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Assessment of Milk Yield and Composition During Bovine Mastitis Caused by a Variety of Pathogens

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Abstract

A better understanding of milk alteration during mastitis at genus-species level of causative pathogen is essential to guide prospective control strategies. The current study aimed to identify the correlation between individual genus or species of pathogens causing bovine mastitis and alteration of milk volume and composition. The study was conducted at individual cow level (n=2,676) from a commercial dairy farm near Mount Gambier, SA, Australia. Milk samples were subjected to conventional microbial culture. A database of individual cow yield production parameters (yield, total milk solids, fat and protein percentage) and somatic cell count (SCC) was obtained from herd testing information. Results showed five individual pathogens at genus or species level isolated from milk. Mixed growth was the most common (30.3%) followed by Coagulase negative Staphylococci CoNS (15.6%), *Staphylococcus aureus* 231 (8.6%), *Streptococcus* spp. 152 (5.7%), *Escherichia coli* 134 (5.1%), *Enterococcus* spp. 122 (4.6%), and there was "No growth" in 807 (30.2%) samples. Milk quality and quantity were affected by all individual pathogens. *S. aureus* showed the highest effect on milk yield. Similarly, *Enterococcus* spp. has the most significant effect on SCC. However, *Escherichia coli* revealed mild to non-significant effect on milk volume and components. Mastitis pathogens varies across regions and times, and the implications of the current study will contribute to the control of bovine mastitis.

Keywords: Mastitis, Cattle, Dairy industry, Milk, Escherichia coli,

Introduction

Mastitis in cattle poses a significant impact on the dairy industry worldwide. Milk quality and quantity can be affected by major and minor mastitis pathogens. Economic consequences of mastitis arise from the reduction in milk supply, milk decomposition, culling of infected cows, cost of veterinary surveillance, challenges of drug residue and potential risks to human health (Seegers et al. 2003, Petrovski et al 2006).

The Australian dairy industry is one industries with a cosmopolitan importantce. Population of Australian dairy cattle is estimated at 2.6 million according to Australian Bureau of Statistics, 2018. The Australian dairy herds reported various intensity of clinical and subclinical bovine mastitis across the continent over the past decades. (Daniel et al, 1982; Gunn et. al. 1999; Shum et al 2009; Al-Farha et. al 2017; Al-Farha et. al 2018). These studies showed various pathogen profiles. It is common knowledge that mastitis pathogens vary across genera and geographies. Therefore, studying their prevalence and correlation with milk components is essential in updating control strategies.

Somatic cell counts (SCC) are widely used as a detector of mastitis. SCC is mainly composed of various immunological cells such as neutrophils, macrophages and epithelial cells (Halasa & Kerkeby, 2020). In addition,

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SCC has been used as an integral component of control strategies for mastitis (Ogola et. al. 2007). Based on previous Australian electronic herd data, SCC averaged 100,000 to 500,000 cell/mL (Dyson et. al. 2022). SCC are often elevated with all pathogenic bacteria for both heifers and multiparous cows (De Hass et. al. 2002).

The drop in milk yield relates to the pathogen-specific mastitis. Based on which bacterial genus or species caused the infection, certain kinds of mastitis pathogens appear to be more virulent than others (Gröhn et al. 2004). Drop in milk yield started two weeks prior to detection of the infection. Some researchers classify mastitis etiological agents to major and minor pathogens (Reyher, et. Al. 2012). Mastitis infection-specific patterns of milk quality and quantity dependent on the causative pathogens has received very little attention.

The current study aimed to investigate prevalence of routinely cultured mastitis-causing pathogens and estimate their effect on milk yield and composition in a single South Australian farm.

Methods

Sample collection

Composite milk samples milk samples (n=2,676) from each functional quarter of individual cows were collected aseptically from a single commercial dairy farm near Mount Gambier, South Australia. Samples originated from cows aged 2 - 10 years of Holstein and Holstein x Jersey cross breeds. Samples were collected on four occasions within 48 hours of the herd testing. Samples were kept on ice and were sent immediately to the PC2 laboratory at The University of Adelaide, Roseworthy Campus.

Milk production and components data

A database of individual cow yield production parameters (yield, total milk solids, fat and protein percentage) and somatic cell count (SCC) was obtained from herd testing information for the 4 herd tests closest to the sampling. SCC was performed for each milk sample using a FOSS Fossomatic 5000 (Hillerød, Denmark), and instrumental milk components assay by a commercial laboratory (NHIS, Cohuna, VIC, Australia).

Microbial culture and identification of mastitis pathogens

In the laboratory, milk samples were subjected to conventional microbial culture using 10 µL aliquots according to National Mastitis Council guidelines [32].

