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Traditional Milking Hygiene Practices and their Effect on Subclinical Mastitis in Holstein-Friesian of Small-Scale Farms

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Abstract | Subclinical mastitis poses significant challenges to dairy productivity, particularly in small-scale farms where traditional milking practices prevail. This study evaluated the impact of traditional milking hygiene practices (TMHP) on subclinical mastitis in Holstein-Friesian cows across 29 smallholder farms in Batu City, Indonesia. Data were collected via direct observation of milking routines, structured interviews, and California Mastitis Test (CMT) assessments to diagnose subclinical mastitis. Results revealed that inadequate hygiene practices such as poor handwashing, shared udder cloths, and insufficient equipment sterilization were prevalent. CMT scores demonstrated a negative correlation between TMHP compliance and mastitis incidence, with rear teats (RR, RL) showing higher susceptibility due to prolonged teat closure times and larger milk volumes. Farmers' habitual practices and educational background play a crucial role in implementing hygienic milking procedures. Key recommendations include pre-milking teat disinfection, equipment sterilization, and farmer training to mitigate mastitis risks. These findings underscore the necessity of integrating Good Dairy Farming Practices (GDFP) in small-scale operations to enhance udder health, milk quality, and farm sustainability.

Keywords | Subclinical mastitis, Traditional milking hygiene, Holstein-friesian, Small-scale dairy farms, Udder health

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INTRODUCTION

The urgency of addressing subclinical mastitis (SCM) is emphasized by its high prevalence and significant economic impact on the global dairy industry. Recent meta-analyses estimate the global prevalence of SCM in dairy cattle to be approximately 42% (Gürbulak and Akçay, 2024). This rate is considerably higher than that of clinical mastitis, which is more visible and thus more promptly managed by farmers (Qolbaini *et al*., 2021). The asymptomatic nature of SCM makes it particularly concerning, as it often goes undetected while causing substantial economic losses (Keyvan, 2023; Mbindyo *et al*., 2020). In Holstein-Friesian dairy cows, a leading breed in milk production, subclinical mastitis can reduce milk yield, alter its nutritional composition, and elevate somatic cell count (SCC) in milk (Antanaitis *et al*., 2021; Fredebeul-Krein *et al*., 2022). Often undetected due to the absence of clinical

symptoms, this condition still negatively impacts livestock productivity and health (Rifatbegović et al., 2024).

In small-scale farms, milking hygiene practices are frequently conducted using traditional methods that neglect modern sanitation standards. This increases the risk of contamination by pathogenic microbes responsible for subclinical mastitis, such as Staphylococcus aureus, Escherichia coli, and Streptococcus species (Antanaitis *et al*., 2021; Sheet *et al*., 2023). Factors such as milking equipment cleanliness, environmental sanitation, and cattle health are critical elements requiring proper management to prevent infections (Jama et al., 2024). Common traditional practices observed in small farms such as failing to wash hands before milking, using the same cloth to clean multiple udders, and inadequate sterilization of milking tools pose substantial contamination risks (Setianingrum *et al*., 2019).

Numerous studies have focused on identifying risk factors for subclinical mastitis and preventive measures. Prior research highlights the importance of equipment hygiene and udder sanitation before and after milking in reducing mastitis prevalence (Vitenberga-Verza *et al*., 2022). Other studies have evaluated the effectiveness of disinfectants, single-use cleaning wipes, and machine milking as alternatives to mitigate infection risks (Antanaitis *et al*., 2022). Concurrently, advancements in metagenomic analysis have accelerated research on pathogenic microbes in subclinical mastitis, enabling comprehensive pathogen identification and insights into evolving antibiotic resistance patterns (Alessandri *et al*., 2023).

However, most studies prioritize large-scale or industrial farms, where resources and technology are more accessible. In contrast, small-scale farms, which dominate the dairy sectors of many developing countries, are often overlooked (Girma and Tamir, 2022). Despite extensive research on subclinical mastitis, several gaps persist, particularly in small-scale farming contexts. A key gap is the lack of in-depth analysis of traditional milking practices among smallholder farmers and their influence on subclinical mastitis prevalence (Zhang et al., 2023). Additionally, the relationship between socio-cultural factors—such as farmers' education levels, access to training, and hygiene perceptions—and the adoption of hygienic practices remains understudied (Jama et al., 2024).

