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Changes in fecal microbiota of dairy cows with and without endometritis

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Abstract

Background Endometritis is a uterine infection caused by bacterial pathogens and has detrimental effects on productive and reproductive performance in dairy cows. A large number of studies have demonstrated the association of gut microbiota with infectious diseases. However, the role of gut microbiota in dairy cows with endometritis is still poorly understood.

Results In the present study, we characterized the fecal microbial populations in the dairy cows suffering from metritis ($n=10$) and healthy cows ($n=9$) using the 16 S rRNA gene sequencing. Results revealed an increased abundance of *Firmicutes* and *Bacteroidetes* in the affected cows indicating the potential role of these two bacterial taxa in the pathogenesis of endometritis. The *Ruminococcaceae_UCG-005* was the predominant genus while *Olsenella* and *Succinivibrio* were the most abundant genera in the cows affected with metritis. Further, the association of specific genera from *Firmicutes* and *Bacteroidetes* indicated three co-occurrence groups indicating the potential interaction of these genera in modulating the immune response, dysbiosis and inflammatory reaction. In addition, a significantly higher abundance of genes involved in the excretory system was observed in affected cows.

Conclusions Our findings provide evidence of changes in gut microbiota composition in cows suffering from metritis and advocate the need to explore the effect of commensal gut bacteria specifically co-occurring taxa in uterine inflammation and infection.

Keywords Endometritis, Cow, Gut microbiome, 16S rRNA sequencing

Background

Endometritis is a big concern for the dairy industry worldwide because it causes huge economic losses due to reduced milk production, lower conception rates, early culling, increased use of antibiotics, and treatment costs [1–3]. For example, the annual economic losses from cow uterine infections amounted to $1.4 billion in Europe and $650 million in the United States [4]. Bacterial contamination of the uterine lumen following parturition has long been documented as the main cause of endometritis. To date, various pathogens, including *Escherichia coli*, *Arcanobacterium pyogenes*, *Fusobacterium necrophorum*, *Prevotella melaninogenicus*, *Bacteroidetes*

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spp., *Pseudomonas spp.*, *Streptococcus spp.*, and *Staphylococcus spp.*, have been identified in a variety of combinations from cows diagnosed with postpartum metritis to support the bacterial hypothesis of the pathogenesis of these uterine infections [5–7].

A better understanding of the microbial communities in the gastrointestinal tract is critical for developing efficient therapeutic interventions for endometritis. It is well established that gut microbiota plays a key role in the maintenance of health and development of disease [8–10]. To date, a large number of studies have reported the composition and function of the uterine bacterial community in animals, such as cattle [11], sheep [12], and humans [13]. Even though the onset of many infectious diseases including endometritis is known to be associated with gut microbiota, very few studies are available on this aspect in dairy cows. Therefore, it is imperative to understand the association of the gut microbiome with endometritis in dairy heifers.

With the advent and widespread use of next-generation sequencing technologies, conducting deep sequencing on samples from specific environments became feasible and allows investigation of the relationship between microbial community and disease. This strategy has been widely used in many animals, such as cattle [14], sheep [15], goat [16], buffalo [17], and humans [18]. Moreover, it also allows the study of the association between gut bacterial community and endometritis by 16 S rDNA sequencing is feasible. The present study aimed to characterize the gut microbiota and its functional diversity in cows affected with metritis.

Table 1 Statistics information of 16 S rRNA sequencing for 19 samples

Group	Sample	Raw tags	Clean tags	Effective (%)	OTUs
M	M1	200,639	192,548	0.959673842	1012
	M2	285,080	269,894	0.946730742	1311
	M3	274,448	261,636	0.953317204	1450
	M4	225,617	214,148	0.949166065	1309
	M5	230,511	222,096	0.96349415	1218
	M6	128,758	119,347	0.926909396	1165
	M7	190,210	181,599	0.954728984	1214
	M8	128,366	123,438	0.961609772	1212
	M9	76,081	71,337	0.937645404	1215
	M10	113,544	107,690	0.948442894	1127
H	H1	93,164	88,406	0.948928771	1092
	H2	59,748	56,962	0.953370824	1350
	H3	133,493	128,431	0.962080409	1312
	H4	41,468	40,654	0.980370406	1188
	H5	133,893	128,170	0.957256914	1347
	H6	74,571	72,569	0.973153102	1306
	H7	85,792	81,305	0.947699086	1348
	H8	133,474	128,611	0.963565938	1382
	H9	76,703	73,982	0.964525507	1379

Results

Sequence information

The sequence statistics of the 16 S rRNA gene for 19 samples are listed in Table 1. In total, 2,685,560 raw sequences were generated from all the samples, with a mean number of 141,345.26 raw tags for each sample. After filtering, a total of 2,562,823 clean tags were obtained, indicating that a total of approximately 95.54% clean raw data was available. Most clean tags were mainly distributed in the range of 400–440 bp (Fig. 1A). Cluster analysis of clean reads yielded a total of 2,620 OTUs, of which 2,174 OTUs were common between the metritis and healthy groups (Fig. 1B). In addition, a total of 195 and 251 OTUs were specific to metritis and healthy groups, respectively. Moreover, we identified a total of 311 core OTUs shared among the samples (Fig. 1C).

