE) in October 2013 (4-month prior to start of calving); they were injected intra-muscularly with a commercial vaccine (Bovilis IBR Marker Inactivated, MSD Animal Health, Buckinghamshire, England).

Management of calves

The experiment involved two first feed maternal colostrum treatments in which the calves were fed according to their BW at birth: (1) 5% of BW of colostrum at birth (5 BW) and (2) 10% of BW of colostrum at birth (10 BW). The birth of each calf was vigilantly observed and calves were separated from their dam approximately 15–20 min post parturition to prevent suckling occurring. Calves were weighed and placed in a straw-bedded pen where they were blood sampled and received their first feed of colostrum, according to the colostrum treatment group to which they were assigned (5% BW versus 10% BW). Calves were randomly assigned to colostrum treatment group depending on calf gender, and fed their own dams colostrum via oesophageal tube within 2.5 hours post birth. Nine males were allocated to each group and 7 and 12 females were allocated to 5 BW and 10 BW groups, respectively. Calves in 5 BW and 10 BW groups averaged 39.8 and 40.8 kg, respectively. For the first colostrum feed, the two groups received 5% and 10% of their body weight of first milking colostrum for 5 BW and 10 BW treatments, respectively. Subsequently, the calves in 5 BW treatment were fed 5% of their BW of second milking colostrum (from their own dam) 12 hours post calving via a teat feeder. Whereas the calves in 10 BW group received colostrum from the first milking post birth (stored at 4°C and heated to body temperature before feeding). Following the first and second colostrum feeds, all calves were fed 12.5% of their BW of colostrum from their own dam for the first 4 days of life. Calves were individually penned for the first 4 days of life, and on day 5 were placed in a group pen where they were fed ‘Calf Gold milk replacer (MR)’ (Bromfield, Dumfries, UK) from an automatic milk feeder (Forster Technik vario, Germany). Calves were gradually weaned (day 56) by the step-down method. All calves were offered ad libitum concentrate and clean water from 5 days of age. Disbudding of calves took place at approximately 5–6 weeks of age. Post weaning calves were removed from their original pen to another group pen on day 63 and fed grass silage plus 2 kg concentrate per day until 70 days of age when the experiment was complete.

Calf vaccination

All calves in the study were immunized at 3 weeks of age against Parainfluenza type 3 virus (PI3 virus), BRSV and Mannheimia (Pasteurella) haemolytica serotype A1 via subcutaneous injection using a commercial vaccine called Bovipast RSP (MSD Animal Health, Buckinghamshire, UK), a booster was then administered 4 weeks later. All calves were also immunized against BoHV-1 at 6 weeks of age, using one dose of a live commercial vaccine (Bovilis IBR marker, MSD Animal Health, Buckinghamshire, UK); this was administered via intranasal route at 6 weeks of age.
Blood sample collection
Blood samples were collected from the tail vein of cows using a 10 mL serum vacutainer (BD, Oxford, UK) at weeks −5, −3, −1 pre calving and +2 post calving. Blood samples were collected from the calf via the jugular vein using a 10 mL serum vacutainer tube (BD, Oxford, UK), at 0, 24, 48 and 72 hours post birth and days 7, 14, 21, 35, 49, 56 and 70 post birth. All blood samples were left at room temperature for 1 hour to allow clotting to occur; samples were then centrifuged at 1764×g for 15 min, serum was decanted into three aliquots and stored at −20°C until specific antibody analysis.

Additional blood samples were collected from the calf via the jugular vein using a 10 mL serum vacutainer tube (BD, Oxford, UK) pre and post weaning on days 49, 53, 56, 57 and day 60. All blood samples were left at room temperature for 1 hour to allow clotting to occur. Samples were then centrifuged at 1764×g for 15 min, serum was decanted into three aliquots and stored at −20°C until blood metabolite analysis.

Sample analysis
Bovine herpesvirus 1 (gE) antibody. Bovine herpesvirus 1 gE antibody was analysed using an ELISA kit for BoHV-1 gE antibody from IDEXX (Hoofddorp, The Netherlands). All kit reagents were brought to 18–26°C and mixed gently through inverting and swirling, and test procedure was followed according to manufacturer’s instructions. The OD values were recorded using a microplate spectrophotometer with a 650 nm filter. Samples were assayed in duplicate, with an inter-assay CV < 0.15. The concentration of BoHV-1 (gE) antibody was calculated by dividing the absorbance of the sample by the mean absorbance of the negative control which resulted in an S/N value. The quantity of antibodies to gE was inversely proportional to the absorbance and the S/N value. If the S/N value was less than or equal to 0.60, the sample was classified as positive for antibodies to the gE antigen of BoHV-1. If the S/N was greater than 0.70, the sample was classified as negative for antibodies to the gE antigen of BoHV-1. Any samples that were greater than 0.60 but less than or equal to 0.70 were retested.

