

Use of a novel adjuvant to enhance the antibody response to vaccination against *Staphylococcus aureus* mastitis in dairy heifers.

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Abstract

A novel adjuvant (Immunoboost[®]) to enhance antibody titer response to a commercial vaccine (Lysigin[®]) against *Staphylococcus aureus* mastitis in dairy heifers was evaluated. In Phase 1, hyper-immunization with Lysigin[®] to enhance serum titers did not result in titers that exceeded conventional immunization. In Phase 2, anti-*S. aureus* titers in heifers immunized with Lysigin[®] + Immunoboost[®] tended to be elevated ($P = 0.10$) over heifers immunized with Lysigin[®] alone by day (D) 7 continuing through D14. By D21, titers in the Immunoboost[®] group were elevated ($P = 0.05$) over conventional vaccinates through D35, returning to baseline by D42. After booster injections on D42, the Immunoboost[®] group exhibited increased ($P = 0.05$) titers over conventional vaccinates on D49 through D63, remaining elevated through D84. Findings suggest that Immunoboost[®] enhanced antistaphylococcal titer responses to commercial vaccination, and support the use of immunization to control *S. aureus* mastitis in dairy heifers.

Introduction

S. aureus mastitis is prevalent in unbred and bred dairy heifers, which may serve as sources for infecting the milking herd (Fox, 2009). Such intramammary infections (IMI) in heifers are associated with local inflammation, induration, reduced mammary development, and extremely high SCC, and have been diagnosed as early as 6 months of age (Boddie et al., 1987). Although administration of intramammary therapy to heifers during gestation (Owens et al., 1994) has been successful, the key to controlling this disease is via prevention.

Vaccination has been attempted to increase immunity to *S. aureus* and to prevent establishment of these bacteria in the bovine mammary gland. While conventional vaccination of dairy heifers with the commercial bacterin Lysigin[®] has been shown to reduce the new infection rate at the time of calving, the antibody response has been less than optimal, and titers never exceeded control values by more than 2 fold and were not sustained after boosting (Nickerson et al., 1999). Alternatively, immunization may be enhanced by incorporating adjuvants, such as

Immunoboost[®], an immune-modulator shown to enhance neutrophil antibacterial activity (Ogden et al., 2002). The purpose of this study was to determine if Immunoboost[®] would enhance the antistaphylococcal titers in heifers vaccinated with Lysigin[®].

Materials and Methods

Phase 1 (a priming and multiple booster trial) compared the titer response between A) Lysigin[®] (Lysigin[®], Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA) administered according to label dosage and B) hyperimmunization with Lysigin[®]. Phase 2 compared the response in the same previously vaccinated heifers between A) Lysigin[®] with B) Lysigin[®] + 2 mL Immunoboost[®] (Vetrepharm, Athens, GA), a Mycobacterium cell wall fraction nonspecific immunotherapeutic, shown to enhance the immune system and reduce death loss and clinical signs associated with *E. coli* diarrhea in calves. The 2 phases were conducted sequentially over a period of 6 months. All animals were commingled by age and placed on pasture with feed bunks equipped with head gates for restraining, vaccinating, and bleeding.

For Phase 1, pre-trial blood samples were collected via jugular venipuncture from 12 Holstein heifers (5-8 mo) and processed to determine serum antibody titers. To qualify, each heifer was required to exhibit an antibody titer of no more than 1:1600 against *S. aureus* antigens.

Qualifying heifers (n=8) were allotted to: Group A) conventionally vaccinated with Lysigin[®] (4 heifers) or Group B) hyper-immunized with Lysigin[®] (4 heifers) and balanced by serum titers, averaging 1:1000. Vaccine injections (5 mL) were administered into the right semimembranosus muscle of the rear leg, and subsequent booster injections alternated on left and right sides. Both groups were immunized on D0 and boosted on D14; on D42, Group B received a second booster injection (hyper-immunization). Blood samples were collected weekly during the trial through D77 to determine serum antistaphylococcal titers.

For Phase 2, the same 8 heifers were used (9-12 mo) and were assigned to treatment groups balanced by serum titers, which averaged 1:1200. Treatments were: Group A) Lysigin[®] only (n=4) and Group B) Lysigin[®] + 2 mL of Immunoboost[®] (n=4). On D0 and D42, Group A heifers were injected with 5 mL of Lysigin[®] only, and Group B heifers were injected with a preparation of 5 mL Lysigin[®] + 2 mL of Immunoboost[®]. All serum antistaphylococcal titers were

determined via ELISA as described in Ryman et al., (2013). Data were analyzed statistically and treatment means separated using SAS (SAS, 2013). The significance level was set to $P < 0.05$ and a trend was defined at $P < 0.10$.

