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Research paper

Comparison of interferon and bovine herpesvirus-1-specific IgA levels in nasal secretions of dairy cattle administered an intranasal modified live viral vaccine prior to calving or on the day of calving☆

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ABSTRACT

Thirty-two Holstein cows were allocated to receive intranasal vaccination with modified live bovine herpesvirus-1 (BHV-1), bovine respiratory syncytial virus (BRSV) and parainfluenza type 3 virus (PI3 V) vaccine either two weeks prior to their projected calving date, or within 24 h after calving. Nasal secretions were collected twice at a 12-h interval on the day prior to vaccination (day 0) and at 2, 4, 7, 10 and 14 days post vaccination to measure interferon (IFN) alpha, IFN-beta, IFN-gamma, and BHV-1-specific IgA by ELISA. Serum neutralizing antibody titers to BHV-1 and BRSV were measured on days 0, 7, and 14.

There was a significant treatment effect ($p < 0.0004$) and interaction ($p < 0.05$) on nasal BHV-1 IgA levels, with higher IgA levels in cows vaccinated within 24 h after calving. There was a significant treatment effect on nasal IFN-gamma concentration ($p < 0.05$) and on nasal total IFN concentration ($p < 0.05$), with higher IFN-gamma and total IFN concentrations seen in cows vaccinated within 24 h after calving. There was no significant treatment or interaction effect on nasal IFN-alpha or IFN-beta concentrations, or on serum neutralizing titers to BRSV.

In spite of prior viral vaccination during the previous lactation, cows vaccinated on the day of calving responded to an intranasal viral vaccination with increased concentrations of IFN-gamma and increased titers of IgA following vaccination which was significantly higher than cows vaccinated precalving. This study is the first to examine respiratory mucosal responses in immunologically mature dairy cattle vaccinated intranasally before and after calving.

1. Introduction

Immunosuppression in periparturient dairy cows has been well documented. Immunologic dysfunction of both lymphocytes and neutrophils (Kehrli et al., 1989; Kimura et al., 1999; Löfstedt et al., 1983) in both periparturient cows and sows, has been demonstrated. This suppression of the immune system has been associated with increased susceptibility to infectious diseases in many organ systems; respiratory, reproductive and gastrointestinal (Mordak and Anthony, 2015; Cai et al., 1994; Pelan-Mattocks et al., 2000; Kimura et al., 2002; Burvenich et al., 2007). While increased immune suppression can be measured beginning three weeks prepartum (Comline et al., 1974; Szekeres-Bartho et al., 1983; Harp et al., 1991), broad immune suppression is more dramatic in the first two weeks post partum (Guidry et al., 1976; Ishikawa, 1987; Ishikawa et al. 1994; Nagahata et al., 1988, 1992; Saad et al., 1989; Stabel et al., 1991; Dosogne et al., 1999; Detilleux et al., 1995; Heiser et al., 2015). While the exact causes of immune suppression in these animals are not clear, hormonal and metabolic changes are thought to influence the suppression of in the immune response.

Little research has characterized the mucosal respiratory immune

Abbreviations: IFN, interferon; BRSV, bovine respiratory syncytial virus; BHV-1, bovine herpesvirus-1; PI3V, parainfluenza type 3 virus

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response of adult cattle in the peripartum period. The majority of mucosal immunity studies in adult cattle, which are few, have focused on the mammary gland and the uterus. Studies in calves have shown measureable increases in interferon and specific anti-viral nasal IgA following intranasal vaccination with vaccines containing viral respiratory pathogens (Todd et al., 1971; Zygraich et al., 1971, 1974a,b, 1978; Gerber et al., 1978; Todd, 1983). To our knowledge similar studies have not been performed in older cattle. This study evaluated the immune response of mature dairy cows following intranasal administration of a modified live viral vaccine containing bovine respiratory syncytial virus (BRSV), bovine herpesvirus −1 (BHV-1) and parainfluenza virus type 3 (PI3 V) (Inforce-3, Zoetis). Systemic and nasal antibody response to BHV-1, systemic antibody responses to BRSV, and nasal interferon release was measured in cows vaccinated 10–14 days prior to calving or on the day of calving.

2. Material and methods

2.1. Animals

Thirty-two Holstein cows (second parity or higher) at the University of Georgia Tifton Campus Dairy Research Center were enrolled. Cows in this herd had not been vaccinated with the intranasal modified live viral vaccine used in this study and no exposure to BHV-1 has been documented. The cow herd and all cows enrolled int his study had received the following vaccinations prior to administration of the intranasal vaccine vaccination program is as follows:

1.)
Fourteen to twenty-one days post calving, cows are vaccinated with vaccine containing modified live BHV-1, BVDV types I and II, PI3 V, BRSV and inactivated *Leptospira canicola, grippotyphosa, hardjo, icterohaemorrhagiae,* and *Pomona* (Bovi-Shield GOLD FP 5 L5 HB, Zoetis), and an *E. coli* bacterin (Envirocor J-5).
2.)
Sixty days prior to calving, cows are vaccinated with inactivated vaccine containing BHV-1, bovine viral diarrhea virus (BVDV) types I and II, PI3 V and BRSV (Triangle 5, Boehringer Ingelheim); an *E. coli* bacterin (Envirocor J-5, Zoetis), and a *Clostridium chauvoei, septicum, haemolyticum, novyi, sordellii,* and *perfringens* Types C & D bacterin-toxoid (Ultrachoice 8, Zoetis).
3.)
Twenty-one days prior calving, cows are vaccinated with an *E. coli* bacterin (Envirocor J-5), and they receive a dose of selenium/vitamin E (MuSe, Merck Animal Health).

