



PEDRO FELIPE RODRIGUES E OLIVEIRA

**Infectious diseases of importance in dairy cattle farming:
approaches to metritis and mastitis**

LAVRAS-MG

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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciências Veterinárias, área de concentração em Sanidade Animal e Saúde Coletiva, para a obtenção do título de Mestre.

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PEDRO FELIPE RODRIGUES E OLIVEIRA

Infectious diseases of importance in dairy cattle farming: approaches to metritis and mastitis

Doenças infecciosas de importância na pecuária leiteira: abordagens para metrite e mastite

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciências Veterinárias, área de concentração em Sanidade Animal e Saúde Coletiva, para a obtenção do título de Mestre.

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Dedico este trabalho à minha família, gigantes em cujo os ombros me apoiei.

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RESUMO

Este trabalho integra estudos sobre duas doenças infecciosas de relevância para a produção de leite: a endometrite e a mastite, cujos efeitos combinados comprometem o desempenho dos rebanhos e ampliam a necessidade de uso de antimicrobianos, com implicações para a saúde pública e a sustentabilidade dos sistemas de produção. Neste contexto, o presente trabalho reúne dois estudos complementares, baseados em abordagens epidemiológicas e microbiológicas, com o objetivo de aprofundar o entendimento sobre a ocorrência, os fatores de risco, a dinâmica dos principais agentes envolvidos nessas doenças, combinando análises epidemiológicas, laboratoriais e experimentais para fornecer subsídios à vigilância, diagnóstico e manejo sanitário em sistemas de produção bovina. No estudo da endometrite, conduziu-se investigação microbiológica e citológica de endometrite em vacas alojadas em *compost barn* (312 animais, 20 fazendas), definindo um limiar ótimo de 8,3% de células polimorfo-nucleares (PMN) para diagnóstico de endometrite subclínica e identificando associações significativas entre presença de determinados gêneros bacterianos (*Prevotella* spp; *Enterococcus* spp e *Histophilus* spp) e resposta inflamatória; as características físico-químicas da cama não se correlacionaram com níveis de PMN. Em conjunto, os estudos enfatizam a natureza polimicrobiana dessas enfermidades, a necessidade de critérios diagnósticos contextualizados e a importância de estratégias integradas que conciliem saúde animal, uso racional de antimicrobianos e práticas de manejo. O estudo da mastite, foram analisadas 60.863 amostras de leite (2009–2021) provenientes de três laboratórios, o que permitiu caracterizar a prevalência e distribuição espaço-temporal dos agentes de mastite na região Sudeste do Brasil; observaram-se elevada participação de agentes contagiosos (notadamente *Staphylococcus aureus* e *Streptococcus agalactiae*), ocorrência de coinfeções e influência do regime pluviométrico sobre a ocorrência de patógenos ambientais (*Escherichia coli* e *Klebsiella* spp).

PALAVRAS-CHAVE: Mastite bovina; Epidemiologia; *Compost barn*; Microbiologia; Saúde da glândula mamária; Produção de leite.

ABSTRACT

This work integrates studies on two infectious diseases of major relevance to dairy production: endometritis and mastitis. Their combined effects impair herd performance and increase the need for antimicrobial use, with implications for public health and the sustainability of production systems. In this context, the present work brings together two complementary studies, based on epidemiological and microbiological approaches, with the aim of deepening the understanding of the occurrence, risk factors, and dynamics of the main agents involved in these diseases. By combining epidemiological, laboratory, and experimental analyses, the work provides support for surveillance, diagnosis, and sanitary management in bovine production systems. In the endometritis study, microbiological and cytological investigations were conducted in cows housed in compost-bedded pack systems (312 animals from 20 farms), defining an optimal threshold of 8.3% polymorphonuclear cells (PMN) for the diagnosis of subclinical endometritis and identifying significant associations between the presence of specific bacterial genera (*Prevotella* spp., *Enterococcus* spp., and *Histophilus* spp.) and the inflammatory response. The physicochemical characteristics of the bedding material were not correlated with PMN levels. Taken together, the studies emphasize the polymicrobial nature of these diseases, the need for context-specific diagnostic criteria, and the importance of integrated strategies that reconcile animal health, rational antimicrobial use, and management practices. In the mastitis study, 60,863 milk samples (2009–2021) from three diagnostic laboratories were analyzed, allowing the characterization of the prevalence and spatio-temporal distribution of mastitis pathogens in southeastern Brazil. A high participation of contagious agents (notably *Staphylococcus aureus* and *Streptococcus agalactiae*), Occurrence of coinfections, and the influence of rainfall patterns on the occurrence of environmental pathogens (*Escherichia coli* and *Klebsiella* spp.) were observed.

KEYWORDS: Bovine mastitis; Epidemiology; *Compost barn*; Microbiology; Udder health; Milk production.

INDICADORES DE IMPACTO

As doenças infecciosas que acometem vacas leiteiras, especialmente a mastite e a endometrite, representam desafios sanitários e econômicos significativos, com impactos diretos sobre a produtividade, a eficiência reprodutiva e a sustentabilidade dos sistemas de produção. A mastite bovina destaca-se como a enfermidade de maior impacto econômico na pecuária leiteira, sendo responsável por redução na produção, prejuízos na qualidade do leite e aumento dos custos com tratamento, manejo e descarte de animais. Por sua vez, a endometrite, comum no período pós-parto, compromete o desempenho reprodutivo, elevando o intervalo entre partos e os custos operacionais das propriedades. Sob a perspectiva epidemiológica, os estudos evidenciam o caráter multifatorial e polimicrobiano dessas doenças, envolvendo a interação entre agente, hospedeiro, ambiente e práticas de manejo. A análise de mais de 60 mil amostras de leite ao longo de mais de uma década demonstrou predominância de patógenos contagiosos, como *Staphylococcus aureus* e *Streptococcus agalactiae*, além de elevada frequência de coinfeções e influência de fatores ambientais, como o regime de chuvas, na ocorrência de patógenos ambientais. Esses resultados indicam a persistência de desafios estruturais no controle da mastite e reforçam a necessidade de estratégias diferenciadas conforme o perfil epidemiológico. No caso da endometrite, a definição de parâmetros diagnósticos mais precisos, baseada em critérios citológicos, aliada à identificação de associações entre microrganismos e resposta inflamatória uterina, contribui para maior acurácia diagnóstica e intervenções mais eficazes. Nesse contexto, o uso de dados microbiológicos e laboratoriais consolida-se como ferramenta estratégica para a vigilância sanitária, permitindo identificar padrões espaço-temporais, monitorar tendências e avaliar a efetividade das medidas de controle, fortalecendo a tomada de decisão baseada em evidências. Os impactos do estudo enquadram-se principalmente nas áreas temáticas de Saúde e Tecnologia e Produção, com interfaces em Trabalho e Meio Ambiente, conforme a Política Nacional de Extensão. Em consonância com os Objetivos de Desenvolvimento Sustentável da ONU, a pesquisa contribui diretamente para o ODS 2 (Fome Zero e Agricultura Sustentável), ODS 3 (Saúde e Bem-Estar), ODS 8 (Trabalho Decente e Crescimento Econômico), ODS 12 (Consumo e Produção Responsáveis) e ODS 15 (Vida Terrestre). Dessa forma, os resultados desta dissertação transcendem o campo acadêmico, oferecendo suporte concreto para o aprimoramento de políticas públicas, inovação em diagnóstico e manejo sanitário, e promoção de sistemas de produção mais sustentáveis, alinhados aos princípios da Saúde Única.

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INTRODUÇÃO

As doenças infecciosas que acometem fêmeas leiteiras figuram entre os principais desafios sanitários dos sistemas de produção, tanto pela alta prevalência quanto pelos impactos diretos sobre o desempenho reprodutivo, produtivo e econômico. Entre essas enfermidades, endometrite e mastite se destacam não apenas pela sua frequência, mas também pela complexidade de sua etiologia, pelas interações entre microrganismos e hospedeiro e pela forte influência das condições de manejo e ambiente sobre sua ocorrência.

A endometrite, caracterizada pela inflamação persistente do endométrio após o parto, está associada à redução da fertilidade, aumento do intervalo entre partos, maior número de inseminações e custos elevados com terapias. Sua etiologia é multifatorial, envolvendo a dinâmica da microbiota uterina, a resposta imune local e fatores de manejo. Em sistemas *compost barn*, amplamente adotados no Brasil pela melhoria no conforto e bem-estar animal, permanece a necessidade de compreender como características físico-químicas da cama, higiene e densidade animal influenciam a colonização uterina e a inflamação pós-parto.

A mastite, por sua vez, é reconhecida mundialmente como a enfermidade de maior impacto econômico na bovinocultura leiteira, sendo responsável por perdas na produção, piora na qualidade do leite e aumento nos custos com medicamentos e mão de obra. Sua epidemiologia varia de acordo com a região, condições ambientais e práticas de manejo, com a participação de patógenos contagiosos e ambientais que podem atuar isoladamente ou em coinfeções. A carência de dados sistematizados sobre a distribuição dos agentes etiológicos no Brasil limita a implementação de programas de controle mais assertivos.

Diante desse cenário, este trabalho reúne dois estudos complementares, ambos voltados à compreensão aprofundada das principais enfermidades infecciosas de importância para a pecuária leiteira. O primeiro aborda a saúde uterina de vacas alojadas em sistemas *compost barn*, investigando a composição microbiana uterina, fatores determinantes da inflamação e o estabelecimento de um ponto de corte diagnóstico para endometrite subclínica. O segundo realiza uma ampla análise epidemiológica dos principais patógenos causadores de mastite na região Sudeste do Brasil, avaliando prevalência, coinfeções e tendências temporais. Em conjunto, os estudos contribuem para o avanço do conhecimento aplicado ao diagnóstico, prevenção e manejo sanitário em rebanhos leiteiros.

Os estudos foram redigidos em formato de artigo, com intuito de serem submetidos às revistas indicadas após correções sugeridas pela banca.

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REPRODUCTIVE HEALTH IN DAIRY COWS: MICROBIOLOGICAL INVESTIGATION OF ENDOMETRITIS IN COMPOST BARN SYSTEMS

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ABSTRACT

This study investigated the relationship between uterine inflammation, microbiological composition, and housing conditions in dairy cows managed in compost-bedded pack barn (CBP) systems. A total of 312 cows from 20 herds were clinically evaluated 3–45 days postpartum, and two endometrial samples were collected, one for cytology and other for bacterial isolation using microbiological culture followed by MALDI-TOF MS. Bedding material was analyzed for physicochemical properties, including moisture, pH, temperature, and organic matter. The optimal threshold for diagnosing subclinical endometritis was determined at the point where the decrease in Akaike Information Criteria (AIC) began to plateau, as identified by linear regression analysis. Subclinical endometritis was diagnosed at a Polymorphonuclear cells (PMN) cutoff of 8.3 %. No bedding physicochemical characteristic were significantly associated with PMN levels, indicating that CBP systems — under

appropriate management conditions — do not inherently increase uterine inflammatory risk. Microbiological analysis revealed a diverse uterine microbiota typical of the postpartum period. Four bacterial genera showed significant associations with inflammation in the mixed-effects model: *Prevotella* and *Arthrobacter* were positively correlated with PMN counts, whereas *Enterococcus* and *Histophilus* showed negative associations. These findings highlight the complexity of the postpartum uterine ecosystem and emphasize the value of integrating microbiological profiling with cytological evaluation. Establishing a PMN threshold of 8.3 % provides a practical diagnostic reference for herds housed in CB systems. Further studies are needed to clarify the functional roles of non-classical taxa in uterine health.

1. INTRODUCTION

Livestock production plays a key role in the global economy, accounting for approximately 40 % of total agricultural output, with both production and consumption increasing steadily in developing countries (FAO, 2025). In this context, dairy farming stands out not only as a vital source of high-quality protein, calcium, and essential micronutrients for human nutrition, but also as a central driver of rural development and economic stability in many regions (FAO, 2010). Milk production supports millions of smallholders and commercial farmers worldwide, generates employment across the supply chain, and contributes significantly to food security (Garcia; Osburn; Cullor, 2019).

Among the challenges faced by the dairy industry, infectious diseases such as metritis and endometritis are among the most important ones (Paiano et al., 2022). Endometritis is a condition that affects dairy cows during the postpartum period, leading to several negative consequences for animal health and productivity (Sheldon et al., 2006). Indeed, metritis/endometritis contributes to reduced milk yield, premature culling, decreased fertility, repeated estrus, increased veterinary and treatment costs, and the milk waste due to antimicrobial therapy (Wang et al., 2018). Estimates suggest that each case can result in total losses ranging from USD 267 to USD 511, considering both direct costs and associated productivity losses (S. L.; Bhakat; Mohanty, 2025). Although scientific studies on the topic are still limited, some authors have reported a disease prevalence around 53 % in individual animals and between 37 % and 74 % at the herd level (Gilbert et al., 2005).

In addition to compromising animal health and causing economic losses, the use of antimicrobials to treat these conditions can have unintended environmental consequences (Džermeikaitė; Krištolaitytė; Antanaitis, 2024). Endometritis is a multifactorial condition that lacks a standardized diagnostic protocol, which difficult early detection and effective management of this illness in dairy herds (Espadamala et al., 2018; Gilbert, 1992). In this context, a deeper understanding of the pathogens involved, their epidemiology, and the mechanisms by which they impact uterine health is essential. The identification of the microorganisms most associated with the disease, and how these agents influence the inflammatory response and reproductive performance of the animal, can significantly improve diagnostic accuracy and prognostic evaluation (Paiano et al., 2022). This knowledge supports more rational therapeutic decisions, avoiding unnecessary treatments, minimizing economic losses, and reducing the indiscriminate use of antimicrobials, thereby helping to restrain the development of antimicrobial resistance.

Considering the economic burden of endometritis in dairy production systems and the growing need to reduce antimicrobial usage, especially in the context of more sustainable and welfare-oriented housing systems, attention has increasingly turned to alternative management strategies. The compost bedded barn (CBP) has emerged as an alternative housing system designed to improve cow comfort, health, and welfare while maintaining productive performance (Black et al., 2013). However, the organic bedding material and increased stocking density can pose microbial and sanitary challenges, requiring careful management of compost moisture, aeration, and microbial load (Albino et al., 2018). Given its growing adoption, a better understanding of how CBP environments interact with cow health, particularly uterine health, remains essential for optimizing both welfare and productivity outcomes.

Therefore, the aim of this study was to investigate the main uterine pathogens causing endometritis in dairy cows allocated in CBP systems, as well as animal and farm level epidemiological factors potentially associated with uterine inflammation.

2. MATERIAL AND METHODS

2.1. Ethics statement

The experimental activities were performed in strict accordance with Brazilian law on use of animal in research and teaching (Lei n° 11.794/2008) and approved by the Ethical Committee for Animal Use (Comitê de Ética para Uso de Animais) of Brazilian Agricultural Research Corporation Dairy Cattle (CEUA/EGL) under protocol 8308220322 approved in December 2022.

2.2. Dairy farms

The study involved the voluntary participation of 20 dairy farms that use the CBP system, 19 of which [19/20 (95 %)] are located in the Brazilian state of Minas Gerais and 1 [1/20 (5 %)] in the state of Goiás. Sampling was carried out using the convenience sampling method, since, in addition to the participation agreement, the selection criteria included housing all animals in confinement systems, including cows in the pre- and post-partum periods, a practice rarely adopted on Brazilian dairy farms and which limited the number of eligible properties.

