Analysis of Mastitis Cases on Modern Dairy Farms in Blitar Regency, Indonesia

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Abstract. This study aims to determine: i) the relationship between lactation level and clinical mastitis, ii) bacteria that cause clinical mastitis and iii) the incidence rate of the disease based on Days in Milk (DIM). The research location was carried out at a modern dairy farm in Blitar Regency, Indonesia. Clinical mastitis dairy divided into nine lactation periods including: lactation period (LP) 1 (168 heads); LP 2 (224 heads); LP 3 (153 heads); LP 4 (102 heads); LP 5 (51 heads); LP 6 (14 heads); LP 7 (7 heads); LP 8 (1 head); LP 9 (3 heads). Data were analyzed using simple regression and descriptive analysis. There was a linear increase in the percentage of mastitis incidence at the lactation period in modern dairy farm, $y = 1.9483x + 14.938$ following $R^2 = 0.2315$. The worst incidence occurred during the sixth lactation (43.75 %). *Streptococcus uberis* was identified as the most common bacteria causing mastitis (55.19 %). The highest incidence of mastitis occurred in Days in Milk (DIM) > 150 d (48.55 %), followed by DIM < 75 d (21.44 %). Furthermore, the second lactation with DIM > 150 d (15.63 %) had the greatest mastitis incidence.

Keywords: Black and white cow, disease control, friesian holstein, minimize economical losses, sand bedding.

1 Introduction

Mastitis is an inflammatory condition affecting the mammary gland or udder, which can present with varying degrees of severity. Several resarch investigations have demonstrated that this condition can result in significant economic losses for modern dairy farms,

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primarily due to reduced milk output and quality, augmented veterinary and medicinal costs, early culling, and mortality [1, 2].

Previous reports have identified two distinct types of mastitis: clinical and subclinical. Clinical mastitis is typified by obvious signs such as udder swelling, redness, and elevated temperature, along with alterations in milk color and consistency [3]. Meanwhile, subclinical mastitis does not exhibit any visible changes in the udder or milk but is characterized by the presence of bacteria and leukocytes in the milk [4]. Several research investigations have reported a higher incidence of subclinical mastitis compared to clinical mastitis [3, 4].

Mastitis in dairy cattle is primarily caused by bacterial agents, including *Streptococcus agalactiae* Lehmann & Neumann 1896*, Streptococcus dysgalactiae* (Diernhofer 1932) Garvie *et al*. 1983 emend Vandamme *et al.* 1996*, Streptococcus uberis* Diernhofer 1932, and *Streptococcus zooepidermicus*. Other microbes that can induce the disease include *Escherichia coli* (Migula 1985) Castellani & Chalmers 1919*, E. feundeii, Aerobacter aerugenes* Beijerinck 1900*, Klebsiella* sp, coronobacteria, salmonellae, mycobacteria, mycoplasmas, viruses, and fungi [5, 6]. Moreover, the bacterial species responsible for clinical mastitis infections include *Corynebacterium* spp., non-aureus staphylococci, as well as pathogenic bacterial communities found in the dairy cattle's environment, such as coliform bacteria [7]. According to Zalizar *et al*. [8], the prevalence of mastitis in smallholder farms ranged from 62 % to 68 %. This phenomenon has been attributed to inadequate sanitation practices, suboptimal dairy management, and farmers' limited understanding of the significance of preserving dairy cattle health.

In modern dairy farms, mastitis incidence is typically poor owing to the use of sand for bedding, improved cage sanitation, and the adoption of automatic milking technologies. A comfortable and hygienic environment within the stables is critical for maintaining optimal dairy herd health. Soft and smooth bedding material is provided to ensure cows' comfort while sleeping, and the bedding must be clean and dry to prevent the occurrence of mastitis cases on farms [9, 10]. Several research have found that the sand is the ideal bedding material for dairy cows [11, 12].