This study aims to identify traditional milking hygiene practices employed by smallholder dairy farmers in Batu City and evaluate their impact on subclinical mastitis in Holstein-Friesian cows. By enhancing understanding of this relationship, the research seeks to provide practical recommendations for improving milking hygiene, udder and teat health, and ultimately milk quality and yield (Antanaitis *et al*., 2021; Fredebeul-Krein *et al*., 2022; Vitenberga-Verza *et al*., 2022).

MATERIALS AND METHODS

Study Location

This study was conducted among smallholder dairy farms in Toyomerto Village, Batu Sub-District, Batu City, East Java Province, Indonesia. Batu City was selected due to its substantial population of smallholder dairy farmers who practice traditional milking methods.

Research Design

The study employed a descriptive survey design with a cross-sectional approach to collect data on milking hygiene practices and subclinical mastitis scores in Holstein-Friesian dairy cows.

Population and Sample

The study population comprised 29 smallholder dairy farmers in Batu City rearing Holstein-Friesian cows. A purposive sampling method was applied, with the following inclusion criteria:

- Farmers owning at least two Holstein-Friesian dairy cows.
- Willingness to participate in the study.
- Use of traditional milking practices.

Data Collection

Data were collected through two primary methods:

Direct observation:

Observational assessment of milking hygiene practices, focusing on:

- Cleanliness of the milking environment.
- Milkers' hand hygiene.
- Teat cleaning procedures prior to milking.
- Sterilization status of milking equipment.
- Post-milking milk handling practices.
- Post-milking teat disinfection (dipping).

Structured interviews: A standardized questionnaire was administered to gather data on farmers' knowledge of milking hygiene, existing practices, and factors influencing subclinical mastitis prevalence.

Subclinical Mastitis Testing

Subclinical mastitis was diagnosed using specialized tools, including a California Mastitis Test (CMT) paddle, CMT reagent, and milk samples from each teat of the udder quarters. The procedure involved:

- Collecting approximately 2 cc of milk from each udder quarter.

- Adjusting the sample volume to ensure it reached the outer line of the CMT paddle when tilted vertically.
- Adding an equal volume of CMT reagent to each paddle well.
- Gently rotating the paddle in circular motions for ≤10 seconds to ensure thorough mixing.
- Immediately interpreting results (within 20 seconds) by visually assessing gel formation, with higher scores indicating stronger reactions.
- Rinsing the CMT paddle after each test to maintain hygiene (Burton, 2021; Rifa'i *et al*., 2024).

Data Analysis

Data were analyzed descriptively and inferentially:

Descriptive analysis: Characterized the distribution of Traditional Milking Hygiene Practices (TMHP) and subclinical mastitis scores.

Inferential analysis: Integrated correlation and regression analyses, conducted using R statistical software (version 4.4.0) with the PerformanceAnalytics package. Data visualization was performed using the ggplot2 package to evaluate relationships between TMHP and subclinical mastitis incidence.

RESULTS AND DISCUSSION

General Conditions of the Study Location

The smallholder dairy farms in Toyomerto Village, Batu Sub-District, Batu City, East Java, are situated on the northern slopes of Mount Panderman at an elevation of 900–1000 meters above sea level. The village receives an annual rainfall of 2000–3000 mm and has an average temperature of 24–26°C, conditions that are ideal for dairy farming (Nardin, 2019).

Educational Background of Dairy Farmers

The educational levels of the farmers ranged from elementary school to bachelor's degrees. Adequate education has been shown to enhance farm management capabilities and operational performance (Murwanto, 2008; Nurdiyansah *et al*., 2020). According to Mitha et al. (2014), farmer profiles can be assessed through factors such as education level, which reflects the quality of human resources in rural areas. Long-term experience in dairy farming also deepens farmers' understanding of livestock management. Smallholder dairy farmers in rural regions face challenges requiring innovative thinking and hard work to improve milk production.

Traditional Knowledge and Practices

In Tuyomerto Hamlet, dairy farming methods and cattle management practices are primarily inherited through generations. This aligns with Haloho *et al*. (2013), who noted that smallholder farms often rely on simple, traditional technologies and knowledge passed down from parents. Nainggolan (2017) further emphasized that prolonged farming experience enhances farmers' skills in effective livestock management.

