

Short Communication

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*Corresponding author:

Eun Tae Kim
Dairy Science Division, National Institute of Animal Science, Rural Development Administration, 114, Sinbang 1-gil, Seonghwan-eup, Seobuk-gu, Cheonan 31000, Korea
Tel: +82-41-580-3399
Fax: +82-41-580-3419
E-mail: etkim77@korea.kr

ORCID:
<https://orcid.org/0000-0001-7486-5638>

†These authors contributed equally to this study.

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Dynamics of bacterial communities in vaginas and feces between pre and postpartum of dairy cows

Jun-Kyu Son^{1,†}, Dong-Hyeon Kim^{1,†}, Jihwan Lee¹, Sang-Bum Kim², Beom-Young Park¹, Myunghoo Kim³, Sungill Lee⁴, Tai-Young Hur¹, Eun Tae Kim^{1,*}

¹Dairy Science Division, National Institute of Animal Science, Rural Development Administration, Cheonan 31000, Korea

²Rural Development Administration, Jeonju 54875, Korea

³Department of Animal Science, College of Natural Resources and Life Science, Pusan National University, Miryang 50463, Korea

⁴Division of Applied Life Science (BK21Plus) & Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 52828, Korea

The reproductive tracts have an intimate relationship with reproduction because there are bacterial communities that can affect reproductive health. The differences in the bacterial community of periparturient dairy cows were investigated. Vaginal and fecal samples were collected seven days before and after calving, and DNA was extracted to sequence the V3-V4 regions of the 16S rRNA genes. In the postpartum vaginas, operational taxonomic units, Chao1, Shannon, and Simpson were decreased, and phyla *Fusobacteria* and *Bacteroidetes* were increased. In summary, bacterial abundance can affect the periparturient biological differences in dairy cows, suggesting a susceptibility to infection within one week after calving.

Keywords: vagina; feces; microbiota; prepartum; postpartum

The important challenge in the livestock industry is reproductive losses, which can lead to economic losses. This issue has led researchers to find strategies to improve reproductive efficiency. One of the strategies is the microbiomes that are involved in the physiological functions of the host. This study focused on the microbiota in the gut and vaginas. The microbiota in the reproductive tracts has an intimate relationship with reproduction because the bacteria communities can affect reproductive health and fertility [1]. Vaginal delivery in human studies has shown a positive effect on developing a newborn's immune system as the bacteria pass through the vaginas, promoting the transmission of maternal microbiota [2]. The high diversity of bacteria as a dysbiosis can affect the establishment and maintenance of pregnancy [3]. This is because the metabolites produced by the bacterial community can affect the host and hormone production [4] and regulation [5]. For example, chemical reactions through the intestinal stimulation, such as stress, affect the bacterial communities, and its metabolites may induce hormone release [6]. In addition, the gut microbiota can degrade hormones and alter gene expression in the host, leading to reproductive improvement [7]. Several studies reported that dysbiosis in the intestinal tract increased the risk associated with various health issues, including inflammatory bowel disease and obesity [8]. Despite many studies on the significance of the bacterial communities on the re-

productive tract, including humans, there have been few cattle studies. In particular, studies on changing the microbial diversity in vaginas are still in their infancy. Therefore, this study characterized the microbial diversity in the vaginas and feces of dairy cows and the differences between pre and postpartum.

All experiments involving animals were conducted using the approved animal care protocols from the Animal Care Committee Institutional Animal Care and Use Committee of the National Institute of Animal Science, Rural Development Administration, Republic of Korea (approval number: NIAS-2019107). Three prepartum Holstein multiparous cows (42 ± 13.2 months old) were used for the experiments. All cows were moved at day-21 before expected calving to one pen and were fed a diet once daily. The cows received a typical close-up diet total mixed ration (13 kg DM/cow/day, crude protein, 11.1%; neutral detergent fiber, 48.2%; acid detergent fiber, 26.9%; calcium, 0.4%; phosphorus, 0.15%). Water was available ad libitum. All the samples were collected on day seven before calving (PRE) and day seven after calving (POST), and the differences in bacterial communities were analyzed. Both vaginas and fecal samples were collected using cotton swabs. For vagina sampling, the vulvar area was totally cleaned with water and then decontaminated with 70% (vol/vol) ethyl alcohol before sampling. A stainless steel collector fitted sterilized cotton-tipped swab covered with a sleeve (A.I/E.T Sanitary sheaths, L = 533 mm, 21", IMV Technologies, France) to minimize contamination was inserted mildly into the vaginas, and a sample was collected from the vaginal wall. The samples were placed immediately in the tube (Swab Collection and Preservation System, Cat. No. 45670, Norgen, Biotek Corp., Canada) containing preservatives. Fecal samples were taken from the rectum after vaginal sampling and

