Research Article

High Subclinical Mastitis in Dairy Goats Tied to Hygiene Gaps and Environmental Bacteria

Fauzia Nadia, Hilda Susanty*, Eli Ratni

Department of Animal Production Technology, Faculty of Animal Science, Universitas Andalas, Padang 25163, West Sumatra, Indonesia.

Abstract | Subclinical mastitis is a common yet often undetected condition in dairy goats that negatively affects milk quality and farm productivity. This study aimed to determine the prevalence of subclinical mastitis and characterize the bacterial profile in lactating Peranakan Etawah (PE) goats on a smallholder dairy farm in West Sumatra, Indonesia, while assessing hygiene-related management practices on the farm. A cross-sectional survey was conducted on all lactating PE goats (n = 12) in the herd. Subclinical mastitis was screened using the IPB-1 reagent on a California Mastitis Test-style paddle. Milk from IPB-1 positive teats was aseptically collected (n = 17) and cultured on Blood Agar and MacConkey Agar, followed by Gram staining and biochemical tests. Farm management practices were evaluated using a semi-quantitative scoring system (0–4) for animal hygiene, housing sanitation, milking technique, post-harvest handling, dry-off management, record keeping, and manure/environment management. The prevalence of subclinical mastitis was 83.3% (10/12 goats), with 17 of the 24 teats testing positive (70.8%). The bacterial isolates were predominantly Gram-positive cocci, which were presumptively identified as environmental Micrococcus-like organisms based on their phenotypic and biochemical characteristics. The Gram-negative isolates were limited and not identified as *Escherichia coli*. *Staphylococcus aureus* was not isolated from the cultured samples under the culture conditions used in this study. The findings indicate a high occurrence of subclinical mastitis associated with poor hygiene practices and the presence of Gram-positive cocci in the environment. Improving udder hygiene and milking management is critical for enhancing milk quality in smallholder PE goat systems.

Keywords | Dairy goats, Mastitis, Milk yield, Subclinical infection, Udder hygiene

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***Correspondence** | Hilda Susanty, Department of Animal Production Technology, Faculty of Animal Science, Universitas Andalas, Padang 25163, West Sumatra, Indonesia; **Email:** hsusanty@ansci.unand.ac.id
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INTRODUCTION

Peranakan Etawah (PE) goats are an important crossbred dairy breed in Indonesia, valued for their adaptability to tropical climates and ability to produce substantial amounts of milk. They serve as a key source of income for smallholder farmers, who typically manage three to ten goats on small plots of land or even without dedicated farmland (Cyrilla *et al*., 2016). Small-scale PE dairy enterprises contribute to local agribusiness chains and support rural livelihoods, particularly in regions such as West Sumatra. As these smallholder systems grow, ensuring productivity, animal health, and reproduction requires careful attention to management and hygiene practices (Nyokabi *et al*., 2021; Widyastuti *et al*., 2023; Zanon *et al*., 2024).

Goat milk differs from cow milk in its nutritional profile, often containing higher levels of certain minerals and being better tolerated by individuals with lactose

intolerance (Nayik *et al*., 2022; Verma *et al*., 2025). These qualities, along with growing consumer awareness of its functional properties, have increased the demand for goat milk in Southeast Asia (Alkaisy *et al*., 2023). Evidence also suggests that regular consumption of goat milk offers health benefits, further stimulating interest among local consumers (Dhasmana *et al*., 2021; Nayik *et al*., 2021; Chen *et al*., 2023).

Mastitis, particularly in its subclinical form, is a major challenge to the quality and safety of dairy products (Hasan *et al*., 2022). Unlike clinical mastitis, subclinical mastitis does not produce visible signs, such as udder inflammation or abnormal milk appearance, making it difficult to detect without screening tests. Nevertheless, it can reduce milk yield, alter its composition, and cause economic losses to commercial or smallholder farmers (Pakrashi *et al*., 2023; Corrêa *et al*., 2024). In tropical production systems, subclinical mastitis is often underestimated because of limited monitoring and inconsistent milking hygiene practices.

The etiology of subclinical mastitis is multifactorial, with environmental hygiene and farm management being critical points in its development. Bacteria from the environment can contaminate teats and milking equipment, especially when sanitation, manure management, and milking routines are inconsistent. Gram-positive environmental organisms, including *Micrococcus* spp., are commonly associated with bedding, dust, skin, and milking these bacteria are generally considered minor pathogens, repeated exposure may be associated with an increased risk of intramammary inflammation under suboptimal hygienic conditions. Although *Micrococcus* spp. are generally regarded as minor or opportunistic organisms, strong reactions in indirect mastitis tests, such as IPB-1, reflect an inflammatory response and do not necessarily indicate an active intramammary infection caused by a single bacterial species.