Subclinical Mastitis Scores and Milking Hygiene Practices

The California Mastitis Test (CMT) is a simple diagnostic tool for identifying subclinical mastitis in dairy cows. The CMT estimates somatic cell count (SCC) in milk, providing an initial indication of subclinical mastitis (Blowey and Edmondson, 2010). The test involves mixing milk with a CMT reagent containing arylsulfonate, which reacts with leukocyte DNA to form a gel. The gel's consistency or agglutination quality reflects the leukocyte count in the milk (Surjowardojo *et al*., 2008) and indicates a positive result for subclinical mastitis (Sharma *et al*., 2010; Namira *et al*., 2022). While CMT scores do not quantify specific bacterial contamination, they categorize subclinical mastitis severity as high or low based on SCC ranges (Pole *et al*., 2023) Table 1.

Table 1: CMT scoring system.

CMT Score	Description	SCC Range (cells/mL)
N(negative)	No thickening of the mixture	0 – 200,000
T (trace)	Slight thickening of the mixture. Trace reaction seems to disappear with the continued rotation of the paddle.	200,000 – 400,000
1	Distinct thickening of the mixture, but no tendency to form a gel. If the CMT paddle is rotated for more than 20 seconds, thickening may disappear.	400,000 – 1,200,000
2	Immediate thickening of the mixture, with a slight gel formation. As the mixture is swirled, it moves toward the center of the cup, exposing the bottom of the outer edge. When the motion stops, the mixture levels out and covers the bottom of the cup.	1,200,000 – 5,000,000
3	Gel is formed and the surface of the mixture becomes elevated (like a fried egg). The central peak remains projected even after the CMT paddle rotation is stopped.	> 5,000,000

Sumber: Burton, 2021.

Figure 1 is a scatterplot depicting the relationship between Traditional Milking Hygiene Practices (TMHP) scores and teat health scores for four udder quarters: Front Right

(FR), Front Left (FL), Rear Right (RR), and Rear Left (RL). Each data point represents a farm's TMHP score (x-axis) and teat health score (y-axis). Blue linear regression lines illustrate the negative trend between TMHP compliance and teat scores, with rear teats (RR, RL) showing tighter clustering and narrower confidence intervals (gray shading) compared to front teats (FR, FL). The figure visually reinforces the stronger correlation between hygiene practices and rear teat health.

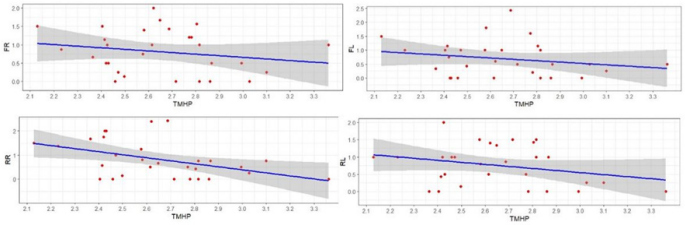

Figure 1: Regression of TMHP with teat health.

The presented figure illustrates the relationship between TMHP (Traditional Milking Hygiene Practices) and four other variables: FR (Front Right teat), FL (Front Left teat), RR (Rear Right teat), and RL (Rear Left teat). This relationship likely indicates that hygiene levels during milking influence the condition of each part of the cow's udder. In other words, hygiene practices in traditional milking may affect the health and cleanliness of individual teats, potentially increasing the risk of subclinical mastitis or other health problems. The figure is likely a visual representation, such as a scatterplot, depicting the association between TMHP and the four teat conditions. The relationship is visualized with blue linear regression lines to illustrate trends between the variables. The relationship is negative, meaning that as TMHP values increase, the values of the other variables (FR, FL, RR, and RL) tend to decrease. For example, higher TMHP scores correlate with lower FR values, and a similar pattern is observed for FL, RR, and RL.

Additionally, the regression line is surrounded by a gray shaded area representing the confidence interval. This interval reflects the uncertainty of the regression model, indicating the reliability of the regression line's predictions. A narrower gray shading suggests higher confidence that the regression line accurately represents the true relationship between variables, while a wider shading implies greater uncertainty. The figure shows a high uncertainty interval for FR (Front Right Teat), whereas FL (Front Left teat), RR (Rear Right teat), and RL (Rear Left teat) exhibit narrower intervals, indicating lower uncertainty and higher data validity. Thus, the results not only demonstrate the direction and strength of the relationship between TMHP and the other variables but also provide insights into the reliability of the linear regression model's predictions.

