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Effects of injectable trace minerals on humoral and cell-mediated immune responses to *Bovine viral diarrhea virus*, *Bovine herpes virus 1* and *Bovine respiratory syncytial virus* following administration of a modified-live virus vaccine in dairy calves

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ABSTRACT

Our objective was to evaluate the effect of an injectable trace mineral (ITM) supplement containing zinc, manganese, selenium, and copper on the humoral and cell mediated immune (CMI) responses to vaccine antigens in dairy calves receiving a modified-live viral (MLV) vaccine containing BVDV, BHV1, PI3V and BRSV. A total of 30 dairy calves (3.5 months of age) were administered a priming dose of the MLV vaccine containing BHV1, BVDV1 & 2, BRSV, PI3V, and an attenuated-live *Mannheimia-Pasteurella* bacterin subcutaneously (SQ). Calves were randomly assigned to 1 of 2 groups: (1) administration of ITM SQ (ITM, n = 15) or (2) injection of sterile saline SQ (Control; n = 15). Three weeks later, calves received a booster of the same vaccine combination SQ, and a second administration of ITM, or sterile saline, according to the treatment group. Blood samples were collected on days 0, 7, 14, 21, 28, 42, 56, and 90 post-vaccination for determination of antibody titer, viral recall antigen-induced IFN-γ production, and viral antigen-induced proliferation by peripheral blood mononuclear cells (PBMC). Administration of ITM concurrently with MLV vaccination resulted in higher antibody titers to BVDV1 on day 28 after priming vaccination compared to the control group ($P = 0.03$). Calves treated with ITM showed an earlier enhancement in PBMC proliferation to BVDV1 following vaccination compared to the control group. Proliferation of PBMC after BVDV stimulation tended to be higher on day 14 after priming vaccination in calves treated with ITM than in the control group ($P = 0.08$). Calves that received ITM showed higher PBMC proliferation to BRSV stimulation on day 7 after priming vaccination compared to the control group ($P = 0.01$). Moreover, calves in the ITM group also had an enhanced production IFN-γ by PBMC after stimulation with BRSV on day 21 after priming vaccination compared to day 0 ($P < 0.01$). In conclusion, administration of ITM concurrently with MLV vaccination in dairy calves resulted in increased antibody titer to BVDV1, and greater PBMC proliferation to BVDV1 and BRSV recall stimulation compared to the control group, suggesting that ITM might represent a promising tool to enhance the humoral and CMI responses to MLV vaccines in cattle.

1. Introduction

Bovine respiratory disease (BRD) has a major impact on the profitability of the dairy and beef industries in North America, resulting in substantial economic losses (Griffin, 1997; McVey, 2009). The infectious agents most consistently implicated in BRD include

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Bovine viral diarrhea virus (BVDV), *Bovine herpes virus 1* (BHV1), *Bovine respiratory syncytial virus* (BRSV), *Parainfluenza 3 virus* (PI3V), *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*. Effective transfer of maternal antibodies against these agents in conjunction with appropriate biosecurity measures and vaccination programs are crucial to prevent and control BRD.

Several factors have been reported to affect the immune response following vaccination (nutritional status, stress, weather extremes, passive transfer of maternal antibodies, vaccination route, vaccine handling). Nutritional status, and particularly mineral levels, have been demonstrated to impact cattle health and performance (Enjalbert et al., 2006; Galyean et al., 1999; Underwood and Suttle, 1999). Trace minerals such as Zinc (Zn), Manganese (Mn), Copper (Cu), and Selenium (Se) are important for optimal immune function (Chirase et al., 1994; Percival, 1998; Underwood and Suttle, 1999) and growth (Spears and Kegley, 2002) in cattle, particularly in highly stressed, and newly received feeder calves (Duff and Galyean, 2007).

Zinc contributes with the structure and function of more than 2500 enzyme systems involved in metabolism (Andreini et al., 2009; Cousins and King, 2004). Zinc activates the enzyme superoxide dismutase, which plays a crucial role in stabilizing cell membranes against reactive oxygen species (ROS) (Bonaventura et al., 2015; Haase and Rink, 2014). Zinc is involved in DNA replication through the actions of ribonucleotide reductase, and is necessary for lymphocytes proliferation and differentiation. Zinc's major roles in the immune response involve signaling and adhesion of neutrophils and macrophages (Bonaventura et al., 2015), production of pro-inflammatory cytokines by monocytes (Rink and Kirchner, 2000), regulation of IL-2 secretion, signal transduction for T cell activation, clonal expansion, differentiation and T_H cells polarization (Haase and Rink, 2014), B-cell function, and antibody production (Pinna et al., 2002; Tomlinson et al., 2008).

