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Stimulation and analysis of the immune response in calves from vaccinated pregnant cows

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ABSTRACT

The effect of vaccinating pregnant cows with an inactivated vaccine against *Mannheimia haemolytica*, BRSV and PI3V infections on selected immune responses in their offspring was examined. Blood samples were collected weekly for 12 weeks from six newborn calves from each of vaccinated (experimental) and unvaccinated (control) dams. Specific antibodies to *M. haemolytica*, BRSV and PI3V and mean values of IgA, IgG concentrations were significantly higher in the experimental calves compared with the controls. However, specific antibody titres to adenovirus type 3, BHV1 and BVDV in the experimental calves had constant levels while the control group levels changed. The IgM, Hp and SAA concentrations generally increased until week 8 in the experimental group, but the control group titres became higher after week 9. This study demonstrates that specific immunisation of cows pre-partum significantly stimulated parameters associated with immunity and it also controlled the acute phase response intensity in their offspring. Therefore the vaccination of dams may provide additional antibody protection against infection to their offspring.

1. Introduction

The development of the innate immune system is essential for new-born animals to survive, especially when exposed to infectious diseases that are responsible for high morbidity and mortality. During the first few months of life new-born calves have a weakened immunity as the granulocyte function and complement activity are low (Cervenak and Kacskovics, 2009; Cortese, 2008); and the calves also lack specific immunity (Boysen et al., 2006; Stefaniak et al., 2012). Protection obtained from colostrum falls as the immunoglobulins decline usually from the seventh day of life. This is at a time when their specific antibodies are at a low concentration; which then develops as the calves grow (Stefaniak et al., 2011). During this period of low immunity the animals are more susceptible to infections including bovine respiratory disease (BRD). BRD is a complex and multifactorial disease caused by bacteria and viruses and is one of the most important diseases of cattle. Estimated losses to the cattle industry from BRD is more than US$3 billion every year (Griffin, 1997). The main causative bacteria are: *Mycoplasma bovis*, and *Mycoplasma dispar* for which there are currently no commercial vaccines; and the *Pasteurellaceae* family of which the most pathogenic is *Mannheimia haemolytica* (Singh et al., 2011). Viruses including PI3V and BRSV have an important role in BRD, not only in the pathogenesis of the disease but also by suppressing the host immunity (Cusack et al., 2003). Calves are usually vaccinated during the first weeks of life. However, calf immunisation may be adversely affected by interference from maternal antibodies or unfavourable environmental conditions. Therefore, the effective vaccination of pregnant cows and the subsequent colostrum intake by their offspring can enhance the immune response of new-born calves.

The aim of the study was to investigate the level of passive immunity acquired by calves that have been fed colostrum derived only from their own dam which was vaccinated pre-partum for *M. haemolytica*, PI3V and BRSV, or from their own unvaccinated dam in the control group.

2. Material and methods

2.1. Experimental procedure

To investigate the effect of stimulating the calf's immune system by vaccinating their dams, pregnant cows were vaccinated with BRSV-PI3V-*M. haemolytica* antigens. The immune response in the calves was analysed and multiple parameters measured as described later.

2.2. Cattle

Twelve pregnant Holstein Friesians cows were sourced from a commercial 2000 head dairy farm. Two groups of six cows were

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housed separately. Experimental procedures and animal management protocols were carried out in accordance with the requirements of the Local Ethics Committee on Animal Experimentation.

2.3. Vaccination of pregnant cows

One group of six cows were vaccinated with 5 ml of inactivated vaccine composed of BRSV-PI3V-*M. haemolytica* antigens (Bovilis®Bovipast RSP, MSD Animal Health, The Netherlands). These subcutaneous vaccinations were in the lateral neck region and were done twice, at 8 and 4 weeks, before the expected delivery dates. The same experimental design was carried out for the six remaining cows administrating PBS.

2.4. The calves

Directly after parturition the new-born calves were separated from their dams and kept in the separate groups from vaccinated–'experimental' and unvaccinated dams. The calves were kept according to the standard breeding regulations and fed colostrum derived from their own dams by oral pathway (in a dose of 2 L per calf three times a day) for three consecutive days after delivery. The total level of colostrum immunoglobulins was determined using a colostrometer for the measurement of specific gravity in bovine colostrum. After that the calves were kept in individual pens and fed with raw milk without the presence of any contaminated infectious agents for 1 month and then fed milk replacer. Rectal temperatures, general condition and the presence of respiratory signs were recorded daily. Blood samples for laboratory analysis were collected from *vena jugularis externa* of calves at weekly intervals for 12 weeks.

2.5. The analyses of immune parameters

Mannheimia haemolytica specific antibody levels were determined using an in-house ELISA as described by Makoschey et al. (2012).

A commercial respiratory ELISA kit (Bio-X Diagnostics, Belgium) was used to obtain the antibody levels to five bovine viruses (BRSV, PI3V, adenovirus type 3, BHV-1 and BVDV). The optical densities in the microwells were read at 450 nm. The signal read for each sample well was divided by the corresponding positive control serum signal and multiplied by 100 to express a result as a percentage (Val). The sample was considered as positive for BRSV by Val > 10.51, for PI3V by Val > 9.56, for adenovirus type 3 by Val > 11.86, for BHV-1 by Val > 10.08 and for BVDV by Val > 10.27, respectively. The values expressed as a percentage (Val) correspond with a degree of positivity of each serum described in the table in the quality control procedure (Quality control, Bio-X Diagnostics, Belgium). The ranges of positivity degrees for each viral agent are described in the figure legends (Figs. 1–5 respectively).