The novelty of this research lies in the exploration of mastitis prevalence and causative factors in modern dairy farms that adhere to good dairy farming practices by using sand bedding in low temperature areas. Specifically, this study aims to investigate: i) the association between lactation stage and clinical mastitis incidence, ii) the identity of bacteria responsible for the disease, and iii) mastitis incidence as determined by Days in Milk (DIM). This research is expected to provide recommendations for mitigating the prevalence and etiology of mastitis in modern dairy farms, with the ultimate goal of minimizing economic losses resulting from reduced milk production, medical expenses, premature culling, and mortality.

2 Material and methods

2.1 Location and time

The research was conducted from mid-March 2022 to December 2022 at GFL farm, a modern dairy farm located in Blitar Regency, East Java Province, Indonesia. The geographical coordinates of the farm are 112°14' to 112°28' east longitude and 8°2' to 8°8' south latitude.

2.2 Materials

This research was approved and registered by the Ethical Clereance No: E.5a/085/KEPK-UMM/III/2022 on March 27th, 2022, issued by Faculty of Medicine, University of Muhammadiyah Malang. Data collected from 4 100 dairy cows showed 723 heads with mastitis, which were reared on the farm. The cows were of the Fries Holland breed and represented various lactation periods ranging from the first to ninth. The first lactation comprised 168 heads, the second lactation had 224 heads, the third lactation consisted of 153 heads, the fourth lactation had 102 heads, the fifth lactation included 51 heads, the sixth lactation was represented by 14 heads, the seventh lactation had 7 heads, the eighth lactation had 1 head, and the ninth lactation was represented by 3 heads. The entire herd was in a closed housed-type free stall barn.

2.3 Method

This research is a case study approach, observation, and survey. Secondary data were obtained from laboratory examination results of 4 100 dairy cows screened between January 2018 and February 2022, which included positive mastitis cases (723 heads).

The prevalence rate and predominant bacterial species responsible for mastitis were analyzed using data obtained from DHIA (Dairy Herd Information Analysis). Factors influencing the occurrence of mastitis were obtained from observations and interviews in the field. The causative bacteria were identified by analyzing laboratory test reports of milk samples from mastitis-affected cows cultured using four primary media. First, blood agar (general media) following the research procedure of Artdita *et a*l. [13]. Second, Mac Conkey agar (screening for gram-negative bacteria) following the research procedure of Artdita *et al.* [13]. Third, Vogel-Johnson agar (screening for gram-positive Staphylococcus) following the protocol of Kolanus and Dompeipen [14]. Fourth, Edward`s media (screening gram-positive for the genus Streptococcus) following the execution procedure of Velasco-Bolaños *et al*. [15].

Data were analyzed using simple regression with Microsoft Excel 2013 and explained using descriptive analysis. Simple regression following this Equation (1) [16]

$$Y = a + \mathbf{b}x \quad (1)$$

Where,
b : regression coefficient
a : intercept
y : mastitis incidence rate
x : lactation period

3 Results and discussions

3.1 Relationship between lactation rate and clinical mastitis incidence rate

The relationship between the lactation rate and the incidence of clinical mastitis is presented in Figure 1.

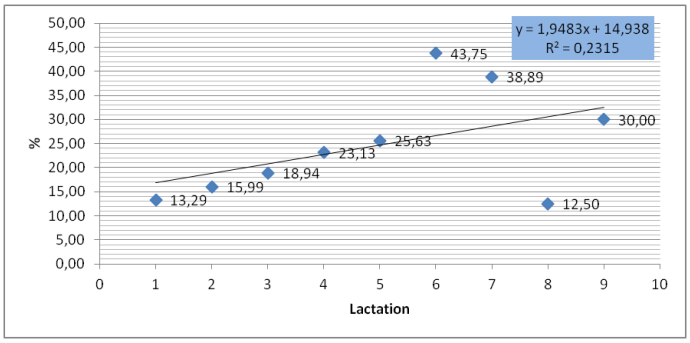

Fig. 1. Relationship between lactation rate and clinical mastitis incidence rate.

