



Prevalence of Subclinical Mastitis in Holstein-Friesian Cow Dairy Among Small-Scale Farms in Batu, Indonesia

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ABSTRACT

Subclinical mastitis continues to be a common issue for small-scale dairy cow farmers in Indonesia. This disease poses significant challenges for farmers as it can lead to a reduction in milk production, resulting in severe economic losses. This study involved 126 dairy cows with lactation periods ranging from 1 to 5, sourced from 29 different farmers, totalling 504 quarters examined. The research utilized a case study method with field exploration conducted in Toyomerto Village, Batu Sub District, Batu City, East Java, Indonesia. The analysis of subclinical mastitis was carried out using the California Mastitis Test (CMT). The findings of the study revealed a prevalence of subclinical mastitis in Toyomerto Village at 69.84% (n=88). Among all the tested teats, 41.47% of them were detected with subclinical mastitis, with the highest prevalence observed in the right hind teats, totalling 27.27%. The prevalence of subclinical mastitis in dairy cows from small farms in Toyomerto Village remains notably high. The continued lack of attention to sanitation and cleanliness remains a primary factor in the high prevalence of mastitis.

Key words: Dairy cows, Prevalence, Subclinical mastitis.

INTRODUCTION

Inflammation of the internal tissues in the udder of dairy cows, commonly known as mastitis (Fatmawati et al. 2019) is considered a local inflammation caused by the invasion of exogenous pathogens, resulting in microbiota and metabolite dysbiosis in milk (Wang et al. 2022). Mastitis is one of the most commonly found diseases in dairy cows worldwide, affecting milk production and quality (Velasco-Bolanos et al. 2021), livestock health (Bobbo et al. 2023), well-being, longevity and performance (Pakrashi et al. 2023), thus leading to long-term and challenging-to-control economic losses (Wang et al. 2023). Economic losses resulting from decreased milk quantity and quality, discarded milk, compromised conception, premature culling, recurring cases and the costs of disease treatment create a significant economic burden for dairy farmers each year (Gonçalves et al. 2018; Mohsin et al. 2022; Meçaj et al. 2023).

Mastitis is classified into two types: clinical mastitis (CM) and subclinical mastitis (SCM) (Hoekstra et al. 2019). Subclinical mastitis represents a significant burden and challenge to the management modern dairy production (Bhandari et al. 2021). Compared to clinical mastitis,

subclinical mastitis is more common and believed to be a cause of greater economic losses in the dairy industry (Ranasinghe et al. 2021). This is because subclinical mastitis can lead to a reduction up to 70% of the overall milk production of the dairy cows (Surjowardojo et al. 2008).

The prevalence of subclinical mastitis in smallholder dairy farms often causes concern among milk producers. Infection of subclinical mastitis in milk quarters can progress to clinical mastitis, thereby increasing the occurrence of new infections on the farm (Ayano et al. 2013; Wicaksono and Sudarwanto 2016). Many farmers may not aware of subclinical mastitis because dairy cows suffering from this disease do not exhibit abnormalities, swelling, heat and there are no visible changes in the physical appearance of the milk (Anggraeni and Nurfuadi 2021). Subclinical mastitis is generally recognise by an increase in the number of somatic cells in milk and an increase in bacterial count (Fatmawati et al. 2019).

The calculation of prevalence and evaluation of factors causing subclinical mastitis in dairy cows are crucial to maintaining quality and quantity of milk production and determining the most appropriate control measures (Anggraeni and Nurfuadi 2021). Prevalence data for

subclinical mastitis in dairy cows in small scale dairy farms in Toyomerto Village, Batu Sub District, Batu City, East Java, Indonesia, during observation period are still very limited. This study aims to provide an overview of the prevalence of subclinical mastitis in small dairy farms in the City of Batu.

MATERIALS AND METHODS

Research design and sampling techniques

This research method utilizes a case study with field exploration (field research). Sample determination is done through purposive sampling from lactating cows during the lactation period 1-5. The sample size for estimating the prevalence is calculated using the formula from Dohoo et al. (2003) as follows:

$$n = z\alpha^2 \times p \times q / L^2$$

$z\alpha^2$ = 4 (confidence interval 95%)
 p = estimated prevalence
 Q = $1 - p$
 L = margin of error

With confidence interval 95%, estimated prevalence 72%, and margin of error 8%, Hence, a sample size of 126 lactating dairy cows was obtained.