Early detection relies on indirect tests that estimate somatic cell responses in milk. The California Mastitis Test (Pyorala, 2003; Roberts, 2024; Ramuada *et al*., 2024) and its local adaptations, including the IPB-1 reagent, are widely used as rapid, low-cost field tools for mastitis detection (Susanty *et al*., 2024). Positive reactions typically prompt bacteriological examinations to identify causative agents and differentiate contagious pathogens from environmental contaminants. Culture-based identification combined with simple biochemical profiling remains practical in resource-limited, smallholder settings.

Despite the importance of PE goats in Indonesia, data on the prevalence of subclinical mastitis and associated bacterial profiles under smallholder management remain limited. Few studies have integrated microbiological findings with systematic assessments of farm hygiene and technical management. This study aimed to determine the prevalence of subclinical mastitis in lactating PE goats at a smallholder farm in West Sumatra, characterize bacterial isolates from IPB-1 positive milk, and identify hygiene-related management gaps that may be associated with environmental contamination. By linking epidemiological data with practical management insights, this study provides information to support improved udder health, milk quality, and economic sustainability of smallholder PE goat dairy systems. Other important predisposing factors for subclinical mastitis in smallholder systems, such as nutritional status, parasitic burden, and animal stress, were not evaluated in the present study and should be addressed in future studies.

MATERIALS AND METHODS

Study area and period

The study was conducted from November to December 2024 at a smallholder dairy goat farm in Payakumbuh City, West Sumatra, Indonesia. The farm maintained 64 heads of Peranakan Etawah (PE) goats reared under a semi-intensive management system. Laboratory analyses, including bacteriological culture, gram staining, and biochemical tests, were performed at the Veterinary Laboratory of Balai Veteriner Bukittinggi (BVB), West Sumatra, Indonesia.

Animals and study design

A descriptive cross-sectional design was used. All lactating PE goats present during the study period (n= 12) were included using a total sampling approach, representing different parities and lactation stages of the goats. Farm-level information on housing, hygiene, and milking practices was obtained through direct observation and informal interviews with farmers. This study focused on detecting subclinical mastitis, bacteriological examination of positive milk samples, and assessment of hygiene-related practices.

Screening for subclinical mastitis

Subclinical mastitis was screened using the IPB-1 reagent following a California Mastitis Test-type procedure (Susanty *et al*., 2024). Each udder half was washed and dried, and the foremilk was discarded. Approximately 2 mL of milk was mixed with an equal volume of IPB-1 reagent in a paddle well and gently rotated for 15–30 s. Reactions were scored as negative (–), trace or weakly positive (+), moderately positive (++), or strongly positive (+++), based on the gel formation (Praja *et al*., 2023). A goat was considered positive if at least one teat showed a reaction to the test. Animal-level prevalence was calculated

as the number of positive goats divided by the total number of lactating goats and expressed as a percentage.

Milk sampling

Milk was aseptically collected from IPB-1 positive teats (n= 17, 20 mL each) into sterile tubes after cleaning and flaming the teat orifice with 70% alcohol. Care was taken to avoid contamination from the skin, hands, or the environment. After collection, each sample was correctly labeled and immediately stored in a sterile cooling box (3-4°C). Samples were transported to the laboratory in a cooled container and stored at refrigeration temperature until they were analyzed (Ratni *et al*., 2024).

Bacteriological culture and isolation

Milk samples were homogenized and streaked onto Blood Agar for Gram-positive bacteria and MacConkey Agar for Gram-negative bacteria using the T-streak method. The plates were incubated aerobically at 37°C for 24–48 h. Colony morphology, hemolysis, and growth characteristics were also recorded. Representative colonies were selected for further analysis.

Gram staining and preliminary identification

Gram staining was performed to observe the bacterial morphology and Gram characteristics. The procedure included fixation of the smear on a glass slide using sterile distilled water and air-drying for 30 s, followed by staining with crystal violet for 1–2 min, treatment with Lugol's iodine solution for 30 s, decolorization with 96% alcohol, and counterstaining with safranin for 2 min. The slides were then rinsed and dried before being examined under a light microscope at 100× magnification using immersion oil (Sarudji *et al*., 2017). The isolates were categorized according to their Gram reaction and cell morphology (cocci, bacilli, or coccobacilli). Gram-positive cocci consistent with *Micrococcus* or *Staphylococcus*, and Gram-negative rods suspected as coliforms, were further analyzed with biochemical tests.

Biochemical characterization

Biochemical tests were performed following standard procedures. Gram-positive isolates were tested using TSIA, catalase, oxidase, motility, indole, urease, citrate utilization, carbohydrate fermentation (glucose, lactose, sucrose, and mannitol), methyl red (MR), Voges–Proskauer (VP), oxidative–fermentative (O/F), and gelatin hydrolysis tests. Gram-negative isolates were evaluated using TSIA, gas and H_2S production, motility, indole, urease, citrate, MR, and VP tests. Presumptive identification was based on gram reaction, colony morphology, and biochemical profiles, with *Staphylococcus* spp., *Escherichia coli*, and environmental bacteria (e.g., *Micrococcus* spp.) considered.