Infectious bovine rhinotracheitis and respiratory syncytial virus antibody. IBRV and BRSV were analysed using indirect ELISA kits from Svanova (Uppsala, Sweden). All kit reagents were brought to 18–26°C and the test procedure was followed according to manufacturer’s instructions. The OD values were recorded using a microplate spectrophotometer with a 450 nm filter. Samples were assayed in duplicate, with an inter-assay CV of 0.15. 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 OD value of the sample or negative control by the corrected OD value of the positive control and multiplying by 100.

Blood metabolites. Serum non-esterified fatty acids, beta-hydroxybutyrate (BHBA), haptoglobin (Hp) and urea concentrations were analysed using the Audit Sapphire 800 Analyser (Audit Diagnostics, Cork, Ireland).

Statistical analysis
Statistical analyses were performed using GenStat, 16th edition (VSN International, 2015). Statistical differences were considered significant at P < .05. REML linear mixed models were performed to detect significant differences among experimental groups. Pre-vaccination antibody titre and day of vaccination were used as covariates and colostrum treatment groups were fitted as fixed effects. Blood metabolites were analysed using repeated measures; colostrum treatment group was fitted as a fixed effect.

Results
The number of weeks relative to parturition had an effect (P = .03) on the mean concentration of antibodies to the gE antigen of BoHV-1 in the dam’s blood (Figure 1). The number of weeks relative to parturition had no effect (P > .05) on the total IBRV antibody concentration (Figure 2).

Peak levels of maternally derived total IBRV antibody were recorded in the calves’ sera during the first 2 days post birth subsequent to colostrum consumption, and the MDA specific to IBRV showed a gradual decline thereafter (Figure 3). Colostrum treatment had no significant effect on the percentage positivity of both antibodies to IBRV and BRSV. However, time did have a significant effect on percentage positivity of antibodies to total IBR (P = .001) and BRSV (P < .001). Total antibodies to IBR peaked at 48 h post birth and decreased thereafter, whereas BRSV antibodies also peaked at 48 hours, decreasing until day 49 and then started to increase.

Overall, 24.3% and 37.8% of calves were treated for pneumonia in the neonatal period and 28–70 days period, respectively, whereas 37.8% of calves were treated for diarrhea in the neonatal period and 0% of calves were treated in the 28–70 days period.

The colostrum feeding regime (5% versus 10% BW) had no effect (P > .05) on the mean concentration of antibodies to the gE antigen of BoHV-1 circulating in the calves sera from birth to 70 days of age (Figure 4). Both treatment groups tested negative to gE antibody (Figure 4).

We observed that during the time of primary vaccination against BRSV at 3 weeks of age there was no increase in antibody titre to the agent circulating in the vaccine in the calves’
sera. We found no significant difference between colostrum treatments or incline in antibodies post primary vaccine at day 21 ($P = .35$) (Figure 5).

We found no difference ($P > .05$) between colostrum treatment groups in terms of response to vaccine at 35, 49, 56 and 70 days post birth. The number of days post vaccination had a significant effect on the level of circulating BRSV in the calves’ sera independent of treatment group ($P < .001$); we observed BRSV antibody titre to be significantly greater at days 56 and 70 than at days 35 and 49 (Figure 6).

Colostrum treatment had no effect ($P > .05$) on BHBA, non-esterified fatty acids (NEFA), urea and Hp concentration in the calves’ blood pre, during and post weaning. However, we found the age of calf to have an effect ($P < .05$) on these blood metabolites. The BHBA concentration was lowest pre-weaning and increased at weaning and thereafter, whereas NEFA concentration decreased at weaning and thereafter. We observed the urea and Hp levels to be greatest in the days post weaning (Table 1). We found no difference ($P > .05$) between treatment group and calf feeding behaviour, that is, visits to the feeder without reward of milk replacer (MR) (Figure 7).

However, the number of visits to the feeder without reward of MR peaked for both treatment groups at approximately day 55 (4 days post weaning). The step-down weaning method decreasing MR volume offered and consumed from day 35 with a direct increase in the amount of concentrate consumed (Figure 8).

