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Assessing Milk Production and Quality during Mastitis Caused by a Variety of Pathogens in Dairy Cows

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Abstract
To affect future management efforts, understanding how mastitis affects milk at the genus-species level of the causing pathogen is critical. The goal of the current study was to establish a connection between a specific genus or species of pathogens that cause mastitis in cows and variations in the quantity and composition of their milk. A lone cow from a commercial dairy farm near Mount Gambier, South Australia, Australia, was the focus of the experiment (n = 2,676). On samples of milk, a standard microbiological culture was conducted. Using data from herd testing, production indicators for each cow, including yield, fat, total milk solids and protein percentages, and somatic cell count, were gathered into a database. The outcomes revealed isolated five different pathogens from milk at the genus or species level. The objective of the current study was to determine a relationship between a certain genuses or species of pathogens that cause mastitis in cows and changes in the amount and makeup of their milk. The quantity and quality of milk were impacted by each specific infection. S. aureus had the greatest impact on milk production. Similarly, Enterococcus species have the biggest impact on SCC. Escherichia coli, however, had a minor to insignificant impact on the amount and composition of milk. Because mastitis pathogens vary between geographic regions and historical periods, the findings of the current study will have an impact on how bovine mastitis is managed.

Keywords: Escherichia coli, Milk, Mastitis in cattle, Dairy sector

Introduction

The Multiple environmental and microbiological predisposing variables are among the numerous etiological agents that contribute to mastitis. Mastitis alone causes the dairy industry in the USA to lose 1.7 billion US dollars annually. Physical, chemical, and microbiological changes in milk, such as variations in the udder's color and hardness, changes in the milk's composition, and the presence of pathogenic microorganisms, can be used to diagnose clinical mastitis (1). The frequency of subclinical mastitis (SM) varies depending on the breed, age, lactation stage, and immunological state of the cows. It is commonly accepted that variations in milk production linked to SM rely on the type of bacteria that invades the mammary glands milk secretory epithelia (2). Breed, age, the health of the mammary glands, the stage of lactation, nutritional management, and season all affect the content of milk. Changes in milk protein and fat concentration are brought on by dietary control and compositional changes. Some key elements influencing milk's fat and protein composition include dry matter consumption, the amount of fiber available, and the diet's energy: protein ratio (3). By path of infection can be split into two major categories: infectious and environmental. Bovine submaxillary mucin (BSM) is typically manifested as an

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inflammation of the mammary gland and udder tissue as an immune response to the invasion of microorganisms, such as bacteria, yeasts, algae, and viruses, from the environment and passed from cow to cow (4).

Research had been done in the area to create evaluation tools for scientifically based well-being indices. Several years later, indicators for animals not included in Welfare Quality, such as horses, donkeys, turkeys, sheep, and goats, were established (5). Mastitis is the most common production disease in dairy herds globally and is well known to have a significant economic impact on dairy farms. In secretory cells of mammary alveoli, milk components are biosynthesized. Structure and function work together seamlessly. Milk production and the secretion of its constituent parts depend on the biochemistry and cellular structure at the level of the mammary alveoli. It is believed that the loss of alveolar cell integrity, cell sloughing, inducing apoptosis, and a rise in poorly differentiated cells are all symptoms of bacterial infection in mastitis cases (6). The most prevalent condition affecting dairy cattle, mastitis, results in significant direct and indirect losses. Mastitis, which can affect a variety of species of animals reared for milk production, is described as an inflammatory reaction brought on by an infection of the udder tissues. Mastitis can directly affect the quality of milk by altering both its chemical and physical characteristics. Initially, only Staphylococcus aureus and Streptococcus agalactiae were thought to be the etiological factors causing mastitis; however, subsequent research has revealed the presence of other bacterial species, fungi, mycoplasmas, and algae (7). The most common illness affecting dairy cows is SM, which can be recognized by an elevated somatic cell count (SCC), altered milk content, and reduced milk production. Short-term SM is more common than long-term SM, which is more often known as chronic subclinical mastitis (8).

The most expensive illness affecting dairy herds worldwide is mastitis. Mastitis also has an impact on animal welfare and results in financial losses since it reduces milk output, compromises milk quality, results in early culling, increases veterinary costs, and wastes milk due to antibiotic treatment. As a natural defense mechanism, the immune system is the primary source of milk somatic cells (9). Mastitis, which is characterized as mammary gland inflammation, is frequently a complex disease with multiple aetiologies. Mastitis, a somewhat common disease in dairy cattle, typically results from harm brought on by various pathogenic germs and natural bacteria. Mastitis can be categorized as either clinical or subclinical depending on the intensity of the inflammatory reaction. Clinical mastitis not only poses a threat to the health and wellness of animals but also significantly lowers milk output and quality, costing the dairy industry a significant amount of money. However, because there are no symptoms, subclinical mastitis instances are underreported. Additionally, estimates indicate that for every documented clinical case, there are 15 to 40 subclinical occurrences (10). Despite advances in technology and veterinary treatments, mastitis, an infection of the mammary gland, remains one of the major health problems and a substantial source of financial loss for cow farmers. Making the proper choices and implementing the right policies to lower internal and external factors that increase the risk of intramammary infection are necessary to protect high-yield dairy cows' mammary glands (11). The reliability of EV analysis concerning sampling time-point and spontaneous diseases is not well understood,