Based on Figure 1, there was a linear increase in the percentage of mastitis incidence with lactation period. This suggests a positive correlation between the two variables, as indicated by the regression equation y = 1.9483x + 14.938 and R^2 = 0.2315. The greater percentage of mastitis cases occurred during the sixth lactation, representing 43.73 % of all cases. This finding is consistent with Penev *et al.* [17], who reported that the greater impact of lactation period on disease occurrence was observed during the third and fourth lactation of milk collection, as compared to the first and second. The second-greater incidence rate of mastitis occurred during the seventh lactation of milk collection, representing 38.89 % of cases.

The incidence of clinical mastitis during the first lactation can be attributed to difficulties in milking cows (heifers). Incomplete milking can result in milk remaining in the udder, providing a favorable environment for microorganisms to grow and trigger mastitis. Microorganisms can quickly develop in the udder through teat openings that do not close promptly or completely after milking, or due to poor nipple conditions [18].

In the second to seventh lactation, the percentage of incidence increased, but decreased in the eighth lactation. This was presumably due to the aging of cows, deteriorated udder shape, and frequent milking, which caused the sphincter openings to close for long periods. Ball and Peters [19] and Abebe *et al.* [20] stated that mastitis was more common among older cows. Furthermore, this was due to the deterioration of old cattle condition, decreased ability to heal from infection or illness, and decrease in the mechanism for closing the sphincter openings. Gonçalves *et al.* [21] showed that aged cows were more at risk of mastitis compared to younger cows. Another factor that affected the rate of infection was age, where older cows were more susceptible due to wider or partially open nipple ducts caused by frequent milking [22]. Figure 2 exhibiting the udder of a normal dairy cow prior to clinical onset of mastitis.

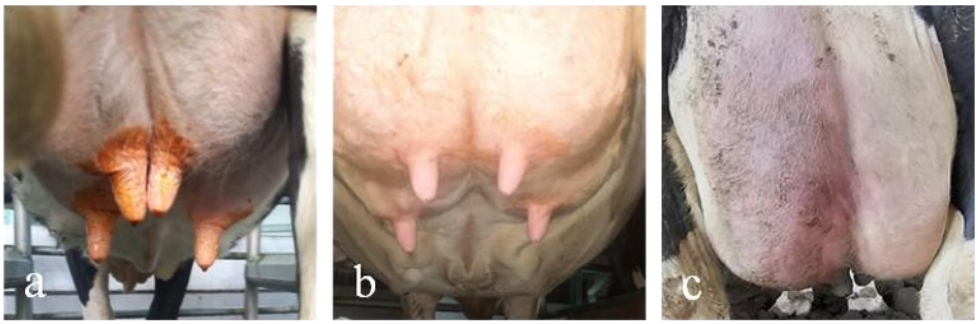

Fig. 2. a) normal udder; b) udder exhibiting subclinical mastitis; and c) clinical mastitis udder.

The total incidence rate of mastitis during this study was 17.29 % with a monthly average of < 2 % due to the use of sand bedding by modern farms. Furthermore, sand bedding is the best material for dairy cows and can prevent infection from mastitis. This is in line with previous studies by Patel *et al.* [12] that it can be used to prevent mastitis infection due to its ability to inhibit bacterial growth. The best type of sand for bedding contains little or no silt and soil. The material used must be dry and clean to prevent the occurrence of mastitis among cattle. A previous study revealed that sand was the best inorganic bedding for livestock because it can inhibit bacterial growth [23].

The second treatment that was thought to reduce the incidence of clinical mastitis in modern dairy cattle farms involved the use of antibiotics during the dry period of the pen. The application of antibiotics intramammary can reduce the rate of mastitis and prevent new infections during lactation and the dry period of the cage. A previous study revealed that these drugs have 90 % to 93 % and 70 % to 80 % effectiveness against *S. agalactiae* and *S. aureus*, respectively. Furthermore, effectiveness of 70 % to 90% was observed against environmental bacteria and Streptococcus groups [20, 24].