2.3 Animal samples

Endometrial samples from 312 dairy cows between the first and sixth week post-partum were collected between 2023 to 2024. The sampling limit for each farm was (20) animals, calculated using Epitools HerdPlus application to reach at least 70 % of farm sensitivity for endometritis diagnosis, considering a maximum proper size of 350 lactating cows (Damasceno, 2025). Herds that exceeded the maximum number of animals for sampling were selected based on the following criteria: continuous housing in the CBP system during pre- and post-partum periods and not being under currently antimicrobial treatment or being submitted to it in the previous 10 days.

Endometrial samples were collected after the cows had been restrained in a chute or handling corridor, perineal hygiene was performed with 70 % ethylic alcohol and drying with paper towels (Paiano et al 2022). Samples were collected using a stainless-steel semen applicator adapted to a cytological brush (Kolplast, Brazil) wrapped in an insemination sheath (WTA, Brazil), introduced into the vagina and guided through the cervix per rectum, as described by (Pascottini et al., 2020). Two endometrial samples were collected from each animal, one for evaluation of endometrial inflammation and molecular parameters and the other for microbiological analysis.

2.4 Bed samples

Bedding samples were collected from pre- and post-partum pen lots immediately after turning the bedding, in the morning or afternoon, using a soil digger, as described by (Oliveira et al., 2023), at a depth of 20 cm and stored in 50 mL conical polypropylene tubes to biochemical analysis. All the farms sampled used wood-derived material in the beds and performed two daily turnings to promote composting of the bedding.

2.5 Epidemiological information

Individual information on the animals and sampled properties was also collected. This included animal history containing breed, parity, history of retained placenta, abortion, stillbirths, metritis, days in lactation, and average milk production. Herd-level information was also collected encompassing number of lactating cows, average production per cow, presence of dry cows in the barn, total daily milk production, barn area, and type of bedding material used in the bedding system.

2.6. Microscopic identification

Endometrial samples of each animal was rolled on a glass slide, air-dried and stained using the rapid panoptic method. Reading was performed using a microscope (Leica, Germany) under oil immersion (1000x). At least 300 cells were counted, with identification of epithelial cells, neutrophils (polymorphonuclear) and other cell types (lymphocytes and macrophages), from this was established the proportion of polymorphonuclear cells (PMNs) (Kasimanickam et al., 2004; Sheldon et al., 2006). The animals were considered sick based on the PMN.

2.7. Microbial Identification

Another endometrial sample of each animal was placed in tubes containing 6 mL of anaerobic transport medium and kept refrigerated until laboratory processing. The microbial identification from endometrial samples was performed as previously described (Paiano et al., 2022). Briefly, samples were agitated in 2 mL of *Brucella* broth to release adherent microorganisms. From this broth, 10 μ L aliquots were plated on culture media containing *Brucella* agar supplemented with 7 % defibrinated horse blood, hemin (5 mg/L), and menadione (1 mg/L); and plated on MacConkey agar, CHROMagar Orientation, and Columbia blood agar with 5 % defibrinated sheep blood, both incubated aerobically at 37 °C for up to 48 hours.

Colonies with distinct morphology were selected and subjected to bacterial identification via MALDI-TOF mass spectrometry using the Microflex system (Bruker Daltonik). Mass spectra were analyzed using MALDI BioTyper 3.0 software, following the manufacturer's guidelines: scores ≥ 2.0 were considered reliable for species-level identification, while scores between 1.7 and 2.0 were accepted for genus-level identification.

2.6. Biochemical analysis

Biochemical analyses of the bedding samples were performed at Engequisa – Environmental Analysis Laboratory (Betim, MG, Brazil). In the laboratory, samples were homogenized and fractionated according to standard operating procedures. Moisture content was determined gravimetrically by drying approximately 10 g of fresh material in an oven at 105 °C until constant weight. pH was measured in a 1:5 suspension in deionized water after shaking for 30 min, using a previously calibrated pH meter. Ammonium was extracted with 2 M KCl and quantified by the phenate (indophenol) colorimetric method. Total organic carbon (TOC) and total nitrogen (TN) were determined following standardized methods for organic matrices, and the C:N ratio was calculated from these values. Organic matter was estimated by loss on ignition at 550 °C. These physicochemical parameters – moisture, pH, nitrogen, carbon– are widely used for characterizing bedding quality in compost bedded pack barn systems, as

they are known to directly influence microbiological activity and the composting process (Oliveira et al., 2023).

2.7. Statistical Analysis

To evaluate the association between animal-level and farm-level variables and the proportion PMNs in uterine cytology smears, a linear mixed model (LMM) was fitted. The response variable was transformed using the parametric power transformation of Box and Cox (1964). This transformation allows the assumptions of linear modeling to be better satisfied (Sakia, 1992). After transformation, the response variable was defined as the logarithm of the PMN proportion added to a constant [$\log(\text{PMN} + 0.5)$].

To determine the most appropriate polymorphonuclear neutrophil (PMN) threshold for characterizing uterine inflammation, mixed models were fitted using a series of cutoff values, and their respective Akaike Information Criteria (AIC) were compared. This approach allowed the identification of the point at which increasing the PMN threshold no longer resulted in a meaningful improvement in model fit.

The model included fixed effects for animal breed (with three categories: Girolando, Holstein, and “other breeds”) and for the presence or absence of bacterial genera isolated from endometrial sample. A random intercept for “farm” was included to account for clustering of animals within farms and potential variability in management and environmental conditions.

Variable selection was performed using stepwise backward elimination based on the Akaike Information Criterion (AIC). All analyses were conducted in R software (R Core Team, 2024), using the “tidyverse” package for data manipulation, “MASS” package for transformation (Venables and Ripley 2002) and the “lme4” (Bates, Bolker, and Walker 2015) and “lmerTest” packages for model fitting and inference (using Satterthwaite’s method for calculating degrees of freedom and p-values).

The linear model was constructed to evaluate the effect of farm-level characteristics on PMN values. For this analysis, the predicted PMN means for each farm — derived from the previously fitted mixed-effects model — were used as the response variable. A linear model was then fitted in which the farm-level predicted PMN value served as the dependent variable, and the chemical composition variables of the bedding material (pH, moisture, organic carbon, total nitrogen, and C:N ratio) were included as explanatory predictors. Statistical significance was assessed using a threshold of $\alpha = 0.05$.

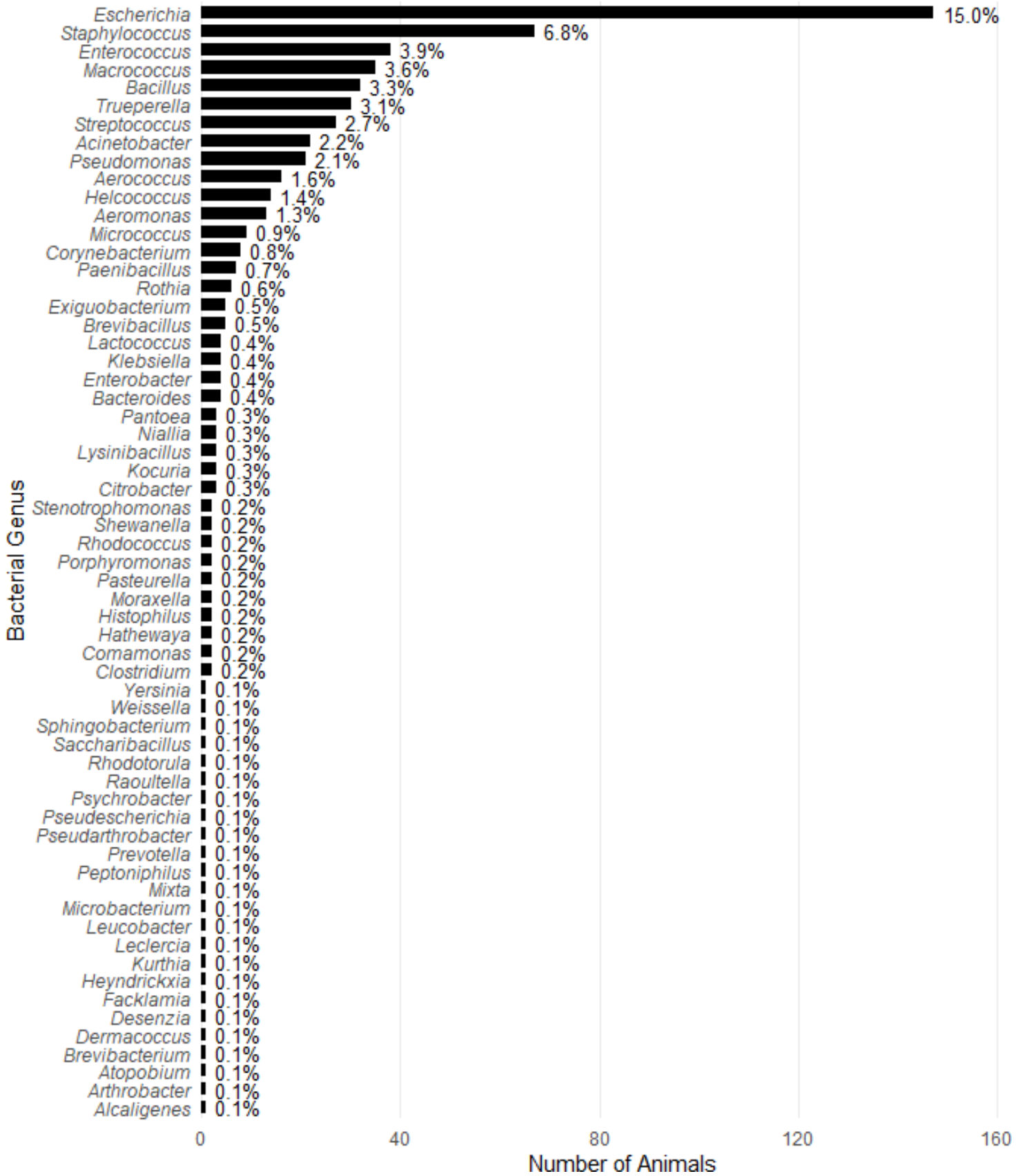
3. RESULTS

The epidemiological results showed that out of 312 animals collected of the 20 farms, the predominantly sampled breed were Holstein with 166 animals [166/312; 53.20 %], followed by the Girolando with 138 animals [138/312; 44.23 %], and eight 8 [8/312; 2.56 %] samples from other breeds. Regarding the number of animals collected per farm, an average of 15.60 ± 4.77 (median 17.00 [IQR 14.25–19.25]) animals was collected per farm; the average vulvar score was 1.84 ± 0.76 (median 2.00 [IQR 1.00–2.00]); the average milk production was 31.12 ± 11.64 (median 30.05 [IQR 23.32–38.53]) L/day among individuals and 5803.10 ± 2915.65 (median 4430.00 [IQR 3780.00–8337.00]) L/day across the farms. 13 [13/20; 65 %] farms used wood shavings as bedding material, four [4/20; 20 %] used a combination of wood shavings and sawdust, and three [3/20; 15 %] used sawdust alone.

A total of 312 endometrial samples were processed for bacteriological culture and identification. The results demonstrated microbiological growth in 92.3 % of the endometrial samples (288/312), of which 98 samples [98/312 (31.4 %)] showed growth of a single bacterial genus and 190 samples [190/312 (60.9 %)] showed growth of two or more bacterial genera. And also, 24 samples [24/312 (7.70 %)] showed no bacterial growth in the tests performed.

Regarding bacterial growth, *Escherichia* spp. was the most frequently detected genus, being isolated from 147 animals [147/312; 47.1 %]; other commonly recovered genera included *Staphylococcus* [67/312; 21.5 %], *Enterococcus* [38/312; 12.2 %], *Macroccoccus* [35/312; 11.2 %], *Bacillus* [32/312; 10.3 %] and *Trueperella* [30/312; 9.6 %] (Figure 1a). The distribution of the genera among the different farms is presented in the figure 2. At the species level, *Escherichia coli* was the predominant taxon [147/312; 47.1 %], followed by *Trueperella pyogenes* [30/312; 9.6 %], *Enterococcus hirae* [26/312; 8.3 %], *Macroccoccus caseolyticus* [24/312; 7.7 %] and *Staphylococcus equorum* [23/312; 7.4 %] (Figure 1b). A wider variety of environmental and opportunistic taxa, e.g., *Pseudomonas*, *Acinetobacter*, *Aerococcus* and *Helcococcus*, were also recovered at lower frequencies.

a



b

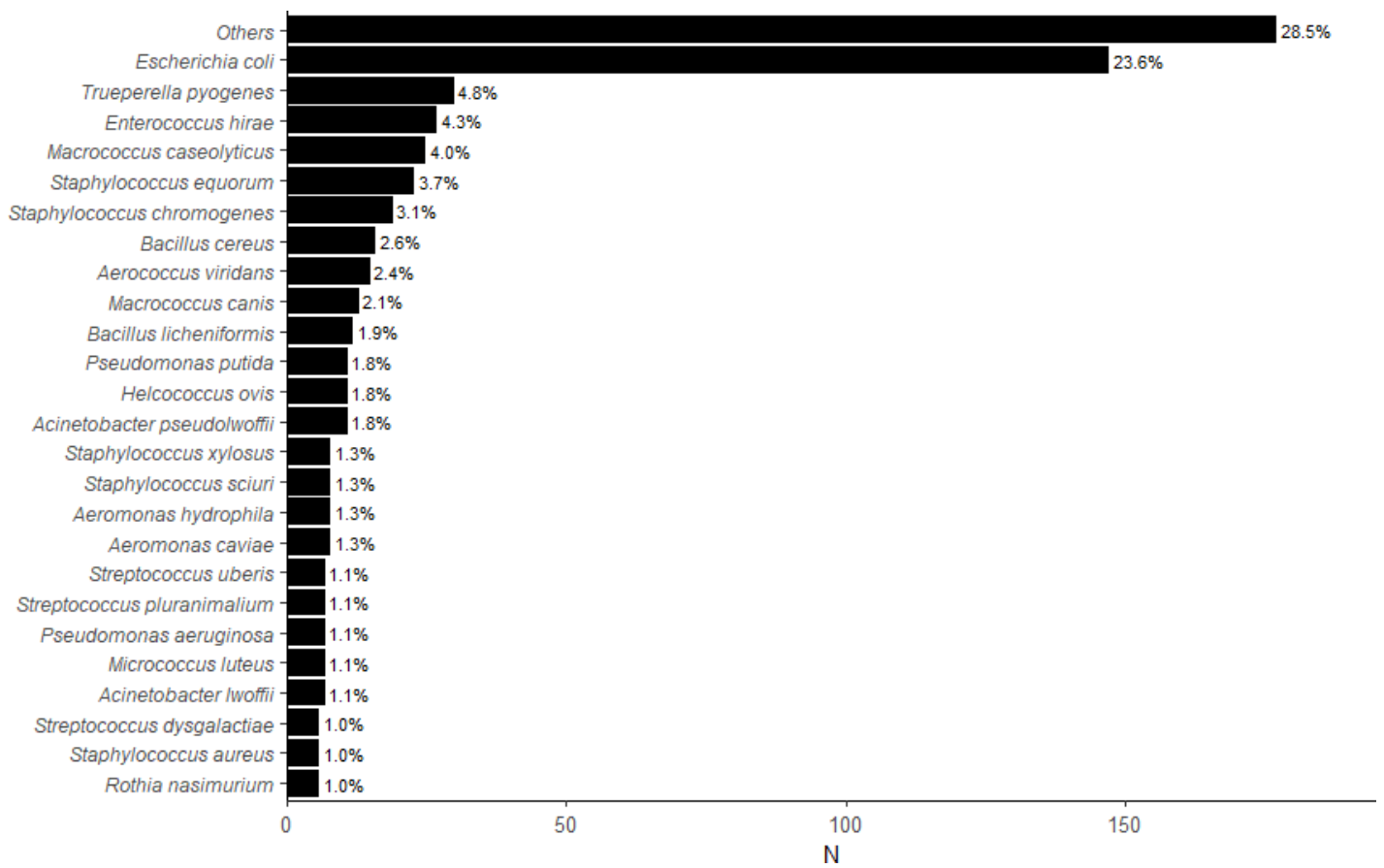


Figure 1: Prevalence of uterine microbiota in dairy cows identified via MALDI-TOF MS. Horizontal bar charts illustrate the most frequent bacterial taxa isolated from the uterine environment. (a) Distribution of bacterial genera, where the X-axis represents the number of animals and labels indicate relative frequency (%). (b) Distribution of bacterial species; the "Others" category includes less expressive species (with fewer than 5 findings). The prevalence of bacterial species found.