This finding aligns with the views of Surjowardojo (2014) and Pribadi *et al.* (2020), who emphasized that milking hygiene plays a critical role in the incidence of subclinical mastitis in dairy cows. Wulansari *et al.* (2017) further explained that pathogens causing subclinical mastitis can spread from infected cows to healthy ones due to unhygienic milking practices. Such transmission is often attributed to inadequate cleanliness of milking equipment, milker's hands, or clothing used during milking.

In the scatterplot depicting the relationship between TMHP and FR/FL, a negative correlation is observed, where improved hygiene practices correspond to lower scores for FR and FL. However, the scattered data distribution suggests a weaker correlation compared to TMHP's relationship with RR and RL. Outliers, or data points deviating from the general trend, also weaken the linear association. Meanwhile, the relationship between TMHP and RR/RL similarly shows a negative trend, where higher hygiene standards inversely correlate with scores for the rear teats. The data points for RR and RL are more clustered than those for FR and FL, indicating a stronger negative correlation for the rear udder quarters.

Overall, milking hygiene practices negatively affect scores across all udder quarters, but the strength of this relationship varies, with stronger correlations observed for the rear teats compared to the front ones. The results suggest that the rear quarters (RR and RL) are more susceptible to subclinical mastitis. This is attributed to the larger volume of the rear udder in dairy cows, which leads to higher milk production from the rear teats. Consequently, the rear teats require more time to close post-milking, providing microbes with an opportunity to enter the teat canal and increasing mastitis risk (Hanggara and Surjowardojo, 2022). According to the FAO and IDF (2011) and Utami *et al.* (2014), milking must be conducted under hygienic conditions, including maintaining cleanliness in the milking area and surrounding environment. Milking hygiene is part of Good Dairy Farming Practices (GDFP), encompassing the cleanliness of milkers, milking areas, equipment, and dairy cattle. Unhygienic milking can cause infection or inflammation of the udder, which ultimately reduces milk quality. The relationship between milking hygiene and udder health is well-documented, with studies indicating that poor hygiene practices lead to increased somatic cell counts (SCC) in milk, a key indicator of mastitis and udder inflammation (Zigo *et al.*, 2021; Hristov *et al.*, 2023). Specifically, the presence of pathogens such as Staphylococcus aureus in milk samples has been linked to inadequate milking hygiene, suggesting that such infections can significantly compromise milk quality (Deddefo *et al.*, 2023). Furthermore, the implementation of proper hygiene procedures during milking has been shown to reduce the incidence of mastitis, thereby improving the overall micro-

biological quality of milk (Hristov *et al*., 2023; Mihajlović *et al*., 2022).

Traditional Milking Hygiene Practices (TMHP) exhibit a negative relationship with all four variables (FR, FL, RR, RL). This negative association suggests that improved TMHP tends to reduce teat scores, potentially revealing specific mechanisms influencing udder health. These insights are crucial for further research, as they may clarify the link between TMHP and udder condition. However, deeper statistical analyses are required to identify the specific effects of each variable. Additionally, addressing outlier data is essential to ensure result validity and accuracy. Such steps will enhance the reliability and comprehensiveness of understanding the examined relationships.

The Relationship Between Traditional Milking Hygiene Practices and Teat Health

Milking hygiene practices are a critical aspect of dairy cattle health management. Numerous studies indicate that cleanliness during milking influences milk quality and teat health. In this study, the analyzed data include variables related to teat health: TMHP (Traditional Milking Hygiene Practices), FR (Front Right teat), FL (Front Left teat), RR (Rear Right teat), and RL (Rear Left teat) (Figure 2).

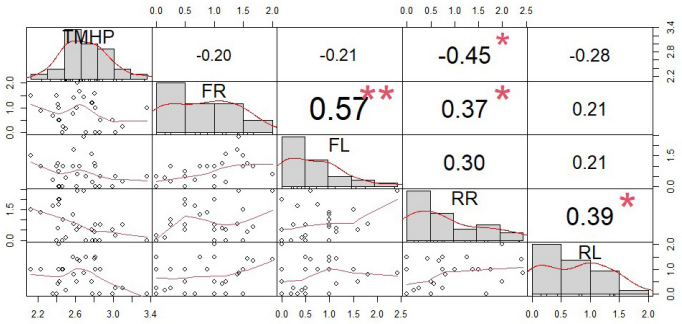

Figure 2: Correlation of TMHP with teat health.

