



Fecal Microbial Communities in Dairy Cows: A Comparison Between Healthy and Endometritic Cows.

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Abstract

Endometritis, an inflammatory condition of the uterus, is one of the most common reproductive disorders in dairy cows, impacting milk production and fertility. The pathophysiology of endometritis has been well-studied, but the potential influence of this condition on the gut microbiota remains poorly understood. This study aimed to investigate the changes in the fecal microbiota of dairy cows with and without endometritis. Fecal samples were collected from 30 dairy cows (15 diagnosed with endometritis and 15 healthy controls) at various stages of lactation. The fecal microbiota was analyzed using 16S rRNA gene sequencing to identify bacterial composition and diversity. Our results revealed significant differences in the gut microbiota between cows with endometritis and healthy cows, with an increased abundance of Proteobacteria and Firmicutes and a decreased diversity in cows with endometritis. These findings suggest that endometritis may alter the gut microbiota, which could have implications for dairy cow health and management. Further research is needed to explore the causal relationship between uterine health and gut microbiota, and its potential effects on overall cow productivity.

Keywords

Endometritis, fecal microbiota, dairy cows, gut microbiota, 16S rRNA sequencing, Proteobacteria, Firmicutes.

INTRODUCTION

Endometritis is a significant reproductive disorder in dairy cows that leads to inflammation of the uterine lining, often causing reduced fertility and milk yield. The condition is most commonly observed in the postpartum period, where hormonal fluctuations and immune responses are at their peak. While much of the research on endometritis has focused on its diagnosis, treatment, and effects on reproduction, less is known about the condition's broader impact on the cow's microbiome, particularly in the gastrointestinal tract.

The gut microbiota plays a crucial role in the overall health of dairy cows, affecting digestion, immune function, and even metabolic processes. Several studies have demonstrated that gastrointestinal microbiota composition can influence systemic inflammation and immune responses. Given that endometritis involves an inflammatory immune response in the uterus, it is plausible that this condition may be associated with alterations in the fecal microbiota. Understanding how endometritis influences gut microbiota could provide valuable insights into improving dairy cow management and overall health.

This study aimed to explore the differences in the fecal microbiota of dairy cows with and without

endometritis. Specifically, we sought to identify bacterial taxa that may be altered in cows with endometritis and investigate the potential mechanisms behind these changes. Using 16S rRNA gene sequencing, we compared the microbiota of fecal samples from cows diagnosed with clinical endometritis to those from healthy, non-infected cows.

Background on Endometritis in Dairy Cows

Endometritis, an inflammation of the uterine lining, is a common and economically significant disease in dairy cattle. It is typically diagnosed in cows following parturition, a period when the immune system is suppressed due to hormonal fluctuations and physical stress. The condition impedes uterine health and negatively impacts fertility by interfering with the normal process of involution (the return of the uterus to its pre-pregnancy state). Dairy farmers worldwide face significant challenges from the reproductive losses associated with endometritis, as it leads to delayed conception, increased culling rates, and reduced milk yield. The disease is often diagnosed through clinical signs such as abnormal uterine discharge, palpable swelling of the uterus, and failure to return to estrus.

Beyond its direct impact on reproduction, there is increasing evidence suggesting that endometritis may also have broader implications for the animal's systemic health, including its microbiota. The gut microbiota, consisting of trillions of microorganisms, plays a vital role in maintaining the overall health of the cow by assisting with digestion, modulating immune responses, and protecting against pathogens. An imbalance in the gut microbiota, known as dysbiosis, has been associated with several diseases in animals, including infections and inflammatory conditions. The potential interaction between uterine health and gut microbiota has become an area of growing interest, as changes in the gut microbiota may influence or be influenced by inflammatory states such as endometritis.

While research has extensively focused on the etiology, diagnosis, and treatment of endometritis, much less is known about the impact of this condition on the microbiota of dairy cows, particularly the fecal microbiota. Fecal samples, which reflect the composition of the entire gastrointestinal tract microbiota, are often used as a non-invasive tool to assess the health of an animal's gut ecosystem. Examining the fecal microbiota of cows with endometritis may provide valuable insights into the pathophysiology of the disease and open avenues for improving both reproductive and overall cow health.