Analysis of subclinical mastitis

The analysis of subclinical mastitis involves the use of specific equipment, including CMT Paddle, CMT Reagent, milk from each quarter of each cow. The procedure consists of taking 1 teaspoon (2cc) of milk from each quarter, an amount that corresponds to the residual milk in the cups when the CMT Paddle is held almost upright, with the milk reaching the external line on the cup's bottom in this nearly vertical position. An equal amount of CMT solution is then

added to each cup of the paddle. The CMT paddle is rotated in a circular motion for no more than 10 seconds to ensure thorough mixing. After that, the test is read quickly, and the visible reaction disintegrates after about 20 seconds. Finally, the reaction is scored visually, with a higher score indicating more gel formation and rinsed the CMT paddle after each test (Burton 2021).

Data analysis

The prevalence of subclinical mastitis is analyzed using the formula from Raymundo et al. (2008) as follows:

$$P = \frac{\text{Number of individuals infected at observation time}}{\text{Population at risk at observation time}} \times 100\%$$

RESULTS AND DISCUSSION

From a total of 126 cows belonging to 29 different farmers, the overall prevalence of subclinical mastitis at cow level as determined by CMT was 69.84% ($n=88$). The distribution of subclinical mastitis among local farmers can be seen in Fig. 1. The high prevalence of subclinical mastitis in smallholder dairy farms is attributed to several factors, including suboptimal implementation of sanitation and hygiene practices in the milking parlor and dairy cattle management. Damp or dirty dairy barns make it easier for udder infection to occur, resulting in a positive diagnosis of subclinical mastitis (Surjowardojo 2011; Ji et al. 2020).

Out of the 504 quarters examined, 41.47% ($n=209$) showed signs of subclinical mastitis. Among all the teats affected by mastitis, the majority affected by subclinical mastitis were the right hind teats, accounting for 27.27% ($n=57$). While the least affected teats were the right front and the left rear teats, both of which had the same rate of 23.92% ($n=50$) as shown in Fig. 2. The tendency of cows lie down on the right side leads to a higher frequency of

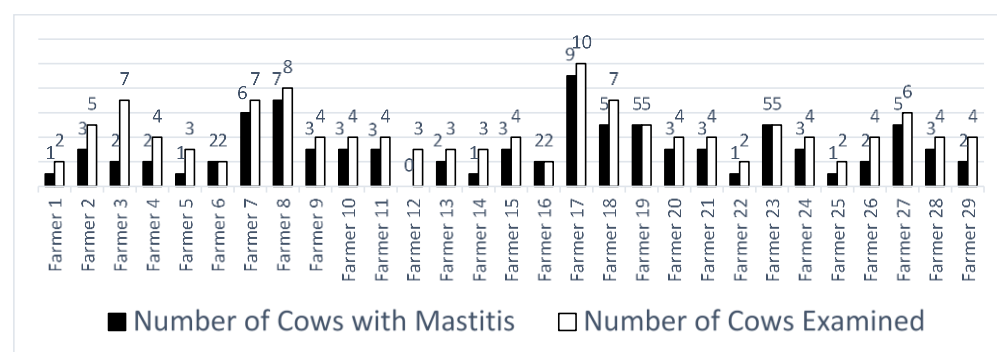


Fig. 1: Number of cows with mastitis/farmer among small-scale farms in Batu, Indonesia

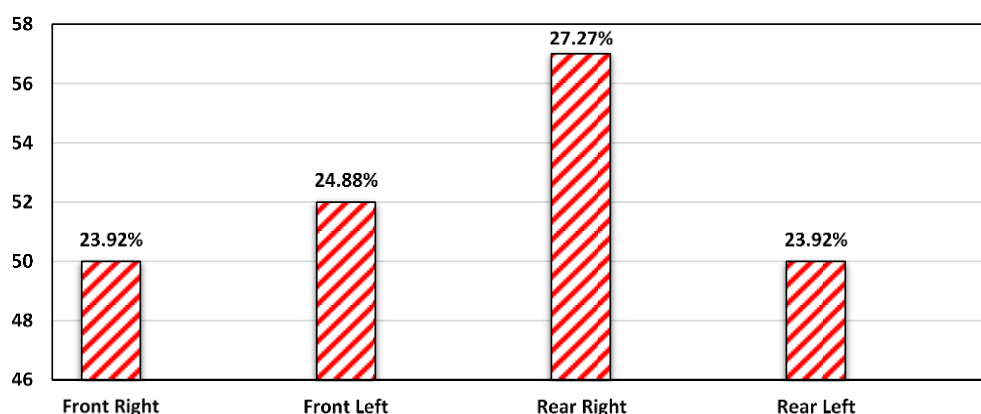


Fig. 2: Number (%) of quarter out of 209 with mastitis in Holstein-Friesian cow dairy among small-scale farms in Batu, Indonesia

contact between the right teats and the stall bedding, especially when the bedding has not been cleaned. This can increase the risk of transmission of pathogenic bacteria causing subclinical mastitis from the barn environment (Pisestyani et al. 2017) which can enter through the streak canal in the teats (Surjowardojo et al. 2008).

