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Incidence and Effected Factors of Clinical Mastitis on Dairy Cows Raised in Farms in Central and East-Southern Vietnam

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ABSTRACT

A total of 20,823 Holstein × Lai Sind crossbred dairy cows from three farms in Central and East-Southern Vietnam were examined to clarify the prevalence of clinical mastitis and evaluate its effects from July to October 2022. The results indicated that clinical mastitis in cows was present at a relatively low rate (7.05%), and the high-density farms had a higher rate of clinical mastitis in cows than in other farms. In a hot, humid month, the incidence of clinical mastitis in cows (7.78%) was higher than in other months in this study, especially in the high-density farm. Cows at the first parity were clinical mastitis (32.58%) higher than at other parities, followed by cows at the second parity. As indicated, farm scale, months, and parity affected the incidence of clinical mastitis in cows in those survey farms. Clinical mastitis caused the fluctuation in milk yield, which was determined at 15.45±8.67 to 18.44±0.72 kg/cow/day; moreover, the milk yield of clinical mastitis cows in the large-scale farm showed a significant fluctuation. Among examined bacterial pathogens, *Streptococcus agalactia* (34.65%) was the most detected from milk samples of clinical mastitis cows, followed by *Klebsiella* (14.47%) and *Escherichia coli* (10.09%), but *Staphylococcus* spp. was present at the lowest rate (0.88%). The treatment followed the veterinarian's guidelines and was adequate for clinical mastitis cows in this study within five days (79.15%). Thus, mastitis management in dairy farms is significantly required to prevent disease spreading in farms, especially in high-density herds.

Key words: Bacterial pathogens, Clinical mastitis, Dairy cows, Milk yield, Vietnam

INTRODUCTION

Livestock farming, especially dairy farming, has been increasingly expanding and developing in quantity and scale in Vietnam. According to estimates from the General Statistics Office of Vietnam, as of July 2021, the total of cow herds in the country was estimated to reach more than 6.3 million heads, of which there were about 331 thousand dairy cows, an increase of 4.29% and fresh milk output reached nearly 1 million 49 thousand tons, an increase of about 6.4% over the same period in 2020. However, the problems encountered in dairy farming were infectious diseases and other internal diseases, such as mastitis, that affected milk output and quality (Nguyen et al. 2020; Nguyen et al. 2023). Mastitis is a common disease of dairy cows, accompanied by physical, chemical, pathological, and bacteriological changes in milk and glandular tissue (Tezera and Ali 2021; El-Demerdash et al. 2023). Mastitis reduces the milk output of cows by 20-30%, significantly affecting livestock productivity, especially subclinical and clinical forms of mastitis (Bekuma and Galmessa 2019; Nguyen et al. 2020; Mohsin et al. 2022; Nguyen et al. 2023). If the mastitis disease in cows continues to exist without prompt and timely treatment, the udder leaves will undergo atrophy, resulting in the inability to produce milk. It will cause a negative impact on the productivity and quality of milk in the subsequent offspring and could potentially lead to the elimination of those cows.

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(Chandra et al. 2011; Zigo et al. 2021; Meçaj et al. 2023). Research by Khasanah et al. (2021) indicated that 68.18% of dairy cows in East Java, Indonesia, had subclinical mastitis. In Pakistan, Ali et al. (2021) reported that 17 and 57% of cows in the Northwest area had clinical and subclinical mastitis.