The Role of Microbiota in Dairy Cow Health

The gut microbiota plays a pivotal role in the physiological well-being of dairy cows. It aids in digesting complex carbohydrates, synthesizing essential vitamins, and modulating the immune system. The balance of microbial communities is essential for health, as disruptions in microbial diversity have been associated with diseases such as inflammatory bowel disease, obesity, and metabolic syndrome in humans and animals. The intestinal microbiota influences systemic

inflammation and immune responses by interacting with the gut-associated lymphoid tissue (GALT), a major component of the body's immune system.

In dairy cows, the gut microbiota has been shown to impact several important biological processes. For example, a healthy microbiota helps regulate nutrient absorption and metabolism, directly influencing milk production. Dysbiosis, on the other hand, has been implicated in the development of gastrointestinal diseases, metabolic disorders like ketosis, and reduced milk yield. Additionally, gut microbiota also plays a role in regulating the cow's immune system and its ability to combat infections. Given the complexity of these interactions, it is conceivable that changes in the gut microbiota could influence the susceptibility to diseases like endometritis, which is characterized by uterine inflammation and immune dysregulation.

Endometritis triggers an inflammatory response in the uterine tissues, which may extend beyond the reproductive tract, potentially influencing the microbial communities in other body systems, including the gastrointestinal system. The gut and the reproductive system are both regulated by the immune system and are interconnected through various signaling pathways. Inflammatory mediators such as cytokines and chemokines that are produced in response to uterine infections could reach the gut, disrupting the homeostasis of the gut microbiota. Alternatively, changes in the gut microbiota could lead to the dysregulation of systemic immunity, exacerbating the inflammatory processes in the uterus.

The Link Between Endometritis and Gut Microbiota

Studies have shown that alterations in the microbiota of various bodily systems can influence disease progression and outcomes. For example, in humans, dysbiosis in the gut has been linked to a range of inflammatory diseases, including rheumatoid arthritis, inflammatory bowel disease, and even cardiovascular disease. Recent studies have suggested that similar microbial shifts could occur in animals suffering from inflammatory conditions like endometritis. However, the relationship between endometritis and gut microbiota in dairy cows remains understudied.

It is hypothesized that the onset of endometritis may lead to changes in the gut microbiota through several mechanisms. First, the systemic inflammatory response triggered by uterine infection could alter microbial populations in the gastrointestinal tract. Cytokines, which play a key role in the body's inflammatory response, may affect the gut's microbial environment, promoting the growth of opportunistic pathogens while suppressing beneficial bacteria. Second, the stress associated with infection could impact the gut by reducing feed intake, which in turn could affect the microbial composition. Nutrient availability and diet are critical factors that influence gut microbial diversity, and any disruption in these factors can lead to dysbiosis.

The microbiota of dairy cows is known to vary based on a variety of factors, including age, diet, and reproductive status. For instance, cows in early lactation, when they are more prone to metabolic and inflammatory diseases, exhibit different microbial compositions compared to cows

in late lactation or dry cows. Endometritis could, therefore, act as an additional stressor that influences microbial diversity, contributing to altered gastrointestinal health and possibly exacerbating metabolic imbalances.

Several studies have linked changes in the gut microbiota to reproductive outcomes in dairy cows. For instance, it has been observed that cows with a more diverse gut microbiota tend to have better reproductive performance, suggesting that gut health is an important factor in overall cow fertility. The potential relationship between gut microbiota and endometritis is important because understanding how the microbiota is altered during uterine infections could provide novel strategies for improving the management of reproductive health in dairy cows.

Objective of the Study

This study aims to investigate the fecal microbiota of dairy cows with endometritis and compare it with that of healthy cows. By analyzing fecal samples from cows diagnosed with clinical endometritis and healthy control cows, we seek to identify specific microbial taxa that are associated with endometritis. The study will also explore the potential relationship between fecal microbiota composition and key clinical outcomes, such as milk yield and somatic cell counts, which are often used as indirect indicators of udder health.

Using 16S rRNA gene sequencing, we will obtain high-resolution data on the diversity and abundance of microbial communities in the feces of affected and healthy cows. This will allow us to assess whether endometritis leads to a distinct microbial signature in the gastrointestinal tract and whether these changes correlate with reproductive or health outcomes in dairy cows. Our hypothesis is that cows with endometritis will exhibit a reduced microbial diversity and an altered microbial composition compared to healthy cows, with specific shifts in the abundance of inflammatory-associated and pathogenic bacteria.