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nevertheless. Then, using small RNA-seq, we examined daily variations in miRNA cargo (12). Bovine mastitis is a serious condition that threatens the world's dairy sector and animal riches, causing significant financial losses. Gram stain, biochemical assays, colony shape, colony hemolytic activity, and microbiological cultures were used to identify and distinguish the isolates from milk samples (13). The calving interval and lengthening of the service time, both of which have an impact on the subsequent lactation's milk output due to their abnormal lengths, are mostly to blame for the financial losses. Controlling the occurrence of infections, especially mastitis, is one of the fundamental tasks of farmers. Another goal is to manage the reproduction of dairy cows (14).

A number of bacteria have the capacity to induce an immunological response that frequently results in spontaneous bacteriological clearance but can also result in persistent subclinical infections, leading to clinical mastitis. The use of antimicrobial therapy is particularly advantageous in circumstances when the pathogens involved have high rates of therapeutic cures but low rates of spontaneous healing (15). More than 150 species of pathogenic bacteria can cause bovine mastitis, a frequent illness seen on dairy farms. Streptococcus agalactiae, a frequent pathogenic bacterium that can be dangerous to both people and aquatic life, is another. S. agalactiae research is currently primarily concentrated in northern China, with sporadic studies conducted in the southeast and southwest. To direct the therapeutic use of antibiotics, drug-resistance genes, and susceptibility tests were found (16). Mastitis is a condition that is known to reduce productivity significantly and have a significant financial impact. Bovine mastitis is a topic on which there isn't much recent knowledge in the study area. In order to isolate the main pathogenic bacteria and evaluate the prevalence of bovine mastitis overall as well as its risk factors (17). To look for subclinical mastitis, aseptic milk samples from 395 dairy cows that had undergone the California mastitis test (CMT) were collected. With the aid of traditional bacterial isolation and identification techniques, samples that were both CMT-positive and CMT-negative were examined (18). Between 2010 and 2019, we gathered Gordonia microbes that were identified from dairy cows with mastitis milk samples. They discuss the frequency, virulence factors, and sensitivity to antimicrobial agents of these microorganisms as well as their growth characteristics (19). It is crucial to understand the mastitis-causing bacteria's properties in relation to their susceptibility to antibiotics. Data on the cows and the herds of origin, as well as clinical mastitis samples, were gathered between 2013 and 2018 (20). Assessing Milk Production and Quality during Mastitis Caused by a Variety of Pathogens in Dairy Cows is the topic of discussion in this section.

Materials and Methods

Sample gathering

Composite milk samples were taken aseptically from each functional quarter of individual cows at a single industrial dairy farm in South Australia, Mount Gambier (n = 2,676). The samples came from Holstein, and Holsteinx Jersey crossbred cows between the ages of 2 and 10. Four distinct samples were taken 48 hours after the herd testing. Samples were kept in the

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freezer and sent right away to the PC2 lab at the Roseworthy Campus of The University of Adelaide.

Details on milk production and its constituent parts

An archive of precise cow output yield markers was created using information from the four closest herd tests, including yield, sample, fat, total milk solids, and protein percentages, as well as somatic cell count. Using a FOSS Fossomatic 5000, SCC was carried out on each milk sample, and a commercial lab used instrumental milk component analysis. Mastitis pathogens are identified by microbial culture. According to National Mastitis Council recommendations, 10 l aliquots of milk samples were used in the laboratory for traditional microbial culture.

Statistic evaluation

Statistical Analysis Software, Cary, North Carolina, USA, version 9.4, was used for all statistical analyses. Some data were modified before analysis.

1. A natural log was used to log-transform the somatic cell numbers. Use a mixed model to investigate the impact of the pathogen found in milk samples on the factors affecting milk output, as shown by Equation 1 in PROC MIXED.
2. Analysis of the impact on milk parameters was done without mixed isolates (n = 812; 30.3%; Table (1)).
3. With mixed isolates excluded from the analysis (n = 812; 30.3%; Table 1), the impact on milk parameters was examined.