A binary map illustrating the distribution of bacterial genera detected in endometrial sample from cows housed in 20 dairy farms (A–T). The figure highlights heterogeneity in the microbial profiles across farms, with some genera—such as *Escherichia*, *Enterococcus*, and *Staphylococcus*—showing widespread occurrence, while others appear sporadically or are restricted to specific locations

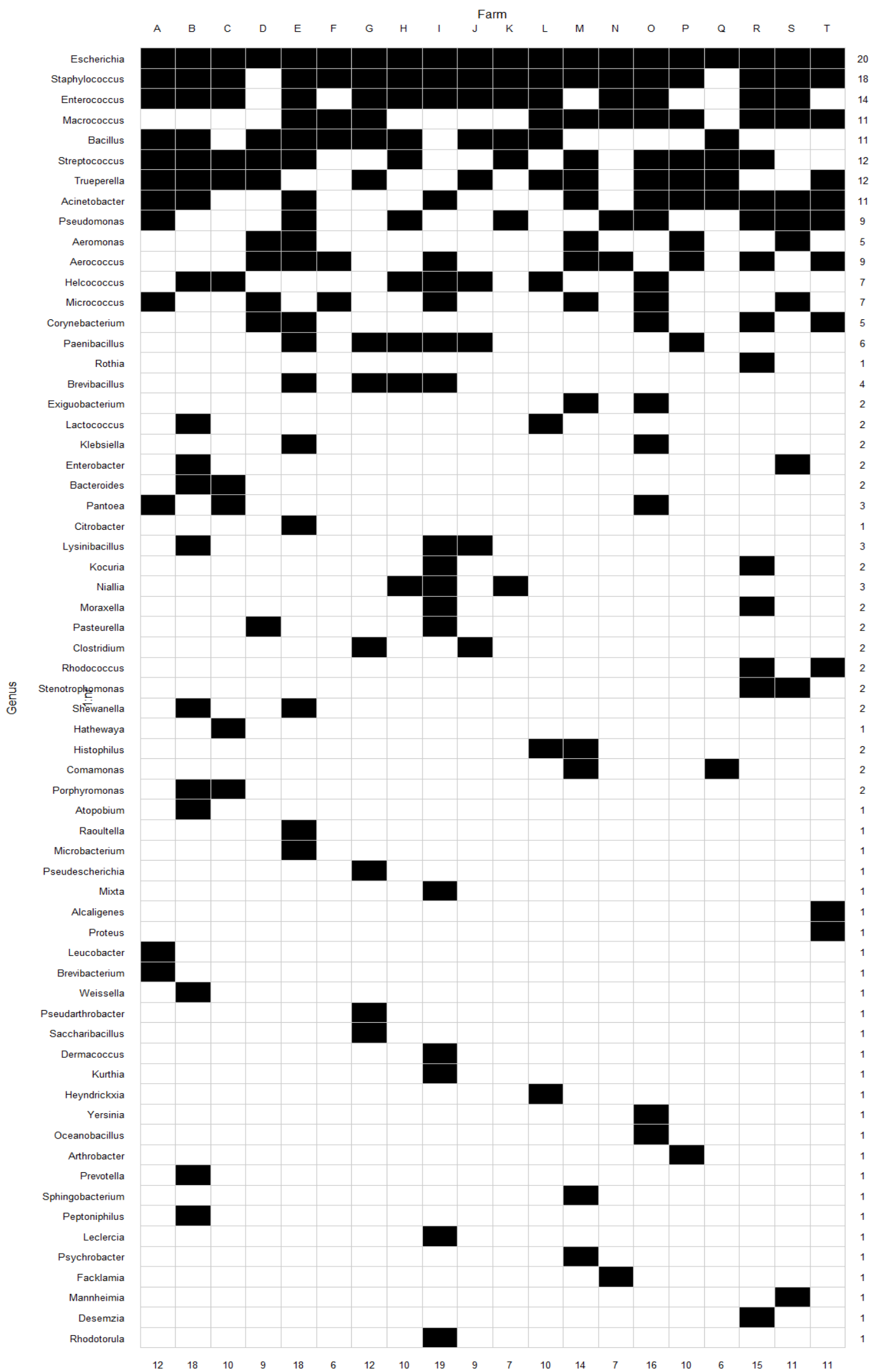


Figure 2: Presence–absence binary map of bacterial genera identified across dairy farms. Each row represents a bacterial genus identified by MALDI-TOF, and each column corresponds to a farm. Black cells indicate presence, and white cells indicate absence of the respective genus within each farm.

The compost-bedded pack material was alkaline and moderately moist. Prepartum samples ($n = 20$) exhibited a mean pH of 9.01 ± 1.05 (median 9.22 [IQR 8.72–9.59], range 6.38–10.20). Moisture content averaged 38.6 ± 9.1 % (median 37.0 % [IQR 32.5–43.3], range 22.0–57.3 %). Organic carbon concentrations were high, with a mean of $51,051 \pm 9,416$ mg·kg⁻¹ (median 50,238 [IQR 46,023–54,309], range 34,374–74,885). Total nitrogen showed variability, averaging 13.802 ± 33.477 mg·kg⁻¹ (median 5,784 [IQR 4,357–7,773], range 2,603–155,739). The resulting C:N ratio was therefore heterogeneous, though generally low across farms.

Postpartum samples ($n = 20$) showed slightly higher alkalinity and increased moisture. The mean pH was 9.63 ± 0.57 (median 9.79 [IQR 9.44–10.15], range 8.19–10.36), while moisture averaged 41.6 ± 7.7 % (median 40.6 % [IQR 36.8–44.4], range 27.0–60.4 %). Organic carbon concentrations were similar to prepartum values (mean $51,877 \pm 7,072$ mg·kg⁻¹; median 51,357 [IQR 49,691–53,022], range 35,674–71,004). Total nitrogen was lower and less variable than in prepartum samples (mean $6,397 \pm 3,551$ mg·kg⁻¹; median 5,681 [IQR 4,692–6,670], range 73–15,071). The resulting postpartum C:N ratio was comparable to prepartum values, remaining close to 8 on average.

A continuous reduction in AIC values was observed as PMN thresholds increased, indicating a progressive improvement in model performance up to a certain point (Figure 3a). When the analysis was restricted to the lower threshold range (Figure 3b), a marked decline in AIC was detected up to approximately 8.3 % PMN, beyond which the curve stabilized.

To objectively estimate this inflection point, two independent linear regressions were fitted to the decreasing and plateau segments of the AIC curve (Figure 3c). The intersection of these regressions occurred at approximately 8.3 % PMN, after which further reductions in AIC were negligible. Therefore, 8.3 % PMN was defined as the optimal threshold for discriminating cows with uterine inflammation, representing the balance between model fit and parsimony. At this threshold, 60 animals were classified as having endometritis.

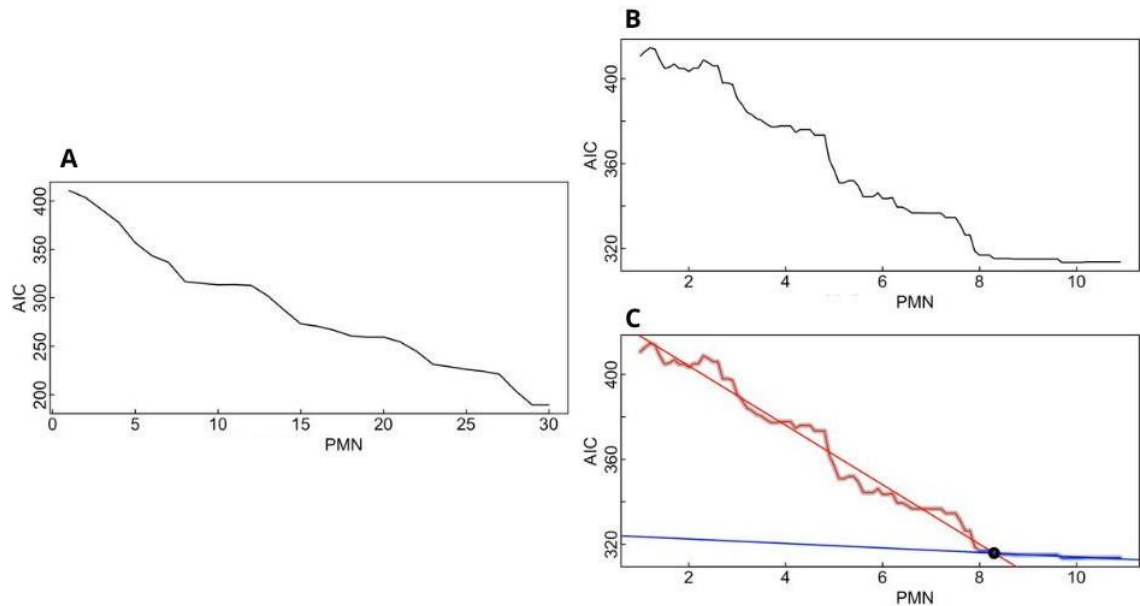


Figure 3. Determination of the optimal PMN threshold based on Akaike Information Criterion. (A) Relationship between increasing PMN thresholds (0–30 %) and corresponding AIC values. (B) Detailed evaluation of lower PMN thresholds (0–11 %), highlighting the region where the decline in AIC is most pronounced. (C) Estimation of the optimum diagnostic point using two linear regressions fitted to the decreasing (red line) and stable (blue line) segments of the curve. The intersection between the regressions (black dot) identifies the optimal threshold, corresponding to 8.3 % PMN.

To further characterize the microbial patterns associated with uterine health status, we assessed the distribution of bacterial genera and species exclusively detected in healthy cows, exclusively in sick cows, or shared between both groups. At the genus level, 6 genera were identified exclusively in cows with uterine inflammation (*Arthrobacter*, *Dermacoccus*, *Heyndrickxia*, *Prevotella*, *Psychrobacter* and *Raoultella*), whereas 30 genera appeared exclusively in healthy animals. A substantial overlap of 26 genera was observed between groups, indicating a broad core microbiota shared across clinical categories (Figure 4a).

At the species level, the pattern was similar but more pronounced: 19 species were detected only in sick cows, 76 species were exclusive to healthy cows, and 37 species were shared by both groups (Figure 4b). The genera and species belonging to each group are listed in the Supplementary Table S1.

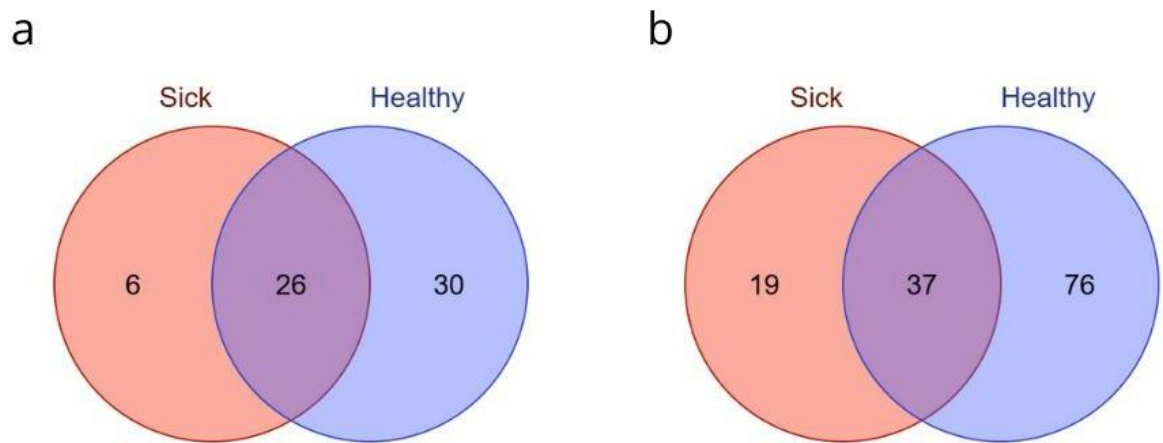


Figure 4: Venn diagrams illustrating the distribution of bacterial taxa identified through MALD-TOF in endometrial sample of dairy cows classified as healthy or sick based on PMN thresholds. Healthy (blue) and Sick (red) groups were categorized according to the empirically determined threshold of 8.3 % PMN. a: Distribution of bacterial genera; b: Distribution of bacterial species.

Bacterial co-isolation significantly influenced PMN counts, being associated with a reduction in PMN levels ($\beta = -0.438$; $p = 0.037$). The number of days in lactation (DEL) also showed a significant negative effect ($\beta = -0.032$; $p < 0.001$), indicating a decline in the inflammatory response as lactation advanced. Other animal-related variables were not significantly associated ($p > 0.05$).

The presence of specific bacterial genera was associated with a significant increase in PMN levels. The genus *Arthrobacter* showed a positive association with PMN count (Estimate = 3.12; SE = 1.44; $p = 0.0316$), indicating that a higher abundance of this taxon was related to a significant increase in the inflammatory response. Similarly, *Prevotella* was also positively associated with the number of PMNs (Estimate = 3.20; SE = 1.45; $p = 0.0282$).

On the other hand, two genera showed a negative association. *Enterococcus* showed a significant inverse effect on PMN count (Estimate = -0.70; SE = 0.25; $p = 0.0066$), suggesting that its presence was related to a lower intensity of the inflammatory response. Similarly, *Histophilus* showed a negative association with the outcome (Estimate = -2.05; SE = 1.02; $p = 0.0440$). The remaining bacterial genera included in the model showed no significant associations ($p > 0.05$).

A second linear model, which evaluated the mean productive parameters per farm, showed no overall significance ($F_{(3,16)} = 1.52$; $p = 0.248$), with a low adjusted coefficient of determination ($R^2_{adj} = 0.075$).

Additionally, the effect of bedding type used in the facilities (1 = wood shavings; 2 = mixture of sawdust and shavings; 3 = sawdust) on the predicted mean PMN values per farm was evaluated. The fitted model did not detect significant differences among bedding types ($F_{(2,17)} = 0.42$; $p = 0.666$), confirming that this management factor had no measurable influence on PMN levels.

4. DISCUSSION

This study provides an integrated evaluation of the uterine microbiota and inflammatory dynamics of dairy cows housed in CB systems, generating evidence relevant to both diagnostic refinement and herd health management. The results obtained reinforce the importance of establishing an empirically adjusted PMN threshold for diagnosing subclinical endometritis. Previous studies have suggested varying cut-off points: for example, values of $\leq 4.75\%$ PMN (Andrade et al., 2021) or 5.5% (Santos et al., 2009) have been proposed for detecting uterine inflammation. Other studies have considered higher limits, such as $>10\%$ at 34–47 days postpartum or $>18\%$ at 20–33 days postpartum (Kasimanickam et al., 2004). In this heterogeneous context, the optimal value found of 8.3% PMN represents an important contribution, as it works as a tool in predicting uterine inflammation under real field conditions. Although the establishment of an empirically determinate PMN optimal value to identify subclinical endometritis, it can be argued that this was the most relevant found of the present study.