At the beginning of the study, all eligible cows scheduled to calve within a four month period were allocated to either receive an intranasal vaccination (Inforce-3, Zoetis) approximately two weeks prior to their projected calving date (Dry Vac), or within 24 h after calving (Fresh Vac). The distribution was by block randomization (with blocks of size 6) based on the order of the projected due dates. Within a block, 3 cows were randomly allocated to each treatment group. This was done to maintain approximately equal group sizes over time while providing for random allocation. Cows with obvious signs of disease (e.g. mastitis, or retained placenta) were excluded from the study.

2.1.1. Sample collection

On the day of enrollment nasal secretions were collected twice at an approximately 12-h interval to determine baseline concentrations of interferon (IFN) alpha, beta and gamma, and BHV-1-specific IgA. Serum was collected for measurement of serum neutralizing antibodies to BRSV and BHV-1. After the second (12 h) sampling the modified-live viral vaccine was administered to each cow via intranasal administration. Nasal secretions were also collected from each cow at 2, 4, 7, 10, and 14 days post vaccination for measurement of interferon and BHV-1-specific IgA concentrations. The laboratory did not have the ability to perform PI3 and BRSV IgA concentrations on nasal secretions. Nasal secretions were collected by placing a soft sponge (Identi-Plug Plastic Foam Sponges) into the nostril for 3 min, then expressing the secretions from the sponge into a polystyrene tube. A blood sample was also collected on day 0, 7, and 14 for measurement of serum neutralizing antibodies to BHV-1 and BRSV.

2.1.2. Measurement of BHV-1-specific IgA in nasal secretions

Bovine herpesvirus-1 (Cooper strain), grown to a viral titer of $\log10^{7.2}$ TCID and inactivated with 2 μM binary ethyleneimine, was utilized as the reference strain in the IgA ELISA. This virus preparation is routinely used by the laboratory for cellular and ELISA assays. Plates were coated with a 1:400 dilution (approximately 0.5 μg/ml of protein) of the antigen in pH 9.7 carbonate buffer overnight and washed three times with PBS containing 0.05% Tween 20. Nasal secretions were diluted 10-fold with phosphate buffered saline (PBS) containing 0.05% Tween 20 (ELISA wash buffer) and vigorously vortexed before testing to ensure fully homogenized samples. All samples were plated as 4 replicates at each dilution tested. Following a preliminary assessment of serial10-fold dilutions of a representative set of nasal secretion samples collected, the initial samples (day 0: T0 and T12) were assessed using serial 2-fold dilutions starting at a 1:100 dilution and later samples (day 2, 4, 7, 10 and 14) were assessed using a 2-fold dilution series starting at 1:1000. Samples exceeding the range were retested at a higher starting dilution and samples that were negative at all dilutions were retested at a lower starting dilution.

Titers were reported as the inverse of the last dilution yielding greater than or equal to twice the mean optical density of the negative control (low IgG FBS, Gibco) at 1:100. The IgA was detected with commercial HRP conjugated rabbit anti-bovine IgA (Bethyl Laboratories), and revealed using 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS, Sigma).

2.1.3. Measurement of IFN-alpha, IFN-beta, and IFN-gamma in nasal secretions

Interferon-gamma, IFN-alpha and IFN-beta were measured using commercially available antibody pairs sold as ELISA development reagents (Kingfisher Biotech Inc.). For each assay the plates were coated with 0.2 μg/ml of antibody in PBS over night in 0.1 M carbonate buffer. The plates were washed three times using the same wash buffer as for the BHV-1 IgA assay. A standard curve from 5000–78 pg/ml (or to 9.75 for IFN-gamma) was plated in duplicate. Interferon–alpha concentrations were converted to ng/dl. Nasal secretions were diluted 1:2 and mixed very well to attempt to disperse the gel portions of the sample. Samples were plated in duplicate. Samples were incubated for 2 h at room temperature and the plates were washed three times. Biotin conjugated anti-IFN antibody was added to the wells (1:10,000 dilution for IFN-gamma, 1:13,000 for INF-alpha, and 1:5000 for IFN-beta). The detection antibody was incubated for 1 h at room temperature. Streptavidin conjugated to HRP was added to each well and incubated for 30 min. A tetramethylbenzidine substrate (TMB, R & D Systems) was added to each well and developed until the highest standard had strong color. The reaction was stopped with acid stop solution (0.5 M sulfuric acid) and the color was recorded at 450 nm. The values for each IFN were calculated using the equation generated by commercially available software (GraphPad Prism) for the composite of the standard curves for the assay set.

2.1.4. Statistical analysis

All response variables were analyzed using a linear mixed model (SAS). A repeated measures model was used with treatment group (pre-