Use a mixed model, as shown by Equation 1 in PROC MIXED, to investigate the impact of the pathogen found in milk samples on the factors affecting milk output.

$$Milk\ production\ parameter\ =\ genus/species\ of\ pathogen\ isolated \qquad (1)$$

Where the cow is the cows estimated genetic worth. The preliminary model examined the impact of the sample day but was excluded from the final model because it was determined that it was not a significant confounder. The example produced related standard errors, least-square means, and changes between least-square means, among other things. The significance threshold was chosen at P < 0.05. Equation 2 exemplifies how to utilize PROC GLIMMIX's linear regression function to evaluate how the milk somatic cell count is impacted by the pathogen found in milk samples.

$$Log(somatic\ cell\ count\ score) = genus/species\ of\ pathogen\ isolated \qquad (2)$$

Although they were shown to be insignificant confounders, the predicted cow genetic value and the sampling day were also evaluated in the preliminary model.

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Results and discussion

One dairy farm in South Australia had a history of treatment failure; the current analysis was carried out. The study's objective was to determine how frequently mastitis-causing bacteria cultures occur in relation to the makeup of milk. Each functional quarter's four different microbiological culture of milk tests yielded results that are displayed in Table (1). Following mixed growth, CoNS came in second with n=418. The least amount of Enterococcus spp. was produced, with n=122. Because we had highlighted them, our study did not include any conclusions related to mycoplasma mastitis. According to routine milk microbiology tests, the overall incidence of mastitis was 59.4% at the cow level. The current study's findings were similar to those of prior Australian research and less so to those of studies conducted recently on other continents. But it's important to recognize that mastitis pathogens evolve over time and across geographic boundaries, mostly as a result of modifications to mastitis management techniques and milking practices.

Table (1): Results of microbial culture distribution

Pathogen	Number	Percentage	Percentage from Speciated isolates
Mixed growth	812	30.3	-
Streptococcus aureus	231	8.6	-
Escherichia coli	134	5.1	-
Enterococcussspp	122	4.6	-
Staphylococcus spp	152	5.7	-
Coagulase-negavtivestaphylocacci	418	15.6	-
No expansion	807	30.1	68.6
Total number of pathogen species and absence of growths	2676		

A recent study found that bacterial infections that are mixed, or more than one isolate per sample of milk, are to blame for roughly one-third of mastitis instances. Additional analysis and surveys that reflect the disease's advanced stage reinforce this finding. Some researchers, however, disregarded the combination infection mastitis and thought it was contamination. These studies, however, do not provide data that correlates with the component status of milk. The failure of multiple mastitis treatments on this farm is most likely interpreted by the co-infection proliferation of two or more pathogens. The collecting of composite samples at the cow level might have also led to mixed growth because various quarters would have developed unique illnesses. The determination of the pathogen profile is essential for the management and treatment of mastitis. The mastitis-causing bacteria were found to be most prevalent in CoNS, followed by mixed infections. Despite some patients having clinical mastitis indications, routine milk culture did not produce growth in 30.2% of the cases in the current investigation. A lower incidence of no growth in clinical instances has been recorded

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by others. The current study found that mastitis-causing bacteria and milk supply and composition were positively correlated in affected cows.

Table (2): Factors affecting milk production and somatic cell numbers

Pathogen	**Parameter**	**Milk fat**	**Milk protein**	**Milk volume**	**Milk solids**	**Somatic Cells**
	Unit	**percent**	**percent**	**litre**		**Cells(thousands)/ml**
Escherichia coli		3.23	3.2	37.69	2.32	147
Enterococcussspp		2.76	3.33	28.55	1.77	273
Staphylococcus spp		3.81	3.51	32.29	2.14	167
Coagulase-negavtivestaphylocacci		3.35	3.26	34.78	2.23	125
No growth		3.01	3.16	36.38	2.11	48
Streptococcus aureus		3.19	3.55	21.48	1.35	258

Table (2) provides the 1,864 cows and the associated 95% confidence intervals for somatic cell counts and milk production metrics for those whose milk samples contained particular infections at the genus or species level or did not demonstrate growth. It was considered that no growth results came from sick cows. Figure (1) shows the comparison of milk volume and milk solids in litres.

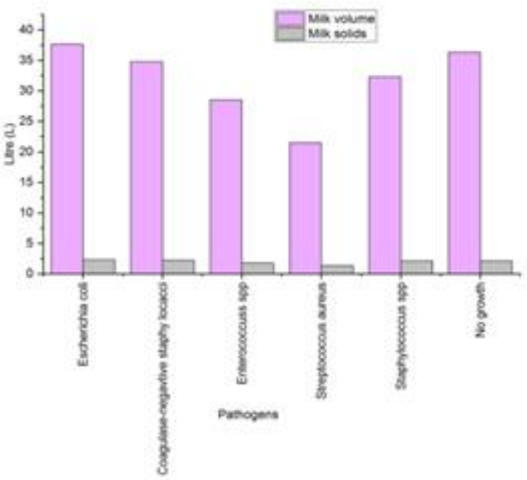

Figure (1): Comparison of Milk volume and Milk solids

It is difficult to understand how the mastitis pathogen invasion affects milk output. Numerous pathogens- or host-related factors, such as microbial load, alveoli exposure, alterations in the cellular metabolism of mammary gland cells, blood vessel damage, hormonal imbalance, milk decomposition caused by the presence of pathogens and leukocyte-released enzymes, and disruption of epithelial integrity, can have an impact on the composition of milk. Figure (2) depicts the Percentage of milk fat and milk protein.