placed in 50 mL conical tubes. All the samples were stored in a -80°C freezer for further analysis.

For metagenomics, DNA was extracted using the PowerSoil[®] DNA Isolation Kit (Cat. No. 12888; MO BIO, USA) according to the manufacturer's protocol. The extracted DNA was further sequenced, processed, and analyzed by Macrogen (Macrogen Inc., Korea). The V3-V4 regions of the 16S rRNA gene were amplified by PCR using the primers containing an ILMN pre-adapter + sequencing primer + specific locus primer: V3 (5'-TCGTCGGCAGCGTC + AGATGTGTATAAGAGACAG + CCTACGGGNGGCWGCAG-3'; forward) and V4 (5'-GTCTCGTGGGCTCGGA + GATGTGTATAAGAGACAGG + ACTACHVGGGTATCTAATCC-3'; reverse). The filtered reads were clustered as operational taxonomic unit (OTU) sequences at 97% similarity using the CD-HIT-out. Chimeric sequences were identified and removed using rDnaTools. The sequences were classified using the Ribosomal Database Project.

Statistical analyses of the biodiversity and bacterial community were performed with samples at an even sequence depth.

The mean Shannon and Simpson indices and the relative abundance of the bacterial communities (phyla and genera levels) were compared using a t-test in SAS (SAS version 9.4; SAS Institute Inc., USA) to determine the significant differences between the PRE and POST dairy cows. A *p*-value of < 0.05 compared to the PRE group was considered significant.

Sequencing of the 16S rRNA genes produced 84,371 and 71,857 reads in the vaginal and fecal samples, respectively, and it was rarefied across samples to the lowest sample depth. The OTUs, Chao1, Shannon, and Simpson, which are the microbial community richness and evenness as representative indices, showed highly diverse microbial diversity except for the vagi-

Table 1. Alpha-diversity of the vaginal and fecal microbiota using 16S rRNA gene sequence

Items	PRE	POST	<i>p</i> -value
Vaginas			
Number of OTUs	610 \pm 26.5	37.3 \pm 1.70*	< 0.01
Chao1	660 \pm 33.9	38.8 \pm 1.31*	< 0.01
Shannon	7.39 \pm 0.41	2.45 \pm 0.40*	< 0.01
Simpson	0.982 \pm 0.01	0.725 \pm 0.07*	0.030
Good's coverage	0.999 \pm 0.002	1.00 \pm 0.0001	0.102
Feces			
Number of OTUs	513.0 \pm 29.7	554.0 \pm 34.7	0.274
Chao1	590.5 \pm 42.0	650.0 \pm 36.4	0.203
Shannon	7.21 \pm 0.12	7.18 \pm 0.19	0.847
Simpson	0.986 \pm 0.001	0.986 \pm 0.002	0.918
Good's coverage	0.992 \pm 0.004	0.994 \pm 0.001	0.499

PRE, one week before calving; POST, one week after calving; OTU, operational taxonomic unit.