These findings are consistent with Pritchard's study [25] that the use of antibiotics in the dry cage period can reduce the incidence of clinical mastitis by 13 % from the first to seventh lactation. A 23 % decrease was also observed at the start of lactation to DIM 120. Programs that can prevent mastitis include proper milking procedures, such as using good equipment, dipping nipples before and after milking as quickly, cleaning udders during milk collection, and culling cattle with chronic infection [26, 24].

3.2 Bacterial causes of mastitis in modern dairy farms

Intra-mammary bacterial infection was considered the main cause of mastitis in dairy cows. Several bacterial species have been identified as the causative agents of this disease in cattle. Furthermore, mastitis can be classified into two types based on the origin of the infectious bacteria and the environment [27]. The top five bacteria that cause mastitis in modern dairy farms are presented in Table 1.

Table 1. Bacterial causes of mastitis in modern dairy farms.

No	Bacteria	Count	%
1	*Streptococcus uberis*	399	55.19
2	*Eschericia coli*	68	9.41
3	*Klebsiella* sp.	54	7.47
4	*Staptococcus* spp.	40	5.53
5	*Staphylococcus* spp.	29	4.01

The results showed that most of the mastitis infections were caused by *S. uberis*, accounting for 55.19 % of all cases. Furthermore, the causative microorganisms that often attack dairy cows included Streptococcus and Staphylococcus groups. Organisms that can cause mastitis were *S. aureus, S. agalactiae, Corynebacterium bovis* Bergey *et al.,* and Mycoplasma species [28, 29, 30]. Méndez-Vilas [31], Wellnitz and Bruckmaier [32] and Abureema *et al*. [33] reported that *S. uberis* was the major cause of clinical and subclinical mastitis in several farms in the world. It was also the main cause of the disease in dairy cows during the dry period of the stable. During the dry period of the cage, bacterial infection cannot be transmitted contagiously. However, mastitis came from the pure environment; especially from straw and other organic materials with lots of *S. uberis*. Other environmental sources of the disease the bacteria included soil, water, and grazing fields [34]. Milk samples obtained from mastitic cows were subjected to culture using the four primary media as depicted in Figure 3.

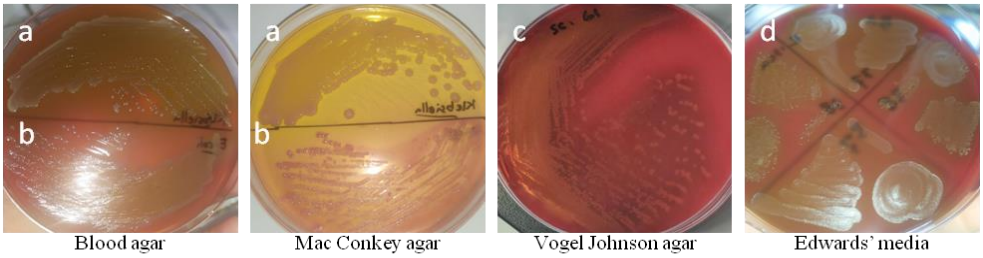

Fig. 3. a) *Klebsiella* sp. bacteria, b) *E. coli* bacteria, c) *S. uberis* bacterial colonies, while d) represents a group of *Staphylococcus* sp.

E. coli is the most common gram-negative bacteria, which can invade the udder through the teats, where it multiplies and initiates an inflammatory response. This bacterium was also considered a normal flora that can be found in the environment around dairy cows, such as bedding, especially in wet conditions [35]. *E. coli* can survive in the mammary glands, where it causes recurrent mastitis infections, which are difficult to treat. This was possibly due to its ability to produce biofilms at different levels [36].