Fecal bacterial community structure and composition

To compare the fecal bacterial composition of healthy and affected cows, we performed the 16 S rRNA gene amplicon sequencing. The alpha diversity analysis was used to compare the richness and diversity of bacterial communities between healthy and affected cows. The results revealed the lower values ($P < 0.05$) of observed species, Chao1, and Shannon indices in the affected cows (Fig. 2A), indicating a lower gut bacterial richness. However, no difference in the Simpson index was observed between the two groups revealing comparable levels of species diversity (Fig. 2A). The rarefaction curve indicated that the sequencing data were reliable and the abundance of different taxa varied depending on the sample (Figure S1). Further, we determined the beta diversity using the partial least squares discrimination analysis which indicated that gut bacteria in affected cows clustered separately from healthy cows (Fig. 2B).

The OTUs were assigned to 15 phyla, 26 classes, 42 orders, 74 families, and 210 genera. At the phylum level, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Tenericutes*, *Proteobacteria*, *Saccharibacteria*, and *Spirochaetae* were the major taxa with the relative abundance of more than 0.5% of all bacteria; *Firmicutes* was the predominant phylum, representing more than 68% and 71% of total bacteria present in affected and healthy cows, respectively (Fig. 2C). At the genus level, a total of 27 bacterial genera were present in affected cows, while the healthy cows had 32 genera with an abundance of more than 0.5% (Table S1). Notably, *Ruminococcaceae_UCG-005* was the predominant genus in the affected and healthy groups representing 18.51% and 15.83% of all bacteria, respectively (Fig. 2D).

To differentiate significant bacterial taxa between affected and healthy cows, Wilcoxon-test and LefSe analysis were used. The results revealed that the relative abundance of *Olsenella*, *Succinivibrio*, *Atopobium*,

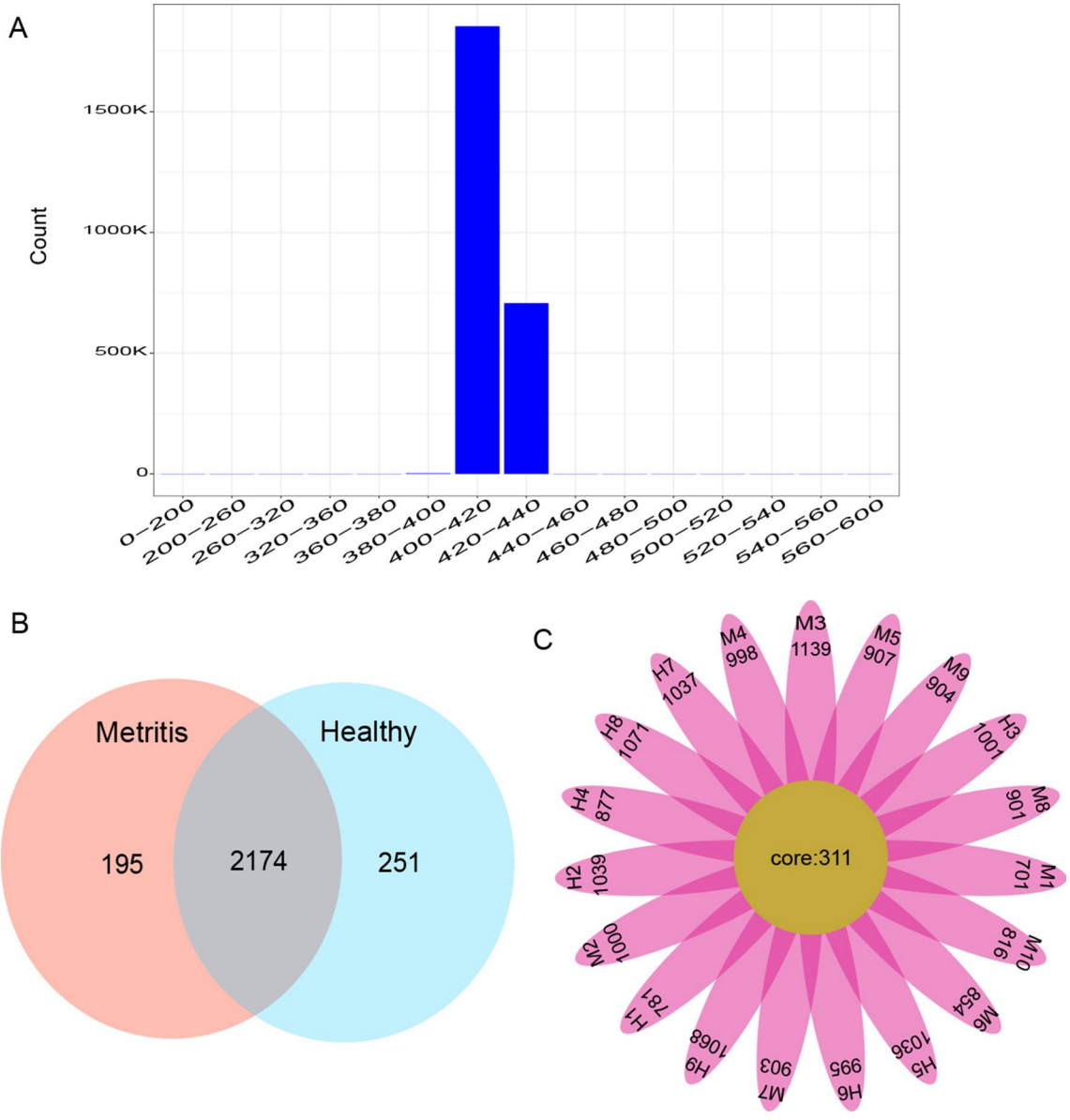

Fig. 1 Distribution of clean tags (**A**) and Venn analysis of OTUs between groups (**B**) or samples (**C**)