**p* < 0.05 compared with control group.

nas of the POST dairy cows (Table 1). The OTUs (610 ± 26.5 vs. 37.3 ± 1.70), Chao1 (660 ± 33.9 vs. 38.8 ± 1.31), Shannon (7.39 ± 0.41 vs. 2.45 ± 0.40), and Simpson (0.982 ± 0.01 vs. 0.725 ± 0.07) in the vaginas of the POST dairy cows were significantly lower than in the PRE cows. In contrast, in the feces, Chao1 (≥ 513.0), Shannon (≥ 7.18), and Simpson (≥ 0.986) showed highly diverse microbial communities regardless of the sampling period. Taxonomic analysis of the reads revealed six main phyla (relative abundance $\geq 1\%$) from the vaginas of the PRE dairy cows: *Bacteroidetes* (27.3 ± 3.95), *Firmicutes* (47.2 ± 3.46), *Tenericutes* (2.42 ± 1.69), *Proteobacteria* (6.37 ± 3.88), *Actinobacteria* (6.79 ± 7.89), and *Verrucomicrobia* (2.03 ± 1.04) (Table 2). On the other hand, the relative abundance and composition of these predominant phyla showed a difference from the vaginas of the POST dairy cows. Five main phyla from the vaginas of POST dairy cows included *Fusobacteria* (41.4 ± 3.07), *Bacteroidetes* (46.5 ± 5.81), *Firmicutes* (7.60 ± 2.18), *Tenericutes* (1.98 ± 1.39), and *Proteobacteria* (1.69 ± 1.79). In particular, in the postpartum vaginas, the relative abundance of *Fusobacteria* (0 vs. 41.4 ± 3.07) and *Bacteroidetes* (27.3 ± 3.95 vs. 46.5 ± 5.81) increased dramatically, and *Firmicutes* (47.2 ± 3.46 vs. 7.60 ± 2.18) decreased. Taxonomic analysis revealed 17 and seven main genera from the vaginas of the PRE and POST dairy cows, respectively (Table 3). *Fusobacterium* and *Sneathia*, as phylum *Fusobacteria*, and *Bacteroides* and *Prophyromonas*, as phylum *Bacteroidetes*, increased in the

vaginas of the POST dairy cows, whereas *Alistipes* and *Paludibacter*, as phylum *Bacteroidetes*, and *Intestinimonas*, *Papillibacter*, *Pseudoflavonifractor*, and *Flintibacter*, as phylum *Firmicutes*, decreased in the vaginas of the POST dairy cows. Taxonomic analysis of the reads revealed six main phyla from the feces of PRE and POST dairy cows except *Lentisphaerae* in POST dairy cows. Taxonomic analysis presented 16 main genera from feces of PRE dairy cows and 22 main genera from feces of POST dairy cows, but there was no significant difference (Table 4).

The most abundant bacterial phyla in the vaginas identified, regardless of sampling time, were *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Tenericutes*, and *Verrucomicrobia*, which concurs with these being the dominant phyla in both pre and postpartum vaginal communities, except for *Fusobacteria*. Clemmons et al. [9] also reported that the dominant bacteria in the vaginas were the same, except for *Fusobacteria*. These phyla in the vaginas are most commonly in host-microbiome relationships in many species, and the ratios and relative abundance are correlated with the changes in host physiology [5,10]. The mechanisms that describe these relationships are not well characterized, but there is a clear relationship between the host phenotype and the existence, abundance, and diversity of several bacterial communities [5]. Increases of the phyla *Fusobacteria* and *Bacteroidetes* are commonly associated with bovine necrotic vulvovaginitis [11]. Bicalho et al. [12] reported that a

Table 2. Impact of pre and postpartum on bacterial communities in vaginas and feces of dairy cows at the phylum level (relative abundance > 1%)

Items	PRE	POST	p-value
Vaginas			
<i>Fusobacteria</i>	0.00	$41.4 \pm 3.07^*$	< 0.01
<i>Bacteroidetes</i>	27.3 ± 3.95	$46.5 \pm 5.81^*$	0.02
<i>Firmicutes</i>	47.2 ± 3.46	$7.60 \pm 2.18^*$	< 0.01
<i>Tenericutes</i>	2.42 ± 1.69	1.98 ± 1.39	0.79
<i>Proteobacteria</i>	6.37 ± 3.88	1.69 ± 1.79	0.22
<i>Actinobacteria</i>	6.79 ± 7.89	0.84 ± 0.33	0.40
<i>Verrucomicrobia</i>	2.03 ± 1.04	0.00	0.11
Others	7.89 ± 2.21	$0.01 \pm 0.01^*$	0.04
Feces			
<i>Bacteroidetes</i>	44.8 ± 2.87	44.3 ± 2.56	0.87
<i>Firmicutes</i>	36.5 ± 2.48	39.8 ± 3.68	0.36
<i>Proteobacteria</i>	2.99 ± 0.50	3.02 ± 1.02	0.97
<i>Verrucomicrobia</i>	2.81 ± 0.36	2.13 ± 1.07	0.47
<i>Spirochaetes</i>	2.63 ± 0.79	2.62 ± 1.50	0.99
<i>Lentisphaerae</i>	1.22 ± 0.33	0.71 ± 0.13	0.14
Others	9.06 ± 0.99	7.41 ± 0.47	0.13