Assessment of technical management and hygiene

Farm management practices were evaluated through observations and farmer interviews. Nine aspects were assessed: animal cleanliness, cleaning methods, barn sanitation, milking technique, post-harvest handling, kid management, dry-off management, record keeping, and manure/environmental management (FAO, 2011). Each aspect was scored on a semi-quantitative scale from 0 to 4, with higher scores indicating better practice.

Data analysis

The data were analyzed descriptively. The prevalence of subclinical mastitis was calculated at the animal and teat levels. The bacteriological findings are summarized in tables based on culture, Gram staining, and biochemical profiles. Management scores were presented as mean values for each aspect and were interpreted in relation to the occurrence of mastitis.

RESULTS AND DISCUSSION

Farm hygiene and management assessment

The assessment of farm hygiene and management practices indicated a moderate level of implementation, with a mean indicator score of 2.78 (Tables 1 and 2). The highest scores were recorded for goat hygiene procedures, particularly cleaning methods, and for kid and replacement stock management (score 4 for each), reflecting relatively good compliance in these domains. Intermediate scores (score 3) were observed for post-milking handling, farm recording systems, and manure and environmental management, suggesting that these practices were in place but not yet optimally implemented. In contrast, lower scores were predominantly associated with routine hygiene measures and milking execution, which are critical control points for minimizing bacterial contamination during milk harvesting and preventing intramammary infections.

Farm management practices were assessed using nine technical parameters and a semi-quantitative scoring system ranging from 0 to 4. The implementation score for each parameter represents the most frequently observed practice, as determined through direct on-farm observations and structured interviews with farmers. The lack of routine daily cleaning of both goats and barns was one of the main factors associated with environmental milk contamination.

Deficiencies in hygiene-related practices were likely associated with increased exposure of the teat canal to environmental bacteria, particularly presumptive Micrococcus-like organisms, during and after milking. Key hygiene measures, including the consistent implementation of hygienic milking procedures, pre- and post-milking

Table 1: Assessment criteria and implementation scores of technical management practices in dairy goat farming.

No.	Aspect factor	Technical alternative	Specification	Score	Implementation score
1.	Goat hygiene (routine cleaning)	Before milking, twice a day	Preventing new contamination of milk from feces and urine	4	
		After milking, twice a day		3	
		Once a day		2	2
		Rarely		1	
		Not practiced		0	
2.	Goat hygiene procedure (cleaning method)	All areas around the udder	Reducing milk contamination by bacteria present around the udder	4	4
		Udder area only		3	
		All body parts washed and cleaned		2	
		All body parts rinsed only		1	
		Not cleaned		0	
3.	Housing sanitation (barn cleaning)	Before milking, twice a day	Maintaining an odor-free housing environment and ensuring goat comfort	4	
		After milking, twice a day		3	
		Once a day		2	2
		Rarely		1	
		Not practiced		0	
4.	Milking procedure/technique	Correct and proper	Minimizing the risk of udder mastitis due to improper milking practices	4	
		Correct but not optimal		3	
		Proper but not correct		2	2
		Not proper and not correct		1	
		Incorrect		0	
5.	Post-milking handling (post-harvest)	Correct and proper	Handling fresh milk and its derived products in accordance with food safety procedures	4	
		Correct but not optimal		3	3
		Proper but not correct		2	
		Not proper and not correct		1	
		Incorrect		0	
6.	Kid and replacement stock management	Correct and proper	Optimizing doe replacement and herd regeneration to sustain milk production	4	4
		Correct but not optimal		3	
		Proper but not correct		2	
		Not proper and not correct		1	
		Incorrect		0	
7.	Lactation management (dry-off practice)	Two months before kidding	Preparing pregnant goats for parturition and optimal milk production	4	
		One and a half months before kidding		3	
		One month before kidding		2	2
		Less than one month before kidding		1	
		No dry period		0	
8.	Farm recording system	Available, complete, and well maintained	Comprehensive recording of all farm activities	4	
		Available, complete, and poorly maintained		3	3
		Available, incomplete, but well maintained		2	
		Available, incomplete, and poorly maintained		1	
		Not available		0	
9.	Manure and environmental management	Processed into biogas	Managing feces, urine, and feed waste to improve animal health and generate income	4	
		Processed into organic fertilizer		3	3
		Disposed of in the garden		2	
		Disposed of into the river		1	
		Treated as waste		0	

Note: Scores ranged from 0 to 4, where 0 = not implemented, 1 = very poor, 2 = poor/limited, 3 = moderate, and 4 = good/well implemented.

Table 2: Technical management and husbandry practices at the study farm (semi-quantitative score, 0–4).