Cobirka et al. (2020) reported that dairy farmers must prioritize the prevention of mastitis infections by diligently following a mastitis control program. Unfortunately, in many cases, farmers tend to address the issue only after mastitis has already occurred. However, few reports of the prevalence of mastitis in dairy cows have been published recently in Vietnam, especially in Central and East-Southern Vietnam, where the herds have been increasing significantly in the last decade. On the other hand, some severe clinical mastitis cows could weaken and lead to death. Mastitis has many different causes, in which bacteria invade and cause clinical mastitis. In addition, subclinical mastitis could also lead to clinical mastitis, affecting the economic efficiency of dairy farming. Several factors have been implicated in clinical mastitis in dairy cows, including bacterial, mycoplasmal, and yeast pathogens (Egwu et al. 1994). Moreover, cows with chronic positives caused by several pathogens had a significant decrease of at least 24.5% in milk yield and 22.4% in total solids yield (Martins et al. 2020). Bacterial pathogens were significant reasons involved in the etiology of mastitis. Among them, *Staphylococcus* spp. was the primary etiological agent of clinical and subclinical mastitis in cows. At the same time, *S. aureus* and *E. coli* were frequently isolated pathogens from clinical mastitis (Contreras et al. 2003). More than 135 bacterial species had been related to bovine mastitis, and 20 pathogens were most frequently detected in mastitis dairy animals (Bradley 2002; Gao et al. 2017). Ali et al. (2021) revealed that *Staphylococcus* spp. (34%) were the most predominant bacterial pathogens isolated from mastitic milk, followed by *Escherichia coli* (19.4%), *Streptococcus* spp. (9%), and *Klebsiella* spp. (8%). Those pathogens caused diseases in animals and affected the quality of milk. The isolation and identification of those pathogens have been essential for treatment and prevention in dairy farms.

Therefore, this study aimed to clarify the incidence of clinical mastitis and the prevalence of pathogens in dairy cows in Central and East-Southern Vietnam. These results provided valuable information about treating and preventing clinical mastitis in those regions and Vietnam.

MATERIALS AND METHODS

Sample collection

A total of 20,823 Holstein × Lai Sind crossbred dairy cows of all ages were examined in this study, including Farm 1 (n=4,275) and Farm 2 (n=16,194) in Central Vietnam and Farm 3 (n=354) in East-Southern Vietnam from July to October 2022 (rainy season in Vietnam). The cows were housed in a free stall barn and fed a total mixed ration silage throughout the year.

Cows were machine milked at 9:00 a.m. and 3:00 p.m., and milk yield was individually recorded. Those mastitis cows were recorded after clinical examinations and CMT tests (California Mastitis Test) at those farms following manufacturers' instructions (DeLaval, USA). Those records were summarized to determine the milk yield of clinical mastitic cows in those farms. Mastitis cows in Farm 2 and Farm 3 were chosen to examine the milk yield in this study.

The Animal Ethic Committee, Nong Lam University, Ho Chi Minh City, and Can Tho University, Can Tho City accepted animal experiment procedures.

Isolation and identification of bacterial pathogens in the mastitic milk

Each milk sample (25mL) was collected after cleaning the surface of the udder, and the foremilk was discarded before collecting the sample. Milk samples were taken manually from four udders and then mixed to produce a composite sample. All the samples were kept on ice during laboratory transportation and stored at –20°C until required for further analyses.

In this study, a total of 228 milk samples were collected from clinical mastitic cows in Farm 1 to detect the major bacterial pathogens, including *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis*, *E. coli*, and *Klebsiella* spp. Those milk samples were collected and transported to the laboratory to detect bacteria within 24 hours.

Then, milk samples were cultured on Blood Agar with 5% defibrinated horse blood (BA, Oxoid, UK), Mannitol Salt Agar (MSA, Merck, Germany), and MacConkey agar (MC, Merck, Germany) to detect those bacteria. After incubations at 37ºC and 24h, suspicious colonies were cultured on Nutrient Agar (NA, Merck, Germany) and tested for biochemical properties of those bacteria following the guidelines of Barrow and Feltham (2003).

Evaluation of treatment of mastitis cows

The clinical mastitic cows in Farm 1 were treated following the veterinarians' guidelines, including antibiotics, anti-inflammation drugs, and supplements. Those cows were observed for ten days, and the recovery of those cows was recorded. The effectiveness of the treatment was evaluated by checking clinical examinations and milk samples with the CMT test for negative results.

Statistical analysis

In statistical analysis, data were analyzed by one-way ANOVA, Tukey pairwise comparisons, and Chi-Square Test to examine the effect of factors in Minitab 19.0 software. The statistical differences were significant at $P<0.05$.

