J. Dairy Sci. 100:1–16
https://doi.org/10.3168/jds.2016-11623

Dynamics of the microbiota found in the vaginas of dairy cows during the transition period: Associations with uterine diseases and reproductive outcome

M. L. S. Bicalho,* T. Santin,† M. X. Rodrigues,† C. E. Marques,† S. F. Lima,† and R. C. Bicalho†[1]
*Department of Clinical Sciences, and
†Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

ABSTRACT

We investigated the microbiota found in the vaginas of Holstein dairy cows during the transition period and described the differences in bacterial composition and total bacterial load (TBL) associated with disease and fertility. Vaginal swabs were collected at −7, 0, 3, and 7 d relative to parturition from 111 dairy cows housed on a commercial dairy farm near Ithaca, New York. Microbiota were characterized by next-generation DNA sequencing of the bacterial 16S rRNA gene, and TBL was determined by real-time quantitative PCR. We applied repeated-measures ANOVA to evaluate the associations of uterine disease and related risk factors with the microbiota and TBL. We estimated phylum-specific bacterial load by multiplying the TBL by the relative abundance of each phylum observed in the metagenomics results. We confirmed the validity of this approach for estimating bacterial load by enumerating the number of bacteria in an artificial sample mixed in vitro and in clinical and healthy vaginal samples. Phyla associated with uterine disease and related risk factors were *Proteobacteria*, *Fusobacteria*, and *Bacteroidetes*. Cows with retained placenta and healthy cows had similar TBL at the day of parturition, but at d 7 postpartum, cows with retained placenta showed a significantly higher TBL, mainly driven by higher estimated loads of *Fusobacteria* and *Bacteroidetes*. Cows diagnosed with metritis had a significantly higher estimated load of *Proteobacteria* at d −7 and at calving and higher estimated loads of *Fusobacteria* in the postpartum samples. Additionally, the estimated load of *Bacteroidetes* at d 7 postpartum was higher for cows diagnosed with endometritis at 35 days in milk. Higher estimated loads of *Fusobacteria* and *Bacteroidetes* were also evident in cows with postpartum fever, in primiparous cows, in cows with assisted parturition, and in cows that gave birth to twins. Our findings demonstrated that microbiota composition and TBL were associated with known periparturient risk factors of uterine diseases and reproductive failure, including parity, assisted parturition, and retained fetal membranes.

Key words: microbiota, bacterial load, metritis, endometritis

INTRODUCTION

Recent developments in DNA sequencing technology enable comprehensive, culture-independent characterizations of dairy cow uterine microbiota, with the goal of identifying a core microbiological guild and associations of specific taxa with uterine disease status. Previous metagenomics studies describing the diversity of bacterial populations in the uterine microbiota of postpartum cows have reported dramatic differences in composition between healthy cows and cows with reproductive diseases (Santos et al., 2011; Jeon et al., 2015). Furthermore, infection by pathogenic strains of *Escherichia coli* was found to disrupt the natural balance of the uterine microbiota (Dohmen et al., 2000; Santos et al., 2011), facilitating subsequent infection by *Trueperella* and *Fusobacteria* species (Bicalho et al., 2012). Such bacterial dysbiosis after parturition is of particular interest, because uterine infection is an important contributor to economic loss in high-producing dairy cows (Overton and Fetrow, 2008). However, alterations in microbiota composition in the vaginal tract of dairy cows and how the diversity changes before uterine disease is established are underexplored at the microbial population level. The detection of early contamination in reproductive-tract infections would be extremely beneficial in allowing for early intervention and prevention of disease development.

Recently, studies using culture-independent methods have indicated that prepartum vaginal colonization of apparently healthy cattle appears to be influenced by the gastrointestinal community and by low abundance

Received June 15, 2016.
Accepted December 16, 2016.
[1]Corresponding author: rcb28@cornell.edu

of lactobacilli, a known inhibitor of many vaginal pathogens among women of reproductive age (Swartz et al., 2014; Laguardia-Nascimento et al., 2015). However, those studies were performed in clinically healthy cattle at different stages of lactation and did not investigate the association between microbiota in the vaginas of dairy cows and incidence of reproductive diseases. The major challenge for the prevention and treatment of uterine disease is the well-known multifactorial etiology of metritis and clinical endometritis (**CE**) in conjunction with their highly complex pathogenesis.