No.	Indicator	Score
1	Goat hygiene (routine cleaning)	2
2	Goat hygiene procedure (cleaning method)	4
3	Housing sanitation (barn cleaning)	2
4	Milking procedure/technique	2
5	Post-milking handling (post-harvest)	3
6	Kid and replacement stock management	4
7	Lactation management (dry-off practice)	2
8	Farm recording system	3
9	Manure and environmental management	3
	Average	2.78

teat sanitation, proper handling of milking equipment, and maintenance of clean housing and milking areas, are essential control points for preventing environmental mastitis (FAO, 2011). Inadequate implementation of these practices is associated with an increased risk of subclinical intramammary inflammation in dairy cows. In addition, routine visual inspection of the udder and teats before milking and strict consistency in daily milking routines are emphasized as fundamental components of good milking hygiene to support the early detection of mastitis and reduce bacterial contamination (FAO, 2023).

Prevalence of subclinical mastitis

Subclinical mastitis screening using the IPB-1 reagent yielded reaction scores ranging from negative (–) to strongly positive (+++). In the left udder halves, the reaction distribution was negative (n = 4), weakly positive (+) (n = 1), moderately positive (++) (n = 3), and strongly positive (+++) (n = 4), whereas in the right udder halves, the distribution was – (n = 3), + (n = 3), ++ (n = 1), and +++ (n = 5). Overall, strong gel formation (+++) was observed more frequently than intermediate reactions in both udder halves, indicating that a considerable proportion of the sampled udder halves exhibited elevated somatic cell–associated reactions according to the IPB-1 scoring criteria.

Screening of 12 lactating Peranakan Etawah (PE) goats revealed a high prevalence of subclinical mastitis, with 83.33% (10/12) of the animals testing positive. At the teat level, 70.83% (17/24) of the teats showed positive reactions, ranging from weak (+) to strong (+++) (Table 3). The highest prevalence was observed in goats with parity 1–2, particularly during early lactation (1.5–7 months post-partum). Most affected teats exhibited moderate to strong reactions, suggesting a substantial subclinical inflammatory response within the mammary gland. This finding aligns with previous reports in dairy goats, where 68.51 % of tested milk samples were positive for subclinical mastitis in West Sumatran smallholder herds (Susanty *et al.*, 2025).

Table 3: Subclinical mastitis screening results using IPB-1 reagent (CMT-type paddle) in lactating Peranakan Etawah (PE) goats (n = 12).

PE goat ID	Parity (lactation no.)	Month in lactation	Left teat	Right teat
1	1	3.5	++	+
2	1	3.5	+	-
3	1	9.0	+++	+
4	2	7.0	-	++
5	2	7.0	-	-
6	5	10.0	++	+
7	5	10.0	++	+++
8	2	10.0	+++	+++
9	5	1.5	+++	+++
10	1	1.5	+++	+++
11	1	7.0	-	-
12	1	7.0	-	+++

Note: IPB-1 reaction score interpretation: "-" = negative, "+" = weak, "++" = moderate, "+++" = strong.

The high prevalence of subclinical mastitis observed in this smallholder herd was likely associated with suboptimal hygiene and milking practices in the herd. Farm observations indicated that udder cleaning was performed only once prior to milking, with no post-milking teat disinfection. Under such conditions, environmental bacteria present on the teat skin, milker hands, milking equipment, and barn surfaces may enter the teat canal and be associated with intramammary inflammation. Furthermore, inadequate barn sanitation, high humidity, and wet bedding create an environment conducive to bacterial survival and transmission. These findings are consistent with previous reports indicating a higher prevalence of mastitis in smallholder farming systems with limited hygiene practices than in more intensive dairy operations (Megersa *et al.*, 2010; Praja *et al.*, 2023).

Bacteriological characteristics of isolates

Seventeen milk samples collected from IPB-1–positive teats were cultured on Blood Agar and MacConkey Agar. Colony morphology was predominantly white (n= 9), followed by yellow, gray, and pink (n= 3 each). Gram staining revealed a clear predominance of Gram-positive bacteria (n= 15), while Gram-negative organisms were infrequently detected (n = 3). Based on cellular morphology, cocci were the most common (n = 14), followed by bacilli (n = 3) and coccobacilli (n = 1). Overall, these findings indicate that the bacterial population isolated from milk samples was largely composed of gram-positive coccoid organisms.

Table 4: Colony morphology and preliminary characterization of bacterial isolates recovered on Blood Agar from IPB-1 positive milk samples of lactating Peranakan Etawah goats (ordered according to Table 2; n = 17).