The correlation plot reveals that traditional milking hygiene practices (TMHP) exhibit varying relationships with the health of individual teats. The correlation coefficient between TMHP and FL is 0.57, indicating a moderate to strong positive relationship between milking hygiene practices and the condition of the Front Left teat. This positive correlation suggests that improved hygiene practices may contribute to better teat health. Mastitis control and prevention can be achieved through several measures. Surjowardojo *et al*. (2019) highlight effective strategies, including maintaining clean and dry barn floors, ensuring sterile hands and clean nails during milking to avoid bacterial contamination, and conducting regular screenings to detect mastitis in cattle. Recent studies reinforce these strategies, emphasizing the importance of maintaining hygienic environments to reduce the incidence of mastitis. Cheng and Han (2020) discuss the critical role of environmental cleanliness in preventing both clinical and subclinical mastitis, noting that a clean and dry barn significantly reduces the risk of bacterial infections. Similarly, Soleimani-Rahimabad *et al*. (2023) found that proper barn hygiene was significantly associated with lower bacterial contamination in milk, underscoring its importance in mastitis prevention.

Previous research emphasizes that good hygiene practices, such as handwashing and equipment sterilization before milking, reduce the risk of mastitis a bacterial infection of dairy cow teats (Heikkilä et al., 2018). Mastitis is a common disease in dairy cattle that directly impacts milk productivity and quality (Ruegg, 2017). The significant correlation underscores the importance of hygiene in preventing teat infections, particularly in the front teats.

This analysis reinforces the critical role of TMHP in udder health management. The findings align with existing literature, emphasizing the need for stringent hygiene protocols to mitigate mastitis risks and enhance overall milk production outcomes. Further studies could explore the mechanisms underlying the observed correlations and evaluate the long-term effects of TMHP on herd health.

DISCUSSION

This study reveals that inadequate milking hygiene significantly increases the risk of subclinical mastitis in Holstein-Friesian dairy cows. Farms that neglect teat cleaning or employ ineffective cleaning methods exhibit higher prevalence of subclinical mastitis, underscoring the critical importance of Traditional Milking Hygiene Practices (Sudono *et al*., 2003; Surjowardojo, 2011). Mastitis transmission can occur via milker's hands due to contact with pathogenic bacteria between the milker and the cow. Thus, handwashing before and after milking is strongly recommended to prevent bacterial transfer from infected udders to healthy ones through contaminated hands. Effective measures to reduce mastitis spread include pre-milking udder cleaning and isolating infected cows to avoid transmission (Hanggara and Surjowardojo, 2022) (Figure 3).

Figure 3: Traditional milking.

Traditional milking practices by farmers often overlook hygiene aspects, particularly handwashing between cows, increasing the risk of mastitis due to bacterial contamination from infected cows (Surjowardojo *et al*., 2008). Sudono *et al*. (2003) emphasize that milker hygiene is vital, as hands can directly transmit mastitis-causing pathogens. Therefore, handwashing before and after milking is essential to prevent bacterial spread from infected to healthy udders. Improving milking hygiene is paramount. Hayati *et al*. (2019) state that mastitis incidence is closely linked to risk factors such as unhygienic milking management and incomplete milking. Farmer negligence toward these aspects contributes to high mastitis rates. Hygienic milking practices include pre-milking teat cleaning, sterilized equipment (Abdullah *et al*., 2023), and post-milking teat dipping with antiseptics (Surjowardojo, 2011). Small-scale farmers often use iodine-based solutions for teat dipping (Surjowardojo and Susilorini, 2016). This practice minimizes microbial entry into open teat canals, reducing mastitis risk (Susilorini *et al*., 2024).

These findings align with Rifa'i *et al*. (2024), who reported a 69.84% prevalence of subclinical mastitis in small-scale dairy farms in Toyomerto Village, Batu City, based on California Mastitis Test (CMT) results. Poor TMHP increases cows' exposure to mastitis-causing pathogens. Amejo (2024) reinforce this, noting that constraints such as time limitations, labor shortages, equipment scarcity, and cow behavior hinder the use of warm water for udder cleaning. These challenges reflect conditions in small-scale Batu farms, where awareness and access to proper hygiene practices remain limited due to knowledge gaps, resource constraints, or lack of technical support.