and *Eubacterium oxidoreducens* group was increased in affected cows, while the abundance of *Treponema2*, *Ruminiclostridium9*, *Lachnospiraceae UCG001*, *Caproiciproducens*, and *Papillibacter* was increased in the healthy group (Fig. 3A). The LefSe analysis indicated that the relative abundance of *Olsenella*, *Lachnospiraceae NC2004*, and *Succinivibrio* were increased in the affected cows, while the abundance of *Lachnospiraceae FCS020*, *Treponema2*, and *Pseudoramibacter* was higher in healthy cows (Fig. 3B). Interestingly, both methods confirmed that *Olsenella* and *Succinivibrio* had a higher relative abundance in the affected cows compared to healthy ones.

Co-occurrence of bacterial genera

As shown in Fig. 4, we identified three major co-occurrence groups (COGs) of bacterial communities that included genera belonging to *Firmicutes* and *Bacteriodetes*. One COG group is constituted of three genera including *Paeniclostridium*, *Clostridium_sensu_stricto_1*, and *Prevotellaceae_UCG_003*. While second COG group consisted of four genera including *Bacteroids*, *Alistipes*, *Rikenellaceae_RC9_gut_group*, and *Ruminococcaeae_UCG_010*. The third COG group had four genera including *Tyzzerella_4*, *Ruminococcaeae_UCG_005*, *Eubacterium coprostanoligenes_group* and *Christenallaceae_R7_group*.