**Discussion**

A newborn calf passively receives MDA via their dam’s colostrum and these are critical in the neonatal immune response (Niewiesk 2014). Vaccination of the young calf is often practised 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 MDA decay (Chase et al. 2008). This study was performed to determine whether the colostrum feeding volume had any effect on their response to BRSV and BoHV-1 (IBRV) vaccination in the early weeks of life, and the effects of passive transfer on blood metabolites at weaning.

**Neonatal immune response (birth to 28 days)**

It has been well documented that calves that receive adequate volumes of good quality colostrum shortly after birth achieve adequate passive transfer (APT) in terms of their serum IgG.

**Figure 2.** Mean total antibody concentration to IBR circulating in dam’s blood pre and post parturition.

**Figure 3.** Mean (±SEM) level of maternal total antibody to IBR circulating in the calves’ blood post parturition.

**Figure 4.** Mean (±SEM) ratio of IBR gE antibody circulating in the calves’ blood post calving when fed 5 or 10% of their BW in colostrum at first feed. Arrow indicates time of vaccine administration at 42 days of age. A value above 0.7 indicates animals are negative for the presence of circulating gE-specific antibodies.

**Figure 5.** Mean (±SEM) total IBR antibodies circulating in calves’ blood post birth when fed 5 or 10% of BW in colostrum at first feed.
concentration post feeding. However, a study by Chamorro et al. (2014) confirmed calves with adequate IgG concentration to be still highly vulnerable 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 (Chamorro et al. 2014). 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 is the antibody concentration, the longer time it takes for MDA to decay. This was not apparent in our study as we observed no differences between treatment groups of calves which showed different levels of passive transfer. This may be due to the high quality and timely manner in which calves were fed colostrum at their first feed. Many of the calves in the 5 BW group achieved APT, and therefore at 3 weeks of age it is likely that not all of the MDA had decayed.

The majority of vaccinated dams involved in this study tested negative for antibodies to the gE antigen of BoHV-1 during the dry period. 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 calves’ blood post colostrum intake and calves also scored negative to the gE antibody. This demonstrated the transfer of the BoHV-1 specific

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| Metabolic variables | Treatment* | Days relative to weaning (d 0) | SED | P-value | Day(D) | D × T |
|--------------------|------------|-------------------------------|-----|---------|--------|-------|
| BHBA               | S BW       | 0.21                          | 0.01| 0.16    | 0.22  | 0.24  | 0.02 | 0.02 <0.001 | 0.38 |
|                    | 10 BW      | 0.22                          | 0.01| 0.19    | 0.22  | 0.24  | 0.02 | 0.02 <0.001 | 0.38 |
| NEFA               | 0.14       | 0.13                          | 0.008| 0.17   | 0.15  | 0.13  | 0.10 | 0.11 0.02 | 0.33 | <0.001 0.30 |
|                    | S DW       | 0.15                          | 0.008| 0.13   | 0.15  | 0.13  | 0.10 | 0.11 0.02 | 0.33 | <0.001 0.30 |
| Urea               | 2.68       | 3.02                          | 0.31| 2.66   | 2.75  | 2.84  | 2.77 | 2.64 3.52 | 0.22 | 0.18   0.01 0.17 |
|                    | 2.75       | 3.01                          | 0.31| 2.66   | 2.75  | 2.84  | 2.77 | 2.64 3.52 | 0.22 | 0.18   0.01 0.17 |
| Haptoglobin        | 0.35       | 0.35                          | 0.01| 0.28   | 0.32  | 0.38  | 0.38 | 0.41 0.34 | 0.32 | 0.02   0.66 0.02 0.54 |

* Treatments: 5 or 10% of BW in colostrum at first feed.

BHBA: Beta hydroxybutyrate.
NEFA: Non-esterified fatty acids.
SED: Standard error of the difference (SED).

Figure 6. Mean (±SD) BRSV antibody circulating in calf sera post birth. Solid arrow indicates time of primary vaccination (day 14) and dashed arrow indicates time (day 42) of booster vaccination with a multivalent respiratory vaccine.

Figure 7. Effect of days on feeder on the number of visits to the feeder without reward of milk replacer (MR). Dashed arrows indicate time point where milk is stepped down and solid arrow indicates time of weaning.
antibodies from vaccination to the calves and consequently it could be verified that this came from the marker vaccine given to the dam as the calves were also gE-negative when tested by ELISA.

