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Clinical response after chitosan microparticle administration and preliminary assessment of efficacy in preventing metritis in lactating dairy cows

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ABSTRACT

The objectives were to evaluate the clinical response to intrauterine administration of chitosan microparticles (CM) and to assess efficacy for preventing metritis in dairy cows. Holstein cows (n = 104; 40 primiparous and 64 multiparous) at increased risk for metritis (cows that had abortion, dystocia, twins, stillbirth, or retained placenta) were randomly assigned to 1 of 2 treatments at 1 d in milk (DIM; 24 h postpartum): CM group (n = 52), daily intrauterine infusion of 8 g of CM dissolved in 40 mL of sterile water for 5 d; control (CON) group (n = 52), daily intrauterine infusion of 40 mL of sterile water for 5 d. Clinical response was assessed by evaluation of parameters associated with inflammation (rectal temperature and plasma haptoglobin concentration) and metabolism [plasma nonesterified fatty acid (NEFA) and β-hydroxybutyrate (BHB) concentrations] up to 14 DIM, and daily milk yield up to 30 DIM. Uterine discharge pH was evaluated at 4, 7, 10, and 14 DIM as an indicator of bacterial load and acid byproduct production. The cumulative incidence of metritis was evaluated up to 4, 7, 10, and 14 DIM. Continuous and dichotomous outcomes were evaluated with mixed linear and logistic regression analysis, respectively. Treatment with CM did not affect rectal temperature (39.17 ± 0.04 vs. 39.14 ± 0.04°C), haptoglobin (1.10 ± 0.05 vs. 1.07 ± 0.05 mg/mL), NEFA (0.64 ± 0.04 vs. 0.63 ± 0.04 mmol/L), BHB (0.61 ± 0.03 vs. 0.57 ± 0.03 mmol/L), or milk yield (30.3 ± 0.92 vs. 30.1 ± 0.97 kg/d) compared with CON. An interaction between treatment and time showed that NEFA concentrations were lower for CM than CON at 10 DIM (0.46 ± 0.06 vs. 0.64 ± 0.06 mmol/L). Treatment with CM resulted in greater uterine discharge pH than CON (6.91 ± 0.03 vs. 6.83 ± 0.02). Cows that developed metritis had increased concentrations of haptoglobin and BHB, and decreased uterine discharge pH and milk yield. Treatment with CM resulted in decreased incidence of metritis up to 7 DIM compared with CON (46.2 vs. 65.4%); however, no differences were found at 4 (11.5 vs. 17.3%), 10 (61.5 vs. 73.1%), and 14 DIM (63.5 vs. 73.1%) for CM versus CON, respectively. In conclusion, CM did not alter clinical parameters of cows at risk for metritis, and may merit further investigation for prevention of metritis. However, the duration of treatment may have to be extended to effectively reduce the incidence of metritis during the high-risk period.
Key words: metritis, chitosan microparticles, dairy cow

INTRODUCTION

Contamination of the female reproductive tract following parturition is ubiquitous in dairy cows (Sheldon and Dobson, 2004; Jeon et al., 2015) leading to high prevalence (~20%) of metritis. The incidence of metritis in cows at increased risk for metritis such as cows that had dystocia, twins, retained placenta (**RP**), or stillbirth is >50% (Markusfeld, 1984; Curtis et al., 1985; Martinez et al., 2012). Besides having welfare implications because of the pain associated with this condition (Stojkov et al., 2015), metritis leads to economic loss because of decreased milk yield, decreased fertility, and increased culling (Overton and Fetrow, 2008). Although uterine bacterial contamination is ubiquitous postpartum, *Escherichia coli*, *Trueperella pyogenes*, *Fusobacterium necrophorum*, and *Bacteroides* spp. are thought to

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be the main causative agents of metritis (Sheldon and Dobson, 2004; Bicalho et al., 2010; Jeon et al., 2015). Recent work has highlighted the importance of *E. coli* (Bicalho et al., 2010; Sheldon et al., 2010), especially the fact that *E. coli* predisposes to infection with other pathogenic bacteria such as *F. necrophorum* and *T. pyogenes* (Bicalho et al., 2012), increases the likelihood of developing metritis, and decreases the likelihood of pregnancy (Bicalho et al., 2010, 2012).