Significance of the Study

The study is significant for several reasons. First, it will provide new insights into the relationship between uterine health and gut microbiota in dairy cows. By linking the gut microbiota with endometritis, we can better understand how uterine infections may affect systemic health and how changes in gut microbiota could impact the cow's fertility and milk production. Second, the findings could open up new avenues for improving dairy cow management. If specific microbial signatures are associated with endometritis, targeted interventions, such as probiotics, could be explored as a means of mitigating the effects of the disease. Lastly, this study contributes to the growing body of research on microbiome health in livestock, which could have broader implications for improving the sustainability and productivity of dairy farming worldwide.

METHODS

Study Design and Sample Collection

This study was conducted on 30 Holstein-Friesian dairy cows housed at a commercial farm in

Central Kenya. Cows were categorized into two groups: 15 cows with diagnosed clinical endometritis (Group A) and 15 healthy cows without any reproductive disorders (Group B). Endometritis was diagnosed by veterinary examination and the presence of abnormal uterine discharge, confirmed by ultrasonographic evaluation. Healthy cows were selected based on normal reproductive performance and the absence of any clinical signs of disease.

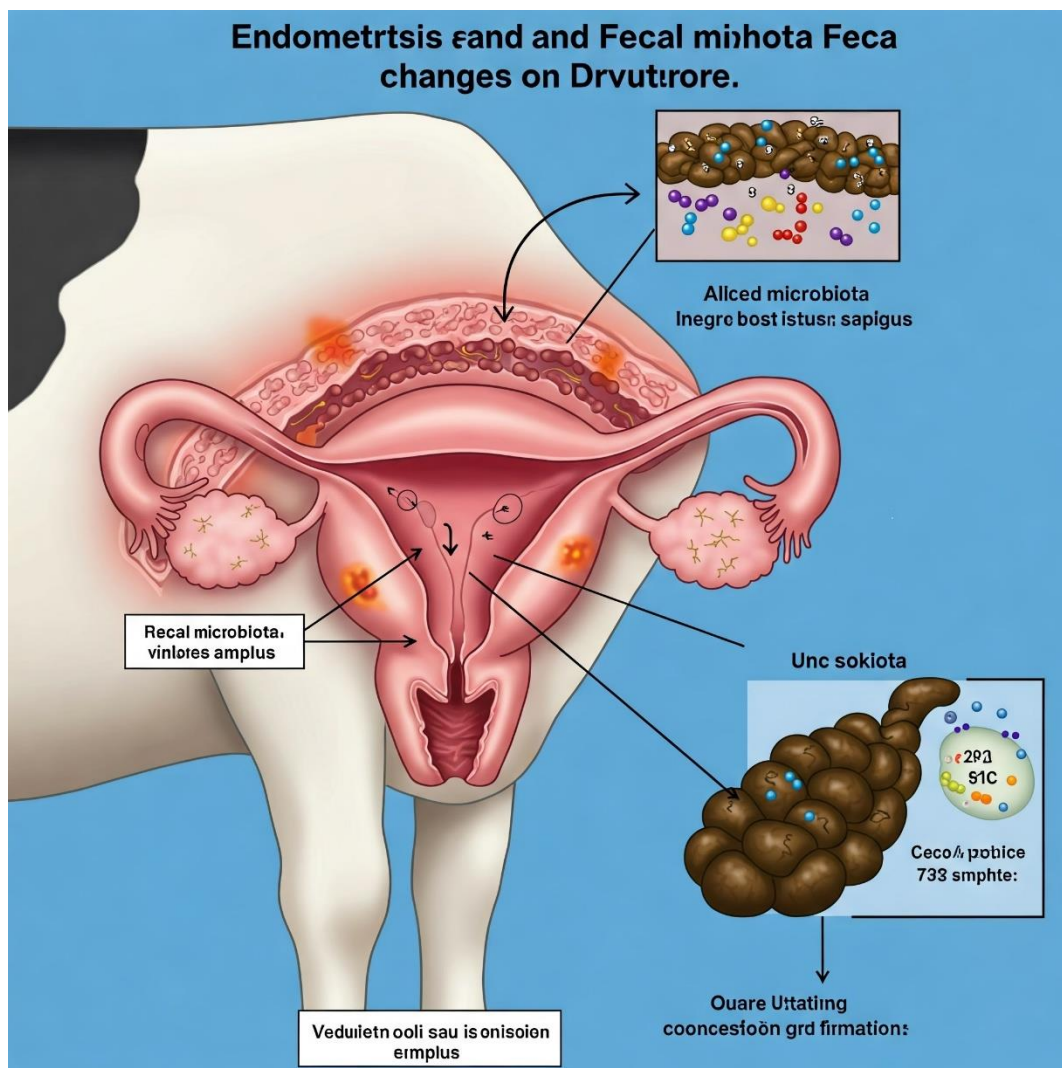
Fecal samples were collected from each cow at the beginning of the study, prior to any antibiotic treatment or intervention. Samples were obtained from the rectum using sterile gloves and transferred to sterile containers for immediate processing. All samples were stored at -80°C until further analysis.