Coliform bacteria, such as *E. coli* and *Klebsiella* sp. can also cause mastitis, as well as account for 9.41 % and 7.47 % of all cases, respectively. These bacteria were often obtained from dirty environmental conditions. Furthermore, they were classified as dangerous and deadly bacteria for infected livestock and can cause culling. *E. coli* bacterial species were included in the category of deadly bacteria causing several infections [37]. *Klebsiella* sp. often comes from the environment, especially from wet beds and grazing fields. A previous study revealed that there has been an increase in mastitis cases caused by this bacterium. It has also become a major problem among livestock in California, including in farms that do not use bedding or graze cattle [38].

Coliform bacteria were found in the environment and can cause mastitis in dairy cows. Overpopulation density, contaminated floors, wet beds, as well as hot and humid climates were factors that can increase the growth of pathogens and the occurrence rate of the infection [20, 39, 40]. Several studies revealed that mastitis can be transmitted from one cow to another through milking [5]. Furthermore, pathogenic bacteria, such as *S. aureus*, *S. agalactiae,* and Corynebacterium mostly lived in the udder of cows as well as the skin of the teats, where they colonize and grow into the nipple canal. A previous study revealed that these bacterial communities have the ability to cause mastitis through an increase in SCC (Somatic Cell Count) [22].

3.3 Mastitis incidence rate based on Day in Milk (DIM)

The number of clinical mastitis incidents on modern dairy farms is presented in Figure 4, while the number of cases based on DIM is shown in Figure 5.

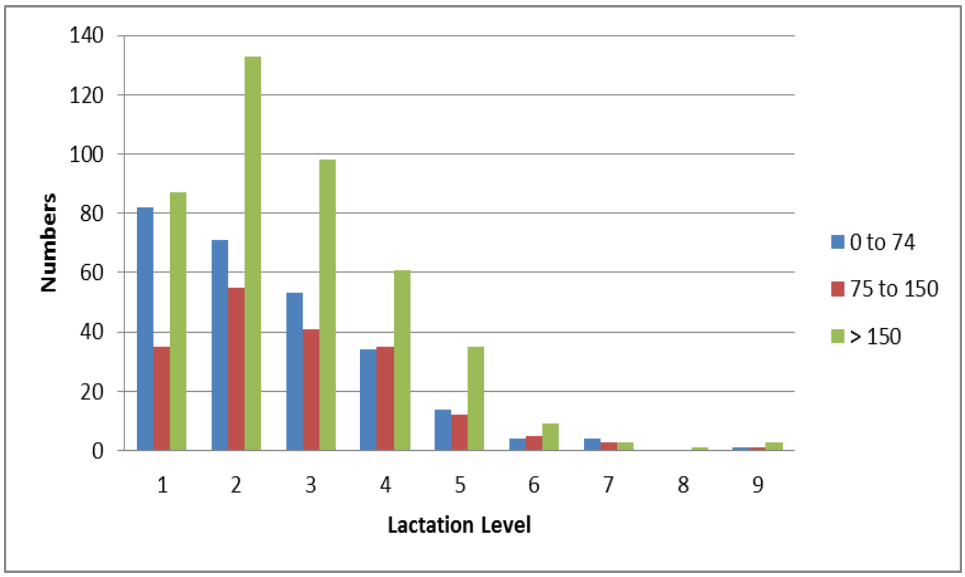

Fig. 4. Number of clinical mastitis based on DIM.

