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Effects of injectable trace minerals on the immune response to *Mannheimia haemolytica* and *Pasteurella multocida* following vaccination of dairy calves with a commercial attenuated-live bacterin vaccine

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ABSTRACT

The objective was to evaluate the effects of an injectable trace mineral (ITM) supplement containing Zn, Mn, Se, and Cu on the humoral and cell mediated immune responses to vaccine antigens in dairy calves receiving an attenuated-live bacterin vaccine containing *Mannheimia haemolytica* and *Pasteurella multocida*. Thirty 3-mo-old dairy calves received 2 doses (21 d apart) of an attenuated-live *M. haemolytica* and *P. multocida* bacterin vaccine (Once PMH, Merck Animal Health, Summit, NJ), and a 5-way modified-live-virus vaccine (Express 5, Boehringer Ingelheim, Vetmedica, St. Joseph, MO). On the day of primary vaccination, animals were randomly assigned to 1 of the 2 treatment groups (n = 15 per group): ITM (ITM administration) or control (sterile saline injection). Treatments were administered concurrently with vaccinations. Blood samples were collected for determination of antibody titers against *M. haemolytica* and *P. multocida* and of antigen-induced proliferation and interferon-γ secretion by peripheral blood mononuclear cells. Serum Se and Mn concentrations were greater ($P < 0.05$) in the ITM group than the control group after ITM use. Serum end-point antibody titers against both bacteria and interferon-γ secretion by peripheral blood mononuclear cells were not different ($P > 0.05$) between groups. The use of ITM with bovine respiratory disease vaccines enhanced ($P < 0.01$) antibody titer fold-change to *M. haemolytica*. Proliferation of peripheral blood mononuclear cells after *P. multocida* stimulation was increased ($P = 0.03$) in the ITM group on d 21 relative to baseline value. In conclusion, ITM administration concurrently with bacterin vaccination improved the immune response to *M. haemolytica* and *P. multocida* and might be a valuable tool to enhance dairy calves' response to bovine respiratory disease vaccination.

Key words: trace minerals, dairy calf, bovine respiratory disease, attenuated-live bacterin vaccine, immune response

INTRODUCTION

Bovine respiratory disease complex (**BRDC**) is considered a major illness that affects North America's cattle industry resulting in substantial economic loses (>$1 billion/yr; Griffin, 1997; McVey, 2009). The complexity of BRDC can be attributed to several factors that potentiate its pathogenesis, including its poly-microbial etiology, stress, immune suppression, failure of passive transfer, weather extremes, or poor biosecurity. The infectious agents commonly involved in BRDC include bovine viral diarrhea virus (**BVDV**), bovine herpes virus-1, bovine respiratory syncytial virus (**BRSV**), parainfluenza 3 virus, *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*. The typically commensal relationship of *M. haemolytica* and *P. multocida* with cattle makes prevention and control of BRDC more difficult (Filion et al., 1984; Confer, 2009). These 2 bacteria are considered normal flora of the cattle upper respiratory tract and on occasion, when animals are immunosuppressed (especially during stress), they colonize the bronchi and lungs causing mild to fatal illness (Dabo et al., 2007; Rice et al., 2007; Confer, 2009). The damage caused by these bacteria in the lower respiratory tract is usually due to the excessive influx of neutrophils and accumulation of fibrin in the lungs, which result in acute respiratory disease and occasionally in death (Dabo et al., 2007; Rice et al., 2007; Confer, 2009).

The authors declare no conflicts of interest, with the exception of L. J. Havenga, who is an employee of Multimin USA.

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Appropriate biosecurity measures and vaccination programs are crucial to prevent and control BRDC. The use of bacterins against *M. haemolytica* and *P. multocida* plays an important role in the prevention of BRDC by reducing the level of colonization and decreasing the likelihood of negative effects on cattle health and performance (Aubry et al., 2001; Larson and Step, 2012). Serum antibodies against bacterial leukotoxin and to specific bacterial surface antigens are considered core in the defense against these pathogens (Shewen and Wilkie, 1988; Crouch et al., 2012), and high concentration of *P. multocida* antibodies in serum at feedlot entry has been correlated with improved beef cattle performance (Fulton et al., 2002).