Copper is important in the mitochondrial metabolic cascades for energy production to supply different organs, including those of the immune system (Failla, 2003). Copper also plays a role in superoxide dismutase activity and neutralization of ROS (Maggini et al., 2007), and contributes to the process of phagocyte killing (Linder, 1991). Ceruloplasmin is a copper-containing enzyme whose production increases dramatically during inflammation in response to the necessity of scavenging oxygen radicals released by immune cells (Percival, 1998). In rodents, copper deficiency is associated with decreased IL-2 production, lymphocyte proliferation and T cells counts (Bala and Failla, 1993; Bonham et al., 2002; Klotz et al., 2003; Linder and Hazegh-Azam, 1996; Minatel and Carfagnini, 2000; O'Dell, 1993; Pan and Loo, 2000; Percival, 1998). Similarly, studies in cattle fed a copper-deficient diet showed a significant reduction in B-lymphocytes and impaired neutrophil activity (Cerone et al., 1998).

Selenium appears to be very important to the migration of neutrophils into tissues and subsequent inflammation (Maddox et al., 1999). Selenium is a component of the enzyme glutathione peroxidase that inactivates ROS production and prevents released ROS from causing cellular damage (Maddox et al., 1999; Neve, 1991). Selenium deficiencies have been associated with depressed neutrophil migration and killing ability, and reduced B-cell response and antibody production. Moreover, Se supplementation enhanced both humoral and cell-mediated and immune responses (Maggini et al., 2007). The level of Se in tissues and blood affected the total IgM levels and BHV1-specific antibody titers after challenge (Reffett et al., 1988). Evidence that Mn plays a role in the immune system is limited. However, Mn has an essential function in removing ROS produced by active phagocytic cells (Tomlinson et al., 2008).

The benefits of administering injectable trace minerals (ITM) on animal health and performance have been previously assessed in dairy (Harrison et al., 1984; Machado et al., 2013), and beef cattle (Arthington et al., 2014; Berry et al., 2000; Genther, and Hansen, 2014; Richeson and Kegley, 2011). However, only a few studies have evaluated the effects of ITM on the immune function of cattle (Arthington and Havenga, 2012; Chirase et al., 1994; Clark et al., 2006; Droke and Loerch, 1989). Arthington and Havenga (2012) assessed the effect of administration of ITM on the humoral immune response after BRD specific MLV vaccination in cattle. That study demonstrated that the ITM given concurrently with viral vaccination enhanced the production of neutralizing antibodies to BHV1 in beef calves. Additionally, recent studies have shown that treatment with ITM concomitantly with MLV vaccination induced a faster BVDV-specific antibody response in newly received, highly stressed calves (Roberts et al., 2015).

A growing body of evidence suggests that both humoral and CMI responses are critical in protection against viral agents involved in BRD (Collen and Morrison, 2000; Howard 1990; Nobiron et al., 2003). A more complete evaluation of the immune responses induced by MLV vaccination requires the use of methods to assess both humoral (antibody response) and cellular effector mechanisms (recall antigen induced proliferation and induction of IFN-γ as the core Th1 cytokine). We hypothesized that administration of ITM improves both humoral and CMI responses to vaccine antigens in dairy calves receiving a modified-live viral (MLV) vaccine containing BVDV1 and 2, BHV1, PI3V and BRSV. The objective of this study was to evaluate the effect of an injectable trace mineral (ITM) supplement containing Zn, Mn, Se, and Cu on the humoral and cell mediated immune (CMI) responses to individual vaccine antigens in dairy calves receiving a MLV vaccine containing BVDV1 and 2, BHV1, PI3V and BRSV.

2. Methods

2.1. Study location and animal husbandry

All cattle used in this experiment were derived from a single commercial dairy farm at Quitman, Georgia. At the farm of origin, fifty calves born during the first week of February in 2014 were placed in individual hutches separated from the rest of the calves on the farm, beginning on the day of birth and maintained until the day of transportation to the experimental farm. Thirty-five healthy calves that had not suffered any clinical disease, or received any vaccines or treatments were selected and transported to the University of Georgia Rose Creek Farm in Oconee County, Georgia, USA for use in the study.

On the day of transportation, the animals received a subcutaneous (SQ) injection of tilmicosin (10 mg/kg of body weight, Micotil® 300, Elanco Animal Health, Indianapolis IN) as a metaphylaxis to prevent the occurrence of respiratory disease associated with shipping. At the experimental farm, the calves were housed in an 8-acre pasture with adequate shade. The calves were cared for in accordance with acceptable practices as outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010). In addition, the research protocol was reviewed and approved by the University of Georgia, Institutional Animal Care and Use Committee (IACUC; protocol number A2014 02-005-Y2-A5).

Throughout the study, the calves grazed Bermuda grass (*Cynodon dactylon*) and Fescue grass (*Festuca arundinacea*) with no access to mineral supplementation. Animals had access to hay (Bermuda grass and Fescue grass) and water *ad libitum*. Additionally, calves received 2.7 kg/head/day of concentrate supplement (Bulk Cattleman's Special; Godfrey's Warehouse; Madison-GA) divided into two meals.