RESULTS

Incidence of mastitis in dairy cows

The incidence of clinical mastitis in cows (Table 1) significantly differed among the studied farms ($P<0.05$). The rate of cows infected with clinical mastitis in Farm 2 was the highest (7.69%); however, there was no difference between Farm 1 (4.94%) and Farm 3 (2.82%). On the other hand, the number of mastitic cows on Farm 2 was higher than in cows on other farms each month ($P<0.05$). It indicated that the high density of cattle on the farm could affect mastitic cows in the herds.

Besides, the rate of cows infected with mastitis was different among those observed months ($P < 0.05$).

Table 1: Incidence of clinical mastitis in dairy cattle in the experimental time

Month	No. of clinical mastitic cows/No. of cows (%)			
	Farm 1	Farm 2	Farm 3	Total
July	50/1,076 (4.65)	290/4,052 (7.16)	3/89 (3.37)	343/5,217 (6.57)^{bc}
August	56/1,089 (5.14)	338/3,895 (8.68)	1/91 (1.10)	395/5,075 (7.78)^{a}
Septemper	53/1,066 (4.97)	339/4,049 (8.37)	2/89 (2.25)	394/5,204 (7.57)^{ab}
October	52/1,044 (4.98)	279/4,198 (6.65)	4/85 (4.71)	335/5,327 (6.29)^{c}
Total	211/4,275 (4.94)	1,246/16,194 (7.69)	10/354 (2.82)	1,467/20,823 (7.05)

Different superscript letters indicate a statistical difference among these rates (P<0.05).

In August, the number of cows infected with clinical mastitis was at the highest rate (7.78%), followed by cows examined in September (7.57%), July (6.57%), and October (6.29%). In detail, at Farm 1 and Farm 3, there was no difference in the prevalence of clinical mastitis in cows during those months (P>0.05); however, the number of clinical mastitic cows in Farm 2 was also at the highest rate (8.68%) in August (P<0.05), followed by cows in September (8.37%), July (6.57%), and October (6.29%).

In general, cows at parity 1 had a clinical mastitic rate (32.58%) higher than other parties (P<0.05) and had a decreasing tendency in the following parties (Table 2). However, cows infected with clinical mastitis in parity 2 were higher than others in Farm 1. Therefore, the parity was a factor that could affect the rate of clinical mastitis infection in cows. During the experimental period, the milk yield of cows in Farm 2 and Farm 3 was at an average of 15.45±8.67 and 18.44±0.72 kg/cow/day (Table 3). They were lower than the average of healthy cows at each farm. The milk yield of cows in Farm 2 showed a significant fluctuation among cows.

The prevalence of bacterial pathogens in clinical mastitic milk samples

The results of the isolation of bacteria in clinical mastitic milk samples (Fig. 1) indicated that those samples were contaminated with *Strep. agalactiae* (34.65%) at the highest rate, followed by *Klebsiella* (14.47%), *E. coli* (10.09%), *Strep. uberis* (8.33%), *Stap. aureus* (0.88%), and other Gram-negative bacteria (1.32%). It revealed that those bacteria were the major pathogens causing clinical mastitis in those cows.

Effectiveness of treatment in clinical mastitis cows

The mastitic cows in Farm 1 were treated according to the veterinarians' guidelines; the results (Table 4) showed that most infected cows recovered after five days of treatment (79.15%). Other cows recovered within ten days (16.59%) and over ten days of treatment (4.27%). Moreover, the recovery rate of clinical mastitic cows varied each month. It could depend on the clinical mastitis types in those cows when examined before treatment. However, these results indicated that the treatment course used at Farm 1 effectively treated clinical mastitic cows.