The genus *Enterococcus* has been identified as a potential probiotic due to its high ecological competitiveness, ability to survive in adverse conditions of the gastrointestinal tract, and production of bacteriocins (enterocins) with antimicrobial activity against various pathogens (Hanchi et al, 2018). Indeed, some strains, such as *E. faecium* SF68 and M74, are already safely used in commercial preparations, showing beneficial effects in modulating the microbiota and the host's immune response. These results reinforce the hypothesis that *Enterococcus* may act as a functionally protective microorganism; however, considering concerns related to the presence of virulence factors and resistance genes (Hanchi et al, 2018), its use should be carefully evaluated on a strain-specific basis, and further studies are needed to confirm its safety and efficacy in different clinical and zootechnical contexts.

Although the present findings suggest a negative association between *Histophilus* and the occurrence of endometritis, this result contrast with the literature. *Histophilus* has been described as an opportunistic pathogen, rather than as a protective member of the uterine microbiota (Sandal; Inzana, 2010). Studies have reported the presence or increased relative abundance of *Histophilus* in cows with reproductive tract disease, including postpartum metritis and subfertility, suggesting a potential pathogenic role or opportunistic overgrowth in dysbiotic uterine environments (Wang et al., 2013; Rodrigues et al., 2015; Tasara et al., 2023).

However, the ecological role of *Histophilus* spp. within the bovine reproductive tract remains complex and context-dependent. While several studies have linked the genus to disease states, *Histophilus* spp. are also recognized as commensal inhabitants of mucosal surfaces, including the reproductive tract (Sandal; Inzana, 2010). Therefore, it remains plausible that under certain microbial community configurations or host immune conditions, the presence of *Histophilus* may not directly contribute to disease and could instead reflect a broader microbial equilibrium.

Regarding, *Prevotella* spp., multiple studies describe as opportunistic pathogens capable of inducing inflammatory cascades and promoting neutrophil recruitment in the bovine endometrium (Cunha et al, 2018). Therefore, the positive association observed in our model, in which increased abundance of *Prevotella* corresponded to higher PMN counts, is consistent with its known pathogenic potential. The fact that other studies also identified *Prevotella* as one of the most prevalent genera in cows with endometritis reinforces the interpretation of this taxon as an indicator of uterine dysbiosis and heightened inflammatory risk in the postpartum period (Cai et al., 2024).

Conversely, *Arthrobacter* does not traditionally feature among pathogens of the bovine reproductive tract. This genus, belonging to the Actinobacteria phylum, is widely distributed in environmental sources and dairy products, often functioning as a marker of hygiene or benign contamination rather than infection. In agreement with this, is reported a higher prevalence of *Arthrobacter* in healthy cows compared to those with endometritis (Liu et al., 2024). Thus, the positive correlation between *Arthrobacter* and PMN levels in our study was unexpected. One plausible explanation is that *Arthrobacter* may proliferate in environments where the uterine barrier was already compromised, or where local dysbiosis favors the secondary expansion of non-pathogenic taxa. In such scenarios, its presence may reflect, rather than drive, inflammatory processes. Nonetheless, because existing literature predominantly associates *Arthrobacter* with healthy uterine conditions, the biological significance of its relationship with inflammation remains uncertain and warrants further investigation.

In the findings of the present study, *E. coli* and *T. pyogenes* were isolated most frequently, consistent with the literature that describes them among the microorganisms most commonly found in cases of metritis and endometritis (Chen et al, 2020; Cunha et al, 2018; Galvão et al, 2019). However, as reported in other studies that also identified them as prevalent, there was no statistically significant correlation between the presence of these species and the disease (Chen et al, 2020; Cunha et al, 2018). In our analysis, neither showed a significant association with increased PMN. These results suggest that the mere detection of *E. coli* or *T. pyogenes* in the uterus may be not sufficient to predict a measurable inflammatory response, highlighting the importance of additional factors, such as bacterial load, strain virulence patterns, host immune status, and interactions with the uterine microbiota, in determining disease development and severity. Furthermore, it should be considered that performing only one sample collection may have influenced the results, since the temporal dynamics of bacterial colonization can alter both the relevance of each microorganism and its association with inflammatory parameters throughout the postpartum period.

5. CONCLUSION

The bacteriological findings reinforce the polymicrobial nature of the postpartum uterus and highlight the predominance of environmental and opportunistic genera within CB systems. *Prevotella* demonstrated a consistent positive association with PMN counts, supporting its role as an indicator of uterine dysbiosis and inflammation. The unexpected positive association between *Arthrobacter* and PMN levels suggests that non-pathogenic or environmental genera may proliferate in disrupted uterine environments, potentially acting as secondary markers rather than primary etiologic agents. The negative associations observed for *Enterococcus* and *Histophilus* indicate that not all detected microorganisms contribute equally to the inflammatory profile, emphasizing the need to interpret microbial presence within a broader ecological and physiological context.

Bedding physicochemical characteristics and bedding type did not significantly influence PMN levels, suggesting that, under adequate management, CB systems do not inherently predispose cows to uterine inflammation. Further studies combining culture-independent sequencing and functional analyses are warranted to clarify the ecological roles of the detected taxa and to support evidence-based reproductive health strategies in CB-based dairy herds.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

- [1] ALBINO, R. L. et al. Comparison of bacterial populations in bedding material, on teat ends, and in milk of cows housed in compost bedded pack barns. *Animal Production Science*, v. 58, n. 9, p. 1686–1691, 2018.
- [2] ANDRADE, J. S.; MOREIRA, E. M.; DA SILVA, G. M.; SCHNEIDER, A.; NUNES, V. R. R.; DA SILVA, R. R.; PFEIFER, L. F. M. Uterine health and fertility of timed AI postpartum Nelore beef cows raised in the Amazon biome. *Livestock Science*, v. 249, p. 104528, 2021.
- [3] Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1). <https://doi.org/10.18637/jss.v067.i01>.
- [4] BLACK, R. A. et al. Compost bedded pack dairy barn management, performance, and producer satisfaction. *Journal of Dairy Science*, v. 96, n. 12, p. 8060–8074, 2013.
- [5] CAI, X.-S. et al. Changes in bacterial community composition in the uterus of Holstein cows with endometritis before and after treatment with oxytetracycline. *Scientific Reports*, v. 14, p. 9511, 2024.
- [6] CHEN, H. et al. Determination of uterine bacterial community in postpartum dairy cows with metritis based on 16S rDNA sequencing. *Veterinary and Animal Science*, v. 10, p. 100102–100102, 20 mar. 2020.
- [7] CUNHA, F. et al. Quantifying known and emerging uterine pathogens, and evaluating their association with metritis and fever in dairy cows. *Theriogenology*, v. 114, p. 25–33, jul. 2018.
- [8] DAMASCENO, M. D. et al. Detection of *Brucella* spp. in compost-bedding pack barn and its relationship with infections in dairy cows during the transition period. *Research in Veterinary Science*, v. 201, p. 106047, 31/12/2025.
- [9] DŽERMEIKAITĖ, Karina; KRIŠTOLAITYTĖ, Justina; ANTANAITIS, Ramūnas. Relationship between dairy cow health and intensity of greenhouse gas emissions. *Animals*, MDPI, 2024.
- [10] Espadamala, A., Pereira, R., Pallarés, P., Lago, A., & Silva-del-Río, N. (2018). Metritis diagnosis and treatment practices in 45 dairy farms in California. *Journal of Dairy Science*, 101(10), 9608–9616. <https://doi.org/10.3168/jds.2017-14296>.

- [11] FAO – FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. Greenhouse gas emissions from the dairy sector: a life cycle assessment. Rome: FAO, 2010.
- [12] FAO – FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. The role of livestock in agricultural development. Disponível em: <https://www.fao.org/4/x0262e/x0262e13.htm#m>. Acesso em: 6 jun. 2025.
- [13] GALVÃO, K. N.; BICALHO, R. C.; JEON, S. J. Symposium review: The uterine microbiome associated with the development of uterine disease in dairy cows. *Journal of Dairy Science*, out. 2019.
- [14] GARCIA, Sara N.; OSBURN, Bennie I.; CULLOR, James S. A one health perspective on dairy production and dairy food safety. *One Health*, 2019.
- [15] GILBERT, R. O. Bovine endometritis: the burden of proof. *Cornell Veterinarian*, v. 82, p. 11–14, 1992.
- [16] GILBERT, Robert O. et al. Prevalence of endometritis and its effects on reproductive performance of dairy cows. *Theriogenology*, v. 64, n. 9, p. 1879–1888, 2005.
- [17] HANCHI, H. et al. The Genus *Enterococcus*: Between Probiotic Potential and Safety Concerns—An Update. *Frontiers in Microbiology*, v. 9, 3 ago. 2018.
- [18] KASIMANICKAM, R. et al. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. *Theriogenology*, v. 62, n. 1–2, p. 9–23, 2004.
- [19] LIU, N. et al. Phenotypic characteristics, antimicrobial susceptibility and virulence genotype features of *Trueperella pyogenes* associated with endometritis of dairy cows. *International Journal of Molecular Sciences*, v. 25, n. 7, p. 3974, 2024.
- [20] OLIVEIRA, C. E. A. et al. Physicochemical Bedding Quality in Compost-Bedded Pack Barn Systems for Dairy Cows: A Systematic Review. *Applied Sciences*, v. 13, n. 17, p. 9832, 2023.
- [21] PAIANO, R. B. et al. Assessment of the main pathogens associated with clinical and subclinical endometritis in cows by culture and MALDI-TOF mass spectrometry identification. *Journal of Dairy Science*, v. 105, n. 4, p. 3367–3376, 2022.
- [22] PASCOTTINI, O. B. et al. Dynamics of uterine microbiota in postpartum dairy cows with clinical or subclinical endometritis. *Scientific Reports*, v. 10, n. 1, 23/07/2020.
- [23] RODRIGUES, N. F. et al. Qualitative analysis of the vaginal microbiota of healthy cattle and cattle with genital-tract disease. *Genetics and Molecular Research*, v. 14, n. 2, p. 6518–6528, 2015.

- [24] S, Gayathri L e BHAKAT M e K, Mohanty T. Metritis in Dairy Cattle: Diagnostic Challenges, Economic Impacts, and Emerging Insights. *Current Journal of Applied Science and Technology*, v. 44, n. 1, p. 87–101, 18 Jan 2025.
- [25] Sakia, R. M. (1992). The Box-Cox transformation technique: a review. In *The Statistician* (Vol. 41).
- [26] SANDAL, I.; INZANA, T. J. A genomic window into the virulence of *Histophilus somni*. *Trends in Microbiology*, v. 18, n. 2, p. 90–99, feb. 2010.
- [27] SANTOS, N. R. et al. Postpartum endometrial cytology in beef cows. *Theriogenology*, v. 71, p. 739–745, 2009.
- [28] SHELDON, I. M.; LEWIS, G. S.; LeBLANC, S.; GILBERT, R. O. Defining postpartum uterine disease in cattle. *Theriogenology*, v. 65, n. 8, p. 1516–1530, 2006.
- [29] TAURAI TASARA et al. Interrogating the Diversity of Vaginal, Endometrial, and Fecal Microbiomes in Healthy and Metritis Dairy Cattle. *Animals*, v. 13, n. 7, p. 1221–1221, 31/03/2023.
- [30] VENABLES, Bill & Ripley, B. (2002). *Modern Applied Statistics With S*. 10.1007/b97626.
- [31] WANG, Meng Ling et al. Uterine microbiota of dairy cows with clinical and subclinical endometritis. *Frontiers in Microbiology*, v. 9, 2018.
- [32] WANG, Y. et al. Microbial diversity in the uterus of healthy and metritic postpartum Holstein dairy cows. *Theriogenology*, v. 80, n. 9, p. 1078–1087, 2013.

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SPATIO-TEMPORAL DISTRIBUTION OF BOVINE MASTITIS-CAUSING PATHOGENS IN BRAZIL USING ROUTINE DIAGNOSTIC LABORATORY DATA, 2009-2021

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ABSTRACT

Bovine mastitis is one of the most important diseases affecting dairy herds, however information on the distribution and frequency of causative pathogens, especially at developing countries, remains limited. This study aimed to describe the frequency and spatio-temporal patterns of mastitis-causing microorganisms in dairy herds from the south-eastern region of Brazil, using routine diagnostic laboratory data. Milk samples (n = 60,863) submitted to three diagnostic laboratories in Minas Gerais between 2009 and 2021 were analyzed using classical bacteriological methods. Descriptive analyses, statistical tests for association, odds ratios, and time series decomposition were performed, and geographic distribution was assessed at the municipality level. The most frequent pathogens were coagulase-negative staphylococci (CoNS) (28.95%; 13,451/46,459), *Staphylococcus aureus* (25.24%; 11,727/46,459), and *Streptococcus agalactiae* (14.74%; 6,849/46,459). Coinfections occurred in 10.27% of samples, with the combination of *S. aureus* and *S. agalactiae* being the most common (18.97%; 1,182/6,230), with samples positive for *S. aureus* presenting a 1.37 (95% CI = 1.23–1.52) times more chance to also be positive for *S. agalactiae*. Isolation of CoNS and *Corynebacterium* spp. in samples demonstrated protective effect against presence of major pathogens. No significant monthly seasonality was detected, albeit when grouped by rainfall pattern, Gram-negative organisms had a higher occurrence during the rainy season. The findings revealed high prevalence of contagious pathogens and widespread coinfections in the analysed data, reflecting ongoing challenges in mastitis control in Brazilian dairy herds and reinforcing the need for correct diagnostic of bovine mastitis and targeted control programs, especially for contagious pathogens.

1. INTRODUCTION

Dairy production is among the most important activities in Brazil, with more than 36 million tons of milk produced in 2023, positioning the country as the fourth largest producer of bovine milk in the world (FAO, 2023). Considering the Brazilian states, Minas Gerais stands out as the main hub of this activity, producing around 27% of total Brazilian milk (9 million tons) (IBGE, 2024). Despite the economic and social importance of milk industry for the country, Brazilian dairy sector still faces several challenges, especially considering infectious diseases such as bovine mastitis (Guimarães et al., 2017; Tomazi et al., 2018). Indeed, mastitis is responsible for considerable economic losses for dairy farming, including expenses with veterinarians and medicines, decreased milk production, and premature culling, in addition to alterations in milk composition and decrease of milk and dairy quality (Fernandes et al., 2021; Aghamohammadi et al., 2018).

Mastitis is defined as the inflammation of the mammary gland, being mainly caused by infectious agents and classified as clinical or subclinical mastitis (Cobirka; Tancin; Slama, 2020). In contrast to the clinical mastitis, subclinical cases do not present visual signs of inflammation in the udder and visible alterations in the milk, presenting only decrease in milk yield, increase in somatic cell count (SCC) and changes in the milk composition (Chen et al., 2022; Zeryehun; Abera, 2017). Mastitis can also be classified according to the origin and epidemiology of the pathogen: environmental mastitis is caused by microorganisms present in the farm environment that can, opportunistically, infect mammary gland and cause severe inflammation; contagious pathogens, on the other hand, are more adapted to the mammary gland and usually cause subclinical cases, and are transmitted between animals during milking process (Dufour; Labrie; Jacques, 2019; Cobirka; Tancin; Slama, 2020). This classification, however, has been less adopted, since microorganisms classically considered as environmental pathogens (such as *Escherichia coli* and *Streptococcus uberis*) has been found demonstrating contagious behaviour; whereas, pathogens more commonly associated with contagious mastitis (e.g. *Staphylococcus aureus*) can also have environmental origin (Ruegg, 2012).