Key factors for quality milk production include maintaining milking sanitation, avoiding raw milk contamination, and ensuring hygienic handling (Kathiriya, 2024). Improper milking practices can lead to pathogenic bacterial contamination, threatening human health (Washabaugh *et al*., 2019; Ngolombe *et al*., 2024). Milk and dairy products may become disease vectors if collected or processed unhygienically (Yulianto *et al*., 2023). This study emphasizes the importance of cleaning milking equipment, as contaminated tools can introduce bacteria, causing udder infections. Udder health is directly linked to safe, high-quality milk production (Akköse and Polat, 2023). Therefore, farmer education on pre-milking udder and teat cleaning, hand hygiene, and equipment sterilization with detergents and disinfectants is critical to reducing subclinical mastitis prevalence. Improved knowledge enhances dairy management efficiency (Zecconi et al., 2018). Asfaw and Negash (2017) recommend using rubber gloves during milking, stripping five milk streams per teat to detect abnormalities, cleaning teats with sanitizers if soiled, pre-dipping with tested disinfectants (applied via dip cups, not sprays), allowing 30 seconds of contact time, and drying teats with separate towels. Teats should be inspected for cracks or lesions that harbor mastitis pathogens.

Enhancing milking hygiene, milk storage, and transport conditions is vital for preserving milk quality (Atigui *et al*., 2023). Additionally, Escherichia coli contamination can arise from milk cans exposed to feces (Cahyono and Sawitri, 2013; Ulfaturromah and Surjowardojo, 2022). Post-milking handling by farmers needs improvement; not all milk is filtered or stored in proper cans, with some using open buckets, accelerating microbial growth (Aziz *et al*., 2020). Efforts to boost awareness and compliance with food safety should prioritize closing knowledge gaps among small-scale farmers through training on safer milk production and TMHP (Kebede and Megerrsa, 2018; Xulu and Naidoo, 2023; Korale-Gedara *et al*., 2023). Implementing Good Dairy Farming Practices (GDFP) is crucial for sustainable dairy operations, ensuring cow health, safe milk, and environmental stewardship (Susilorini *et al*., 2024). Komala et al. (2022) recommend that small-scale farms adopt GDFP to improve management. Farmers in Tuyomerto Hamlet practice manual hand milking using the full-hand technique followed by stripping to complete milking. According to Surjowardojo *et al*. (2008), the whole-hand method minimizes teat injury risks. Rough milking can cause teat lesions, increasing susceptibility to mastitis-causing microorganisms.

Practical Implications

The findings of this study yield several practical recommendations for small-scale dairy farmers in Batu:

Pre-milking teat cleaning: Using clean water and a specific disinfectant applied to teat surfaces before milking can significantly reduce the risk of subclinical mastitis.

Hygienic equipment use: Regularly wash milking equipment with detergent and disinfectant to prevent bacterial contamination. Equipment should be sterilized routinely using steam, boiling water, or antiseptic solutions to eliminate microorganisms resistant to regular cleaning.

Farmer education and training: Enhance farmer awareness and knowledge of proper hygiene practices through targeted training and extension programs.

By adopting improved hygiene practices, farmers can enhance herd health, elevate milk quality, and ultimately boost farm productivity and profitability.

CONCLUSIONS AND RECOMMENDATIONS

This study concludes that Traditional Milking Hygiene

Practices (TMHP) play a critical role in reducing the prevalence of subclinical mastitis in smallholder dairy farms. Results from the California Mastitis Test (CMT) indicate lower subclinical mastitis scores in farms with stringent hygiene practices. Rear teats are more susceptible to mastitis compared to front teats, likely due to prolonged teat closure time and higher milk volume. Optimizing hygiene practices is expected to improve animal health, milk quality, and farm productivity. The findings underscore the importance of implementing Good Dairy Farming Practices (GDFP) at the smallholder level to support operational efficiency and sustainability.

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NOVELTY STATEMENTS

This study uniquely investigates the impact of traditional milking hygiene practices on subclinical mastitis prevalence in Holstein-Friesian cows within smallholder farms. It is the first to quantify the relationship between specific hygiene routines and mastitis occurrence using California Mastitis Test data and offers evidence-based recommendations tailored to traditional farm settings.

AUTHOR'S CONTRIBUTIONS

Tri Eko Susilorini: Critical review and revision.
Poespitasari Hazanah Ndaru: Critical review and revision.
Rifa'i: Data acquisition, Analysis and manuscript drafting.
Puguh Surjowardojo: Conception, Critical review and approved.

Conflict of Interest

The authors declare no conflict of interest.

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