Results and Discussion

Phase 1 results showed no differences in anti-*S. aureus* titers between conventionally vaccinated (Group A) and hyper-immunized animals (Group B) overtime through D49, both of which remained low (<1:4000) (Figure 1). Titers increased only slightly or not at all 1 wk after the first and second immunizations that were given on D0 and D14 to both experimental groups. Likewise, titers did not increase 1 wk after Group B was hyperimmunized on D42. In an earlier study using Lysigin[®], Nickerson et al. (1999) also observed only small increases in titers of vaccinated heifers after the first 3 immunizations. Similarly, Middleton et al. (2009) found that Lysigin[®] produced low titers and was short lived in adult cows immunized against *S. aureus*.

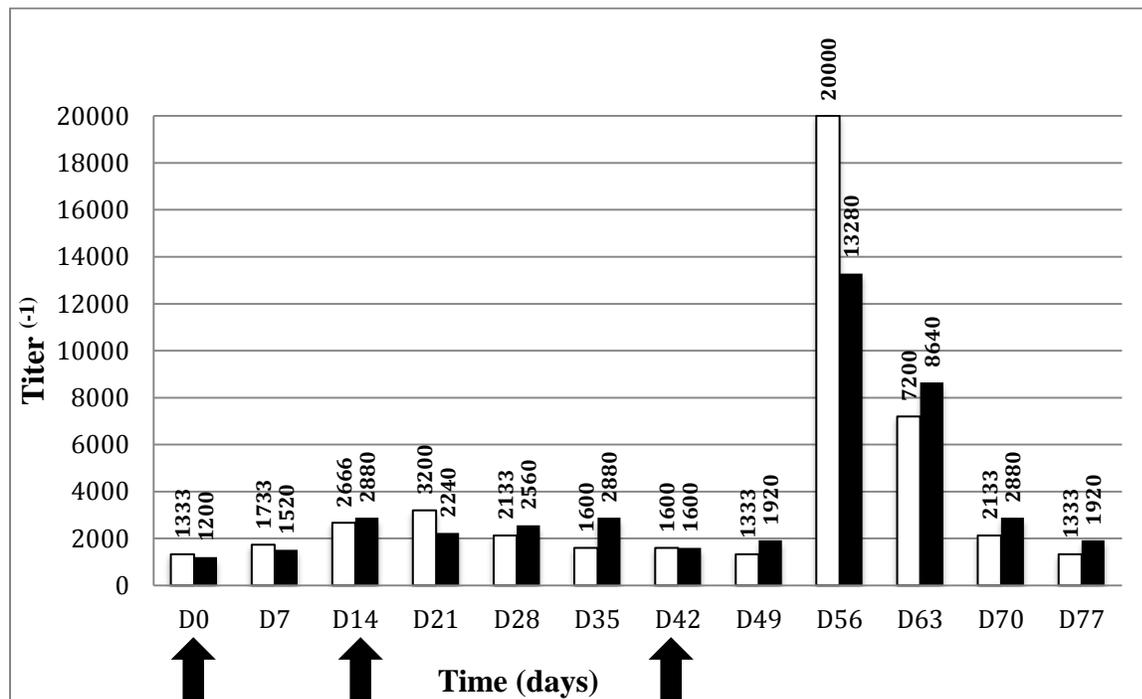


Figure 1: Titer comparison of heifers vaccinated conventionally with Lysigin[®] (Group A) and heifers hyper-immunized with Lysigin[®] (Group B) in Phase 1. White bars represent Lysigin[®] and black bars represent Lysigin[®] hyper-immunization. Arrows indicate injection days. Only Group B received injection on D42 (hyper-immunization).

The titer increases in both groups on D56 in Phase 1 of the present trial were attributed to exposure to an exogenous respiratory or enteric staphylococcal infection, which elevated titers for approximately 1 week (D56 and D63), then declined to baseline levels on D70 and D77. A similar spike in titer was observed by Ryman et al. (2013), which was attributed to exposure to blood-sucking horn flies, vectors in the transmission of *S. aureus*, that initiated staphylococcal infections on teats, leading to IMI and subsequent elevation in anti-staphylococcal titers.

In Phase 2, titers in both groups (A and B) were similar on D0 (1:1200), but on D7, Group B titers increased more than Group A titers (1:1800 vs. 1:2800; $P < 0.10$), a trend that continued through D14 (1:2400 vs. 1:4000; $P < 0.10$) (Figure 2). Group B titers were significantly higher on D21 (1:1600 vs. 1:4000; $P < 0.05$), and remained elevated relative to Group A titers through D35 ($P < 0.05$), decreasing to D0 values by D42 of the trial.