Mastitis is caused by bacterial infections and risk increases with physical injuries to the gland (Radostits et al. 1994; El-Demerdash et al. 2023). Regarding the bacteria causing mastitis in dairy cows, they include *Staphylococcus* sp. (Anggraeni and Nurfuadi 2021), *Escherichia coli* (Bhandari et al. 2021), *Corynebacterium pyogenes*, *Micrococcus* spp. (Daniel et al. 1986), *Streptococcus agalactiae*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Enterococcus* spp., *Serratia marcescens*, *Klebsiella pneumoniae* (Palladini et al. 2023) and *Staphylococcus aureus* (Ren et al. 2020; Yang et al. 2020). *Staphylococcus aureus* is the most common causative agent of mastitis affecting dairy cows on dairy farms worldwide (Hoque et al. 2018; Zayda et al. 2020; Sharifi et al. 2023). These bacteria are thought to be acquired from the cow's environment such as soil, manure, bedding materials, etc. (Hogan and Smith 2003). Subclinical mastitis is challenging to detect without testing because inflammation in subclinical mastitis can occur even without visible abnormalities in the udder and teats (Guerrero et al. 2015).

The management and hygiene of milking are crucial practices on local dairy farms. In conventional hand milking processes, hand hygiene is of utmost importance. This because the transmission of subclinical mastitis can occur through cross-contamination of bacteria between the milker's hand and the udder of the milked cow. Understanding the prevalence and spatial distribution of bovine subclinical mastitis and its associated risk factors will facilitate the prevention and control of the disease (Chen et al. 2022).

Conclusion

The calculated prevalence of subclinical mastitis based on CMT in samples of dairy cow milk on small-scale dairy farms in Toyomerto Village, Batu Sub District, Batu City, is still quite high at 69.84%. The less-than-optimal implementation of sanitation and hygiene management is one of the factors causing the high prevalence of subclinical mastitis.

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Author's Contribution

All authors designed the study, Rifa'i performed the practical procedures, analyzed and interpreted the data, and wrote the manuscript. This manuscript content was

authored, reviewed, and approved by Lilik Eka Radiati, La Choviya Hawa, and Puguh Surjowardojo for publication.

REFERENCES

- Anggraeni HE and Nurfuadi SZ, 2021. Subclinical mastitis prevalence on small scale dairy farming in Bogor. Journal of Applied Veterinary Science and Technology 2(1): <https://doi.org/10.20473/javest.V2.I1.2021.1-4>
- Ayano AA, Hiriko F, Simyalew AM and Yohannes A, 2013. Prevalence of subclinical mastitis in lactating cows in selected commercial dairy farms of Holeta district. Journal of Veterinary Medicine and Animal Health 5(3): 67-72.
- Bhandari S, Subedi D, Tiwari BB, Shrestha P, Shah S and Al-Mustapha AI, 2021. Prevalence and risk factors for multidrug-resistant *Escherichia coli* isolated from subclinical mastitis in the western Chitwan region of Nepal. Journal of Dairy Science 104(12): 12765-12772. <https://doi.org/10.3168/jds.2020-19480>
- Bobbo T, Matera R, Pedota G, Manunza A, Cotticelli A, Neglia G and Biffani S, 2023. Exploiting machine learning methods with monthly routine milk recording data and climatic information to predict subclinical mastitis in Italian Mediterranean buffaloes. Journal of Dairy Science 106(3): 1942-1952. <https://doi.org/10.3168/jds.2022-22292>
- Burton E, 2021. Clinical Veterinary Diagnostic Laboratory. Creative Commons Attribution-NonCommercial 4.0 International License. University of Minnesota. <https://pressbooks.umn.edu/cvdl/>
- Chen X, Chen Y, Zhang W, Chen S, Wen X, Ran X, Wang H, Zhao J, Qi Y and Xue N, 2022. 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 (148):65-73. <https://doi.org/https://doi.org/10.1016/j.rvsc.2022.04.007>
- Daniel R, Barnum D and Leslie K, 1986. Observations on intramammary infections in first calf heifers in early lactation. The Canadian Veterinary Journal 27(3): 112-115. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1680159/pdf/canvetj00591-0030.pdf>
- Dohoo I, Martin W and Stryhn H, 2003. Veterinary epidemiologic research. Charlottetown.
- El-Demerdash AS, Bakry NR, Aggour MG, Elmasry SS, Mowafy RE, Erfan A, Taha MF, El-Gmaal AAAM, Mohamed AAE, Hagag N and Samir M, 2023. Bovine mastitis in Egypt: bacterial etiology and evaluation of diagnostic biomarkers. International Journal of Veterinary Science 12(1): 60-69. <https://doi.org/10.47278/journal.ijvs/2022.161>
- Fatmawati M, Setianingrum A, Haskito AEP and Dameanti FNAEP, 2019. Prevalensi dan Faktor Predisposisi Mastitis Subklinis pada Sapi Perah Rakyat Dusun Bakir, Desa Sukomulyo, Kabupaten Batu. Veterinary Biomedical and Clinical Journal 1(2): 35-41.
- Gonçalves J, Kamphuis C, Martins C, Barreiro J, Tomazi T, Gameiro AH, Hogeveen H and Dos Santos M, 2018. Bovine subclinical mastitis reduces milk yield and economic return. Livestock Science (210): 25-32. <https://doi.org/10.1016/j.livsci.2018.01.016>
- Guerrero A, Dallas DC, Contreras S, Bhandari A, Cánovas A, Islas-Trejo A, Medrano JF, Parker EA, Wang M and Hettinga K, 2015. Peptidomic analysis of healthy and subclinically mastitic bovine milk. International Dairy Journal (46): 46-52. <https://doi.org/10.1016/j.idairyj.2014.09.006>
- Hoekstra J, Rutten VP, Van den Hout M, Spaninks MP, Benedictus L and Koop G, 2019. Differences between *Staphylococcus aureus* lineages isolated from ovine and caprine mastitis but not between isolates from clinical or