Statistical analysis

All statistical analyzes were performed in SAS version 9.4 (Statistical Analysis Software, Cary, NC, USA). Prior to analysis, some data were manipulated.

- Somatic cell counts were log transformed using a natural log.
- Mixed isolates were eliminated from analyzes of the effects on milk parameters (n= 812; 30.3%; Table 1)
- Pathogens identified to genus level were eliminated from analyzes, addressing species level effects (n= 274; 10.2%; Table 1); NOTE: Although not speciated, the coagulase-negative staphylococci (CoNS) were retained

The effect of the pathogen isolated from milk samples on the milk production variables was estimated using a Mixed model in PROC MIXED, as presented in Equation 1:

Equation 1: $milk\ production\ parameter = genus\ /\ species\ of\ pathogen\ isolated_{cow}$

where cow= estimated genetic value of the cow. The preliminary model also tested the effects of sampling day, but was found to be not significant as a confounder and hence, not included in the final model. The outputs of the model were least-square means, their respective standard errors, and differences between least-square means. The level of significance was set ap $P<0.05$.

The effect of the pathogen isolated from milk samples on the milk somatic cell count was estimated using linear regression in PROC GLIMMIX as presented in Equation 2:

Equation 2: $\log(somatic\ cell\ count\ score) = genus\ /\ species\ of\ pathogen\ isolated$

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The preliminary model also tested the effects of estimated cow genetic value and sampling day, but were found to be not significant as confounders. The outputs of the model were the geometric means of the somatic cell count and their respective 95% confidence intervals.

Results and Discussion

The current study was conducted on a single dairy farm in South Australia, where repeated treatment failure was observed. The study aimed to investigate the prevalence of routinely-cultured mastitis-causing pathogens and estimate their effect on milk composition. Isolated pathogens in the current study included CoNS, *Escherichia coli, Enterococcus* spp., *Staphylococcus aureus, Streptococcus* spp., and mixed growth. Table (1) presents the results of microbial culture of milk tests collected from each functional quarter on four occasions. Mixed growth (when two pathogens have been present concurrently) was recorded as the most predominant group n=812 (30.3%), followed by CoNS n=418 (30.3%), whereas *Enterococcus* spp. Were yielded at the lowest rate n=122 (4.6%). Results related to *Mycoplasma* mastitis were excluded from the current study as they were highlighted in our previous work (Al-Farha et. al. 2020a, Al-Farha 2020b, Al-Farha et. al. 2017). Based on routine microbial culture of milk, the overall occurrence of mastitis was 59.4% (1,590/2,676) at the individual cow-level. The findings of the current study were comparable with other Australian studies and less to other recent continents' studies (Mbindyo et al. 2020). However, it is critical to understand that mastitis pathogens vary across time and regions mainly because of the differences in milking practices and mastitis management strategies.

Table 1. Distribution of microbial culture results for the 2,676 samples collected on four occasions from the single farm in South Australia

Pathogen	Number	Percentage	Percentage from speciated isolates
Escherichia coli	134	5.1	11.4
Coagulase-negative staphylococci	418	15.6	NA
***Enterococcus* spp.**	122	4.6	NA
Staphylococcus aureus	231	8.6	19.7
***Streptococcus* spp.**	152	5.7	NA
Mixed growth	812	30.3	NA
No growth	807	30.2	68.9
Total number of speciated pathogens and no growths		**2,676**	**1,590**

Results of the current study indicate that almost one-third of mastitis cases have mixed bacterial infections, i.e. more than one isolate per each milk sample has been yielded. This finding is validated by other studies and surveys (Koop et al 2010; Bandyopadhyay et al. 2015; Pașca et. al 2020) which reflect the advanced phase of the disease. Several studies, on the other hand, ignored the mix infection mastitis and considered it contamination. However, these studies lack correlated data with the milk component status. It is likely that the co-infection growth by two or more pathogens interprets perhaps the failure of repeated mastitis treatment on this farm.

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Mixed growth may also have resulted due to the collection of composite samples at cow level, which means different quarters would have yielded different pathogens.

For mastitis control and management, it is crucial to determine pathogen profiles. CoNS was found to have the highest prevalence of individual mastitis-causing pathogens, after the mix infections. Of the CoNS isolates yielded in the current study, fourteen were speciated as metacillin-resistant and were genomically assessed through whole genome sequence in a previous study (Khazandi et al. 2018). CoNS are responsible for subclinical and mild mastitis cases (Jaskelainen et al. 2013). Pathogenic effect of CoNS in subclinical bovine mastitis can be attributed to enterotoxin gene and other antimicrobial resistant factors (Fry et al 2014; Khazandi et al. 2018).