PRE, one week before calving; POST, one week after calving.

* $p < 0.05$ compared with control group.

Table 3. Impact of pre and postpartum on vaginal bacterial communities of dairy cows at the genus level (relative abundance > 1%)

Phylum	Genus	PRE	POST	p-value
<i>Fusobacteria</i>	<i>Fusobacterium</i>	0.00	39.5 ± 4.30*	0.01
	<i>Sneathia</i>	0.00	2.76 ± 0.16*	0.04
<i>Bacteroidetes</i>	<i>Bacteroides</i>	12.6 ± 2.03	26.6 ± 1.99*	0.02
	<i>Porphyromonas</i>	0.13 ± 0.10	28.4 ± 8.73*	0.04
	<i>Alistipes</i>	3.41 ± 0.37	0.00*	0.01
	<i>Paludibacter</i>	2.08 ± 0.48	0.00*	0.03
	<i>Parapedobacter</i>	1.4 ± 0.65	0.00	0.09
	<i>Paraprevotella</i>	1.14 ± 0.85	0.00*	0.20
	<i>Sphingomonas</i>	1.29 ± 1.73	0.01 ± 0.01	0.40
<i>Proteobacteria</i>	<i>Escherichia</i>	0.09 ± 0.08	1.39 ± 1.93	0.44
	<i>Luteimonas</i>	2.14 ± 1.92	0.16 ± 0.21	0.28
	<i>Micrococcus</i>	1.56 ± 1.58	0.05 ± 0.05	0.31
<i>Actinobacteria</i>	<i>Dietzia</i>	4.09 ± 5.45	0.02 ± 0.02	0.40
	<i>Intestinimonas</i>	7.01 ± 1.79	0.00*	0.03
<i>Firmicutes</i>	<i>Staphylococcus</i>	2.26 ± 2.57	0.01 ± 0.01	0.34
	<i>Papillibacter</i>	6.47 ± 0.93	0.00*	0.01
	<i>Oscillibacter</i>	1.54 ± 0.59	0.00	0.07
	<i>Streptococcus</i>	0.65 ± 0.35	0.01	0.12
	<i>Helcococcus</i>	0.01 ± 0.01	3.26 ± 2.20	0.17
	<i>Pseudoflavonifractor</i>	3.17 ± 0.78	0.00*	0.03
	<i>Flintibacter</i>	2.16 ± 0.64	0.00*	0.04
	<i>Akkermansia</i>	2.02 ± 1.05	0.00	0.11
<i>Tenericutes</i>	<i>Ureaplasma</i>	2.38 ± 1.69	1.99 ± 1.40	0.81
Others	Others	42.4 ± 4.59	5.58 ± 0.71*	0.01

PRE, one week before calving; POST, one week after calving.

* $p < 0.05$ compared with control group.

decrease in the immunity of cows after calving might result in dysbiosis, and opportunistic bacteria, such as *Fusobacteria* and *Bacteroidetes*, which can grow and contribute to infection. The *Fusobacteria* was an unintended result, but the presence of *Fusobacteria* infection, which was not detected in prepartum, was notable in this study. A previous study reported that *Fusobacteria* originate in the feces and may susceptiblely infect the postpartum vaginas [12]. *Fusobacteria* were detected in the feces regardless of pre and postpartum, suggesting that *Fusobacteria* may be an unknown external infection, not necessarily a fecal infection.

