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Egg yolk IgY: Protection against rotavirus induced diarrhea and modulatory effect on the systemic and mucosal antibody responses in newborn calves

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ABSTRACT

Bovine rotavirus (BRV) is an important cause of diarrhea in newborn calves. Local passive immunity is the most efficient protective strategy to control the disease. IgY technology (the use of chicken egg yolk immunoglobulins) is an economic and practical alternative to prevent BRV diarrhea in dairy calves. The aim of this study was to evaluate the protection and immunomodulation induced by the oral administration of egg yolk enriched in BRV specific IgY to experimentally BRV infected calves. All calves in groups Gp 1, 2 and 3 received control colostrum (CC; BRV virus neutralization Ab titer – VN = 65,536; ELISA BRV IgG_1 = 16,384) prior to gut closure. After gut closure, calves received milk supplemented with 6% BRV-immune egg yolk [(Gp 1) VN = 2048; ELISA IgY Ab titer = 4096] or non-immune control egg yolk [(Gp 2) VN < 4; ELISA IgY Ab titer < 4] twice a day, for 14 days. Calves receiving CC only or colostrum deprived calves (CD) fed antibody (Ab) free milk served as controls (Gp 3 and 4, respectively). Calves were inoculated with $10^{5.85}$ focus forming units (FFU) of virulent BRV IND at 2 days of age. Control calves (Gp 3 and 4) and calves fed control IgY (Gp 2) were infected and developed severe diarrhea. Around 80% calves in Gp 1 (IgY 4096) were infected, but they showed 80% (4/5) protection against BRV diarrhea. Bovine RV-specific IgY Ab were detected in the feces of calves in Gp 1, indicating that avian antibodies (Abs) remained intact after passage through the gastrointestinal tract. At post infection day 21, the duodenum was the major site of BRV specific antibody secreting cells (ASC) in all experimental groups. Mucosal ASC responses of all isotypes were significantly higher in the IgY treated groups, independently of the specificity of the treatment, indicating that egg yolk components modulated the immune response against BRV infection at the mucosal level. These results indicate that supplementing newborn calves' diets for the first 14 days of life with egg yolk enriched in BRV-specific IgY represents a promising strategy to prevent BRV diarrhea. Moreover a strong active ASC immune response is induced in the intestinal mucosa following BRV infection after the administration of egg yolk, regardless the specificity of the treatment.

1. Introduction

Rotaviruses (RVs) are responsible for globally significant gastrointestinal disease, primarily in children <5 years of age and the young of other mammalian and avian species, including calves, pigs and South American camelids (Parreno et al., 2001; Ramig, 2004; Saif and

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Parwani, 1994). Diarrhea is the most commonly reported newborn calf disease and the main cause of mortality (Bendali et al., 1999). Bovine rotavirus (BRV) is the most common pathogen involved in neonatal diarrhea in both dairy and beef herds (da Costa Mendes et al., 1993; Garaicoechea et al., 2006). Calves are susceptible to BRV diarrhea up to 8 weeks of age and by the third week of life the susceptibility decreases as the age increases (Bridger, 1994; Dhama et al., 2009). In Argentina, BRV is the main cause of neonatal diarrhea in calves and it was detected in 62.5% (250/400) of calf diarrhea cases in beef and dairy herds during a ten-year-survey from 1994 to 2003 (Garaicoechea et al., 2006). The BRV diarrhea results in greater financial loss to cattle producers than any other infectious disease, particularly due to the reduction of weight gain in affected animals and treatment expenses (Aich et al., 2007; Bellinzoni et al., 1990; Bendali et al., 1999). There is no specific treatment for BRV diarrhea. Affected animals receive therapies to replace lost fluids and electrolytes and antibiotics for secondary bacterial infection. The current strategy for the control of the disease is based on vaccination of the mother to protect the offspring by transference of passive Abs (Bellinzoni et al., 1990; Fernandez et al., 1998; Saif et al., 1987). This strategy reduces severe diarrhea but it does not prevent virus infection or the appearance of clinical signs (Bendali et al., 1999; Parreño et al., 2004). A thorough understanding of the mechanisms of intestinal immunity and their correlation with protection of neonates is critical to develop effective vaccines and complementary or alternative passive immunity strategies. Passive immunization by oral administration of specific Abs from different sources such as immune colostrum or chicken egg yolk IgY could represent effective and economic strategies to prevent gastrointestinal infections in food animals.

