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Dysbiosis of the udder microbiota as a risk factor for mastitis in cows

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Introduction

Bovine mastitis remains one of the most prevalent and economically important diseases in dairy farming, leading to reduced milk yield, lower milk quality, and higher treatment costs. This problem is particularly pronounced in parts of Central Asia, including Kazakhstan, where both clinical and subclinical forms occur at high levels in commercial herds. On dairy farms in East Kazakhstan, clinical mastitis affected up to 35% of cows in some years, with subclinical mastitis prevalence similarly high, indicating that the disease remains widespread despite ongoing prevention efforts (Mukhamadieva et al., 2023). The high incidence of udder inflammation in this region reflects broader challenges in feeding, housing, and herd management, making mastitis a persistent health and economic concern for local dairy farmers.

Recent research has emphasized the important role of the mammary gland microbiota in maintaining udder health and in the development of inflammatory disorders. Advances in next-generation sequencing (NGS) techniques have enabled detailed profiling of microbial communities, including bacteria that cannot be cultured traditionally, providing new insights into the microbial dynamics underlying mastitis (Derakhshani et al., 2018). The present study aimed to investigate changes in the composition and structure of the mammary microbiota in dairy cows across healthy, subclinical, and clinical mastitis conditions.

Material & methods

The study was conducted on one commercial dairy farm located in the Akmola region (Central Kazakhstan). A total of 30 dairy cows were included and divided into three experimental groups: clinically healthy cows, cows with subclinical mastitis, and cows with clinical mastitis (10 cows per group). Milk sampling was performed at the cow level, with one individual milk sample collected from each cow, resulting in a total of 30 milk samples. Clinical mastitis was diagnosed based on veterinary clinical examination, the California Mastitis Test (CMT; DeLaval, Sweden), and direct determination of somatic cell count (SCC) using the fluoro-opto-electronic method in accordance with ISO 13366-2 | IDF 148-2:2006. Subclinical mastitis was defined solely based on SCC, with a threshold value exceeding 400,000 cells/mL. Before milk sampling, the udder was washed with water, treated with a pre-milking disinfectant (Kenopur), and dried using disposable towels. No additional antiseptic treatment of teat surfaces was applied prior to sampling. The first streams of milk were discarded, after which 100 mL of milk was aseptically collected into sterile disposable containers while wearing gloves. Milk samples were transported to the laboratory within 2 hours after collection and stored at 4-5 °C until analysis. Samples were not frozen.

Bacterial identification was performed by direct nucleotide sequencing of the 16S rRNA gene, followed by comparison of nucleotide identity with sequences deposited in the international GenBank database. Phylogenetic trees were constructed using nucleotide sequences of reference strains.

Ethical statement

All experimental procedures involving animals were conducted in accordance with the International Guiding Principles for Biomedical Research Involving Animals (2012) and complied with the ethical standards of Directive 2010/63/EU of the European Parliament and the Council on the protection of animals used for scientific purposes. The study protocol was approved by the local ethics committee (Protocol No. 2, dated 01 November 2023). Milk sampling was performed with the knowledge and consent of the farm management.

Results & Discussion

Analysis of the taxonomic structure at the phylum level revealed pronounced differences among clinically healthy cows, cows with subclinical mastitis, and cows with clinical mastitis (Table 1). In all groups, the dominant phylum was *Firmicutes*, consistent with previous reports (Derakhshani et al., 2018). The relative abundance of *Firmicutes* was highest in cows with subclinical mastitis (73.4%), whereas cows with clinical mastitis showed a lower relative abundance (57.4%). This lower representation of *Firmicutes* in clinical mastitis may reflect a disruption of microbial community stability and a shift toward other taxa, as reduced *Firmicutes* abundance has been associated with dysbiosis in mastitic milk (Falentin et al., 2016).

Table 1. Microbiome composition by mastitis status based on 16S rRNA analysis.

Taxon (Phylum)	Healthy	Subclinical mastitis	Clinical mastitis
Firmicutes, %	68.8	73.4	57.4
Bacteroidota, %	13.1	16.6	5.4
Proteobacteria, %	7.0	1.4	11.2
Actinobacteriota, %	4.0	2.4	11.4
Bdellovibrionota, %	1.7	0.3	13.4
Other phyla, %	5.3	6.0	1.2
Total diversity (number of phyla)	14	14	11

The phylum *Bacteroidota* ranked second in relative abundance in healthy cows (13.1%) and in cows with subclinical mastitis (16.6%), whereas cows with clinical mastitis exhibited a lower relative abundance (5.4%). Members of *Bacteroidota* are frequently associated with commensal microbial communities, and lower relative abundances of this phylum have been reported in association with dysbiosis and inflammatory conditions of the mammary gland (Zhu et al., 2024).

Considerable differences were also observed in the relative abundance of *Proteobacteria*. In healthy animals, *Proteobacteria* accounted for 7.0% of sequences, were less abundant in cows with subclinical mastitis (1.4%), and were most abundant in cows with clinical mastitis (11.2%). Many members of *Proteobacteria* are opportunistic or pathogenic microorganisms, and a higher relative abundance of this phylum has been associated with inflammation and microbial instability in disease.

A similar pattern was observed for *Actinobacteriota*. Higher relative abundances of *Actinobacteriota* have been reported in association with dysbiotic conditions and may reflect either the opportunistic proliferation of specific genera or changes in local microenvironmental conditions associated with inflammation (Derakhshani et al., 2018).

Of particular interest was the phylum *Bdellovibrionota*, which reached a relative abundance of 13.4% in clinical mastitis, compared with 1.7% in healthy cows and 0.3% in subclinical mastitis. Members of *Bdellovibrionota* are often described as bacterial predators in environmental ecosystems, and their presence at elevated relative abundance may reflect restructuring of the microbial community under conditions of dysbiosis. To the best of our knowledge, there are no reports documenting *Bdellovibrionota* in the bovine milk microbiome specifically. However, elevated levels of minor or predatory taxa have been noted in other dysbiotic microbiomes (Lam and Ye, 2022).

Overall taxonomic diversity also differed among health states: 14 bacterial phyla were identified in both healthy cows and cows with subclinical mastitis, whereas only 11 phyla were detected in cows with clinical mastitis. Reduced diversity is a characteristic feature of inflammatory diseases and reflects a loss of resilience and stability in the microbiota (Derakhshani et al., 2018).

Conclusion

The present study demonstrated that bovine mastitis was characterized by distinct differences in the composition and diversity of the mammary gland microbiota at the phylum level. *Firmicutes*, the dominant phylum in healthy and subclinical cows, showed a marked reduction in clinical mastitis. *Bacteroidota*, typically associated with commensal communities, was also reduced in clinical mastitis. In contrast, opportunistic or potentially pathogenic phyla, including *Proteobacteria* and *Actinobacteriota*, were more abundant in clinical mastitis. *Bdellovibrionota*, a phylum of predatory bacteria rarely reported in milk, reached elevated levels in clinical mastitis. Overall, taxonomic diversity declined in clinical mastitis compared with healthy and subclinical states.

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