PE goat ID	Teat	IPB-1 score	Sample code	Colony color	Margin	Gram reaction	Morphology	Further tests
1	LT	++	PE-G1-LT(+2)	Yellow	Entire	Positive	Coccus	Yes
1	RT	+	PE-G1-RT(+1)	White	Entire	Positive	Coccus	Yes
2	LT	+	PE-G2-LT(+1)	–	–	–	–	–
3	LT	+++	PE-G3-LT(+3)	Yellow	Entire	Positive	Bacillus	No
3	RT	+	PE-G3-RT(+1)	–	–	–	–	–
4	RT	++	PE-G4-RT(+2)	Grey	Entire	Positive	Bacillus	No
6	LT	++	PE-G6-LT(+2)	White	Entire	Positive	Coccus	Yes
6	RT	+	PE-G6-RT(+1)	White	Entire	Positive	Coccus	Yes
7	LT	++	PE-G7-LT(+2)	Yellow	Entire	Positive	Bacillus	No
7	RT	+++	PE-G7-RT(+3)	Grey	Entire	Positive	Coccus	Yes
8	LT	+++	PE-G8-LT(+3)	White	Entire	Positive	Coccus	Yes
8	RT	+++	PE-G8-RT(+3)	White	Entire	Positive	Coccus	Yes
9	LT	+++	PE-G9-LT(+3)	White	Entire	Positive	Coccus	Yes
9	RT	+++	PE-G9-RT(+3)	White	Entire	Positive	Coccus	Yes
10	LT	+++	PE-G10-LT(+3)	White	Entire	Positive	Coccus	Yes
10	RT	+++	PE-G10-RT(+3)	Grey	Entire	Positive	Coccobacillus	No
12	RT	+++	PE-G12-RT(+3)	White	Entire	Positive	Coccus	Yes

Note: "–" indicates no observable bacterial growth/recorded colony characteristics on Blood Agar. LT = left teat; RT = right teat. IPB-1 score: "+" = weak, "++" = moderate, "+++" = strong reaction.

Table 5: Colony morphology and preliminary characterization of bacterial isolates recovered on MacConkey Agar from IPB-1 positive milk samples of lactating Peranakan Etawah goats (ordered according to Table 2; n = 17)

PE goat ID	Teat	IPB-1 score	Sample code	Colony color	Margin	Gram reaction	Morphology	Further tests
1	LT	++	PE-G1-LT(+2)	Pink	Entire	Negative	Coccus	Yes
1	RT	+	PE-G1-RT(+1)	–	–	–	–	–
2	LT	+	PE-G2-LT(+1)	–	–	–	–	–
3	LT	+++	PE-G3-LT(+3)	–	–	–	–	–
3	RT	+	PE-G3-RT(+1)	–	–	–	–	–
4	RT	++	PE-G4-RT(+2)	Pink	Entire	Negative	Coccus	Yes
6	LT	++	PE-G6-LT(+2)	–	–	–	–	–
6	RT	+	PE-G6-RT(+1)	–	–	–	–	–
7	LT	++	PE-G7-LT(+2)	–	–	–	–	–
7	RT	+++	PE-G7-RT(+3)	–	–	–	–	–
8	LT	+++	PE-G8-LT(+3)	–	–	–	–	–
8	RT	+++	PE-G8-RT(+3)	–	–	–	–	–
9	LT	+++	PE-G9-LT(+3)	–	–	–	–	–
9	RT	+++	PE-G9-RT(+3)	Pink	Entire	Negative	Coccus	Yes
10	LT	+++	PE-G10-LT(+3)	–	–	–	–	–
10	RT	+++	PE-G10-RT(+3)	–	–	–	–	–
12	RT	+++	PE-G12-RT(+3)	–	–	–	–	–

Note: "–" indicates no bacterial growth/recorded colony characteristics on MacConkey Agar. LT = left teat; RT = right teat. IPB-1 score: "+" = weak, "++" = moderate, "+++" = strong reaction.

Most isolates exhibited growth on Blood Agar and were identified as gram-positive cocci arranged in tetrads or irregular clusters, a morphology consistent with environmental *Micrococcus* spp. (Tables 4–6). Only a small number of gram-negative isolates were recovered, and none showed phenotypic characteristics consistent with

Escherichia coli (Table 7). Notably, *Staphylococcus aureus* was not isolated from the cultured samples under the culture conditions used in this study, suggesting a limited role for contagious mastitis pathogens in this herd.

Table 6: Biochemical profiles of Gram-positive isolates recovered on Blood Agar (n = 11).

Sample code	TSIA	Gas	H_2S	Catalase	Oxidase	Motility	Indole	Urease	Citrate	Lactose	Glucose	Sucrose	Mannitol	MR	VP	O/F	Gelatin	Presumptive ID
PE-G1-LT(+2)	Red/Yellow	–	–	+	–	–	–	+	–	+	+	–	–	–	–	–/–	+	*Micrococcus*-like
PE-G1-RT(+1)	Red/Red	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–/–	–	*Micrococcus*-like
PE-G6-LT(+2)	Red/Yellow	–	–	+	–	+	–	+	–	+	+	–	–	+	–	–/–	+	*Micrococcus*-like
PE-G6-RT(+1)	Red/Red	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–/–	–	*Micrococcus*-like
PE-G7-RT(+3)	Red/Yellow	–	–	+	–	+	+	+	–	+	+	–	–	+	–	–/–	+	*Micrococcus*-like
PE-G8-LT(+3)	Yellow/Yellow	–	–	+	–	–	–	+	–	–	+	+	–	–	–	–/–	–	*Micrococcus*-like
PE-G8-RT(+3)	Yellow/Yellow	–	–	+	–	+	–	+	–	–	–	–	–	+	–	–/–	+	*Micrococcus*-like
PE-G9-LT(+3)	Red/Yellow	–	–	+	–	–	–	+	–	+	+	–	+	–	–	–/–	–	*Micrococcus*-like
PE-G9-RT(+3)	Yellow/Yellow	–	–	+	–	–	–	+	–	–	+	+	–	+	–	–/–	+	*Micrococcus*-like
PE-G10-LT(+3)	Red/Yellow	–	–	+	–	–	–	+	–	–	+	+	–	–	–	–/–	+	*Micrococcus*-like
PE-G12-RT(+3)	Red/Yellow	–	–	+	–	–	–	+	–	–	+	–	–	–	–	–/–	+	*Micrococcus*-like