Mastitis can be caused by a great number of different pathogens and, among the most common worldwide are *S. aureus*, *Streptococcus agalactiae*, *E. coli*, non-aureus *Staphylococcus* (also known as coagulase-negative *Staphylococcus* – CoNS) and *Klebsiella pneumoniae* (Contreras; Rodríguez, 2011; Ruegg, 2017). This etiological diversity makes microbiological diagnosis a cornerstone of mastitis management, as the identification of the causative agent directly influences clinical and managerial decision-making, including the assessment about the need of antimicrobial therapy, the selection of the most appropriate drug

for treatment (if warranted), the adoption of selective treatment protocols, and the implementation of pathogen-specific prevention and control measures (Barkema et al., 2009).

Additionally, knowing the etiology of the disease is essential not only at the herd level but also on a regional and national scale, as it allows a deeper understanding of the challenges faced by producers, and the development of more effective preventive and control strategies. In the Brazilian context, characterized by marked heterogeneity in herd size, management practices, and technological adoption, the analysis of available diagnostic data is essential to estimate the true magnitude of the mastitis challenge, identify priority pathogens and regions, and evaluate how control strategies have evolved over time. Such an approach allows not only the assessment of current epidemiological patterns but also the monitoring of progress achieved in mastitis control, offering an objective basis for refining guidelines, orienting public and private interventions, and strengthening evidence-based policies aimed at improving udder health and sustainability of dairy production in Brazil.

Given that, the objective of this study was to describe the occurrence of mastitis-causing pathogens using routine diagnostic laboratory data, as well as their spatial and temporal distribution. The data used here are results of milk bacteriological culture from three different laboratories in Brazil, from farms in states of the southeastern region, especially in Minas Gerais state, obtained between 2009 and 2021.

2. MATERIAL AND METHODS

2.1. Data source and microbiological procedures

Data used here were obtained from three different laboratories in Minas Gerais (MG) state: Laboratórios Integrados de Sanidade Animal e Saúde Coletiva (LISASC), Cia do Leite Laboratory, both localized in the municipality of Lavras, MG; and Mais Leite Soluções Zootécnicas Laboratory, in São João del-Rei, MG. The methodologies used by each laboratory to identify the different mastitis-causing pathogens are described in Figure 1.

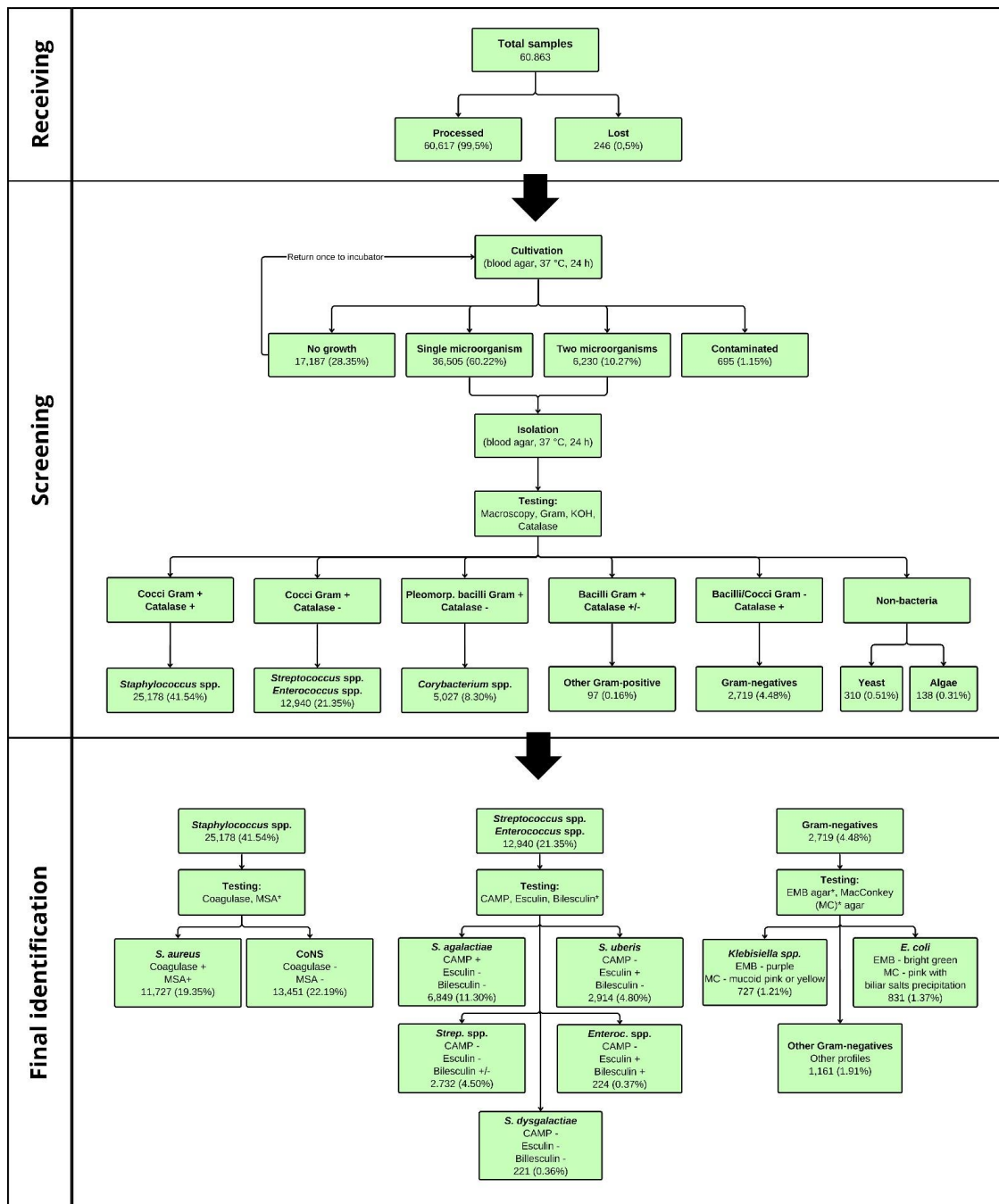


Figure 1 – Flowchart of milk sample processing, microbiological screening, and identification of mastitis-causing microorganisms, including sample classification and the frequency of isolates obtained.

In general, the laboratories used classical microbiology test based on the “Laboratory Handbook on Bovine Mastitis” (National Mastitis Council, 2017), with minor variations. Laboratories were not responsible for collecting the milk samples and the diagnosis was based only in macro and microscopic morphologies of microorganisms. Briefly, milk samples were

plated in blood agar and incubated at 37 °C for 24 h. Colony morphology was analysed and samples with up to two different colonies were isolated and incubated again in blood agar plates at the same conditions. Samples that exhibited growth of three or more different colony morphologies were considered as contaminated. Sample that did not depicted any growth within 24 h, were returned to the incubator for another 24 h (total 48 h).

Subsequent analyses were performed for each isolated colony, with Gram staining and catalase test used for screening and classification of the colonies in the following main patterns for subsequent identification: Gram-positive cocci and catalase positive (staphylococci pattern); Gram-positive cocci and catalase-negative (streptococci or enterococci pattern); Gram-negative bacilli and catalase positive or negative (Gram-negative pattern).

Staphylococci-like colonies were differentiated using coagulase test, being classified as *S. aureus* (coagulase-positive) or CoNS (coagulase-negative). Coagulase-negative staphylococci (CoNS) nomenclature was chosen since most of the laboratories only performed coagulase test for differentiating *S. aureus* and other staphylococci. Cia do Leite laboratory also used mannitol agar for *S. aureus* identification.

Streptococci or enterococci-like colonies were forwarded to Christie–Atkins–Munch-Petersen (CAMP) test, esculin and bile-esculin hydrolysis tests, allowing the identification of the following microorganisms: *S. agalactiae* (CAMP positive, esculin and bile esculin negative); *S. uberis* (CAMP negative, esculin positive and bile esculin negative), and *Enterococcus* spp. (CAMP negative, esculin and bile esculin positive). Patterns different from those were identified as other streptococci (*Streptococcus* spp.) by LISASC laboratory. Cia do Leite and Mais Leite laboratories identified *S. dysgalactie* when the isolate was negative for all tests (CAMP, esculin and bile esculin negative).

Gram-negative bacilli were grown in Eosin Methylene Blue (EMB) in LISASC laboratory or MacConkey (MC) agar in Cia do Leite and Mais Leite laboratories. *E. coli* was identified by presentation of bright green colonies in EMB or pink colonies with biliar salts precipitation in MC; while *Klebsiella* spp. was identified as purple colonies in EMB and mucoid pink or yellow colonies in MC.

If the microorganism did not present any of the above-described patterns, it was considered as indeterminate microorganism. Moreover, Gram-positive bacilli were described as “other Gram-positive”; Gram-positive pleomorphic bacilli were considered as *Corynebacterium* spp.; algae (*Prototheca* spp.) and yeast isolates were identified by characteristic macroscopic and microscopic morphology. Flow diagram for all microorganism identification is represented in Figure 1.

2.2. Statistical analyses

Data from the three laboratories were consolidated using Microsoft Excel software (Microsoft® Excel® for Office 365, USA) containing sample and farm identification, sample receiving date and bacteriological results. Descriptive analyses were performed to present both absolute and percentual numbers for the occurrence of the different pathogens (isolated or in coinfections), as well as considering their spatial and temporal distributions. Chi-square or Fisher exact tests were performed to assess whether there were associations between pathogens (coinfection) and between rainy season and pathogens' occurrence, with a significant level of 5% (Dohoo; Martin; Stryhn, 2012). For the associations that showed significant results at sample level, chi-square test was also performed at herd level. For seasonal analyses, months between October and March were considered as rainy season, according to the climate pattern observed for the studied region (INMET, 2017). Odds ratios were calculated to assess the level of association for the variables that exhibited significant chi-square/Fisher tests results (Dohoo; Martin; Stryhn, 2012). All analyses were performed using the basic package of R software version 4.4.1 (R Core Team, 2025). Plots of monthly distribution by pathogen were built using “tidyr” (Wickham et al., 2019), “ggplot2” (Wickham, 2016), and “patchwork” (Pedersen, 2025) packages in a R software. The “tidyverse” (Wickham et al., 2019), “geobr” (Pereira; Gonçalves, 2024), and “sp” (Pebesma; Bivand, 2005; Bivand et al., 2013) packages were used to plot the distribution map of sample locations, also using the R software.

For time series analysis, data were transformed into a time series format, which was first decomposed into observed data, trend, seasonality, and random effects. Seasonality of the pathogen occurrence was tested using the QS test for seasonality in time series. The “forecast” (Hyndman et al., 2024; Hyndman et al., 2008) and “seastest” (Ollech, 2021) packages of R software were used for these analyses.

3. RESULTS

3.1. Samples description

A total of 60,863 individual samples were received by the three laboratories between January 2009 and December 2021, 51.02% (31,050/60,863) by Cia do Leite Laboratory, 26.07% (15,869/60,863) by LISASC, and 22.91% (13,944/60,863) by Mais Leite Laboratory. Of these, 246 samples could not be processed for different reasons, such as broken or empty vial, lost sample, etc.

The samples were from different states within the southeastern region of Brazil: 71.75% (43,667/60,863) were from the state of Minas Gerais, 1.38% (839/60,863) from São Paulo, and

0.09% (52/60,863) from Rio de Janeiro. A total of 26.79% of the samples (16,305/60,863) did not have information regarding state of origin. Regarding municipalities and herds, analyzed samples were from 131 different municipalities and from 1,273 different dairy herds (Figure 2).

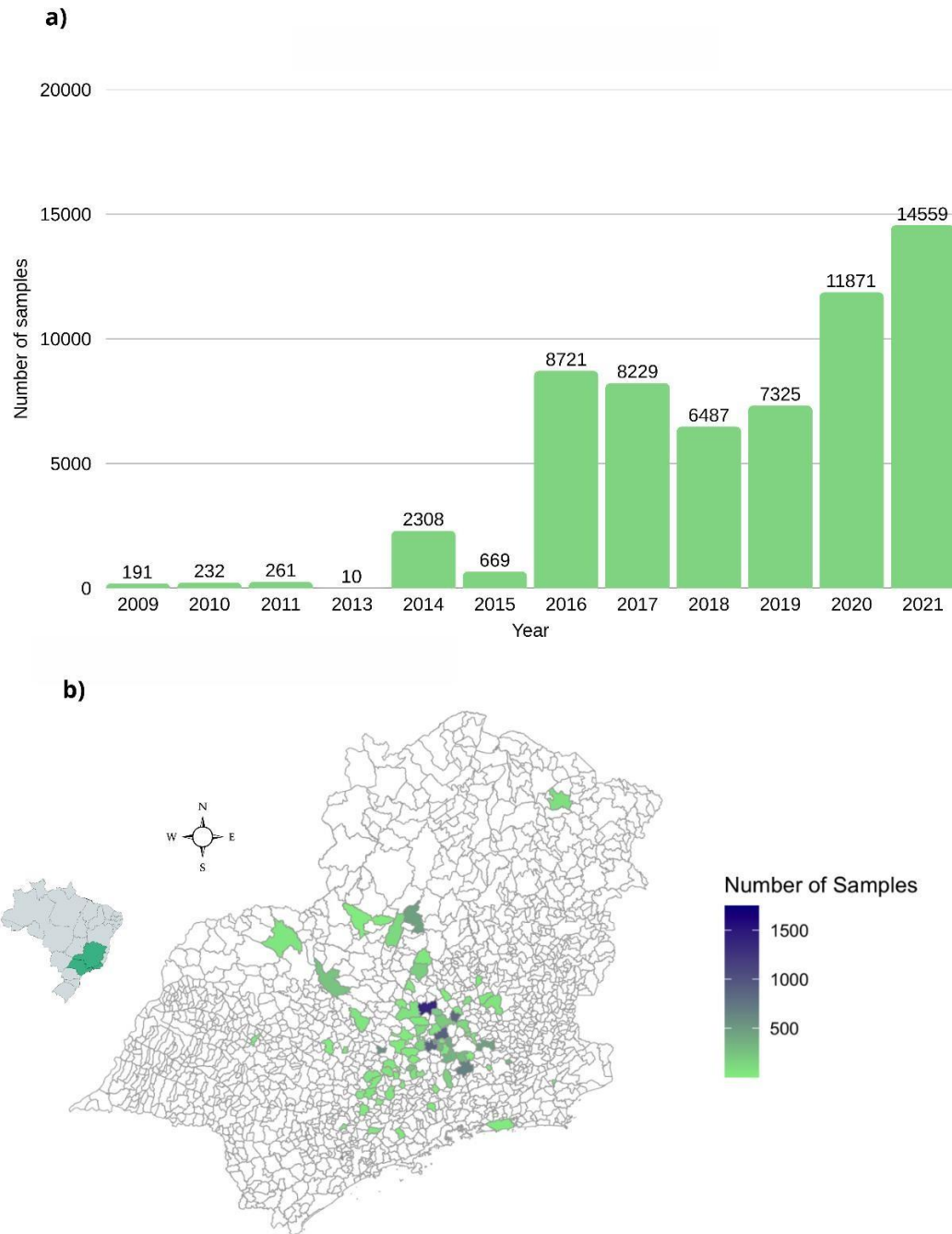


Figure 1 - Temporal and spatial distribution of 60,863 milk samples sent to three different laboratories in Minas Gerais state for bovine mastitis diagnostic, between 2009 and 2021. The bar chart (a) shows the number of samples processed per year, and map (b) illustrates the geographic distribution of samples by municipality in the states of Minas Gerais, São Paulo and Rio de Janeiro, Brazil. Colour intensity indicates the number of samples per municipality.