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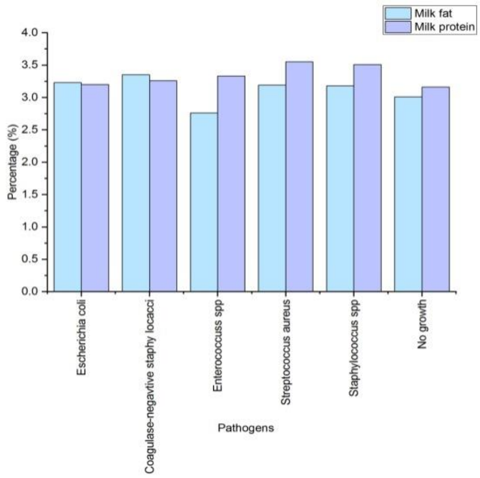

Figure (2): Percentage of milk fat and milk protein

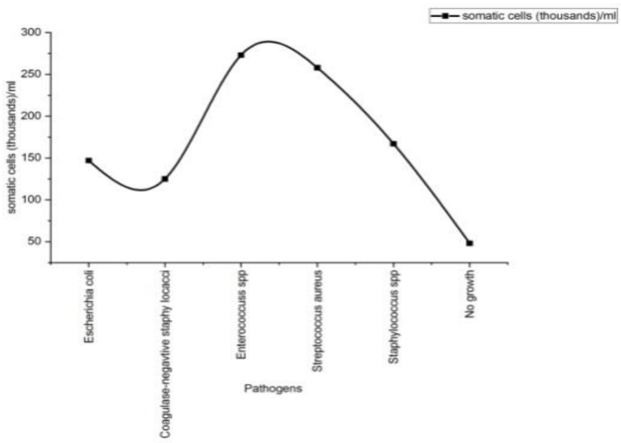

Figure (3): Milk somatic cell count

Curiously, the current study's findings underlined the relationship between speciated infections and milk composition. Figure (3) represents the somatic cell count milk.Similar findings from earlier research have been found. Before a final judgment can be formed, further research must be conducted in this area. E coli are one of the most common organisms that cause acute and chronic mastitis, both of which are sub-clinical as opposed to clinical. When compared to other prevalent mastitis pathogens, E. coli has the least detrimental effects on SCC. The fact that the degree of pathogen invasion, stage of breastfeeding, immunological state, level of energy, and vitamin deficit might all affect E. coli invasion of the mammary gland suggests this. Furthermore, there may have been little to no effect of E. coli-related mastitis on milk components in the current experiment due to the low severity of the E. coli infection and the insignificant mammary tissue inflammatory response. How the disease progresses in E. coli, mastitis depends critically on the host's level of protection. For the infection to be cured and the outcome of E. colimastitis, neutrophils must be able to efficiently eradicate the disease. The investigation's findings showed a connection between all detected mastitis pathogens at the genus and species levels and milk loss caused by

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mastitis. CoNS had a high impact on the milk SCC, comparable to Streptococcus spp. Caused. CoNS have previously demonstrated a variety of effects on changing milk components. These intriguing findings implicate the CoNS in the bigger impacts on milk yield and content as well as the SCC. Studies, where CoNS will not be merged but will instead be presented at the species level are necessary due to the possibility of differences in the pathogenic effect between species. The highest SCC reaction was induced by S. aureus and Enterococcus species. Given that both have been identified as significant mastitis pathogens, this is hardly surprising.

Conclusion

In this study monitoring milk output and quality in dairy cows suffering from mastitis brought on by a range of infections is essential for ensuring the herd's general well-being and productivity. Milk output, milk quality, and financial losses for dairy farmers are all impacted by the widespread and expensive condition known as mastitis, which affects dairy cows worldwide. Farmers can effectively manage mastitis and lessen its effects on milk output and quality through rigorous assessment and monitoring. The exact pathogens causing mastitis are identified, the intensity and duration of the infection are assessed, and the efficacy of various treatment procedures is determined. Regular milk yield monitoring is necessary for evaluating milk supply since mastitis can significantly lower milk production due to decreased milk letdown and gland damage. In order to identify changes in SCC, bacterial contamination, and the presence of unusual milk components like blood or pus, evaluating milk quality is also necessary. The severity of the mastitis, the cow's response to therapy, and any potential effects on the milk's safety and marketability are all shown by these characteristics, which are very useful.

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