Rotavirus preferentially infects the mature enterocytes of the upper small intestine, near the tips of the villi. The severity and localization of intestinal infection vary among animal species and between studies; however, the pathological changes are primarily limited to the proximal small intestine (Candy, 2007; Dhama et al., 2009; Estes, 2001; Varshney et al., 1995). Considering the early susceptibility to the infection, the presence of maternal Abs – IgG_1 and IgA – derived from unabsorbed colostrum and milk in the gut lumen plays an important role in the protection against BRV infection and disease (Fernandez et al., 1998). To boost maternal Ab titers, cows are immunized parenterally with BRV vaccines 60 and 30 days before calving (Dhama et al., 2009; Saif and Fernandez, 1996). High titers of passive circulating Abs in newborn calves play a complementary role in protection against BRV induced diarrhea, because IgG_1 acquired from colostrum is also transferred from serum to the intestine of neonatal calves (Besser et al., 1988). Virus neutralizing (VN) Ab titers against BRV in newborn calf serum may also be an indicator of protection against BRV diarrhea. However, it has been observed that newborn calves frequently developed BRV diarrhea despite moderately high levels of circulating BRV Abs derived from colostrum and, by the third week of life, most of them have been exposed to BRV in the field (Estes, 2001; Kohara and Tsunemitsu, 2000; Parreño et al., 2004). Although serum Abs are essential in dairy calves, which are usually separated from their dams after colostrum intake and subsequently fed with milk replacers lacking Abs, we previously demonstrated that BRV specific circulating Abs derived from colostrum intake suppress the development of BRV-specific Ab secreting cells' (ASC) responses of neonatal calves in a dose dependent manner at both systemic and mucosal levels (Parreño et al., 2004). Following the same line of research, in a second study we also demonstrated that the administration of milk supplemented with BRV immune colostrum for the first 14 days of life induced high protection rates against BRV diarrhea in calves and a positive modulation of the neonatal immune responses towards higher numbers of BRV-specific IgA ASC and greater ASC isotype diversity in the intestinal mucosa. It was also shown that this active immune response protected against BRV challenge after the cessation of the passive Ab supplementation (Parreño et al., 2010).

In this regard, a promising, economically feasible and practical new strategy which has been explored during the past two decades is the supplementation of the milk diet of calves with specific Abs from egg yolk (IgY) (Ikemori et al., 1992, 1997; Kuroki et al., 1997, 1994; Mine and Kovacs-Nolan, 2002). Bovine RV is highly immunogenic in poultry and IgY technology offers several advantages over the classical ways of Abs production. It is a non-invasive technology, since the Abs can be obtained by collecting eggs, thus animals do not need to be bled. The IgY technology is in alignment with the 3R principles of animal welfare: Reduction, Refinement and Replacement of laboratory animals (Schade et al., 1996). The only Ab isotype present in chicken egg yolks is IgY. The isolation of IgY Abs is simple and approximately 1500 mg of IgY can be harvested each month from each laying hen (5–25 mg/egg yolk), with between 2 and 10% being antigen specific IgY, representing a faster and cheaper way of polyclonal Ab production than other sources. Regarding the function, there are four important differences between avian IgY and mammalian IgG: IgY does not bind protein A or G, nor rheumathoid factor; chicken egg yolk immunoglobulins do not interfere with mammalian IgG in serological tests and they do not activate mammalian complement. These differences are advantageous for the application of IgY technology in many areas, from diagnostics to alternatives to antibiotic therapy (Carlander et al., 2000; Schade et al., 1994; Tini et al., 2002).

Passive protection of newborn calves against Group A BRV using specific egg yolk immunoglobulins as a milk supplement within the immediate postnatal period may be a suitable clinical option to control BRV diarrhea. It has been demonstrated previously that treatment of BRV diarrhea using avian egg yolk enriched in BRV specific IgY Abs during the first 2 weeks of life enhanced body weight gain and markedly reduced BRV infection (virus titer in stools as well as the number of calves shedding virus) (Heckert et al., 1999; Kuroki et al., 1997; Touchette et al., 2003). This indicates that egg yolk Abs can be a potential tool against BRV infection when administered daily immediately after birth, when BRV incidence is higher. However, there is no information about how these heterologous Abs and egg yolk itself may modulate the mucosal and systemic immune responses of newborn calves against BRV, which are criti-