3.2. Microbiological results

Among the 60,617 processed samples, 28.35% (17,187/60,617) resulted in milk samples with no growth, whereas for 71.65% (43,430/60,617) at least one microorganism was isolated. From the samples that exhibited growth, 695 [1.60% (695/43,430)] showed isolation of three or more different colony morphologies and thereby considered contaminated, while for the other 42,735 (98.40%) samples one [84.05% (36,505/43,430)] or two [14.34% (6,230/43,430)] different colony morphologies were isolated (Figure 1).

Fourteen different microorganism groups were detected, with Gram-positive bacteria accounting for the majority of isolates (68.02%; 31,609/46,459), followed by Gram-negative bacteria (3.98%; 1,851/46,459), yeasts (0.67%; 310/46,459), and algae (0.40%; 188/46,459).

Among Gram-positive microorganisms, CoNS were the most frequently identified, representing 28.96% (13,451/46,459) of all isolates, followed by *S. aureus* [25.24% (11,727/46,459)], *S. agalactiae* [14.74% (6,849/46,459)], *Corynebacterium* spp. [10.82% (5,027/46,459)], *S. uberis* [6.27% (2,914/46,459)], and other *Streptococcus* spp. [5.88% (2,732/46,459)]. Among Gram-negative bacteria, *E. coli* accounted for 1.79% (831/46,459) of the isolates, followed by *Klebsiella* spp. [1.57% (727/46,459)]. The general frequency of all isolated microorganisms is shown in Figures 1 and 3.

A total of 6,230 [10.27% (6,230/60,617)] samples depicted coinfections (simultaneous presence of two agents at the same sample). The most frequent combination were *S. aureus* and *S. agalactiae* [19.00% (1184/6,230)], followed by association between *S. aureus* and *Streptococcus* spp. [16.61% (1035 /6,230)]. In total, 67 different combinations between microorganisms were identified (Figure 3). Results of all chi-square tests for association between microorganisms, odds ratio (OR) values and confidence intervals are shown in Figure 3 and Supplementary material S1.

Several significant associations of microorganisms were found ($p < 0.05$), with OR indicating both risk ($OR > 1$) and protection ($OR < 1$). Samples containing *S. aureus* had 1.37 times more chance of being coinfecting with *S. agalactiae* (95% CI = 1.23–1.52) and 1.57 more chance of having also other *Streptococcus* spp. (95% CI = 1.40 – 1.75). Conversely, the presence of CoNS was observed as protection factor for the infection with several pathogens, such as *S. aureus* (0.016, 95% CI = 0.013–0.018), *S. agalactiae* (OR = 0.456; 95% CI: 0.408–0.510), *Streptococcus* spp. (OR = 0.673; 95% CI: 0.599–0.754) and *E. coli* (OR = 0.716; 95% CI: 0.524–0.972). In addition, *Corynebacterium* spp. also demonstrated protective effect against *Streptococcus* spp. (OR = 0.068; 95% CI: 0.046–0.098), *S. aureus* (OR = 0.239; 95% CI: 0.203–0.281), *S. agalactiae* (OR = 0.274; 95% CI: 0.225–0.333), *S. uberis* (OR = 0.417; 95% CI:

0.318–0.539), *E. coli* (OR = 0.277; 95% CI: 0.124–0.541), and *Klebsiella* spp. (OR = 0.325; 95% CI: 0.103–0.792).

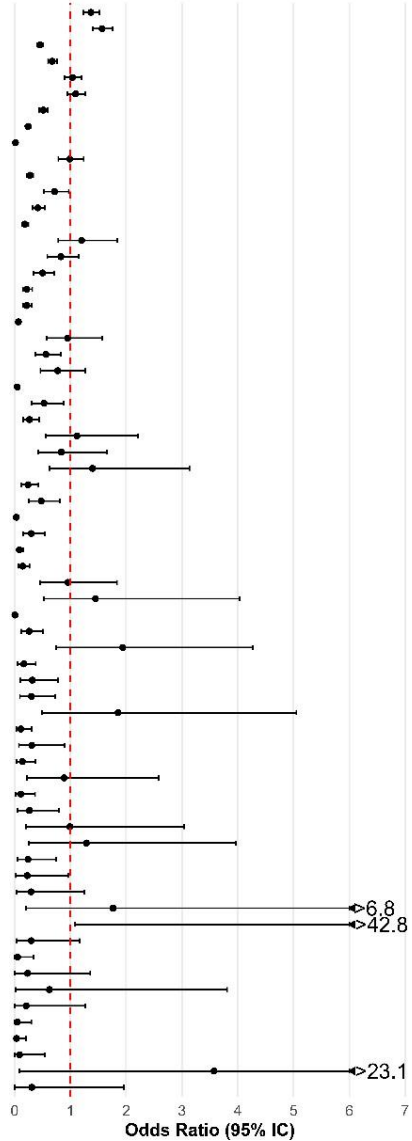
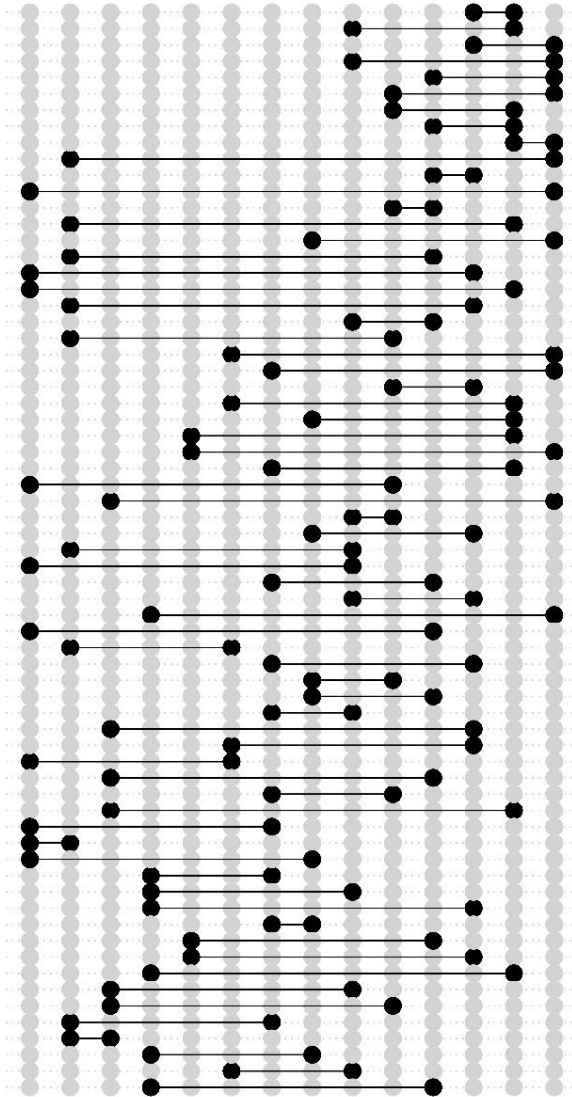
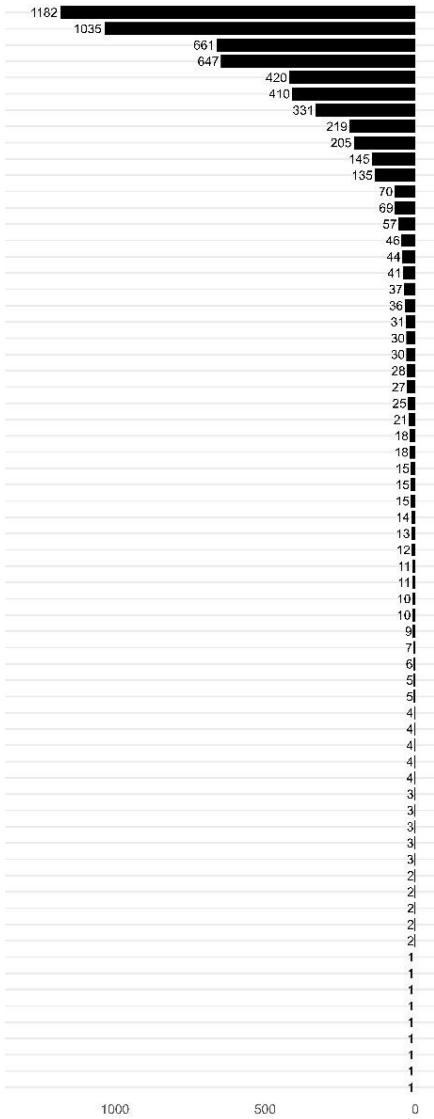
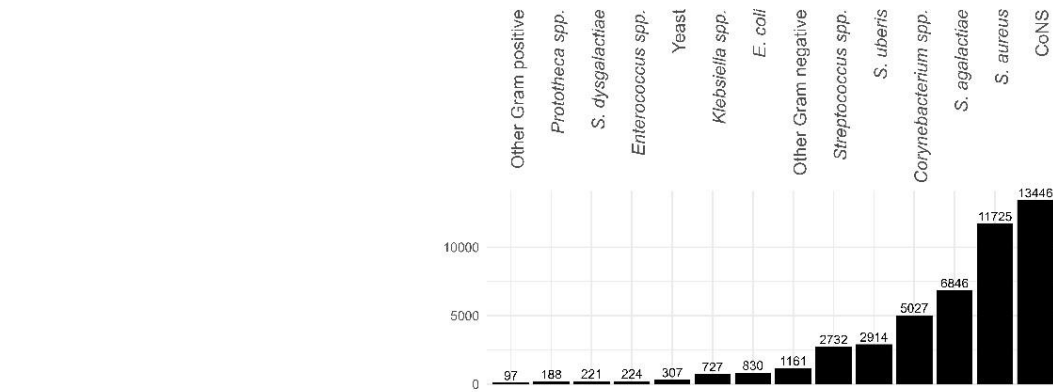


Figure 3 - Associations between mastitis-causing agents in dairy cows based on co-occurrence and odds ratio (OR) analysis. UpSet plot presenting the frequency and intersections of the main microorganisms isolated from milk samples (center). Horizontal bars (left) represent the number of coinfections of each microorganism pair, and vertical bars (top) indicate the total number of isolates per microorganism. The plot on the right displays the OR with 95% confidence intervals for significant associations between microorganisms. The dashed line represents the null value (OR = 1).

The analyzed milk samples were from 1,273 different herds, with 81.22% of the herds showing at least one animal infected by CoNS, followed by 80.6% for *S. aureus* (1,026/1,273), 52.5% for *S. agalactiae* (669/1,273), 50.3% for *Corynebacterium* spp. (640/1,273), 45.2% for *S. uberis* (575/1,273), and 19.3% for *E. coli* (246/1,273). Still considering the herd level, 96.1% of the herds (1,224/1,273) showed the presence of two or more microorganisms, being 25.7% (327/1,273) *S. aureus* + *S. agalactiae* and 14.9% (190/1,273) *S. aureus* + *Streptococcus* spp. Also, a significant positive association at herd level was observed between CoNS and *S. aureus*, in which the presence of one microorganism increased the likelihood of detecting the other by 4.33-fold (OR = 4.33; 95% CI: 3.17–5.93). Similarly, CoNS showed a significant positive association with other pathogens at the farm level, including *Streptococcus* spp. (OR = 5.74; 95% CI: 3.75–8.80), *S. agalactiae* (OR = 2.42; 95% CI: 1.80–3.26), and *E. coli* (OR = 2.30; 95% CI: 1.47–3.60). Furthermore, *Corynebacterium* spp. also exhibited significant risk associations with several mastitis-causing pathogens at the farm level, including *Streptococcus* spp. (OR = 1.45; 95% CI: 1.15–1.83), *S. agalactiae* (OR = 2.29; 95% CI: 1.83–2.86), *E. coli* (OR = 1.77; 95% CI: 1.33–2.35), *S. aureus* (OR = 2.47; 95% CI: 1.85–3.31), *S. uberis* (OR = 3.10; 95% CI: 2.47–3.91), and *Klebsiella* spp. (OR = 2.01; 95% CI: 1.45–2.78). As shown at Supplementary material S2.

3.3. Temporal analysis

The temporal distribution of pathogens was evaluated to identify potential seasonal patterns. The seasonality analysis, performed using the QS test for seasonality in time series based on monthly variation, revealed no significant differences for any of the agents ($p > 0.05$). Chi-square test, on the other hand, demonstrated significant associations of some pathogens

with season when months were grouped according to regional rainfall pattern, classified into dry and rainy seasons. CoNS and all Gram-negative pathogens showed a higher likelihood of occurrence during the rainy season, whereas *S. uberis*, *Corynebacterium* spp., and *Prototheca* spp. showed a significant reduction in occurrence during this period. Monthly distribution of mastitis-causing microorganisms is shown in Figure 4. The OR values for each microorganism are at Supplementary material S3.

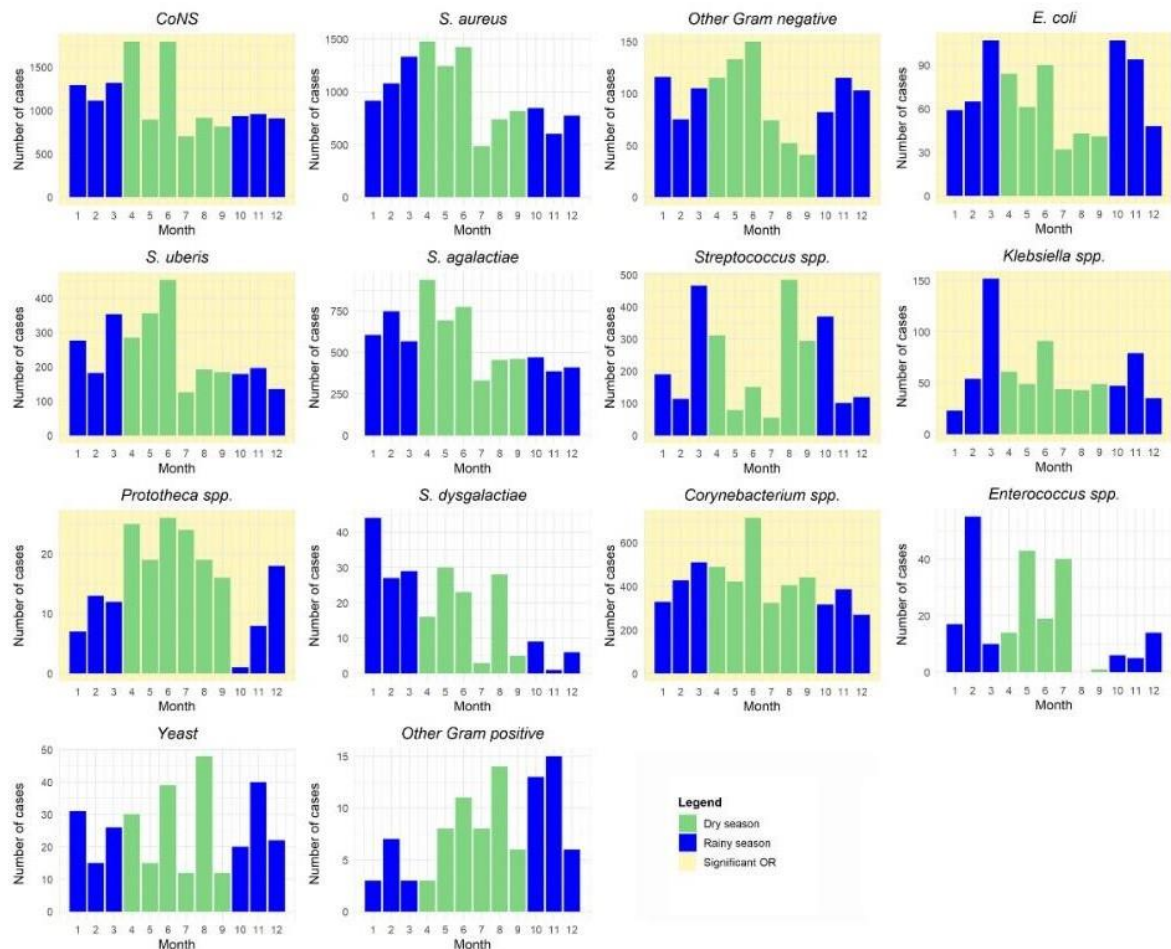


Figure 4 - Monthly distribution of mastitis-causing microorganisms according to the season throughout the years. Bars in blue represent the rainy season, and bars in green represent the dry season. The yellow background highlights graphs with a statistically significant relationship between agent and seasons based on odds ratio (OR) analysis ($p < 0.05$).