DISCUSSION

In this study, the rate of clinical mastitis in cows was relatively low (7.05%) in total of the surveyed farms. However, various factors could affect the prevalence of clinical mastitis in those examined farms. In the high-density farm (Farm 2), the rate of clinical mastitis in cows was higher than in other farms because those cows directly contacted each other in the barns or were contaminated with pathogens in the husbandry environment. Blowey and Edmondson (2000) reported that environmental clinical mastitis could occur due to the larger herd sizes, the pressures on the environment during the housed period, and the higher milk flow rates associated with higher yields. In another research by Workineh et al. (2002), the California Mastitis Test (CMT) and bacterial culturing revealed that 21.5% of the cows were clinically infected and 38.2% had subclinical mastitis in Ethiopian dairies. Moreover, the higher rate of clinical mastitis in cows in Farm 2 was also in each month compared to the two left farms (P<0.05). It indicated that the high-density herd was an essential factor

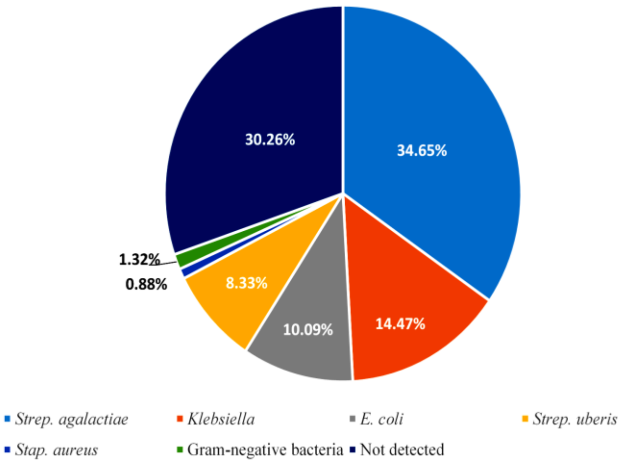

Fig. 1: Prevalence of pathogens in milk samples of clinical mastitic cows (n=228).

Table 2: Incidence of clinical mastitic cows on parity

No. of parity	No. of clinical mastitic cows (%)			
	Farm 1 (n=211)	Farm 2 (n=1,246)	Farm 3 (n=10)	Total (n=1,467)
1	31 (14.69)	444 (35.63)	3 (30.00)	478 (32.58)
2	77 (36.49)	260 (20.87)	3 (30.00)	340 (23.18)
3	55 (26.07)	201 (16.13)	-	256 (17.45)
4	33 (15.64)	142 (11.40)	4 (40.00)	179 (12.20)
5	8 (3.79)	77 (6.18)	-	85 (5.79)
6	7 (3.32)	82 (6.58)	-	89 (6.07)
7	-	40 (3.21)	-	40 (2.73)
				(P<0.05)

Table 3: The milk yield of clinical mastitic cows in this study

Month	Milk yield (Mean±SD) (kg/cow/day)	
	Farm 2 (n=1,246)	Farm 3 (n=10)
July	15.42±7.86	18.27±0.90
August	14.63±8.94	17.31±0.60
September	14.50±8.39	18.65±0.58
October	17.24±9.49	19.62±0.78
Average	15.45±8.67	18.44±0.72
	(P>0.05)	(P>0.05)

Table 4: The effectiveness of treatment in clinical mastitic cows in Farm 1.

Month	No. of treated cows	Result of treatment					
		Treatment ≤ 5 days		Treatment of 5 days < X ≤ 10 days		Treatment > 10 days	
		No. of cows	Percentage (%)	No. of cows	Percentage (%)	No. of cows	Percentage (%)
July	50	45	90.00	4	8.00	1	2.00
August	56	42	75.00	11	19.64	3	5.36
September	53	44	83.02	7	13.21	2	3.77
October	52	36	69.23	13	25.00	3	5.77
Total	211	167	79.15	35	16.59	9	4.27

causing clinical mastitis in cows in those regions at any time. Therefore, it should have a hygiene management system to prevent contamination and infection in dairy farms, especially crowed herds.