Third-generation cephalosporins are approved for treatment of metritis and have been used successfully to prevent metritis (Risco and Hernandez, 2003; McLaughlin et al., 2013). However, the US Food and Drug Administration has prohibited the use of third-generation cephalosporins for prevention of disease in cattle. This prohibition was based on the risk to public health because of increased resistance of *Salmonella* to cephalosporin antibiotics, and the link to cephalosporin use in food animals. This action highlights the need for alternative antibiotics for prevention and treatment of disease in food animals, so we can protect human and animal health. Nonetheless, during the last 2 decades, research into discovering and developing new antibacterial agents has been limited (Courvalin, 2008). This antagonistic reality of increased antimicrobial resistance of bacteria and limited therapeutic availability of antibiotics will likely limit the options for prevention and treatment of metritis and other diseases.

Recent research from our group has demonstrated that chitosan microparticles (**CM**) can be a viable alternative to traditional antibiotics (Jeon et al., 2014). We demonstrated that CM acts by binding to structural components of the bacterial cell membrane and have a dose-dependent broad spectrum of antimicrobial activity (Jeon et al., 2014). Although CM work better at low pH, they work well at neutral pH, which is critical for activity in the lumen of organs. Indeed, we showed that intrauterine infusion of 8 g/d of CM for 5 d was as effective as systemic treatment with 2.2 mg/kg of ceftiofur hydrochloride for 5 d at reducing the number of *E. coli* in the uterus (Jeon et al., 2014). Another important feature of CM is that they exert a broad spectrum of antimicrobial activity without increasing resistance (Ma et al., 2016), probably because it acts by binding to structural components of the bacterial cell wall (Jeon et al., 2014).

Although chitosan is categorized as GRAS (generally recognized as safe) for general use in foods by the Food and Drug Administration, no evaluation has been done of clinical response after intrauterine administration in cows. Therefore, one of the objectives of this study was to evaluate the clinical response after intrauterine administration of CM in dairy cows. We also hypothesized that intrauterine infusion of CM in cows at increased risk for metritis would decrease the incidence of metritis; therefore, the second objective was to evaluate the efficacy of intrauterine CM administration in preventing metritis in dairy cows.

MATERIALS AND METHODS

Cows, Housing, and Feeding Management

All animal procedures were approved by the University of Florida Institutional Animal Care and Use Committee (IACUC# 201207405). This study was conducted in a 4,000 lactating-cow herd located in North Central Florida. A total of 104 (40 primiparous and 64 multiparous) Holstein cows at increased risk for metritis (cows that had abortion, dystocia, twins, stillbirth, or retained placenta were enrolled from January to April 2014. Cows were housed in free-stall barns and were milked 3 times daily. The rolling herd average milk production was approximately 11,000 kg of milk/cow per year. Cows were fed the same TMR, formulated to meet or exceed the NRC (2001) nutrient requirements for lactating Holstein cows weighing 680 kg and producing 45 kg of 3.5% FCM.

Sample Size

The outcome metritis incidence was used for sample size calculation because dichotomous outcomes typically have a larger variance and so require a larger sample than a similar effect size with a continuous outcome (Bhandari et al., 2002). The sample size was calculated to detect a difference of 17 percentage units (28 vs. 45% or 45 vs. 62%) in metritis incidence because this was the weighted average reduction in metritis incidence when ceftiofur was used to prevent metritis in dairy cows at increased risk for metritis (Risco and Hernandez, 2003; McLaughlin et al., 2013). A sample size of 104 cows (52 per group) was calculated (Minitab Inc., State College, PA) for $\alpha = 0.05$ and $\beta = 0.20$, and allowing for up to 10% attrition. With this sample size, we would be able to detect a difference of 0.15 mmol/L in nonesterified fatty acids (**NEFA**), 0.15 mmol/L in BHB, or 0.15 mg/mL in haptoglobin, with a standard deviation of 0.25, α of 5%, and power of 80%.

Inclusion and Exclusion Criteria

Only cows with at least one risk factor for metritis (abortion, dystocia, twins, stillbirth, or retained placenta) were eligible for enrollment in the study. Abortion was defined as expulsion of a dead calf between dry