- subclinical mastitis. *Journal of Dairy Science* 102(6): 5430-5437. <https://doi.org/10.3168/jds.2018-16196>
- Hogan J and Smith KL, 2003. Coliform mastitis. *Veterinary Research* 34(5):507-519. <https://hal.science/file/index/docid/902763/filename/hal-00902763.pdf>
- Hoque MN, Das ZC, Rahman A, Haider MG and Islam MA, 2018. Molecular characterization of *Staphylococcus aureus* strains in bovine mastitis milk in Bangladesh. *International Journal of Veterinary Science and Medicine* 6(1): 53-60. <https://doi.org/10.1016/j.ijvsm.2018.03.008>
- Ji Y, Xiao F, Zhu W, Liu SS, Feng X, Sun C, Lei L, Dong J, Khan A, Han W and Gu J, 2020. LysGH15 effectively control murine mastitis caused by *Staphylococcus aureus*. *Pakistan Veterinary Journal* 40(4): 519-522. <http://dx.doi.org/10.29261/pakvetj/2020.056>
- Meçaj R, Muça G, Koleci X, Sulçe M, Turmalaj L, Zalla P, Koni A and Tafaj M, 2023. Bovine environmental mastitis and their control: an overview. *International Journal of Agriculture and Biosciences* 12(4) 216-221. <https://doi.org/10.47248/journal.ijab/2023.067>
- Mohsin M, Swar SO, Imran M, Ali W, Sultan MD, Asrar R, Abbas RZ, Aleem MT, Aguilar-Marcelino L, Aslam A, Shahab A, Ahsan MA, Shehata AI, Alhoshy M, Habib YJ, Farhan MHR and Yin G, 2022. Chronic mastitis: Leading cause of udder fibrosis and different means of its management. *Agrobiological Records* 8: 13-21. <https://doi.org/10.47278/journal.abr/2022.004>
- Pakrashi A, Ryan C, Gueret C, Berry DP, Corcoran M, Keane MT and Mac Namee B, 2023. Early detection of subclinical mastitis in lactating dairy cows using cow-level features. *Journal of Dairy Science* 106(7): 4978-4990. <https://doi.org/10.3168/jds.2022-22803>
- Palladini G, Garbarino C, Luppi A, Russo S, Filippi A, Arrigoni N, Massella E and Ricchi M, 2023. Comparison between broth microdilution and agar disk diffusion methods for antimicrobial susceptibility testing of bovine mastitis pathogens. *Journal of Microbiological Methods* 212, 106796. <https://doi.org/10.1016/j.mimet.2023.106796>
- Pisestyani H, Sudarnika E, Ramadhanita R, Ilyas AZ, Basri C, Wicaksono A, Nugraha AB and Sudarwanto MB, 2017. Perlakuan celup puting setelah pemerahan terhadap keberadaan bakteri patogen, *Staphylococcus aureus*, *Streptococcus agalactiae*, dan *E. coli* pada sapi perah penderita mastitis subklinis di peternakan Kunak Bogor. *Jurnal Sain Veteriner* 35(1): 63-70. <https://doi.org/10.22146/jsv.29293>
- Radostits OM, Blood DC and Gay CC, 1994. *Veterinary medicine. A textbook of the diseases of cattle, sheep, pigs, goats and horses*. Bailliere Tindall Ltd.
- Ranasinghe R, Deshapriya RMC, Abeygunawardana DI, Rahularaj R and Dematawewa CMB, 2021. Subclinical mastitis in dairy cows in major milk-producing areas of Sri Lanka: Prevalence, associated risk factors, and effects on reproduction. *Journal of Dairy Science* 104(12): 12900-12911. <https://doi.org/10.3168/jds.2021-20223>
- Raymundo L, Work TM, Bruckner AW and Willis B, 2008. A coral disease handbook: Guidelines for assessment, monitoring, and management.