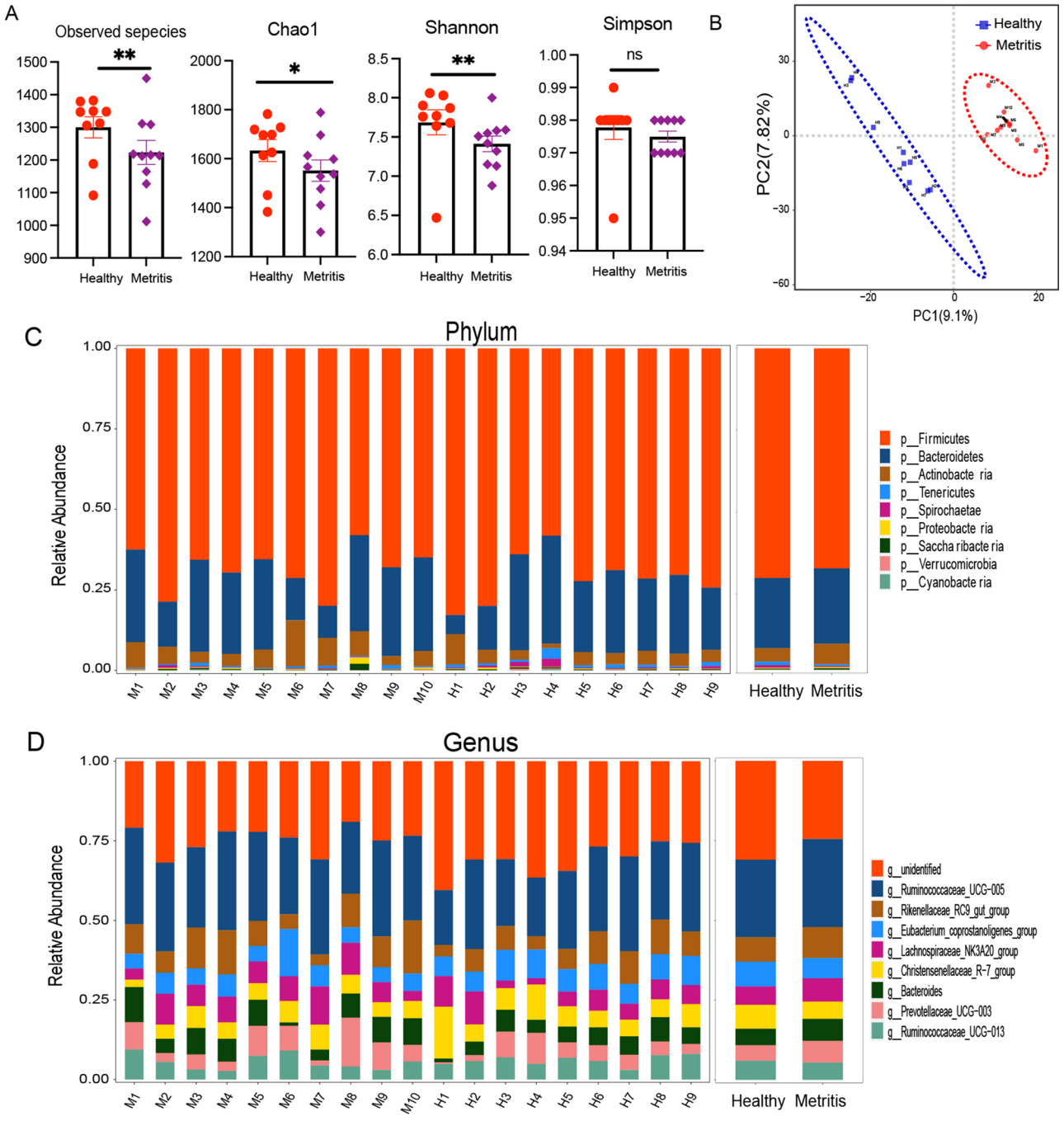

Fig. 2 Comparison of gut microbiota profiles between metritis and healthy cows. (**A**) Alpha diversity in each microbiota was compared between metritis and healthy cows. (**B**) Partial Least Squares Discrimination analysis of beta diversity between metritis and healthy cows. (**C**) Bar chart representing the microbiota compositions at the phylum level between metritis and healthy cows. (**D**) Bar chart representing the microbiota compositions at the genus level between metritis and healthy cows

Functional prediction of gut bacterial communities

To further predict the function of bacteria genes in affected and healthy cows, the PICRUSt2 software was used. In total, the genes were annotated to 6 level-1 and 44 level-2 KEGG pathways (Table 2). The most abundant first-level pathways were metabolism and human diseases. Among them, the genes for carbohydrate metabolism were abundant in the metabolism pathway, while drug resistance (antimicrobial) had a higher relative abundance in the human diseases pathway. The analysis exhibited significant differences ($P < 0.05$) in the relative abundance of genes coding for Signal transduction, Replication and repair, and Excretory system pathways between affected and healthy cows. Moreover,

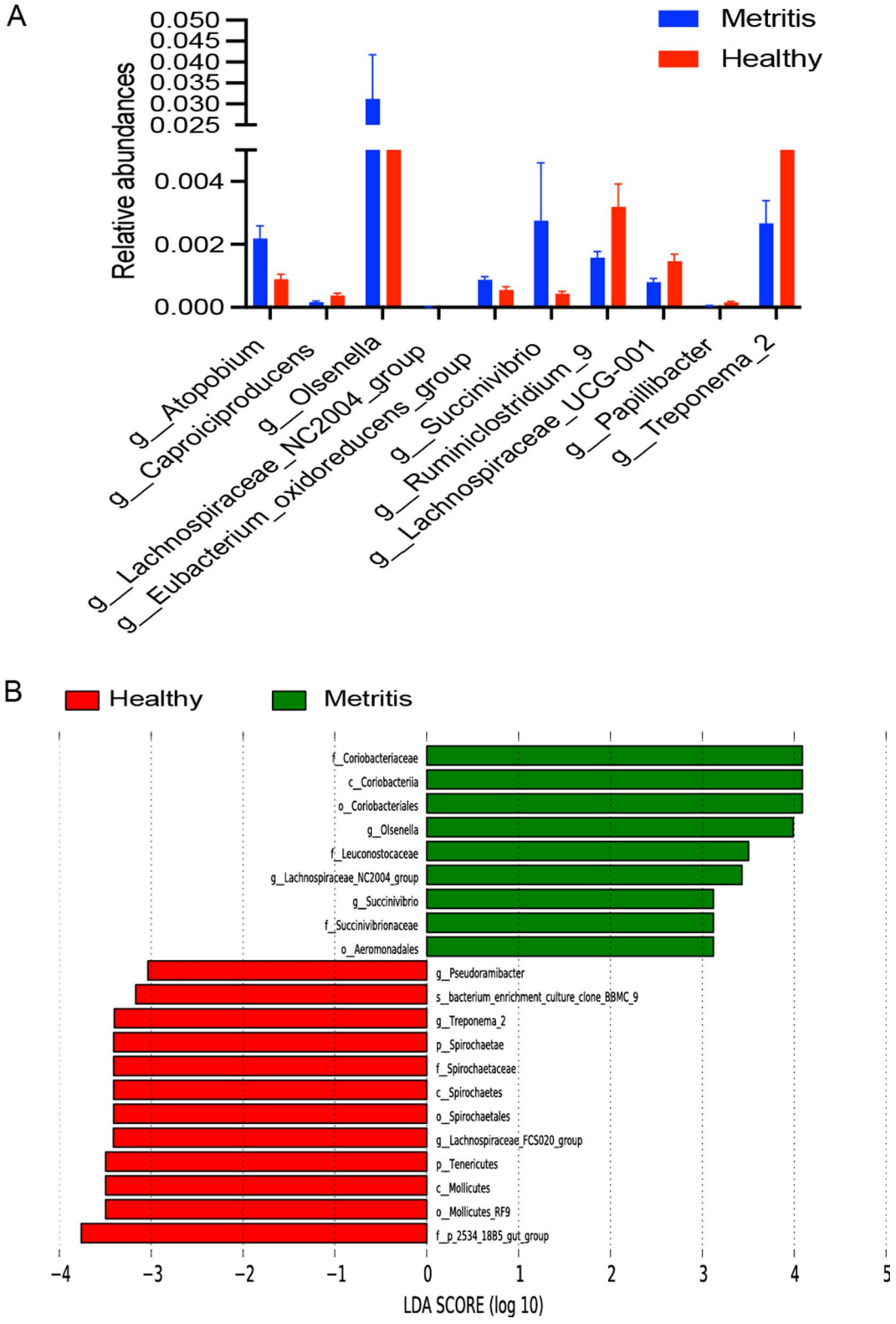

Fig. 3 Differences analysis in the gut microbiota between metritis and health cows using the Wilcoxon test (**A**) and Lefse analysis(**B**)