4. DISCUSSION

Bovine mastitis remains one of the most significant sanitary and economic challenges in the Brazilian dairy industry, with considerable impacts on milk yield, milk quality, and costs associated with management and medication (Guimarães et al., 2017). Although Brazil is one of the world's largest milk producers, information regarding the prevalence and distribution of mastitis pathogens in the country is still limited. This scarcity of data highlights the need for continuous surveillance and region-specific studies to better understand the epidemiological patterns of the disease. In this sense, our results demonstrated a high frequency of *S. aureus* and *S. agalactiae* (80.6% and 52.5%, respectively) in the studied herds, using routine diagnostic laboratory data (2009-2021) from the Southeast region of Brazil. Additionally, these microorganisms were frequently isolated from the same milk sample, being the most common (19%) coinfection combination observed and with significant positive association.

These findings underscore the challenges faced by Brazilian dairy producers in effectively controlling contagious mastitis. The recurrent identification of contagious pathogens such as *S. aureus* and *S. agalactiae* in diagnostic laboratory datasets may, in part, be attributed to a selection bias. Specifically, cases of mastitis caused by environmental pathogens are less frequently submitted for microbiological analysis, while samples from suspected contagious cases are more likely to be sent for laboratory confirmation. As a result, the predominance of these contagious bacteria in laboratory reports may not fully reflect their actual prevalence at the herd level, but rather the submission patterns and diagnostic priorities of the producers and veterinarians involved.

Both agents, *S. aureus* and *S. agalactiae*, are well adapted to the bovine mammary gland, often causing manifestations without evident local signs (Tong et al., 2025). However, their presence is associated with an increase in somatic cell count (SCC), reducing milk quality and leading to significant economic losses for producers (Piepers; De Vliegher, 2018). These characteristics of these pathogens, underlies their role as major mastitis pathogens, efficiently transmitted during milking procedures and often causing subclinical infections, allowing infected cows to act as long-term reservoirs within the herd (Tong et al., 2025). Such characteristics make detection and elimination particularly difficult and favor the continuous circulation of these pathogens in dairy herds. In the Brazilian context, where many production systems - especially smallholder farms - are characterized by limited access to technical

assistance, suboptimal milking hygiene, restricted use of microbiological diagnostics, and delayed culling or segregation of infected animals, these factors may interact to facilitate the dissemination of these pathogens and amplify the economic and sanitary consequences of infection (Serpa et al., 2026, unpublished data). Consequently, the combination of microorganism biology and production system characteristics contributes to the high frequency of contagious mastitis observed and reinforces the central role of *S. aureus* and *S. agalactiae* as the most challenging agents for mastitis control in Brazilian dairy farming.

Staphylococcus aureus is considered the pathogen with greater impact in bovine dairy farming, starting from the challenges in the diagnosis, since this pathogen may exhibit intermittent shedding in milk, reducing the sensitivity of conventional microbiological detection methods (Pumipuntu et al., 2017). Moreover, even when correctly identified, *S. aureus* possesses mechanisms that favor infection persistence, such as the internalization in mammary gland cells, biofilm formation and acquisition of antimicrobial resistance mechanisms, which hinders or prevents the action of antimicrobials for treatment, especially in multiparous cows (Campos et al., 2022). These biological characteristics help to explain the high frequency and persistence of *S. aureus* observed in the results and contextualize the limited effectiveness of antimicrobial treatment, for this reason, culling is the recommended measure for *S. aureus*-positive animals (Dufour; Scholl, 2012). The high frequency of *S. aureus* observed in this study may also be partly associated with the limited culling of positive animals in dairy herds (Barkema; Schukken; Zadoks, 2006). In many cases, producers choose to retain infected cows in the herd or sell them to neighboring farms rather than removing them from production. This situation is further exacerbated by diagnostic limitations and production characteristics typical of Brazilian dairy systems, including the lack of routine testing of animals prior to purchase (Ferreira et al., 2024). Altogether, these factors contribute to the persistence and dissemination of *S. aureus*-associated mastitis in Brazil.

Likewise, *S. agalactiae* is a highly contagious pathogen, efficiently transmitted during milking and frequently responsible for sudden and pronounced increases in SCC (Holmøy et al, 2019). Although its impact on milk quality and udder health is substantial, *S. agalactiae* infections are generally responsive to treatment and can be eliminated from the herd through systematic control programs and antibiotic therapy (Keefe, 1997). In contrast, as mentioned to

S. aureus, it also presents a major therapeutic challenge due to its ability to persist within mammary epithelial cells and to form biofilms, which protect the bacteria from both the host immune system and antimicrobial agents (Tong et al., 2025). The similar pathogenic strategies of *S. aureus* and *S. agalactiae*, combined with their high transmissibility, may explain the frequent detection of coinfections involving these two species, with samples positive for *S. aureus* showing 37% higher odds of also presenting *S. agalactiae* compared with samples negative for *S. aureus*.

On the contrary of the relevance of *S. aureus* and *S. agalactiae* as contagious agents of bovine mastitis, CoNS are often reported without clinical signs and with lower or modest increases in SCC (Reyher; Dohoo, 2011). This group of microorganisms was the most frequently identified in this study, present in 22.19% of the samples and 81.22% of the herds, as well as they were the most reported isolates in other several studies (Pyörälä; Taponen, 2009; Bexiga et al., 2014; Getahun et al., 2024; Taponen; Pyörälä, 2009). However, their usually lower pathogenicity has sparked the debate regarding their true role: opportunistic pathogens capable of causing mastitis and production losses, or commensals with potential modulatory effects (El-Jakee et al., 2013). In fact, CoNS are a very heterogeneous group of bacteria, with some species exhibiting virulence factors and biofilm-forming ability, allowing them to sustain persistent infections and increase SCC (Srednik et al., 2017); while other species appear to compete with more aggressive pathogens, such as *S. aureus* and *Streptococcus* spp., with *in vitro* and *in vivo* studies demonstrating antagonistic effects, which does not rule out their potential pathogenicity (Reyher et al., 2012). Supporting this last hypothesis, our findings demonstrated that, although CoNS were the most frequently identified microorganisms, followed by *S. aureus*, there were very few cases of coinfections between these two pathogens (3.26% - 205/6,231). Indeed, the presence of CoNS seems to decreased the odds of finding *S. aureus* in the same sample by 98.41%; while, at the herd level, the presence of one increased the chance of finding the other by 4.33 times, this may be explained by the fact that both share similar epidemiological niches, but by infecting the same teat, act as competitors (Ruiz-Romero; Vargas-Bello-Pérez, 2023; Touaitia et al., 2025). Similarly, CoNS were also observed as protective factor against other major pathogens, such as *Streptococcus* spp., *S. agalactiae*, and *E. coli*. In all these cases, at the farm level, the relationship between CoNS and the respective bacteria was also of risk, showing

that the presence of one microorganism indicated the possibility of the presence of the other. Similarly to CoNS, another minor pathogen also demonstrated protective factors against major pathogens was *Corynebacterium* spp., which showed an apparent protective effect against *Streptococcus* spp., *S. aureus*, *E. coli*, *S. agalactiae*, and *S. uberis*. Even though, the observed risk at the farm level excludes the hypothesis that the protective factor observed at animal level was due to the farms did not have both pathogens simultaneously in the herd. These results, together with the findings demonstrated by Reyher et al. (2012) in a meta-analysis with observational studies and clinical trials (which demonstrated a protective effect of minor pathogens against subsequent intramammary infections caused by major mastitis pathogens), strongly suggest that CoNS and *Corynebacterium* spp. may exert a protective effect against major mastitis pathogens, by modulating the udder microbiota in a way that reduces the risk of new intramammary infections caused by more virulent organisms. Other possible reasons for the protective effect observed can be attributed to several biological and immunological mechanisms operating within the bovine udder as described by previous works, including interference with the penetration of major pathogens through the teat canal and the metabolites of CoNS affecting the *S. aureus* capacity to adhere to epithelial cells (Reyher et al.; 2012; Toledo-Silva et al; 2023). Colonization by minor pathogens may induce a nonspecific activation of the host immune response, reflected by a small increased somatic cell count and shifts in differential cell populations, which can enhance local defense and reduce the likelihood of subsequent intramammary infections by more virulent microorganisms (Schukken et al., 1999; Pyörälä ; Taponen, 2009). In addition, minor pathogens have also been suggested to inhibit major pathogens through the production of bacteriocins and other antimicrobial substances, as well as by modulating the udder environment, including alterations in fatty acid concentrations and stimulation of anti-staphylococcal antibody production (Brooks; Barnum, 1984; Nickerson; Boddie, 1994; Schukken et al., 1999). Together, these mechanisms may limit major pathogen colonization and penetration through the teat canal, providing a biological basis for the observed protective associations between minor pathogens and major mastitis agents. Nonetheless, since the present study was a cross-sectional with convenience sampling, results about the possible effect of these minor pathogens (CoNS and *Corynebacterium* spp.) on preventing infections by major pathogens must be interpreted consciously and conclusive statement cannot be made.

Other possible explanations for the observed protective effect include failures in bacteriological identification, particularly differentiation between *S. aureus* and CoNS, which may present similar macroscopic colony characteristics and lead to misclassification during routine diagnostics. Indeed, coagulase-negative *S. aureus* strains may be erroneously identified as non-aureus *Staphylococcus* when conventional methods are used (Akineden et al., 2011). Nevertheless, such misclassifications are unlikely to fully account for the findings of this study. The protective effect of CoNS has been consistently observed in previous research, and the present analysis benefits from a large sample size and the inclusion of data from multiple laboratories, which likely reduce the impact of potential diagnostic errors. Another issue that concerns limitations of bacteriology is the potential misclassification of less epidemiologically relevant streptococcal species, such as *Enterococcus* spp. *S. dysgalactiae*, and *S. uberis*, since these microorganisms may exhibit variable overlapping phenotypic profiles. Nonetheless, such diagnostic imprecision has limited practical impact, since the course of action to be taken is the same for both microorganisms (Rossitto et al., 2002).

Regarding the pathogens more associated to clinical mastitis, although no clear seasonal pattern was observed in the monthly analysis, the higher frequency of Gram-negative pathogens during the rainy season - particularly *E. coli* (OR = 1.52; 95% CI: 1.32–1.76) and *Klebsiella* spp. (OR = 1.28; 95% CI: 1.11–1.49) - suggests that rainfall-associated environmental conditions play a key role in shaping mastitis etiology in the region. This association likely reflects the substantial increase in environmental soiling during the rainy season, as cattle tend to lie in wet and muddy areas that favor the accumulation of organic matter and manure on the udder surface. Such conditions not only facilitate the persistence of dirt and contamination around the teat orifice, increasing the risk of environmental mastitis, but also creating a microenvironment conducive to bacterial survival and proliferation (Schreiner; Ruegg, 2003). Definitely, elevated humidity and moderate temperatures enhance the persistence of environmental pathogens in bedding, manure, and milking areas, thereby amplifying the overall exposure and infection risk (Toghdory et al., 2022).

Also, the higher frequency of isolation of CoNS during the rainy season (OR = 1.07; 95% CI 1.03–1.11; $p < 0.01$) suggests that environmental and host-related factors contribute to their occurrence. A possible explanation is the increased temperature and humidity during this

period, which may induce heat stress, with a direct impact on the animals' immune system, compromising their ability to ward off infections and thereby facilitating opportunistic infections or the overgrowth of commensal bacteria residing on the teat skin and within the teat canal (Becker; Collier; Stone, 2020). In this context, De Buck et al. (2021) demonstrated that different CoNS species display distinct ecological niches and virulence traits, with some being well adapted to the mammary gland (e.g., *S. chromogenes*, *S. simulans*), while others are more opportunistic and associated with the surrounding environment (e.g., *S. haemolyticus*, *S. sciuri*). Therefore, the seasonal increase observed here likely reflects the combined effects of environmental stressors and shifts in the balance between commensal and pathogenic CoNS populations, rather than a purely environmental transmission pattern.

Although some limitations of the present study have already been clarified, several additional aspects warrant consideration. First, the use of convenience sampling may introduce representativeness bias, potentially influencing the generalizability of the findings. Second, there was minor methodological heterogeneity across the participating laboratories, which could have affected the consistency of the results. Third, temporal gaps during the collection period may have limited the continuity and completeness of the dataset. Fourth, the lack of detailed information about the farms and animals restricted the ability to perform more complex analyses of risk factors and epidemiological associations. Even with these limitations, when microbiological data are aggregated, they yield valuable insights into the distribution of pathogens, reveal temporal trends, and highlight regional differences. This aggregated approach supports a collective understanding of mastitis that extends beyond the scope of individual production systems. Beyond the immediate impact at the farm level, the systematic use of diagnostic results enables the construction of a broader epidemiological perspective, offering essential information for the development of effective control strategies and for understanding the overall dynamics of mastitis in Brazil.