Note: TSIA results are presented as slant/butt color reactions (Red = alkaline; Yellow = acid). "+" = positive; "–" = negative. Presumptive identification was based on Gram reaction, colony morphology, and biochemical patterns.

Table 7: Biochemical profiles of Gram-negative isolates recovered on MacConkey Agar (n = 3).

Sample code	TSIA	Gas	H_2S	Motility	Indole	Urease	Citrate	MR	VP	Presumptive ID
PE-G1-LT(+2)	Red/Red	–	–	+	–	–	+	–	–	Negative
PE-G4-RT(+2)	Red/Red	–	–	+	–	+	+	–	–	Negative
PE-G9-RT(+3)	Yellow/Yellow	–	–	+	–	–	+	+	–	Negative

Note: TSIA results are presented as slant/butt color reactions (Red= alkaline; Yellow= acid). "+" = positive; "–" = negative. Presumptive identification indicates the isolates did not match the biochemical profile of *Escherichia coli*.

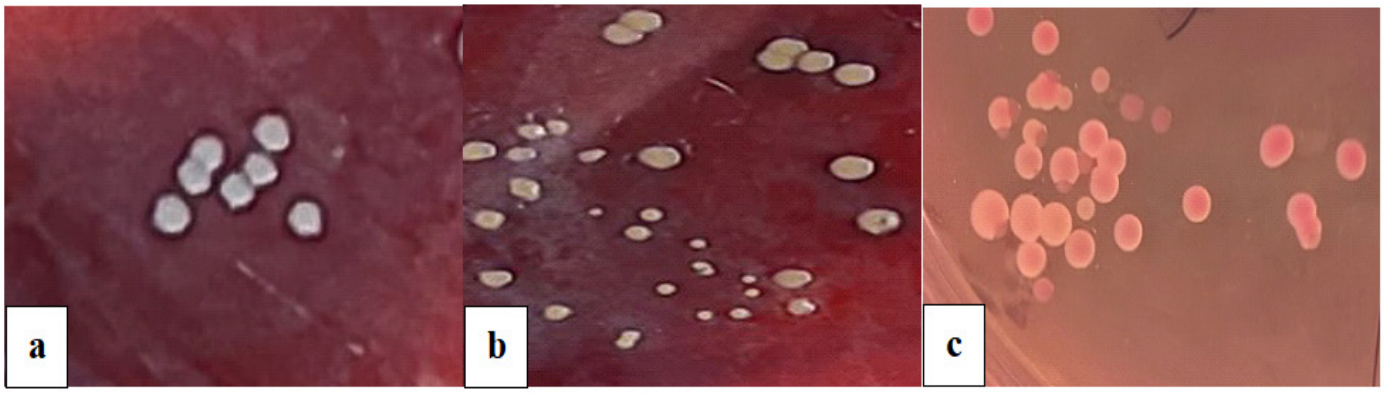

Figure 1: Representative bacterial colonies isolated from milk samples on Blood Agar (a, b) and MacConkey Agar (c). (a) White, circular, convex colonies of Gram-positive bacteria. (b) Yellow, circular, convex colonies of Gram-positive bacteria. (c) Pink/reddish, circular, convex colonies of Gram-negative bacteria.