The experimental period in this study was the rainy season, with a high temperature and humidity in Central and Southern Vietnam. During months, the incidence of clinical mastitis in cows in August (7.78%) was primarily higher than in other months at all surveyed farms. This is a high-temperature month; therefore, the cows could be more stressed or infected with multiple pathogens in the environment. Boujenane et al. (2015) also reported that cows calved from July to September showed the highest number of clinical mastitis cases compared to other months. Jingar et al. (2014) reported in research conducted in India that the hot, humid climate could adversely affect the incidence of clinical mastitis in cows. Rahman et al. (2009) published that 6.8% of quarters in the dry season and 18.7% of quarters in the wet season were affected with mastitis, which depended on the season. However, there were no differences in the prevalence of mastitis in cows among herd sizes in the wet season. In the present study, only the high-density farm (Farm 2) showed different clinical mastitis rates among experimental months. It indicated that other factors, along with high density, could cause disease in this farm, such as hygiene conditions and survival of pathogens in the environment. Animals that produce a high volume of milk are at an increased risk of developing mastitis in the rainy season because of droplet infection, as well as the presence of damp and muddy floors (Sinha et al. 2021). Further research should be conducted to clarify the reasons and influential factors causing clinical mastitis in those farms.

On the other hand, the results of this present study showed that cows at parity 1 (32.58%) had a higher rate of clinical mastitis than other parties ($P<0.05$). It showed that cows at the first parity could be infected with disease due to incomplete growth of mammal glands or less resistance to pathogens surviving in the husbandry environment. Therefore, those cows were more sensitive to pathogens and got diseases. Besides, the feed supplied for cows at the first parity also affects the prevalence of clinical mastitis. Arvidson et al. (2005) reported that feeding factors associated with mastitis of first parity cows were amounts of concentrate given in the period around parturition and feeding-related diseases. In another study, Boujenane et al. (2015) showed that the risk of clinical mastitis was also higher than in cows at first parity. In contrast, Jingar et al. (2014) reported that the mastitis incidence was lower in the first parity than in other parities and increased with the increase in the parity number. Sinha et al. (2021) reported that the percentage of incidence of mastitis was maximum in fourth parity (41.1%) in Karan Fries and Sahiwal cattle at 41.1% and 52.8%, respectively. Several studies agreed with increased clinical and subclinical mastitis in advancing age and parity (Slettbakk et al. 1995; Radostits et al. 2000; Quaderi 2005). It has been shown that high-yielding and aged cows are more prone to mastitis. Thus, the prevalence of clinical mastitis in cows could depend on the health, age, or care conditions at those farms.

Muturi (2020) showed that mastitis significantly affects milk production and leads to losses due to discarded abnormal milk. In this study, there was a decrease in the milk yield of clinical mastitis cows in two surveyed farms. The cows in the high-density farm (Farm 2) showed fluctuations in milk yield in cows in all examined months. It indicated that clinical mastitis substantially affected the quality of milk and milk yield in cows. In the research by Lescourret and Coulon (1994), for cases in early or mid to late lactation, the production at mastitis onset was a determining factor of the amount and pattern of milk production loss induced. Houben et al. (1993) dealt with the effect of several cases within a given lactation to indicate that the actual milk yield loss was divided and attributed to both effects of mastitis occurring in the current and previous lactations. Schmenger et al. (2022) also reported that severe disease progression in relation to animals was observed in two factors: stages of lactation and previous diseases prior to the occurrence of mastitis. It was found that cows in the early stages of lactation experienced more severe cases of mastitis. In this study, most clinical mastitis cases occurred in cows at the first parity; therefore, the milk yield decreased significantly. Thus, it should have a management method to prevent clinical mastitis in cows and ensure the milk yield in production.

From 211 clinical mastitis cows in Farm 1, 288 milk samples clarified the prevalence of bacterial pathogens. In this study, *Strep. agalactiae* was detected at the highest rate (34.65%), followed by *Klebsiella* and *E. coli*. Al-Farha and Petrovski (2022) stated that five individual pathogens at the genus or species level were isolated from mastitis milk in Australia. Mixed growth was the most common (30.3%), followed by coagulase-negative *Staphylococci* CoNS (15.6%), *Stap. aureus* (8.6%), *Streptococcus* spp. (5.7%), *E. coli* (5.1%), *Enterococcus* spp. (4.6%), and not determined (30.2%). Those bacteria could be present in the mammal glands or the husbandry environment. Zhang et al. (2016) reported that mastitis pathogens were isolated from 63.43% of the milk samples, whereas *Strep. agalactiae* accounted for 38.61% of all pathogens, followed by *Strep. dysgalactiae* (28.16%), *Staph. aureus* (19.10%), *E. coli* (6.90%), and other pathogens (7.23%). Moreover, cows were relatively infected by environmental pathogens. Wu et al. (2019) also found that the milk microbiota was related to the bedding and airborne dust microbiota. In this study, the presence of *Staphylococcus* spp. was detected at a low rate in mastitis milk, whereas this pathogen was the most predominant in another research. Workineh et al. (2002)