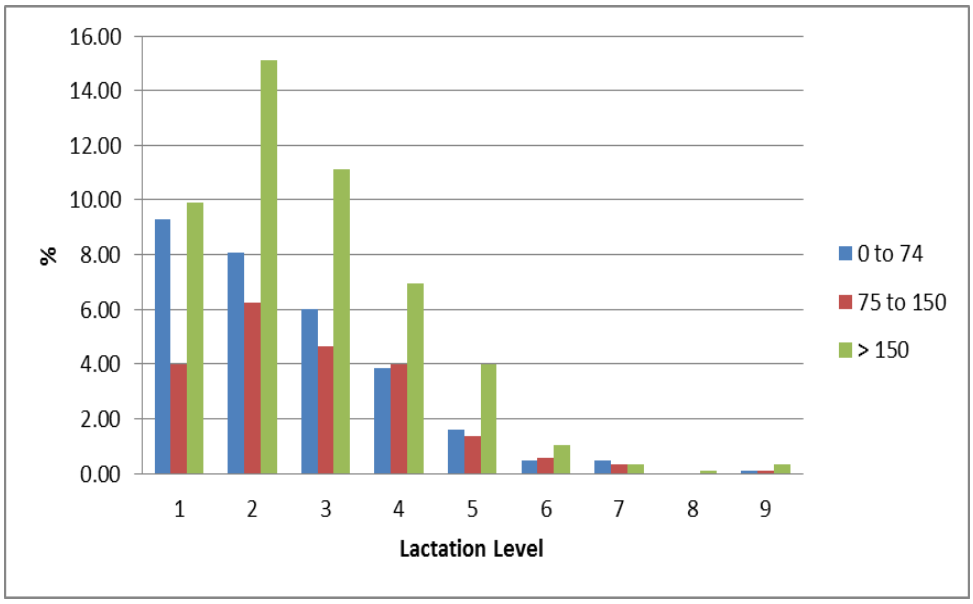

Fig. 5. Mastitis percentage based on DIM.

DIM is the number of days the cows have been milked since the time of birth. Figure 2 and Figure 3 showed that the peak number of mastitis incidents occurred in DIM at the end of lactation of more than 150 d and less than 75 d, namely 48.55 % and 21.44 %, respectively. The incidence of mastitis in late DIM was worst due to the prolonged duration needed for cows to dry out. Furthermore, the longer the cows are milked, the longer it takes for the sphincter openings to close again, and this caused deterioration of the shape of the udder. The structure of the udder also affected the susceptibility to infection. Cattle with

large funnel-shaped teats or pendular-shaped udders after calving have a greater risk of experiencing subclinical mastitis [41].

The second-worst incidence of mastitis occurred in early DIM, which was less than 75 d after calving. This is in line with Cobirka *et al.* [5] that several cases were detected during the first week of lactation. The occurrence of multiple mastitis in early DIM was due to several factors, such as increased production during early milk production until the peak at 60 d to 90 d after calving. Figure 5 showed that the worst incidence of mastitis occurred in the second (15.63 %) and first (10.37 %) lactation with DIM of more than 150 d. Ball and Peters [19] stated that after one week of calving, the mother often produced milk, and this increased gradually and reached a peak at 1 mo to 2 mo or 30 d to 60 d. The decline continued until the dairy cows dried out or stopped producing milk. Furthermore, Wilson *et al.* [42] revealed that at DIM 1 d to 45 d, the production can increase by 7 %, then decrease by more than 20 % at DIM 46 d to 114 d. It also decreased again by more than 17 % at DIM 115 to 199 and by more than 35 % at DIM > 199 d.

According to Vliegher *et al.* [43], there is a higher risk of clinical mastitis in first lactating cows, and more than one lactating cow can be affected throughout the entire lactation. Leelahapongsathon *et al.* [44] also found that early lactation (early DIM) in dairy cattle is associated with an increased risk of clinical mastitis.

4 Conclusions

The study found that there was a linear increase in the percentage of mastitis incidence at the lactation period in modern dairy cattle farm, $y = 1.9483x + 14.938$ following $R^2 = 0.2315$. The greater incidence occurred during the sixth lactation (43.75 %). *S. uberis* was identified as the most common bacteria causing mastitis (55.19 %). The highest incidence of mastitis occurred in DIM > 150 d (48.55 %), followed by DIM < 75 d (21.44 %). Furthermore, the second lactation with DIM > 150 d (15.63 %) and DIM > 150 d in the first lactation (10.37 %) had the greatest mastitis incidence. This research suggests that the use of stall bedding with sand media is able to reduce the incidence of mastitis in dairy cows, particularly in low temperature maintenance areas. Equations should be centred and should be numbered with the number on the right-hand side.

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Conflict of Interest. The author confirms that there was no conflict of interest in this papper.

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