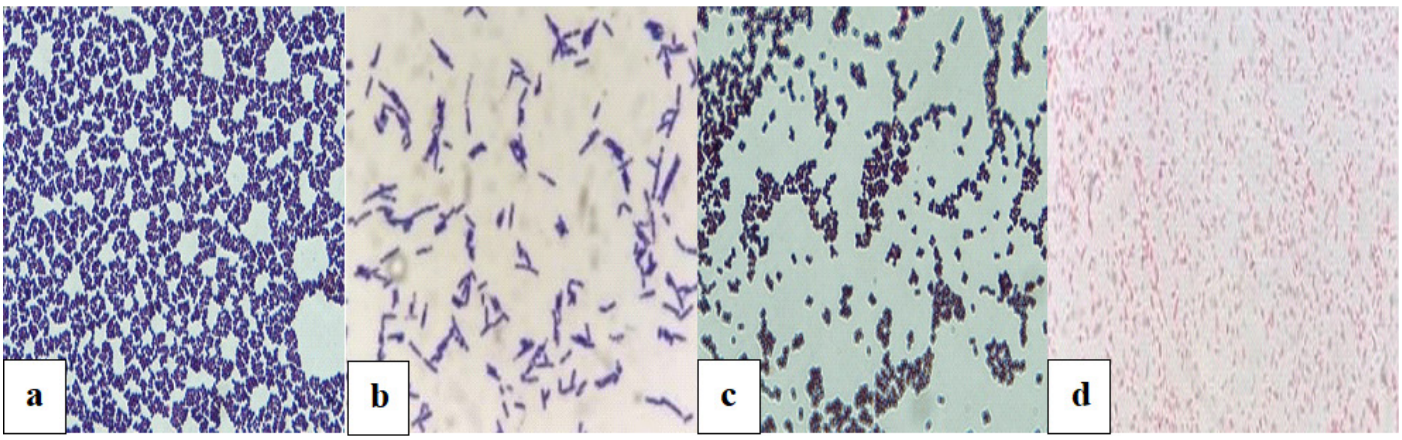

Figure 2: Gram-stained bacteria from milk isolates visualized under oil immersion (1000x magnification). (a) Purple, spherical cells of Gram-positive cocci arranged in clusters/tetrads. (b) Purple, rod-shaped cells of Gram-positive bacilli. (c) Purple, oval-shaped cells of Gram-positive coccobacilli. (d) Pink/red, rod-shaped cells of Gram-negative bacilli.

The dominance of gram-positive environmental bacteria indicates that subclinical mastitis in this herd was more likely associated with environmental exposure rather than direct animal-to-animal transmission. These bacterial groups are commonly found on teat skin, milking equipment, bedding, and housing surfaces, and may enter the mammary gland through inadequate udder preparation or contaminated hands and utensils during milking. This bacteriological profile is biologically consistent with farm-level observations, indicating suboptimal implementation of hygiene- and milking-related practices, which may have facilitated bacterial transfer during milking.

The cultured bacterial colonies exhibited distinct characteristics according to their gram type. Gram-positive bacterial colonies were generally circular, convex or raised, with smooth margins, and appeared white, gray, or yellow in color (Figure 1a, b). In contrast, the gram-negative bacterial colonies were circular and convex with smooth margins and pink to reddish coloration (Figure 1c). *Staphylococcus epidermidis* produces white-pigmented colonies, whereas *Micrococcus* spp. form yellow and white pigmented colonies with circular and convex appearances (Thoyib *et al*., 2007).

In addition to culture observations, Gram staining was performed to identify the Gram characteristics of the isolated bacteria. Gram-positive bacteria appeared purple after staining and were generally cocci in shape, whereas Gram-negative bacteria appeared red or pink and were typically rod-shaped. The results of gram staining of the samples are presented in Figure 2. Based on Figure 2, the gram-positive bacteria exhibited cocci, bacilli, and coccobacilli morphologies, with cocci being the predominant form among gram-positive isolates. Further biochemical tests were performed on 11 samples that were Gram-positive cocci. Isolates showing Gram-positive bacilli morphology were not subjected to further biochemical testing because they were considered likely environmental contaminants (e.g. Bacillus-like organisms) and were not the primary focus of this study on Gram-positive cocci associated with subclinical mastitis.

The gram-negative bacteria identified in this study exhibited bacillary morphology. Three samples showing gram-negative, rod-shaped characteristics were selected for further identification of *Escherichia coli*, whereas the remaining 14 samples could not be subjected to further testing because no bacterial growth was observed. Samples that met the criteria for subsequent analysis were subcultured to obtain pure isolates prior to biochemical testing. Because several bacterial species share very similar phenotypic characteristics, biochemical tests are required to enable more accurate identification and minimize misidentification (MacFaddin, 1980). In addition to supporting bacterial identification, biochemical testing can be used to monitor the presence and dynamics of bacteria in milk, thereby contributing to the establishment of preventive measures against the occurrence of pathogenic bacteria in livestock production systems (Artdita *et al*., 2021).

Biochemical characterization of isolates

Based on preliminary and biochemical testing, *Micrococcus* spp. was the most frequently assigned presumptive identity among the isolates (n= 11), indicating that organisms consistent with *Micrococcus* characteristics dominated the bacterial population recovered in this study. These isolates were gram-positive and showed a consistent biochemical profile, including catalase-positive and oxidase-negative reactions, non-motility, and an inability to utilize citrate as the sole carbon source. Carbohydrate fermentation patterns were variable, with glucose and lactose being fermented more frequently than sucrose and mannitol. Collectively, these biochemical characteristics supported the identification of *Micrococcus* spp. rather than *Staphylococcus* spp.

Three Gram-negative isolates were also recovered and exhibited limited carbohydrate fermentation, variable urease and citrate reactions, and negative indole test results. These profiles were not consistent with those of *Escherichia coli*, and their low frequency indicated a minimal contribution of gram-negative bacteria to subclinical mastitis in this herd.