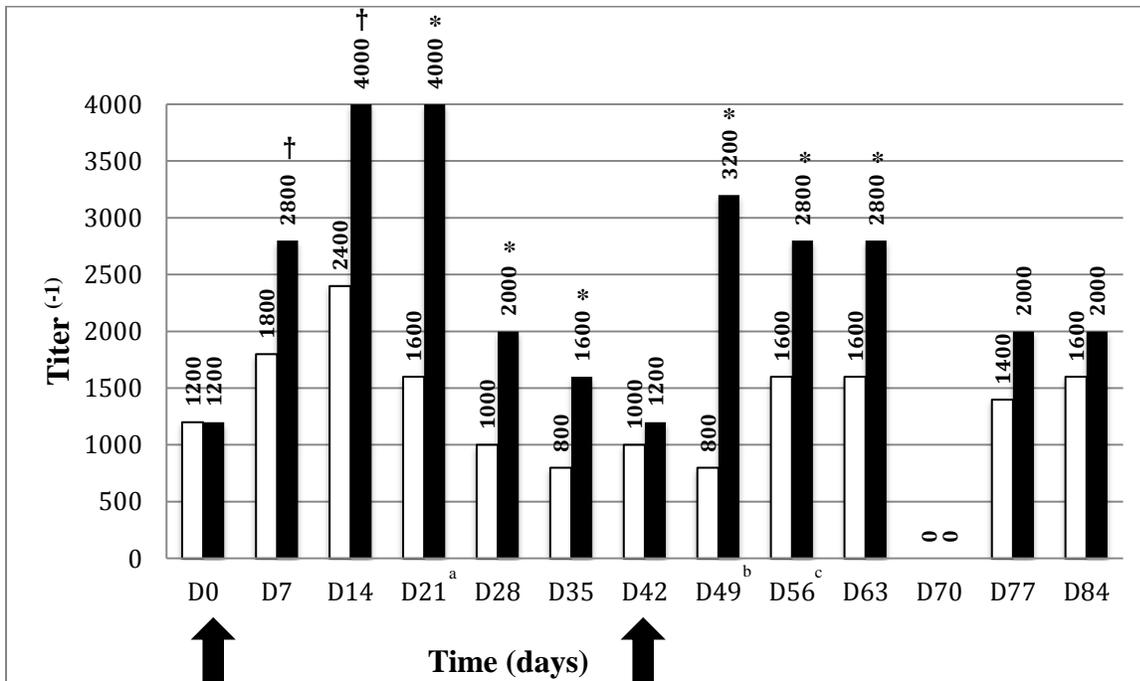


Figure 2: Titer comparison of heifers vaccinated with Lysigin® (Group A) and heifers vaccinated with Lysigin® + 2 ml Immunoboost® (Group B) in Phase 2. White bars represent Lysigin® and black bars represent Lysigin® + 2 ml Immunoboost®. Arrows indicate injection days. Both groups received injections on both injection days. Day 70 was not tested due to inclement weather. *Treatments differ $P < 0.05$. †Treatments differ $P < 0.10$. ^aDiffered from D0 ($P < 0.01$). ^bDiffered from D42 ($P < 0.005$). ^cDiffered from D42 ($P < 0.10$).

After both groups received a second injection on D42, Group B titers (1:3200) became elevated relative to Group A titers (1:800) on D49 ($P < 0.05$) and remained elevated on D56 (1:1600 vs. 1:2800; $P < 0.05$) and D63 (1:1600 vs. 1:2800; $P < 0.05$). Both groups then decreased through D84 to 1:1600 (Group A) and 1:2000 (Group B).

Compared to D0 values, titers increased on D21 ($P < 0.01$), which was attributed to the increase in Group B titers. After the second injection on D42, titers increased on D49 ($P < 0.005$), which was again attributed to the increase in Group B titers. Titers tended to remain elevated on D56 compared with D42 ($P < 0.10$) due to Group B titers; however, thereafter, there were no differences from D42.

Results suggest that antistaphylococcal antibody titers in response to Lysigin[®] are enhanced by incorporating the adjuvant Immunoboost[®] into the vaccine preparation and can be elevated approximately 4-fold compared with Lysigin[®] alone. Likewise, Camussone et al. (2013) found that primigravid Holstein heifers vaccinated with *S. aureus* adjuvanted with ISCOMATRIX[™] responded with significantly higher levels of anti-bacterin and anti-CP5 IgG and IgG₂ in sera than animals given the same vaccine adjuvanted with Al(OH)₃, which is the same adjuvant used in the Lysigin[®] preparation.

Ogden et al. (2002) also found that Immunoboost[®] augmented the immune response of stocker calves vaccinated against a respiratory disease complex compared with unvaccinated controls. Immunized animals exhibited an increase in circulating lymphocytes and elevated serum interferon- γ , indicating that there may be additional stimulation of the immune system using this product over conventional vaccination alone.

Titers in Phase 2 heifers treated with Lysigin[®] + Immunoboost[®] were 4 fold that of heifers treated with Lysigin[®] alone at the highest point in the trial (D49); however, titers were not sustained for more than 35 days after the initial vaccine injection. Similarly, Nickerson et al. (1993) found that the mean antistaphylococcal titer was approximately 4 fold that of controls after immunizing nonlactating cows with an experimental *S. aureus* vaccine developed by Watson (1988); however, titers in this later trial remained elevated for at least 10 weeks.

The elevated antibody response to Lysigin[®] plus Immunoboost[®] in Phases 2 suggests that conventional vaccination may be augmented by incorporation of this adjuvant and supports the continued evaluation of immunization for mastitis control in dairy heifers. Immunizing heifers against mastitis early in their life cycle will hopefully establish immunity as well as immune memory well before the first cycle of milk production.

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