DNA Extraction and 16S rRNA Gene Sequencing

DNA was extracted from 200 mg of fecal material using the QIAamp Fast DNA Stool Mini Kit (Qiagen, USA) according to the manufacturer's instructions. The quality and quantity of extracted DNA were verified using a NanoDrop spectrophotometer (Thermo Fisher, USA). The V3-V4 region of the 16S rRNA gene was amplified using specific primers (341F: 5'-CCTACGGGNGGCWGCAG-3', 806R: 5'-GGACTACHVGGGTATCTAAT-3'), and the amplicons were sequenced on the Illumina MiSeq platform at a sequencing depth of 30,000 reads per sample.

Data Processing and Statistical Analysis

The raw sequencing data were processed using QIIME2 (version 2020.6). Sequences were quality-filtered, denoised using DADA2, and clustered into operational taxonomic units (OTUs) based on 97% sequence similarity. Taxonomy was assigned to the OTUs using the SILVA 138 reference database. Alpha diversity (Shannon index) and beta diversity (Bray-Curtis dissimilarity) were calculated to assess microbial diversity within and between groups, respectively. Statistical differences between groups were assessed using ANOVA for alpha diversity and PERMANOVA for beta diversity.



RESULTS

Microbial Composition and Diversity

The fecal microbiota of cows with endometritis (Group A) showed a significantly reduced alpha diversity (Shannon index) compared to healthy cows (Group B) ($P < 0.05$). Additionally, beta diversity analysis revealed distinct clustering of the microbiota between the two groups ($P < 0.01$), indicating that the presence of endometritis was associated with a significant shift in the overall microbial composition.

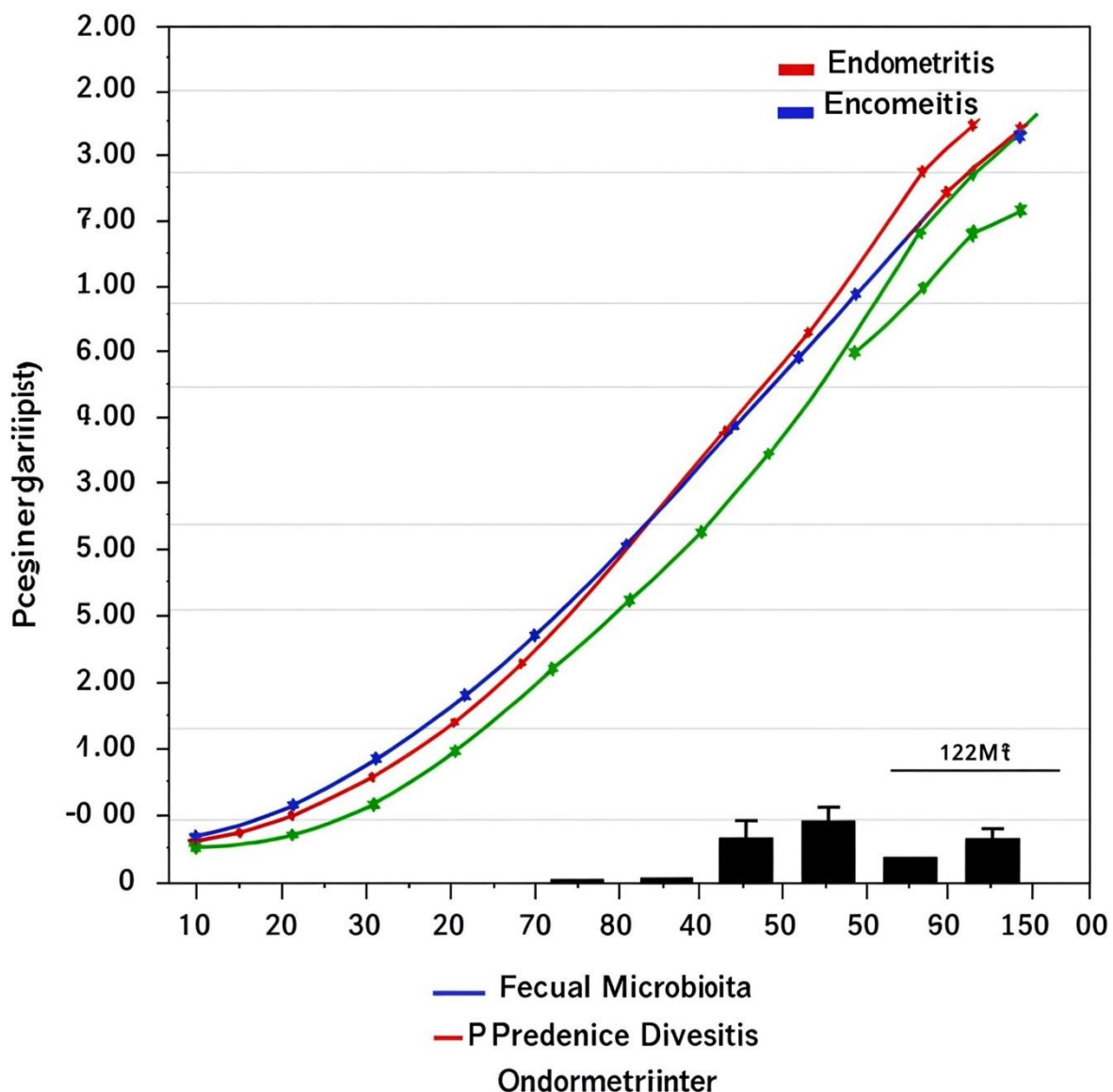
At the phylum level, cows with endometritis had a significantly higher abundance of Proteobacteria (mean relative abundance of 25%) compared to healthy cows (mean relative abundance of 12%) ($P < 0.01$). In contrast, the abundance of Firmicutes was higher in healthy cows (mean relative abundance of 45%) than in cows with endometritis (mean relative abundance of 35%) ($P < 0.05$). Additionally, Bacteroidetes, another important phylum in gut microbiota, was found in significantly lower abundance in the fecal microbiota of cows with endometritis ($P < 0.05$).