- Ren Q, Liao G, Wu Z, Lv J and Chen W, 2020. Prevalence and characterization of *Staphylococcus aureus* isolates from subclinical bovine mastitis in southern Xinjiang, China. *Journal of Dairy Science* 103(4): 3368-3380. <https://doi.org/10.3168/jds.2019-17420>
- Sharifi A, Sobhani K and Mahmoudi P, 2023. A systematic review and meta-analysis revealed a high-level antibiotic resistance of bovine mastitis *Staphylococcus aureus* in Iran. *Research in Veterinary Science* 161: 23-30. <https://doi.org/10.1016/j.rvsc.2023.05.016>
- Surjowardojo P, 2011. Tingkat kejadian mastitis dengan whiteside test dan produksi susu sapi perah friesien holstein. *TERNAK TROPIKA Journal of Tropical Animal Production* 12(1): 46-55. <https://ternaktropika.ub.ac.id/index.php/tropika/article/view/134>
- Surjowardojo P, Suyadi S and Hakim L, 2008. Ekspresi produksi susu pada sapi perah mastitis. *TERNAK TROPIKA Journal of Tropical Animal Production* 9(2): 1-11. <https://ternaktropika.ub.ac.id/index.php/tropika/article/view/File/152/160>
- Velasco-Bolanos J, Ceballes-Serrano CC, Velasquez-Mejia D, Riano-Rojas JC, Giraldo CE, Carmona JU and Ceballos-Marquez A, 2021. Application of udder surface temperature by infrared thermography for diagnosis of subclinical mastitis in Holstein cows located in tropical highlands. *Journal of Dairy Science* 104(9): 10310-10323. <https://doi.org/10.3168/jds.2020-19894>
- Wang M, Bissonnette N, Laterriere M, Dudemaine PL, Gagne D, Roy JP, Sirard MA and Ibeagha-Awemu EM, 2023. Gene co-expression in response to *Staphylococcus aureus* infection reveals networks of genes with specific functions during bovine subclinical mastitis. *Journal of Dairy Science* 106(8): 5517-5536. <https://doi.org/10.3168/jds.2022-22757>
- Wang Y, Nan X, Zhao Y, Jiang L, Wang H, Zhang F, Hua D, Liu J, Yang L, Yao J and Xiong B, 2022. Discrepancies among healthy, subclinical mastitic, and clinical mastitic cows in fecal microbiome and metabolome and serum metabolome. *Journal of Dairy Science* 105(9): 7668-7688. <https://doi.org/10.3168/jds.2021-21654>
- Wicaksono A and Sudarwanto M, 2016. Subclinical mastitis prevalence and milk microbiological evaluation on small scale dairy farms in Boyolali. *Acta Veterinaria Indonesia* 4(2): 51-56. <https://journal.ipb.ac.id/index.php/actave/tindones/article/download/12365/12373/>
- Yang F, Zhang S, Shang X, Li H, Zhang H, Cui D, Wang X, Wang L, Yan Z and Sun Y, 2020. Detection and molecular characterization of methicillin-resistant *Staphylococcus aureus* isolated from subclinical bovine mastitis cases in China. *Journal of Dairy Science* 103(1): 840-845. <https://doi.org/10.3168/jds.2019-16317>
- Zayda MG, Masuda Y, Hammad AM, Honjoh K-I, Elbagory AM and Miyamoto T, 2020. Molecular characterisation of methicillin-resistant (MRSA) and methicillin-susceptible (MSSA) *Staphylococcus aureus* isolated from bovine subclinical mastitis and Egyptian raw milk cheese. *International Dairy Journal* 104: 1-9. <https://doi.org/10.1016/j.idairyj.2020.104646>