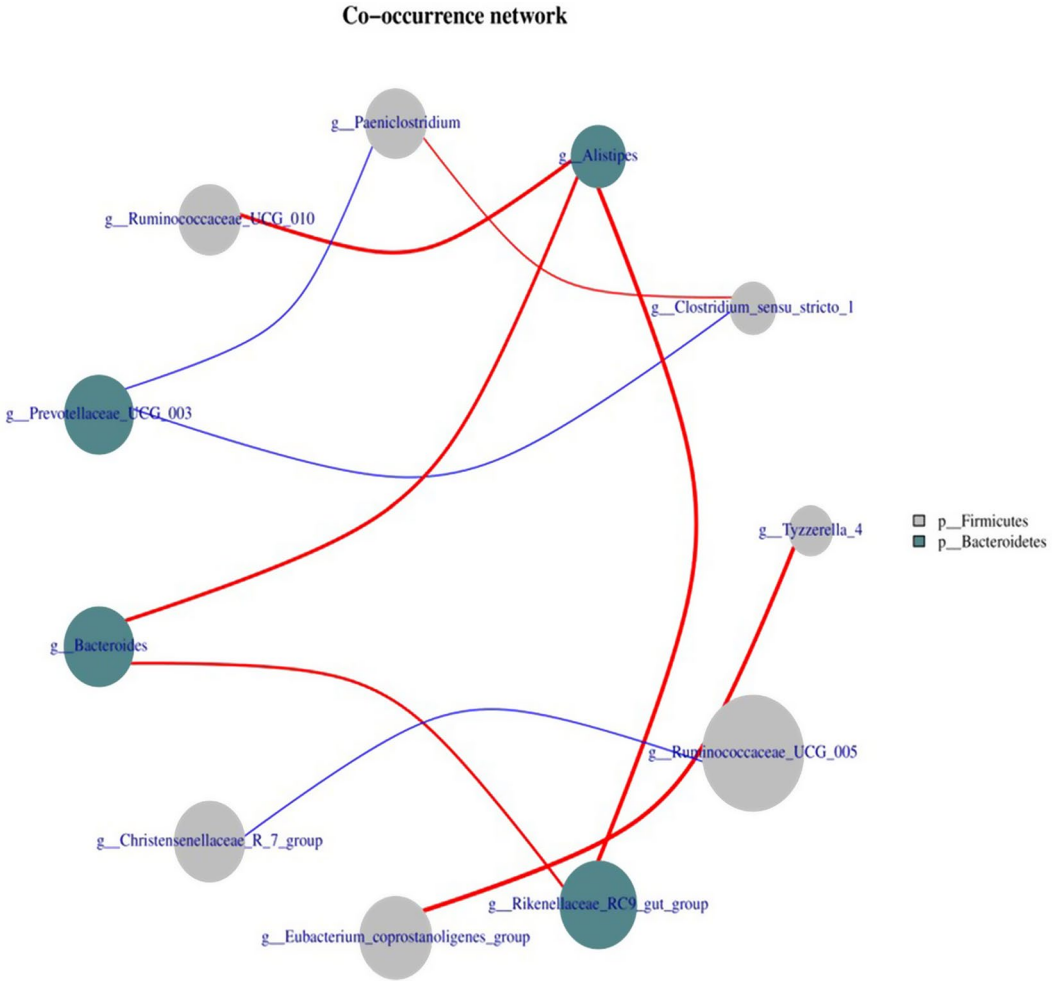

Fig. 4 Co-occurrence network of genera observed in fecal microbiota

using the STAMP analysis, carbohydrate metabolism and excretory system pathways were found to be significantly enriched ($P < 0.05$) between the affected and healthy groups (Fig. 5).

Discussion

Endometritis is highly prevalent in dairy cows and is characterized by the inflammation of endometrial glandular and stromal tissues. It has been reported that the pathogenesis of endometritis is complicated and many causative pathogens have been successively identified, such as the *Escherichia coli*, *Trueperella pyogenes*, *Fusobacterium necrophorum*, and *Staphylococcus aureus* [6]. To date, studies on endometritis pathogens have mainly concentrated on the composition and function of the uterine microbiota in cattle [1, 7, 11]. However, no studies have reported the potential role of changes in gut microbiota in cows suffering from metritis. To the best of our knowledge, this is the first report on the potential role of the gut bacteriome in the onset of metritis in cows compared with the healthy ones. In the present study, an increased abundance of *Firmicutes* and *Bacteroidetes* in cows affected with metritis. Similarly, both *Firmicutes* and *Bacteroidetes* were also found to be abundant in the uterine microbiota in cattle [1, 7, 11]. These results further suggested that gut bacteria might play vital roles in maintaining the homeostasis of the intrauterine environment. However, more work is needed to confirm the potential role of these microbes in the pathogenesis of metritis.

