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# The effect of prepartum intravaginal bacteriophage administration on the incidence of retained placenta and metritis

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## ABSTRACT

The objective of this study was to evaluate the effect of prepartum intravaginal administration of an anti-*Escherichia coli* bacteriophage cocktail on uterine health and reproductive performance of lactating dairy cows. The study was conducted on a commercial dairy farm located near Ithaca, New York, from March to September 2012, and 107 cows were enrolled. The cows were divided randomly into 2 groups (treatment and control); treatment group cows were submitted to 3 intravaginal administrations of 20 mL of a bacteriophage cocktail including 10 different phages in a lysate with titration of approximately $10^9$ plaque-forming units, on d 230, 260, and 275 of gestation. Control cows were not treated with a placebo, and physical examination and sample collection was conducted equally for both groups. Vaginal swabs were collected at d 230, 260, and 275 d of gestation, cervical swabs were collected at 3 ± 1 d in milk, and blood was collected at 275 d of gestation for nonesterified fatty acids and interleukin (IL)-6 serum level measurements and at 10 DIM for β-hydroxybutyrate and IL-6 serum level measurements. The bacteriophage cocktail significantly increased the incidence of retained placenta and metritis. Swab samples were cultured for *E. coli* and no difference was found between treatment and control groups. The lysate contained $6 \times 10^9$ IU of lipopolysaccharide/mL and could have caused prepartum overstimulation of toll-like receptor-4 (TLR4)-chemokine pathway, leading to a weaker immune response. However, IL-6 was not affected by treatment, although sample size was insufficient to prove a treatment effect on the suppression of the chemokine cascade and innate immune system. Bacteriophage treatment had an excellent bactericidal effect in previous in vitro trials; however, special attention must be given to production of the cocktail to obtain a purified solution free of *E. coli* bacterial products and fragments, particularly lipopolysaccharide.

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## INTRODUCTION

Poor reproductive performance is an important production-limiting factor (Gröhn and Rajala-Schultz, 2000). Postpartum uterine diseases, especially metritis, are important for animal welfare reasons, contributing to cow discomfort and elimination from the herd, and for economic reasons, profoundly affecting reproductive performance and reducing the profit potential of dairy farms (Bicalho et al., 2010a). Rajala and Gröhn (1998) reported that second-lactation cows affected with dystocia, retained placenta, or metritis produced 2.2, 1.4, and 1.3 kg/d less milk, respectively, compared with healthy herdmates. Therefore, uterine diseases cause substantial economic losses, which are incurred as a consequence of reduced milk yield and costs of culling and treatment. The total cost per case of metritis is estimated to range between $329 and $386 (Overton and Fetrow, 2008).

The uterine lumen is usually contaminated by bacteria after parturition (Földi et al., 2006). The composition of the bacterial flora in the postpartum uterus of dairy cows differs between healthy cows and those with metritis (Santos et al., 2011). *Escherichia coli*, *Arcanobacterium pyogenes* (now named *Trueperella pyogenes*), and *Fusobacterium necrophorum* are considered important etiological agents of uterine diseases (Miller et al., 2007; Bicalho et al., 2010a; Santos et al., 2011). The presence of *E. coli* in the uterus is associated with uterine inflammation and impaired reproductive performance (Studer and Morrow, 1978; Bonnett and Martin, 1995; Bondurant, 1999). Zerbe et al. (2001) observed that *E. coli* products cause a dominant functional depression of neutrophils and suggested that the earlier appearance of *E. coli* in the uterus may support the coinfection of this organ by other etiologic agents at later times. Bicalho et al. (2010a) investigated virulence factors, including the type 1 fimbrial adhesin fimH, and reported that cows with at least one fimH-carrying *E. coli* strain isolated from the uterus had a 4.6-fold increased odds of metritis when compared with *E. coli* negative cows.

Later, Bicalho et al. (2012) reported that the presence of fimH-carrying *E. coli* at 1 to 3 DIM in the uterus was strongly associated with metritis, and cows that had the fimH-carrying bacteria were 16.2 times more likely to develop *F. necrophorum* intrauterine contamination at 8 to 10 DIM.

Ideally, treatments for uterine diseases should control pathogens in the uterus and result in no or only short-duration milk and meat withdrawal periods. Systemic or intrauterine antibiotic therapy is commonly used as a treatment for metritis, and antibiotic therapy is usually followed by milk and meat withdrawal periods (Azawi, 2008). However, extensive use of antimicrobials in food animal production has contributed to the emergence of antimicrobial resistance among pathogens, which complicates the treatment of infectious diseases (Tollefson et al., 1999).