The predominance of presumptive Micrococcus-like organisms suggests that environmental and skin-associated bacteria were commonly observed among isolates recovered from subclinical mastitis cases. *Micrococcus* spp. are commonly regarded as commensal or opportunistic organisms associated with skin and milking environments (Timm *et al*., 2020). Their frequent isolation from milk samples may therefore reflect incomplete hygiene barriers during milking and repeated exposure of the teat canal to environmental contaminants during milking. Under suboptimal management conditions, such organisms may be associated with persistent subclinical inflammation and elevated somatic cell counts, ultimately compromising the milk quality. The differentiation between *Micrococcus* spp., *Kocuria* spp., and coagulase-negative staphylococci based solely on conventional biochemical tests is limited. Therefore, the isolates in this study were classified only as presumptive environmental Micrococcus-like organisms based on their phenotypic and biochemical characteristics.

Implications for smallholder dairy goat management

The combined pattern of IPB-1 positivity and the predominance of skin-associated gram-positive bacteria indicate that control strategies should prioritize hygiene at critical points directly influencing milk contamination and udder health. Key measures include consistent pre-milking udder preparation, use of clean hands and milking equipment, improved housing sanitation, and effective post-milking teat management. Strengthening these practices is expected to reduce the bacterial load at the teat end and may help lower the frequency of positive IPB-1 reactions over time.

The results of this study suggest that subclinical mastitis in smallholder Peranakan Etawah goats is primarily associated with environmental contamination rather than contagious mastitis pathogens. Consequently, interventions should focus on improving basic hygiene practices that are practical and economically feasible for smallholders. This is in line with previous findings showing that regular screening for subclinical mastitis, pre- and post-milking udder washing, and proper bedding sanitation are strongly recommended to reduce the occurrence of mastitis at the farm level (Fesseha *et al*., 2021). Priority actions include enhancing the frequency and effectiveness of udder cleaning, standardizing milking techniques, implementing post-milking teat disinfection, maintaining dry and clean bedding, and improving overall barn sanitation (Plozza *et al*., 2011).

Improvements in these management practices are expected to reduce environmental bacterial pressure, lower the incidence of intramammary infection, and enhance milk quality, shelf life, and consumer safety. In addition, routine monitoring using simple field-based screening tools, such as the IPB-1 reagent or the California Mastitis Test, can support the early detection of subclinical mastitis and facilitate timely management interventions.

Study limitations and future perspectives

This study was conducted on a single smallholder farm with a limited number of lactating goats, which may have limited the generalizability of the findings. Furthermore, bacterial identification was based on conventional culture, gram staining, and biochemical characterization without molecular confirmation. Despite these constraints, this study provides important baseline information on the prevalence, bacterial profiles, and management-related risk factors of subclinical mastitis in Peranakan Etawah goats under smallholder farming conditions in West Sumatra.

Future research should involve a larger number of farms and animals, incorporate molecular approaches for pathogen identification, evaluate antimicrobial susceptibility patterns, and assess the effectiveness of targeted hygiene interventions and training programs for farmers. Such efforts are essential for improving udder health, enhancing milk safety, and supporting the long-term sustainability of smallholder dairy goat production systems.

CONCLUSION

Subclinical mastitis was highly prevalent in Peranakan Etawah goats under smallholder management in West Sumatra, with presumptive environmental Micrococcus-like organisms being the most frequently recovered isolates. Staphylococcus aureus and Escherichia coli were not isolated from the milk samples under the culture conditions applied in this study. These findings suggest that udder health problems in this herd were primarily associated with environmental contamination and suboptimal milking hygiene rather than contagious mastitis pathogens. These results emphasize the importance of practical and low-cost interventions, including proper udder preparation, consistent milking routines, and improved barn sanitation, to reduce bacterial exposure and support milk quality. This study provides baseline data for designing targeted mastitis control strategies and highlights the need for further studies using broader sampling and molecular identification

to better characterize environmental mastitis agents in smallholder dairy goat systems and support sustainable goat milk production in similar contexts.

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NOVELTY STATEMENT

This study provides the first integrated assessment of the prevalence of subclinical mastitis, environmental bacterial profiles, and technical management implementation scores in smallholder Peranakan Etawah dairy goats in West Sumatra. By combining field-based mastitis screening with a structured evaluation of farm hygiene practices, this study demonstrated that suboptimal hygiene implementation, rather than contagious pathogens, is the primary driver of subclinical mastitis in smallholder systems.

AUTHOR'S CONTRIBUTION

ER conceived and designed the study, coordinated the field and laboratory work, and critically revised the manuscript for its important intellectual content. HS contributed to the study design, performed the bacteriological and biochemical examinations, assisted in data analysis, and contributed to manuscript revision. FN participated in farm visits and data collection, performed IPB 1 testing and milk sampling, organized the dataset, performed descriptive analysis, and prepared the first draft of the manuscript. All authors discussed the results, approved the final version of the manuscript, and agreed to be accountable for all aspects of the work.

Ethical considerations

Ethical approval was not required because milk sampling was considered noninvasive.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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