It is also increasingly recognized that the efficiency of bacterial elimination depends on the level of exposure to the bacterial load at parturition (Sheldon et al., 2002; LeBlanc et al., 2011). Previous studies have investigated associations between bacterial load and uterine infection by examining postpartum bacterial growth density of specific bacteria isolated from the uterine lumen with culture-dependent methods and biochemical approaches (Elliott et al., 1968; Hussain et al., 1990; Williams et al., 2005). However, in complex environments, in which multispecies populations are sampled along with impurities, or where the bacteria are internalized within a matrix, determination of bacterial load by real-time quantitative PCR (**qPCR**) based on the 16S rRNA gene is likely to have far greater sensitivity and precision (Nadkarni et al., 2002).

To our knowledge, the microbiota signature in the vaginas of dairy cows before and after parturition has not been comprehensively characterized. Here we used cultivation-independent, molecular-phylogenetic techniques (next-generation DNA sequencing and qPCR of the 16S rRNA gene) to characterize and compare bacterial populations in the vaginas of Holstein dairy cows sampled 1 week before parturition, on the day of calving, and at 2 time points during the first week of lactation. An additional key feature of this study is that we estimated the total bacterial load (**TBL**) in the vaginal samples using real-time qPCR. We aimed to describe how the microbiota and TBL found in the vaginas of dairy cows are associated with disease and fertility.

MATERIALS AND METHODS

Ethics Statement

This study was carried out in strict accordance with the recommendations of the Animal Welfare Act of 1966 (P.L. 89–544) and its amendments 1970 (P.L. 91–579), 1976 (P.L. 94–279), and 1985 (P.L. 99–198) that regulate the transportation, purchase, care, and treatment of animals used in research. The research protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Cornell University (protocol number: 2011–0111). Sampling procedures and experimental manipulations were authorized by the farm owner, who was aware of the procedure.

Animals, Facilities, and Farm Management

This study was conducted in a commercial dairy farm near Ithaca, New York. The farm milks 3,450 Holstein cows 3 times daily in a parallel rotary 100-stall milking parlor. Primiparous and multiparous 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 nutrient requirements for lactating Holstein cows weighing 650 kg and producing 45 kg of 3.5% FCM (NRC, 2001).

Dry cows were housed in 2 separate groups: (1) a far-off group, consisting of cows from 225 to 255 d of gestation, and (2) a close-up group, consisting of cows >255 d of gestation. Pregnant heifers were moved to the dry cow facility 40 d before their expected due date (at approximately 240 d of gestation). Farm employees were responsible for overseeing the dry cow facility 24 h/d, 7 d/wk. They were trained to detect cows at stage 1 or 2 of parturition and move them from the close-up barn to 1 of 4 maternity pens, which were each 400 m^2 with a deep straw bed. Once in the maternity pen, cows were allowed to give birth without intervention unless it was judged necessary by the maternity pen employee; immediately after parturition, calves were removed from the maternity pen and placed in a dry sawdust-bedded pen that was heated with heating lamps during the winter. Fresh cows were first milked within 4 h of calving in a 5-stall parallel parlor in the maternity area.

Reproductive management used a combination of Presynch (Moreira et al., 2001), Resynch (Fricke et al., 2003), and detection of estrus. For the first service, all cows were inseminated by timed AI following completion of the Presynch protocol. Briefly, cows were injected with 25 mg of $PGF_{2\alpha}$ (Lutalyse Sterile Solution; Pfizer Animal Health, Parsippany, NJ) at 55 ± 3 and 69 ± 3 DIM, and were subsequently submitted to the Ovsynch protocol (Pursley et al., 1995): 100 μg of GnRH (Cystorelin; Merial Ltd., Iselin, NJ) at 81 ± 3 DIM, $PGF_{2\alpha}$ at 88 ± 3 DIM, and GnRH at 90 ± 3 DIM, and then inseminated at a fixed time at 91 ± 3 DIM. Following the first service, cows could be inseminated based on estrus detection. Estrus was detected based only on electronic activity sensors (Alpro, DeLaval, MO) worn around the neck. Cows not