It is well established that changes in the gut microbiota are frequently associated with disease incidence [19–21]. Gut microbiota has an important impact on disease and health, and its mechanism mainly includes the following aspects: immune regulation, nutrient metabolism, inflammation regulation, toxin metabolism. Understanding the mechanisms of how gut microbiota affects disease and health could help us find more effective interventions to maintain the balance of gut microbiota and promote health. Unveiling the diversity of gut microbiota helps in mining the pathogens affecting the disease. In the present study, relatively higher abundance of *Olsenella* and

Table 2 Relative abundance analysis of the functional microbial genes in the metritis and health feces

First level Pathway	Second level Pathway	Metritis	Health	P value
		Relative abundance (%)	Relative abundance (%)	
Cellular Processes	Cell motility	2.028	2.124	0.125
	Cell growth and death	1.310	1.301	0.633
	Transport and catabolism	0.364	0.353	0.660
	Cellular community - prokaryotes	3.420	3.428	0.888
	Cellular community - eukaryotes	3.2E-06	3.3E-06	0.966
Environmental Information Processing	Signal transduction	3.5951	3.675	0.032
	Membrane transport	4.753	4.717	0.822
Genetic Information Processing	Replication and repair	5.355	5.377	0.025
	Folding, sorting and degradation	2.623	2.603	0.354
	Translation	6.2451	6.233	0.591
	Transcription	0.293	0.293	0.892
Human Diseases	Neurodegenerative disease	0.375	0.393	0.148
	Substance dependence	0.000	0.000	0.196
	Infectious disease: parasitic	0.053	0.058	0.251
	Drug resistance: antineoplastic	0.395	0.387	0.278
	Infectious disease: bacterial	1.179	1.194	0.287
	Cancer: overview	0.798	0.806	0.360
	Immune disease	0.060	0.0634	0.3878
	Infectious disease: viral	0.099	0.102	0.571
	Drug resistance: antimicrobial	1.581	1.588	0.649
	Cancer: specific types	0.090	0.089	0.813
	Cardiovascular disease	0.291	0.291	0.907
	Endocrine and metabolic disease	0.374	0.374	0.995
Metabolism	Carbohydrate metabolism	15.534	15.444	0.134
	Xenobiotics biodegradation and metabolism	1.114	1.139	0.155
	Not included in regular maps	0.063	0.065	0.247
	Amino acid metabolism	11.746	11.701	0.256
	Biosynthesis of other secondary metabolites	2.599	2.567	0.449
	Nucleotide metabolism	4.613	4.630	0.556
	Lipid metabolism	2.939	2.955	0.589
	Glycan biosynthesis and metabolism	3.237	3.191	0.675
	Metabolism of other amino acids	1.928	1.934	0.703
	Metabolism of terpenoids and polyketides	1.561	1.557	0.7206
	Energy metabolism	6.779	6.763	0.795
	Metabolism of cofactors and vitamins	6.824	6.816	0.848
Organismal Systems	Excretory system	0.031	0.035	0.029
	Circulatory system	0.000	0.000	0.993
	Aging	0.439	0.449	0.194
	Development and regeneration	0.028	0.0230	0.251
	Digestive system	0.193	0.190	0.613
	Environmental adaptation	0.385	0.388	0.642
	Nervous system	0.329	0.332	0.657
	Immune system	0.500	0.502	0.687
	Endocrine system	1.254	1.256	0.749

Succinivibrio was observed in the affected cows compared to healthy group. Association of *Olsenella* with reduction in gut inflammation has been reported earlier [22], suggesting its potential role in inflammatory conditions and host health. Evidence showed that *Succinivibrio* strains are associated with the production of acetic and succinic acids [23]. Recently, studies have demonstrated that succinic acid was upregulated in cows with endometritis using the metabolomic analysis [24], which indicates that the *Succinivibrio* might play a vital role in regulating the host health.

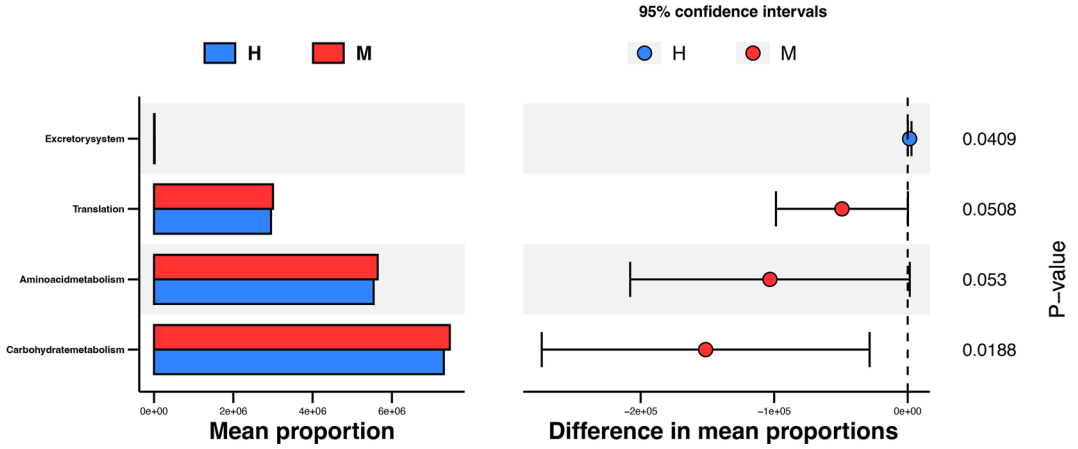

Fig. 5 Abundance profiles of predicted functional gene categories showed significant statistical differences between metritis and healthy cows. The results were filtered using a P-value of 0.05 and effective size of 0.05 threshold in STAMP