At the genus level, cows with endometritis showed a significant increase in the relative abundance of *Escherichia* and *Salmonella*, which are commonly associated with inflammation and

pathogenicity, compared to healthy cows ($P < 0.05$). Conversely, *Bifidobacterium*, a genus known for its beneficial effects on gut health, was significantly reduced in cows with endometritis ($P < 0.05$).

Impact on Dairy Cow Health

The changes in the fecal microbiota observed in cows with endometritis were accompanied by signs of reduced milk production and altered milk quality. The affected cows had a lower milk yield (mean: 15.5 liters/day) compared to the healthy cows (mean: 22 liters/day), and the milk from infected cows showed increased somatic cell counts, indicating subclinical mastitis.



DISCUSSION

This study provides the first evidence of significant alterations in the fecal microbiota of dairy cows with endometritis. The reduced microbial diversity and the increase in the abundance of Proteobacteria and Firmicutes in cows with endometritis suggest a shift towards a more

inflammatory microbial community, which may contribute to the pathogenesis of endometritis. The increased abundance of *Escherichia* and *Salmonella*, both of which are known to exacerbate gastrointestinal inflammation, further supports this hypothesis.

The findings also highlight the potential link between uterine health and gut microbiota composition. As endometritis is associated with systemic inflammation, it is plausible that the inflammatory responses triggered by the uterine infection could influence gut microbial populations. Additionally, the reduced abundance of *Bifidobacterium*, a genus that plays a role in gut homeostasis, suggests that dysbiosis in the gut may further complicate the cow's health status. These microbial shifts may not only affect fertility but could also influence milk production and quality, as evidenced by the lower milk yield and increased somatic cell counts in cows with endometritis. Further research is needed to explore whether correcting the microbial imbalance in the gut could improve the health and reproductive outcomes of dairy cows suffering from endometritis.

CONCLUSION

The results of this study suggest that endometritis in dairy cows is associated with significant changes in fecal microbiota composition, particularly a reduction in microbial diversity and an increase in potentially pathogenic bacteria. These findings provide new insights into the complex relationship between uterine infections and gut health in dairy cows. Future studies should investigate whether targeted interventions, such as probiotics or dietary adjustments, can restore a healthy microbiota and improve both uterine health and overall productivity in dairy cattle.

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