5. CONCLUSION

The present study provides a comprehensive overview of the epidemiological profile of bovine mastitis pathogens in Brazilian dairy herds, revealing a predominance of contagious agents such as *S. aureus* and *S. agalactiae*, as well as a high frequency of co-infections involving these species, which underscores the persistent challenges faced by Brazilian

producers in controlling contagious mastitis. In contrast, the apparent protective effects of coagulase-negative staphylococci (CoNS) and *Corynebacterium* spp. against major pathogens (*S. aureus* and *S. agalactiae*) suggest that certain minor organisms may play a modulatory role in the mammary gland microbiota. Environmental and seasonal influences were also evident, with Gram-negative pathogens showing higher prevalence during the rainy season. These findings reinforce the multifactorial nature of mastitis epidemiology, in which host, pathogen, and environmental factors interact dynamically.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

- [1] AKINEDEN, Ö. et al. A coagulase-negative variant of *Staphylococcus aureus* from bovine mastitis milk. *Journal of Dairy Research*, v. 78, n. 1, p. 38–42, 1/12/2010.
- [2] BECKER, C. A.; COLLIER, R. J.; STONE, A. E. Invited review: Physiological and behavioral effects of heat stress in dairy cows. *Journal of Dairy Science*, v. 103, n. 8/05/2020.
- [3] BEXIGA, Ricardo et al. Dynamics of bovine intramammary infections due to coagulase-negative staphylococci on four farms. *Journal of Dairy Research*, v. 81, n. 2, p. 208–214, 2014.
- [4] BIVAND R, Pebesma E, Gomez-Rubio V (2013). *_Applied spatial data analysis with R, Second edition_*. Springer, NY. <<https://asdar-book.org/>>.
- [5] BRADLEY, A. J. Bovine mastitis: An evolving disease. *Veterinary Journal*, v. 164, n. 2, p. 116–128, 2002.
- [6] BROOKS, B. W.; BARNUM, D. A. The susceptibility of bovine udder quarters colonized with *Corynebacterium bovis* to experimental infection with *Staphylococcus aureus* or

- Streptococcus agalactiae*. Canadian Journal of Comparative Medicine, v. 48, n. 2, p. 146, abr. 1984.
- [7] CAMPOS, B. et al. Diversity and pathogenesis of *Staphylococcus aureus* from bovine mastitis: current understanding and future perspectives. BMC Veterinary Research, v. 18, n. 1, 24/03/2022.
- [8] CHEN, Xuelong et al. Prevalence of subclinical mastitis among dairy cattle and associated risks factors in China during 2012–2021: A systematic review and meta-analysis. Research in Veterinary Science, [S.l.], v. 148, p. 65–73, nov. 2022.
- [9] COBIRKA, M.; TANCIN, V.; SLAMA, P. Epidemiology and Classification of Mastitis. Animals, v. 10, n. 12, p. 2212, 26/11/2020.
- [10] CONTRERAS, G. Andres; Rodríguez, Juan Miguel. Mastitis: Comparative etiology and epidemiology. Journal of Mammary Gland Biology and Neoplasia, v. 16, n. 4, p. 339–356, dez. 2011.
- [11] DE BUCK, Jeroen *et al.* Non-aureus Staphylococci and Bovine Udder Health: Current Understanding and Knowledge Gaps. Frontiers in Veterinary Science Frontiers Media S.A., , 15/04/2021.
- [12] DUFOUR, S. et al. Manageable risk factors associated with the lactational incidence, elimination, and prevalence of *Staphylococcus aureus* intramammary infections in dairy cows. Journal of Dairy Science, v. 95, n. 3, p. 1283–1300, mar. 2012.
- [13] DUFOUR, S.; Labrie, J.; Jacques, M. The Mastitis Pathogens Culture Collection. Microbiology Resource Announcements, v. 8, n. 15, p. 18–19, 2019.
- [14] EL-JAKEE, J. K. et al. Emerging of coagulase negative staphylococci as a cause of mastitis in dairy animals: An environmental hazard. International Journal of Veterinary Science and Medicine, v. 1, n. 2, p. 74–78, dez. 2013.

- [15] FAO. FAOSTAT: Countries by commodity rankings. Available at: https://www.fao.org/faostat/en/#rankings/countries_by_commodity. Accessed on: 5 June 2024.
- [16] GETAHUN, Yared Abate et al. Coagulase-negative staphylococci from bovine milk: Antibigram profiles and virulent gene detection. BMC Microbiology, v. 24, n. 1, 1/12/2024.
- [17] HOLMØY, Ingrid H. et al. A cohort study of the effect of *Streptococcus agalactiae* on milk yield and somatic cell count in Norwegian dairy cows. Journal of Dairy Science, v. 102, n. 9, p. 8385–8399, 1/09/2019.
- [18] HYNDMAN R, et al. (2024). `_forecast: Forecasting functions for time series and linear models_`. R package version 8.22.0, <https://pkg.robjhyndman.com/forecast/>.
- [19] HYNDMAN RJ, Khandakar Y (2008). “Automatic time series forecasting: the forecast package for R.” *_Journal of Statistical Software_*, *27*(3), 1-22. doi:10.18637/jss.v027.i03.
- [20] IBGE. Produção agropecuária: Leite. Available at: <https://www.ibge.gov.br/explica/producao-agropecuaria/leite/br>. Accessed on: 05/06/2024.
- [21] INSTITUTO NACIONAL DE METEOROLOGIA (INMET). *Estação chuvosa em Minas Gerais*. Nota técnica nº 004/17. Belo Horizonte: 5º Distrito de Meteorologia – SEPRE, 2017. Available at: https://portal.inmet.gov.br/uploads/notastecnicas/Nota_tecnica_CEDEC_04_17.pdf. Accessed on: 29/09/2025.
- [22] LEELAHAPONGSATHON, K. et al. Molecular epidemiology of *Streptococcus uberis* intramammary infections: Persistent and transient patterns of infection in a dairy herd. Journal of Dairy Science, v. 103, n. 4, p. 3565–3576, 1/04/2020.
- [23] NATIONAL MASTITIS COUNCIL. Laboratory handbook on bovine mastitis. 3. ed. New Prague: National Mastitis Council, 2017.

- [24] NICKERSON, S. C.; BODDIE, R. L. Effect of Naturally Occurring Coagulase-Negative Staphylococcal Infections on Experimental Challenge with Major Mastitis Pathogens. *Journal of Dairy Science*, v. 77, n. 9, p. 2526–2536, set. 1994.
- [25] OLIVARES-PÉREZ, J. et al. Prevalence of bovine subclinical mastitis, its etiology and diagnosis of antibiotic resistance of dairy farms in four municipalities of a tropical region of Mexico. *Tropical Animal Health and Production*, v. 47, n. 8, p. 1497–1504, 2015.
- [26] OLLECH D (2021). *seastests: Seasonality Tests*. R package version 0.15.4, <https://CRAN.R-project.org/package=seastests>.
- [27] PEBESMA E, BIVAND R (2005). “Classes and methods for spatial data in R.” *R News*, *5*(2), 9-13.
- [28] PEDERSEN, T. L. (2025). *patchwork: The Composer of Plots (Version 1.3.2)* [R package]. Available at: <https://patchwork.data-imaginist.com>
- [29] PEREIRA R, Goncalves C (2024). *_geobr: Download Official Spatial Data Sets of Brazil*. R package version 1.8.2.
- [30] PIEPERS, S. et al. Impact of intramammary infections in dairy heifers on future udder health, milk production, and culling. *Veterinary Microbiology*, v. 134, n. 1-2, p. 113–120, 16/02/2009.
- [31] PUMIPUNTU, N. et al. Screening method for *Staphylococcus aureus* identification in subclinical bovine mastitis from dairy farms. *Veterinary World*, v. 10, n. 7, p. 721–726, jul. 2017.
- [32] PYORALA, S.; TAPONEN, S. Coagulase-negative staphylococci—Emerging mastitis pathogens. *Veterinary Microbiology*, v. 134, n. 1-2, p. 3–8, 16 feb. 2009.
- [33] PYÖRÄLÄ, Satu; TAPONEN, Suvi. Coagulase-negative staphylococci-Emerging mastitis pathogens. *Veterinary Microbiology*, v. 134, n. 1–2, p. 3–8, 16 feb. 2009.
- [34] R Core Team (2024). *_R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

- [35] R Core Team (2025). *_R: A Language and Environment for Statistical Computing_*. R Foundation for Statistical Computing, Vienna, Austria. Available in <https://www.R-project.org/>.
- [36] RĂZVAN-DRAGOȘ ROȘU et al. Occurrence and Antimicrobial Susceptibility of Major Bacterial Pathogens Associated with Subclinical Mastitis in Dairy Cows in Western Romania. *Microorganisms*, v. 14, n. 1, p. 26–26, 21 dez. 2025.
- [37] REYHER, K. K. et al. Examining the effect of intramammary infections with minor mastitis pathogens on the acquisition of new intramammary infections with major mastitis pathogens-A systematic review and meta-analysis. *Journal of Dairy Science*, v. 95, n. 11, p. 6483–6502, nov. 2012.
- [38] REYHER, K. K.; DOHOO, I. R. Diagnosing intramammary infections: evaluation of the National Mastitis Council recommendations. *Journal of Dairy Science*, v. 94, n. 1, p. 212–224, 2011.
- [39] ROSSITTO, P. V. et al. Antibiotic susceptibility patterns for environmental streptococci isolated from bovine mastitis in central California dairies. *Journal of Dairy Science*, v. 85, n. 1, p. 132–138, 2002.
- [40] RUEGG, P. L. A 100-Year Review: Mastitis detection, management, and prevention. *Journal of Dairy Science*, v. 100, n. 12, p. 10381–10397, 1/12/ 2017.
- [41] RUEGG, P. L. New Perspectives in Udder Health Management. *Veterinary Clinics of North America: Food Animal Practice*, v. 28, n. 2, p. 149–163, jul. 2012.
- [42] RUIZ-ROMERO, R. A.; VARGAS-BELLO-PÉREZ, E. Non-aureus staphylococci and mammaliicocci as a cause of mastitis in domestic ruminants: current knowledge, advances, biomedical applications, and future perspectives – a systematic review. *Veterinary Research Communications*, v. 47, n. 3, p. 1067–1084, 25/03/2023.

- [43] SCHUKKEN, Y. H. et al. Experimental *Staphylococcus aureus* Intramammary Challenge in Late Lactation Dairy Cows: Quarter and Cow Effects Determining the Probability of Infection. *Journal of Dairy Science*, v. 82, n. 11, p. 2393–2401, nov. 1999.
- [44] SCHUKKEN, Y. H. et al. Experimental *Staphylococcus aureus* Intramammary Challenge in Late Lactation Dairy Cows: Quarter and Cow Effects Determining the Probability of Infection. *Journal of Dairy Science*, v. 82, n. 11, p. 2393–2401, nov. 1999.
- [45] SREDNIK, M. E. et al. Biofilm formation and antimicrobial resistance genes of coagulase-negative staphylococci isolated from cows with mastitis in Argentina. *FEMS Microbiology Letters*, p. fnx001, 12/01/ 2017.
- [46] TAPONEN, Suvi; PYÖRÄLÄ, Satu. Coagulase-negative staphylococci as cause of bovine mastitis-Not so different from *Staphylococcus aureus*? *Veterinary Microbiology*, v. 134, n. 1–2, p. 29–36, 16/02/2009.
- [47] TOGHDORY, Abdolhakim et al. Effects of Environmental Temperature and Humidity on Milk Composition, Microbial Load, and Somatic Cells in Milk of Holstein Dairy Cows in the Northeast Regions of Iran. *Animals*, v. 12, n. 18, 1/09/2022.
- [48] TONG, X. et al. Virulence of Bacteria Causing Mastitis in Dairy Cows: A Literature Review. *Microorganisms*, v. 13, n. 1, p. 167–167, 15/01/2025.
- [49] TOUAITIA, R. et al. *Staphylococcus aureus* in Bovine Mastitis: A Narrative Review of Prevalence, Antimicrobial Resistance, and Advances in Detection Strategies. *Antibiotics*, v. 14, n. 8, p. 810, 8/08/2025.
- [50] WICKHAM H, et al (2019). “Welcome to the tidyverse.” *Journal of Open Source Software*, 4(43), 1686. doi:10.21105/joss.01686.
- [51] WICKHAM, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag. doi:10.1007/978-0-387-98141-3
- [52] WICKHAM, H.; Bryan, J. (2025). *readxl: Read Excel Files (Version 1.4.5) [R package]*. Posit Software, PBC. Available at: <https://readxl.tidyverse.org>

- [53] WICKHAM, Hadley et al. *Welcome to the Tidyverse*. Journal of Open-Source Software, v. 4, n. 43, p. 1686, 2019.
- [54] ZERYEHUN, T.; Abera, G. *Prevalence and Bacterial Isolates of Mastitis in Dairy Farms in Selected Districts of Eastern Harrarghe Zone, Eastern Ethiopia*. Journal of Veterinary Medicine, v. 2017, p. 1–7, 2017.
- [55] ZHANG, Z. et al. *Detection of Antibiotic Resistance, Virulence Gene, and Drug Resistance Gene of Staphylococcus aureus Isolates from Bovine Mastitis*. Microbiology Spectrum, v. 10, n. 4, 2022.
- [56] AGHAMOHAMMADI, M. et al. Herd-Level Mastitis-Associated Costs on Canadian Dairy Farms. *Frontiers in Veterinary Science*, v. 5, 14/05/ 2018.
- [57] FERNANDES, L. et al. Effect of subclinical mastitis detected in the first month of lactation on somatic cell count linear scores, milk yield, fertility, and culling of dairy cows in certified organic herds. *Journal of Dairy Science*, v. 104, n. 2, p. 2140–2150, feb. 2021.
- [58] COBIRKA, M.; TANCIN, V.; SLAMA, P. Epidemiology and Classification of Mastitis. *Animals*, v. 10, n. 12, p. 2212, 26/11/2020.
- [59] DOHOO, IAN; MARTIN, WAYNE; STRYHN, HENRIK. *Methods in epidemiologic research*. Charlottetown: VER Inc., 2012.
- [60] GUIMARÃES, J.L.; BRITO, M.A.; LANGE, C.C.; SILVA, M.R.; RIBEIRO, J.B.; MENDONÇA, L.C.; MENDONÇA, J.F.M.; SOUZA, G.N. et al. Estimate of the economic impact of mastitis: A case study in a Holstein dairy herd under tropical conditions. *Preventive Veterinary Medicine*, v. 142, p. 46–50, jul. 2017.
- [61] TOMAZI, T.; FERREIRA, G.C.; ORSI, A.M.; GONÇALVES, J.L.; OSPINA, P.A.; NYDAM, D.V.; MORON, P.; DOS SANTOS. Association of herd-level risk factors and incidence rate of clinical mastitis in 20 Brazilian dairy herds. *Preventive Veterinary Medicine*, v. 161, p. 9–18, 1/12/2018.

- [62] BARKEMA, H. W.; SCHUKKEN, Y. H.; ZADOKS, R. N. Invited Review: The Role of Cow, Pathogen, and Treatment Regimen in the Therapeutic Success of Bovine *Staphylococcus aureus* Mastitis. *Journal of Dairy Science*, v. 89, n. 6, p. 1877–1895, jun. 2006.
- [63] FERREIRA, J. S. et al. Biosecurity practices in the dairy farms of southern Brazil. *Frontiers in Veterinary Science*, v. 11, 27/03/2024.
- [64] KEEFE, G. P. *Streptococcus agalactiae* mastitis: a review. *The Canadian Veterinary Journal*, v. 38, n. 7, p. 429, jul. 1997.
- [65] BARKEMA, H. W. et al. Invited review: The role of contagious disease in udder health. *Journal of Dairy Science*, v. 92, n. 10, p. 4717–4729, oct. 2009.
- [66] SCHREINER, D. A.; RUEGG, P. L. Relationship Between Udder and Leg Hygiene Scores and Subclinical Mastitis. *Journal of Dairy Science*, v. 86, n. 11, p. 3460–3465, nov. 2003.
- [67] TOLEDO-SILVA, B. et al. Metabolites of non-aureus staphylococci affect the ability of *Staphylococcus aureus* to adhere to and internalize into bovine mammary epithelial cells. *Veterinary Research*, v. 54, n. 1, 26/10/2023.

CONCLUSÃO

Os resultados deste trabalho evidenciam que tanto a endometrite quanto a mastite são enfermidades multifatoriais, fortemente influenciadas pela interação entre agente, hospedeiro, ambiente e manejo. A investigação em sistemas compost barn estabeleceu um limiar citológico relevante (8,3% de PMN) para o diagnóstico da enfermidade e, de forma complementar, revelou que a microbiota uterina pós-parto é diversa e que determinados gêneros bacterianos apresentam associações específicas com a inflamação uterina, enquanto as características físico-químicas da cama não se mostraram determinantes. A análise de ampla base laboratorial permitiu caracterizar a persistência de patógenos contagiosos e a influência de fatores climáticos sobre microrganismos ambientais causadores de mastite. Tais achados reforçam a importância de estratégias de vigilância epidemiológica, diagnóstico aprimorado e manejo adaptado às condições de produção para promover a saúde mamária e reprodutiva de vacas leiteiras.