The diversity of the gut microbiota is strongly associated with changes in gut microbial functions [25]. In the present study, the most abundant first-level pathways were Metabolism and Human Diseases in the affected and healthy cows. Further, we observed the excretory system pathway was found to be significantly different between affected and healthy groups using STAMP analysis ($P < 0.05$). Previous studies have demonstrated that the excretory system is responsible for not only regulating the water balance in various body fluids but also maintains the chemical composition of body fluids by removing metabolic wastes [26–28]. It can be inferred that gut microbiota function in the intrauterine environment through the excretory system, thereby affecting the host's health.

The present study revealed four COGs of fecal bacteria. First COG exhibiting interaction of *Clostridium* genera with *Prevotellacaea* is in agreement with earlier reports indicating Clostridium is a major genus present in vaginal and fecal microbiota in cows [29, 30]. An increased abundance of *Clostridium* species in fecal samples at pre-breeding in animals that were unable to establish a pregnancy as this spp. can interact with other microbes to induce regulatory T cells or anti-inflammatory commensal bacteria leading to microbial dysbiosis in the vaginal ecosystem. Moreover, *Prevotella spp.* is reported as one of the most common microbes that cause uterine infections and also linked to bacterial vaginosis [31, 32]. So this correlation of Clostrium genera with *Prevotella spp.* seems meaningful regarding their potential involvement in uterine infections including endometritis [29, 33]. The second COG group observed in the present study consisted of four genera including *Bacteroids*, *Alistipes*, *Rikenellaceae_RC9_gut_group*, and *Ruminococcaeae_UCG_010*. These findings are in agreement with earlier studies reporting both *Bacteroids* and *Ruminococcaeae* positively correlated in a COG associated with endometritis in dairy cows [34, 35]. Moreover, the fecal abundance of *Bacteroides*, *Clostridium* and *Alistipes* species can be used as a marker for the prediction of chances to establish a pregnancy in a cow. Additionally, *Bacteroides* species are also associated with negative health status in cattle [36]. Likewise, increased abundance of *Alistipes*, *Ruminococcaceae UCG-005*, *Ruminococcaceae UCG-013*, and *Prevotella* in addition to other genera, was associated with CXCL13 which is a promising marker for chronic inflammation of the endometrium [37]. Moreover, the interaction of *Bacteroides* fragilis with *Burkholderiales* indicated potential immunomodulatory effects through pyrine-caspase1 inflammasome formation subsequently leading to activation of the TLR2/TLR4 signaling pathway [38].

The third COG was constituted of four bacterial genera including *Tyzzerella_4*, *Ruminococcaeae_UCG_005*, *Eubacterium coprostanoligenes_group* and *Christenallaceae_R7_group*. Negative association of *Tyzzerella_4* with serum levels of progesterone and testosterone has been observed in human subjects with postpartum depressive disorder. It should be noted that high levels of estrogen have shown association with pathogenesis of endometriosis which is an estrogen-dependent disease [39]. Due to negative effect of *Tyzzerella_4* with progesterone might lead to increased estrogen levels subsequently affecting onset of metritis.

Findings of the present study revealed that uterine pathogens might interact with each other in avoiding uterine defense and interact to facilitate colonization of the endometrium. Similar interactions of microbial pathogens were also observed in cows with metritis or purulent vaginal discharged [1, 40]. Pathogenic bacteria (such as *Trueperella spp.*, *Fusobacterium spp.*) were also present in the uterus of virgin heifers and of pregnant cows [41, 42]. Collectively, the co-occurrence of uterine pathogens could be considered of major importance in

the development of uterine infection. The cooperative interspecies signaling and mechanism behind synergisms need to be elucidated.

The favorable interactions among bacteria in COG including metabolite exchanges, could promote group survival under adverse conditions like nutrient deficiencies [43]. Variations in bacterial composition in the uterus of healthy and diseased cows indicates effect of nutritional and physiological changes. Identifying the co-occurring microbes and critical nutrients that favor growth of pathogenic bacteria is crucial for understanding the disease pathogenesis and developing novel treatment strategies [44]. Such competitive and cooperative interactions of gut microbes and mechanisms of establishing infection requires further elucidation through future studies involving metabolomic and transcriptomic data.

In this work, we characterized the changes in gut microbiota composition in cows suffering from endometritis in dairy cows. However, the study has limitations in terms of the causal relationship between the gut microbiome and endometritis. Further investigation is necessary to resolve the issue.

Conclusions

The findings of present study revealed the association between the gut bacteriome and endometritis in dairy cows. The *Ruminococcaceae_UCG-005* was the predominant genus while *Olsenella* and *Succinivibrio* were most abundant genera in the cows affected with metritis. Three co-occurrences bacteria group identified in the present study reveal the role of genera from *Firmicutes* and *Bacteroidetes* phyla in involvement in metritis. Our findings indicated the potential association of functional genes related to excretory system pathways with endometritis in dairy cows. This study is of great significance for improving the level of animal reproductive health, and is helpful to provide more effective strategies and measures for the prevention and treatment of obstetric diseases.