Bacteriophages are viruses that infect bacteria and are obligate intracellular parasites lacking their own metabolism. Phages are host specific, able to infect only specific species or even strains of bacteria (Barrow and Soothill, 1997). Optimistic results regarding their use against certain bacterial infections have been revealed by recent randomized clinical trials in mice and cattle. Mice were successfully rescued with a single injection of a phage solution containing $3 \times 10^8$ plaque-forming units (**pfu**) of a certain bacteriophage after inoculation with lethal doses of vancomycin-resistant *Enterococcus faecium* (Biswas et al., 2002). Additionally, Smith and Huggins (1983) reported that bacteriophage therapy was successful when newborn, colostrum-deprived calves were inoculated with *E. coli* and treated with intramuscular injections of a bacteriophage solution.

Our research group isolated environmental bacteriophages using *E. coli* derived from the uterus of postpartum dairy cows (Bicalho et al., 2010b) and evaluated the in vitro antimicrobial activity of a bacteriophage cocktail against intrauterine *E. coli* (Santos et al., 2010). This bacteriophage cocktail had excellent in vitro bactericidal ability against several genetically distinct strains of *E. coli* previously isolated from intrauterine secretions of metritic cows. In a recent study, using 597 cows, we evaluated the effectiveness of an anti-*E. coli* bacteriophage cocktail by intrauterine administration at $2 \pm 1$ DIM. The cocktail included 4 different phages, with a dose of approximately $10^7$pfu, but no effects on uterine health and reproductive performance were found (Machado et al., 2012). As mentioned before, the presence of *E. coli* in the uterus at 1 to 3 DIM is highly associated with uterine disease. Therefore, it is possible that in our previous work (Machado et al., 2012), *E. coli* had already had a detrimental effect on the uterus at the moment of treatment. Sharma et al. (2011) observed that prophylactic peripartal intravaginal administration of a probiotic treatment lowered the incidence of metritis and endometritis in dairy cows. Specific strains of *E. coli* that are more likely to cause uterine diseases may inhabit the lower genital tract of some cows before parturition, predisposing them to metritis, and that bacteriophage treatment might have been more successful if applied prepartum. Additionally, the dosage used for the preparation of the bacteriophage cocktail in Machado et al. (2012) could have been insufficient to treat the bacterial load of the lochial secretion. Therefore, the objective of this study was to evaluate the effect of prepartum intravaginal administration of an anti-*E. coli* bacteriophage cocktail on uterine disease.

## MATERIALS AND METHODS

### Farm and Management

The study was conducted on a commercial dairy farm located near Ithaca, New York, from March to September 2012. This farm was selected because of its long working relationship with the Ambulatory and Production Medicine Clinic at Cornell University. The farm milked 2,800 Holstein cows 3 times daily in a double 52-stall parallel milking parlor. The cows were housed in freestall barns with concrete stalls covered with mattresses and bedded with manure solids. All cows were offered a TMR consisting of approximately 55% forage (corn silage, haylage, and wheat straw) and 45% concentrate (corn meal, soybean meal, canola, cottonseed, and citrus pulp) on a DM basis. The diet was formulated to meet or exceed the NRC nutrients requirements (NRC, 2001) for lactating Holstein cows weighing 650 kg and producing 45 kg of 3.5% FCM. The prefreshening diet had 19.25, 108.59, 0.38, and 63.77 mg/kg of Cu, Mn, Se, and Zn in its composition, respectively; NRC nutrient requirements in prefreshening diet are 13.0, 18.0, 0.30, and 22.0 mg/kg, respectively (NRC, 2001). Furthermore, the lactation diet had 20.02, 72.85, 0.47, and 80.81 mg/kg of Cu, Mn, Se, and Zn in its composition, respectively; NRC nutrient requirements in lactation diet are 11.0, 13.0, 0.30, and 52.0 mg/kg, respectively (NRC, 2001).

The reproductive management used a combination of Presynch (Moreira et al., 2001), Ovsynch (Pursley et al., 1995), Resynch (Fricke et al., 2003), and detection of estrus, with 25 to 30% of cows bred via timed AI and the remainder bred after detection of estrus solely by activity monitors (Alpro; DeLaval, Kansas City, MO).

### Treatment Groups and Case Definition

Statistical power and sample size calculations were performed before initiation of the study. Considering a