Commercial kits were used following the manufacturer's instructions to analyse gamma globulin concentration (Bio-X Diagnostics, Belgium), Ig classes (Bethyl Laboratories, Inc.), haptoglobulin (Hp) and serum amyloid A (SAA) which was measured using two different commercial ELISA kits (Tridelta Development Limited, Ireland). Standard curves for each parameter were determined and optical densities were determined at OD_{450} unless otherwise stated. Dilution series of standard curves were constructed using different standards/calibrators provided in the kits at 234.8 , 156.5, 104.4, 69.6, 46.4, 30.9, 20.6 and 13.7 μg/ml for gamma globulin (the bovine blood serum, Bio-X Diagnostics, Belgium); 500, 250, 125, 62.5, 31.25, 15.625, 7.8 and 0 ng/ml (blank) for IgG (the bovine reference serum, Bethyl Laboratories, Inc.); 1000, 500, 250, 125, 62.5, 31.25, 15.625 and 0 ng/ml (blank) for IgA and IgM (two different bovine reference sera, Bethyl Laboratories, Inc.). 2.5, 1.25, 0.625, 0.312 and 0 mg/ml for Hp (the Hp calibrator, Tridelta Development Limited, Ireland) measured at OD_{630}; and 300, 150, 75, 37.5, 18.8 and 0 ng/ml for SAA (the SAA calibrator, Tridelta Development Limited, Ireland) which were read at OD_{450} and OD_{630} as a reference. If the samples were diluted the result was multiplied by the dilution factor.

For all of the parameters examined the optical densities in the microwells were read using an automated plate reader (Elx800 Microplate Reader, BioTek Instruments, Inc., USA). The data were collected using the KC Junior programme manufactured by BioTek Instruments, Inc. (USA). From previous studies the described parameters have a normal distribution (NN).

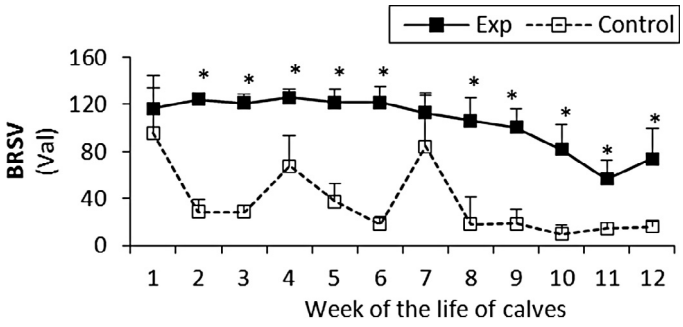

Fig. 1. The percentage (Val) of monoclonal antibodies specific to BRSV in sera of experimental and control calves from the 1st to 12th week of the animal life. *Significant differences ($p < 0.05$) within the two groups of calves. Exp, experimental calves; Control, control calves.

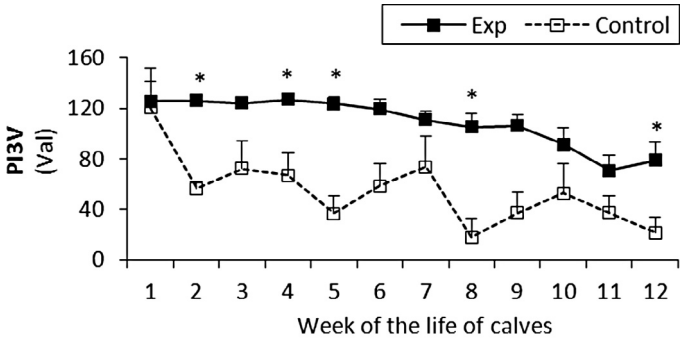

Fig. 2. The percentage (Val) of monoclonal antibodies specific to PI3V in sera of experimental and control calves from the 1st to 12th week of the animal life. *Significant differences ($p < 0.05$) within the two groups of calves. Exp, experimental calves; Control, control calves.

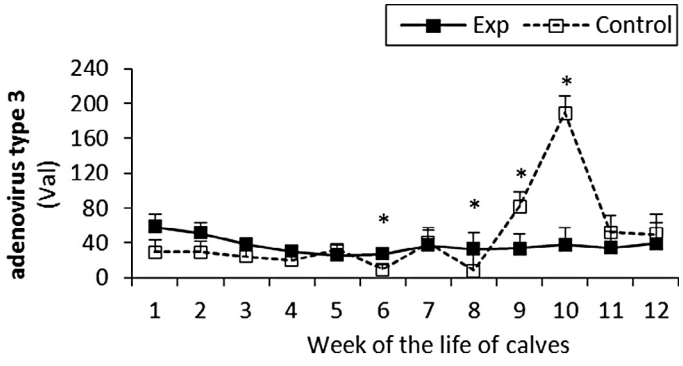

Fig. 3. The percentage (Val) of monoclonal antibodies specific to adenovirus type 3 in sera of experimental and control calves from the 1st to 12th week of the animal life. *Significant differences ($p < 0.05$) within the two groups of calves. Exp, experimental calves; Control, control calves.