Accumulated data has suggested that the composition of fecal microbiota contributes to feed, environmental, and animal factors [13,14]. Changes in the bacterial community affect the production of metabolites, which in turn affects the host through complex interactions between the central nervous system [15]. In the current study, although the same diet was provided regardless of pre and postpartum to reduce the effect of diet, there were no differences in the microbial diversity, regardless of before and after calving.

Overall, these results suggest that an intravaginal infection can occur within one week after calving, but it does not affect the microbial composition of feces in pre and postpartum. In addition, the data support the hypothesis that the bacterial communities in vaginas can be altered through dietary control, which will be tested in a future study. Despite these results, research on the vaginal bacterial community is still lacking, and identifying the correlation between vaginal microbial changes and calf health will be important in future studies.

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Table 4. Impact of pre and postpartum on fecal bacterial communities of dairy cows at the genus level (relative abundance > 1%)

Phylum	Genus	PRE*	POST	p-value
Bacteroidetes	<i>Bacteroides</i>	17.5 ± 1.73	13.9 ± 2.94	0.22
	<i>Alistipes</i>	7.09 ± 1.21	5.22 ± 0.76	0.15
	<i>Paludibacter</i>	3.24 ± 1.05	5.03 ± 0.58	0.12
	<i>Parapedobacter</i>	3.06 ± 0.48	5.28 ± 1.92	0.24
	<i>Anaerophaga</i>	0.15 ± 0.21	1.27 ± 1.78	0.47
	<i>Paraprevotella</i>	2.27 ± 1.86	2.14 ± 0.64	0.93
	<i>Muribaculum</i>	2.49 ± 0.66	1.19 ± 0.45	0.09
	<i>Tannerella</i>	1.65 ± 0.50	1.45 ± 0.86	0.79
	<i>Alloprevotella</i>	0.43 ± 0.25	1.89 ± 1.77	0.36
	<i>Anaerocella</i>	0.22 ± 0.17	1.45 ± 1.74	0.42
	<i>Lutaonella</i>	0.8 ± 0.18	1.24 ± 0.85	0.54
Firmicutes	<i>Intestinimonas</i>	9.92 ± 0.17	8.85 ± 1.21	0.34
	<i>Papillibacter</i>	4.06 ± 0.30	5.45 ± 0.69	0.09
	<i>Pseudoflavonifractor</i>	4.11 ± 0.31	3.50 ± 1.12	0.52
	<i>Colidextribacter</i>	1.58 ± 0.16	1.92 ± 0.39	0.34
	<i>Oscillibacter</i>	1.47 ± 0.17	1.87 ± 0.38	0.28
	<i>Flintibacter</i>	2.54 ± 0.21	1.87 ± 0.47	0.17
	<i>Turicibacter</i>	0.62 ± 0.13	1.77 ± 1.09	0.27
	<i>Ethanoligenens</i>	0.83 ± 0.20	1.37 ± 0.84	0.46
Spirochaetes	<i>Treponema</i>	2.63 ± 0.79	2.60 ± 1.50	0.98
Proteobacteria	<i>Ruminobacter</i>	0.34 ± 0.45	0.92 ± 1.23	0.58
Verrucomicrobia	<i>Akkermansia</i>	2.79 ± 0.36	2.12 ± 1.07	0.48
Proteobacteria	<i>Mailhella</i>	1.82 ± 0.04	1.53 ± 0.62	0.58
Others	Others	28.4 ± 2.99	26.2 ± 0.69	0.41

PRE, one week before calving; POST, one week after calving.

ORCID

Jun-Kyu Son, <https://orcid.org/0000-0002-6266-3606>

Dong-Hyeon Kim, <https://orcid.org/0000-0003-0756-8419>

Jihwan Lee, <https://orcid.org/0000-0002-0040-3104>

Sang-Bum Kim, <https://orcid.org/0000-0002-8187-4134>

Beom-Young Park, <https://orcid.org/0000-0002-0604-7569>

Myunghoo Kim, <https://orcid.org/0000-0002-8444-6952>

Sungill Lee, <https://orcid.org/0000-0002-4621-4333>

Tai-Young Hur, <https://orcid.org/0000-0003-3129-2942>

Eun Tae Kim, <https://orcid.org/0000-0001-7486-5638>

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