Trace minerals play an important role in adequate health, performance, and immune response to viral BRDC pathogens in dairy calves (Teixeira et al., 2014; Palomares et al., 2016). Some trace minerals, including Se, Zn, Cu, and Mn, are fundamental elements in the structure and function of several proteins that participate in general homeostatic processes essential for an adequate immune function. These include the pathways regulating energy production, DNA replication and transcription, and modulators of reactive oxygen species (**ROS**; Failla, 2003; Genther and Hansen, 2014). The use of injectable trace minerals (**ITM**) combined with vaccination demonstrated a positive effect in beef cattle by increasing serum neutralizing antibodies against bovine herpes virus-1 (Arthington and Havenga, 2012; Arthington et al., 2014), reducing morbidity and antibiotic treatment and costs, and increasing ADG in shipping-stressed calves (Richeson and Kegley, 2011). Additionally, in dairy calves with adequate mineral status, administration of ITM has been proven to enhance health status by increasing neutrophil function, glutathione peroxidase activity (Teixeira et al., 2014), as well as serum neutralizing antibody titers and leukocyte proliferation against common bovine respiratory disease (**BRD**) pathogens (Palomares et al., 2016).

In a previous study, we reported that administration of ITM concurrent with a modified-live-virus (**MLV**) vaccine in dairy calves resulted in earlier and more robust antibody titers against BVDV1 and in more robust peripheral blood mononuclear cell (**PBMC**) proliferation after stimulation with BVDV1 and BRSV antigen than for the control group (Palomares et al., 2016). In the present study, we tested the hypothesis that administration of ITM can also improve the antibody and cell mediated immune responses to vaccine bacterial antigens in dairy calves receiving an attenuated-live bacterin vaccine containing *M. haemolytica* and *P. multocida*.

MATERIALS AND METHODS

This trial is part of a larger study performed at the University of Georgia, where we investigated the effects of ITM on the immune response to viral antigens following MLV vaccination (Palomares et al., 2016). The calves were cared for in accordance with acceptable practices as for the *Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 2010). The research protocol was approved by the University of Georgia Institutional Animal Care and Use Committee.

Experimental Design, Animals, and Treatments

A total of 30 weaned dairy bull calves (3.5 mo of age) received 2 mL of an attenuated-live *M. haemolytica* and *P. multocida* bacterin (Once PMH, Merck Animal Health, Summit, NJ) and 5 mL of a MLV vaccine containing bovine herpes virus-1, BVDV1 and 2, BRSV, and parainfluenza 3 virus (Express 5, Boehringer Ingelheim, Vetmedica, St. Joseph, MO). Both vaccines were given subcutaneously as recommended by the manufacturer. On the day of primary vaccination, calves were randomly assigned to 1 of 2 groups (15 calves per group) using a software (Research randomizer, V3.0, Social Psychology Network, Middletown, CT): (1) ITM, administration of injectable trace minerals (1 mL/45 kg of BW; Multimin 90, Multimin USA Inc., Fort Collins, CO) subcutaneously concurrently with vaccination, or (2) control, injection of sterile saline (1 mL/45 kg) subcutaneously at the time of vaccination. Three weeks after initial vaccination, calves received a booster of the same vaccines and a second dose of ITM or saline according to previous group assignment. Administration of ITM provided 15 mg/mL of Cu, 60 mg/mL of Zn, 5 mg/mL of Se, and 10 mg/mL of Mn. Calves were grazing in bermudagrass (*Cynodon dactylon*) and fescue (*Festuca arundinacea*) with ad libitum access to hay and water. A commercial cattle ration (Bulk Cattleman's Special; Godfrey's Warehouse, Madison, GA) containing energy, protein, minerals, and vitamins was offered twice daily as a supplement (around 2.7 kg/d per calf).

Sample Collection

Blood was collected from all the calves via jugular venipuncture into vacuum tubes (Vacutainer, BD Diagnosis, Franklin Lakes, NJ) with and without anticoagulant (acid citrate dextrose) to obtain whole blood and serum, respectively. Blood was collected on d −7, 0 (enrollment), 7, 14, 21, 28, 42, 56, and 90 relative to primary vaccine and ITM administration, for determination of serum trace mineral concentration, serum neutralizing antibody titers to *M. haemolytica* and *P. multocida*, antigen-induced PBMC proliferation, and interferon (**IFN**)-γ production upon stimulation with *P. multocida*. Additionally, trace mineral status was assessed in liver biopsy samples collected from each calf on d −7, 21, and 56 relative to the day of primary vaccination (d 0) as previously described (Palomares et al., 2016).

Blood Sample Processing and PBMC Preparation

Within 2 h after collection, blood samples were transported on ice to the laboratory and tubes without an-

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