reported that *Staphylococcus* constituted 57% of the isolates, of which the predominant cause of bovine mastitis was *Stap. aureus* (40.5%), and other mastitis pathogens isolated, including *Streptococcus* (16.5%), Coliforms (9%), and *Corynebacteria* (5%). Bradley (2002) recorded the same results, in which coagulase-negative *Staphylococcus* predominated (53.5%), followed by *Streptococcus* and *Enterococcus* (16.1%). Moreover, this research and other reports revealed that *E. coli* was commonly detected in mastitis milk. Cervinkova et al. (2013) reported that *Enterobacteriaceae* were found in 10.0% of mastitis samples, most of which (6.6%) were positive for *E. coli*, commonly found in the husbandry environment. The prevalence of environmental pathogens increases at the expense of contagious pathogens causing mastitis in cows (Bradley 2002). Thus, the prevalence of pathogens in the husbandry environment should be controlled to prevent contamination or infection in cows. From that, it could be limited to the prevalence of clinical mastitis cows in those farms.

Those clinical mastitis cows in Farm 1 were treated and observed for ten days. Those results showed that most cases recovered under five-day treatment. It could be that those cows had subclinical mastitis mainly at a young age; therefore, those cows were susceptible to treated drugs. In each month, the same results were recorded in all farms. In the treatment method, antibiotics were necessary to treat clinical mastitis in cows. Oliveira and Ruegg (2014) reported that antimicrobial drugs were recommended for use in all herds and that they received extra treatments. Smulski et al. (2020) showed that various adjunctive therapies, including antioxidants (such as vitamin C and E and β-carotene), lysozyme dimer, or NSAID, had shown potential in enhancing fertility in mastitis cows solely treated with antibiotics. It demonstrated that each supplementary intervention enhanced the efficacy of antibiotics, with the combination of antibiotics and antioxidants proving to be the most successful treatment. A great opportunity exists to improve mastitis therapy on large dairy herds, but more diagnostic methodologies are necessary to guide treatments. Farmers and veterinarians should work together to create protocols based on the herd's needs, considering reduced inappropriate and excessive use of antibiotics. The findings of Wilm et al. (2021) indicated the possibility of enhancing treatment precision by promoting techniques that facilitate quicker and more accurate identification of the causative pathogen. Thus, the knowledge regarding optimal treatment strategies for cases of mastitis caused by Gram-negative bacteria can be effectively implemented. Thus, early detection and suitable medicine were important to treat mastitis in cows.

Conclusion

In those surveyed farms in Central and East-Southern Vietnam, clinical mastitis in cows occurred at a relatively low rate. The herd's size and climate could affect the incidence of clinical mastitis in cows, especially in hot, humid months. Besides, cows at the first parity were highly essential to clinical mastitis disease in this study. The milk yield of clinical mastitis cows decreased, with high division in the high-density farm. Moreover, several pathogens were presented in milk samples of clinical mastitis cows; those bacteria cause animal disease and are transmitted to humans. A suitable protocol and drugs in treatment were essential and effective in treating mastitis. Those results are valuable information in managing and treating clinical mastitis in cows in those farms and Vietnam.

Author Contributions

Conceptualization, Thuong T. Nguyen, Thuan K. Nguyen; methodology, Thuong T. Nguyen, Thuan K. Nguyen, Cuong K. Nguyen; formal analysis, Thuong T. Nguyen, Thuan K. Nguyen, Nhu Q. Ho, Thu N. A. Le, Thanh C. Pham, Cuong K. Nguyen; writing-original draft preparation, Thuong T. Nguyen, Thuan K. Nguyen; writing-review and editing, Thuong T. Nguyen, Thuan K. Nguyen. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data supporting this study's findings are available on request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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