Methods

Sample collection

The fecal samples were collected in Jan 2020 from 15 days postpartum Holstein cows ($n = 19$) housed in the experimental cattle farm of Institute of Animal Husbandry and Veterinary Medicine, Henan Academy of Agricultural Sciences. Incidence of metritis was determined according to method as described previously [45]. Briefly, cows showing clinical signs including red-brown to purulent foul-smelling vaginal discharge; transrectal palpation of a thin-walled, large uterus containing large amounts of fluid or gas, and absence of systemic clinical signs like pyrexia, anorexia or reduced milk production. Cows with none of the above-mentioned clinical signs (uterine size and thickness expected for 5–10 days in milk, a watery red vaginal discharge without foul odor, and no systemic clinical signs) were classified as healthy. No medication was administered prior to sample collection. Put on shoulder-length gloves, lubricate the anus with paraffin oil, grasp the tail with the other hand, raise the head, pick up the fingers together, form a 45–60 degree angle, into the rectum. Discard the first 3 handfuls of manure and collect the fourth handfuls to the RNA-free 50mL centrifuge tube. The samples were flash-frozen on dry ice and stored at – 80 °C until DNA extraction.

DNA extraction

DNA was isolated from each fecal sample using the QIAamp Microbiome Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Concentration and purity of metagenomic DNA were measured by NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The quality of metagenomic DNA was confirmed by 1% agarose gel electrophoresis.

16 S rRNA gene sequencing

The V3 and V4 regions of 16 S rRNA gene were amplified using the primer pairs described by [46]. The polymerase chain reaction (PCR) conditions for amplification were: denaturation at 94 °C for 3 min followed by 30 cycles of 45 s at 94 °C, 60 s at 52 °C, and 60 s at 72 °C, with a final elongation at 72 °C for 10 min. Amplified PCR products were evaluated by 1% agarose gel electrophoresis. The PCR products were purified with the Agencourt AMPure XP (Beckman Coulter, USA) following the manufacturer's instructions. The Nextera XT Index kit (Illumina, San Diego, CA, USA) was used to construct the DNA library for each sample. Normalization was performed with a Nextera XT DNA library prep kit (Illumina, San Diego, CA, USA), and paired-end sequencing of each sample was performed through the Illumina MiSeq platform (Illumina, San Diego, CA, USA).

Bioinformatic Analysis

Raw sequence data for each sample was trimmed and filtered by using FLASH ver1.20 [47] and Trimmomatic ver0.36 [48] software. In addition, the sequences shorter than 120 base pairs were further discarded. Subsequently, the Quantitative Insights into Microbial Ecology (QIIME; version2) pipeline was used for filtering the low-quality tags while retaining effective tags [49]. The filtered reads were clustered as operational taxonomic unit (OTU) at 97% similarity using the Uparse ver. 7.0.1001 software [50]. OTUs were taxonomically assigned using the RDP Classifier algorithm against the Silva database (ver.132) with the confidence threshold of 0.7. A p-value < 0.05 was defined as the significant threshold level.

Statistical analyses

Microbial alpha diversity parameters including Chao1, Shannon, and Simpson were analyzed using the Mothur ver.1.48.0 software [51]. Partial Least Squares Discrimination analysis (PLS-DA) was used to assess microbial beta diversity at the OTU level. Three multivariate statistical tests (ANOSIM, MetaStats, and LefSe) were used to evaluate the differences in species composition and community structure between healthy and affected cows. The functional prediction of 16 S rRNA genes were performed by the PICRUSt2 software [52]. Amino acid sequences were aligned and translated from the gene catalog against the proteins in the KEGG database. The hierarchical Ward-linkage clustering was used to define genus COGs as described previously [53]. The R program and Tutools platform (https://www.cloudtutu.com/) were used for visualizing of the data and statistical analysis.

Abbreviations

COGs	co-occurrence groups
KEGG	Kyoto Encyclopedia of Genes and Genomes
LefSe	Linear discriminant analysis Effect Size
OTU	operational taxonomic unit
PCR	polymerase chain reaction
PLS-DA	Partial Least Squares Discrimination analysis
rRNA	ribosomal Ribonucleic Acid

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-025-04580-8.

Supplementary Material 1

Supplementary Material 2

Acknowledgements

Not applicable.

Author contributions

ZHS conceived of the study, performed the research, and drafted the manuscript. YLL, ZHQ, XZY, YZW and BZ checked the heifers and collected the samples. XYM drafted the manuscript. FH revised the paper. WJW participated in the study's design and assisted with the data analysis. TXD performed and analyzed the data, reviewed and edited the paper. All authors read and approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability

The sequence data have been deposited at NCBI Sequence Read Archive database with accession number PRJNA847011. All data generated or analyzed during this study are included in this published article and its additional files, and the dataset analyzed during the current study is available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Animal Ethics Committee of Institute of Animal Husbandry and Veterinary Medicine, Henan Academy of Agricultural Sciences (Approval number SYXK 2020-0003), China. Experimental protocols for obtaining bovine clinical samples used in this study were carried out in strict accordance with the Animal Ethics Procedures and Guidelines of China.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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