Our findings demonstrated the majority of cows to have total IBRV antibodies circulating in their blood. When the dams were tested using the ELISA method to detect gE antibodies, it was confirmed that the presence of IBRV antibodies in the dams was the result of vaccination and not wild-type infection as all of the animals were gE-negative. Consequently, we found calves to have a considerable level of total IBRV antibodies circulating in their blood post colostral feeding as a result of MDA. In contrast to the current study, research carried out in Ireland during 2009 to estimate dairy and beef herd seroprevalence of BoHV-1 found a high seroprevalence of 74.9% wild-type IBRV (95% C.I. 69.9–79.8%). A small percentage of herds (2%) within the study vaccinated the dams against BoHV-1, which indicates that natural infection in widespread (Cowley et al. 2011).

At the time of the primary BRSV vaccination we observed that there was no increase in antibody titre to the agent circulating in the vaccine in the calves’ blood. This may be due to the presence and interference of MDA from colostrum and the immaturity of the immune system (Tyler et al. 1989). In parallel to this study, Windeyer et al. (2015) investigated the relationship of BRD or artificial immunization with the serologic response in Holstein calves; findings confirmed that the time taken for the anti-BRSV antibody to decay was longer in calves that were treated for BRD than in those that were not. Reports showed that timing of vaccination had no effect on the rate of antibody decay when immunized at 2 or 5 weeks of age, and that using a multivalent respiratory vaccine on calves with APT was not beneficial to the calf, due to the level of MDA present.

**Immune response (4 weeks to 10 weeks)**

A large proportion of calves (37.8%) were treated for pneumonia during the 4–10 weeks period post parturition, and the rate of pneumonia was much greater compared to that during the neonatal period in this current study. This is similar to reports from Van Donkersgoed et al. (1993), and is probably due to the window of vulnerability between MDA decline and immaturity at this time. However, when calves are challenged at a later date by this specific pathogen or given a booster vaccination, the calf is already primed from the primary vaccine. Although post primary vaccination did not result in an increase in antibody titre levels in the blood, it is likely that a cell-mediated response may have occurred post vaccination (Fulton et al. 2004). Consequently, after the booster vaccine (2 weeks later) the calf had an improved ability to produce increased antibody levels in the blood (which is known as an amnestic response to the infection, Woolums, 2014) and demonstrated there was immune priming following the first dose of vaccine.

Van Donkersgoed et al. (1995) reported on the effects of various vaccination protocols on passive and active immunity to Pasteurella haemolytica and Haemophilus somnus in beef calves. The authors established that calves vaccinated at 3 and 4 months of age, when there were still low levels of pre-existing antibodies circulating, had significantly greater antibody titres to P. haemolytica at 5 months of age and to H. somnus at 5 and 6 months of age than calves that were not vaccinated. It has been suggested by Woolums (2007) that vaccinating calves when MDA are still present may be of benefit to the producer in terms of cost and animal welfare; calves are very vulnerable to disease at this young age and vaccines can be very effective in producing a protective immune response to later pathogen challenge. In some cases calves are not fully protected when vaccinated in the face of maternal antibodies and this is thought to be correlated with the age of the animal at vaccination, type of vaccination, amount of MDA in circulation and virulence of the challenging pathogen (Woolums, 2007).

BRSV is a challenging issue in calves as MDA are often present in 1- to 6-month-old calves. This can have a knock on effect on vaccination programmes because these antibodies do not decay until approximately 40 days of age (van der Poel et al. 1999, Chase et al. 2008). Our results mirror this issue, where there was no response to vaccination at 3 weeks of age after the primary 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

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**Figure 8.** Effect of days on feeder on concentrate intake between colostrum treatment groups. Dashed arrow indicates the first step-down phase in MR and solid arrow indicates time of weaning (transition from liquid to solid diet).
received MDA. Vaccination cannot prevent latency of the vaccine itself or natural exposure to wild-type BoHV-1, therefore it is crucial that producers have a vaccination plan where immunization is repeated according to the protocol and other management factors are in place to encourage eradication of this disease.