In the current study, routine milk culture yielded no growth in 30.2% of cases, although some had clinical mastitis signs. Similar to the current findings, Dyson et al. (2022) reported approximately one third of the cases in clinical and subclinical mastitic cows in various regions in south-east Australia. Others have reported lower incidence of no growth in clinical cases (Petrovski et al 2009).

In the current study, infected cows showed a positive correlation between mastitis-causing pathogens and milk yield and composition. Table 2 presents the milk production parameters and somatic cell counts with their respective 95% confidence intervals for the 1,864 cows whose milk samples yielded individual pathogens at genus or species level or no growth. 'No growth' results were assumed to originate from cows that are not infected. The mechanism of milk production alterations during the invasion of the mastitis pathogen is complicated. Several pathogen- or host-related factors can influence milk composition including microbial load, number of alveoli exposed, changes in cellular metabolic activities of mammary gland cells, damage of blood vessels, hormonal imbalance, milk decomposition due to the existence of enzymes released from pathogens and leukocytes, and epithelial integrity interruption (Petrovski 2006). Interestingly, the results of the current study highlighted the correlation between speciated pathogens and milk composition. Previous study has shown similar results (Grohn et al 2005). This is an area that needs further research before a final conclusion can be made.

Table 2. Means with their respective standard errors of the milk production parameters and somatic cell counts with their respective 95% confidence intervals for the 1,864 cows whos milk samples yielded individual pathogens at genus or species level or no growth

Pathogen	**Parameter**	**Milk fat**	**Milk protein**	**Milk volume**	**Milk solids**	**Somatic cells**
	Unit	Percent	Percent	Litre		Cells (thousands) / mL
Escherichia coli		3.17 ± 0.06	3.18 ± 0.02	36.54 ± 1.15	2.25 ± 0.07	147 (118 – 182)
Coagulase-negative staphylococci		3.32 ± 0.03	3.25 ± 0.01	34.09 ± 0.69	2.19 ± 0.04	125 (111 – 142)
***Enterococcus* spp.**		2.68 ± 0.08	3.32 ± 0.01	28.54 ± 0.01	1.71 ± 0.06	273 (218 – 343)

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Staphylococcus aureus	3.15 ± 0.04	3.54 ± 0.01	20.76 ± 0.72	1.34 ± 0.01	258 (219 – 304)
***Streptococcus* spp.**	3.72 ± 0.09	3.48 ± 0.03	31.05 ±1.24	2.07 ± 0.07	167 (136 – 204)
No growth	2.98 ± 0.03	3.15 ± 0.01	35.85 ± 0.53	2.09 ± 0.02	48 (44 – 53)

E coli is one of the predominant pathogens responsible for sub-clinical rather than clinical mastitis (Hingthong et. al. 2017) with two phases, acute and chronic mastitis. However, limited cases of *E. coli* mastitis in the current study were found and effects on milk yield and composition were not severe in single infections. *E. coli* invasion of the mammary gland could be influenced by the intensity of the pathogen invasion, lactation stage, the immune status, energy and vitamin decencies (Cobirka et. al. 2020). A study by de Haas et. al. 2002 indicates that *E. coli* mastitis has the lowest effects on SCC compared to other common mastitis pathogens. Furthermore, in the current study, limited or non-significant influence of *E. coli* related mastitis on milk components may have resulted from the mild severity of *E. coli* infection and the insignificant mammary tissue inflammatory response (Blum et al. 2020). The defense status of the host during *E. coli* mastitis is a critical component in deciding the disease's fate. The ability of neutrophils to effectively eliminate the infection is critical for the cure of infection and the fate of *E. coli* mastitis (Burvenich et. al 2003).

Findings of the current study revealed that milk loss was detected by mastitis associated with all isolated mastitis pathogens at genus and species level. A high effect on the milk SCC was caused by CoNS, similar to the one caused by *Streptococcus* spp. In the past, CoNS have shown various effect on alteration of milk components. This may have occurred due to variability between the species or the immune response by the cow. Indeed. These findings of the higher effects on milk yield and composition, and the SCC, being associated with the CoNS are intriguing. The potential in differences of the pathogenic effect between species require studies where CoNS will not commingled but reported at a species level. *Enterococcus* spp and *S. aureus* resulted in the highest SCC response. This is not surprising having both listed as major mastitis pathogens. Interestingly, 'no growth' resulted in very low SCC of just 48,000 cells/mL that is lower than most previous reports saying non-infected cows have SCC of 100,000 cells/mL or even higher at an average (e.g., Andrews et al. 1983, Barkema et al, 1999).

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