Weaning is often a stressful event for young calves (Schaefer et al. 1997) as demonstrated by visible physiological changes in their health, performance and well-being. There is a strong association with stress and growth, fertility, animal welfare, carcass quality and vulnerability to disease (Schaefer et al. 1997, Ali et al. 2008). In this current study, we found the colostrum treatment group (5% versus 10% BW) to have no effect on the blood metabolite concentrations measured during the weaning period. We believe that this is because calves were all fed identical diets after their second colostrum feed, and therefore the difference in colostrum treatment was too slight to cause a difference between treatment groups at weaning time. At the time of weaning, it is likely that the MDA have declined and the calves immune system has somewhat matured since birth and become more independent in terms of producing endogenous Ig. We observed no difference (P > .05) in immune status after 3 days of age as a result of colostrum feeding within the present study. In addition to this, these calves were gradually weaned using a step-down method and still had access to the milk feeding equipment where they could suckle the artificial teat. The advantage of gradual weaning is that it prepares the calf for weaning through reducing the volume of milk fed and thus creates a greater demand for nutrients. Consequently, the calf consumes an increased amount of solid feed during the step-down phase, and this reduces the likelihood of growth lag during the post weaning period (Khan et al. 2007, Sweeney et al. 2010). These findings are similar to reports from Jasper et al. (2008) where calves that were offered continued access to the milk feeding system and provided with warm water instead of milk had reduced acute response to weaning compared to abruptly weaned calves; abruptly weaned calves were more active and vocalizations were observed at more than 3 times the rate. There have been previous suggestions that other factors besides the withdrawal of milk at weaning can be distressing for dairy calves, such as not being able to access to the milk feeding system and provided with warm water instead of milk had reduced acute response to weaning compared to abruptly weaned calves; abruptly weaned calves were more active and vocalizations were observed at more than 3 times the rate. There have been previous suggestions that other factors besides the withdrawal of milk at weaning can be distressing for dairy calves, such as not being able to suckle the milk feeding equipment (Jasper et al. 2008). The calves in the present study had access to the milk feeding equipment for a minimum of 1 week post weaning. Hill et al. (2012) reported that calves gradually weaned over a 2- to 3-week period that had been fed 0.88 kg DM of MR did not cause any reduction in starter consumption and ADG (Hill et al. 2012). In addition to this, Sweeney et al. (2010) reported a gradual reduction in the volume of milk fed over a 10-day period to be the best step-down method in terms of weight gain performance for calves weaned at 56 days of age.

In the current study, the age of the calf did have an effect on the metabolite concentration levels. In comparison to this, Lynch et al. (2010) found no effects (P > .05) of treatment or treatment × day interactions for serum Hp concentrations when calves were weaned abruptly. However, day of sampling was significant (P = .0002) for Hp in this study, and on day 2 the Hp concentration increased compared with baseline (0.48 mg/mL versus 0.32 mg/mL). In this study, BHBA levels increased post weaning and this finding is comparable to reports from Klotz and Heitmann (2006) and Khan et al. (2011) where BHBA levels increased as the calf got older. This was an indication of change in sources of physiological fuel at the time of weaning when there was a shift from liquid milk to a solid diet. Quigley and Bernard (1992) suggested that this increase in BHBA may be a result of increased solid feed intake and reflect a response to alimentary ketogenesis. In contrast to BHBA concentration, NEFA concentration circulating in calves’ blood decreased at weaning and thereafter. This finding is in agreement with Klotz and Heitmann (2006) who reported a large decline in NEFA concentration between week 5 and week 8, 0.27 and 0.16 mmol/L, respectively. Similar to the current study, the decline in NEFA coincided with the termination of MR at weaning (week 7). Quigley (1996) reported a negative correlation between NEFA concentration and the intake of starter concentrate in the pre-ruminant calf. It has been suggested that this is due to the change in diet composition in terms of the transition from a liquid diet which is high in fat and lower in carbohydrate to a solid diet lower in fat and higher in carbohydrate (Klotz and Heitmann 2006). It has been reported that Hp concentrations increase quickly during inflammatory distress (Conner et al. 1988). The Hp concentration in the calves’ blood appeared to increase at weaning and peak at 2 days post weaning, decreasing to normal levels thereafter. This indicates that the removal of milk from the diet at weaning had a stressful impact on the calves, which results in an inflammatory response.

**Conclusion**

The mean level of antibodies to the gE antigen of BoHV-1 during the dry period tested negative due to the presence of artificial infection via gE-negative marker vaccine. As expected the MDA for the specific respiratory viruses were at their peak levels in the calves sera post colostrum ingestion and decreased thereafter. There were no differences between colostrum treatment groups on the level of BRV antibodies post vaccination at 3 weeks of age and post booster vaccination 4 weeks later. Antibodies to the gE antigen of BoHV-1 tested negative within both treatment groups as a result of pre-parturient immunization. Colostrum treatment group had no significant effect on the levels of stress during the weaning period, in terms of the blood metabolites analysed within this study. Gradual weaning is recommended to minimize the level of stress caused to the calf and allows for a smooth transition from liquid to solid diet. Further research is required in this field to investigate appropriate timing of vaccination regimes to maximize immune response during the neonatal period to reduce the level of BRD within dairy herds.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This work was supported by Department of Agriculture, Food and the Marine; Department of Agriculture and Rural Development in Northern Ireland; AgriSearch (farmer levy).
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