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Prevalence and Risk Factors of Bubaline Subclinical Mastitis in Selected Peninsular Malaysian States

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ABSTRACT

Subclinical mastitis (SCM) is a common disease in dairy buffaloes worldwide, resulting in economic losses due to reduced milk production and quality. There is a dearth of information on the susceptibility to bubaline mastitis and the associated factors at farm and animal levels. This study determines bubaline mastitis's prevalence and risk factors in buffalo farms in Malaysia. A cross-sectional study was conducted at 12 buffalo farms across selected states in Peninsular Malaysia, including Selangor, Kedah and Penang. California Mastitis Test (CMT) was used to identify infected and non-infected buffaloes, whereas on-farm assessment and cross-sectional survey were conducted to collect farm and animal-based data. Data analysis was conducted using descriptive statistics, Chi-square test, and binary logistic regression. Overall, the prevalences of SCM at the quarter and animal levels were 29.7% (95% CI: 6.3%–49.0%) and 40.1% (95% CI: 0.0%–66.7%), respectively. While the animal-level prevalence of SCM was not significantly different across states, a significant difference was observed in the quarter-level prevalence (Selangor; 28.2% [95% CI 9.9–40.1], Kedah; 22.1% [95% CI: 6.3–33.3], Penang; 46.3% [95% CI: 43.2–49.0]). The prevalence of SCM bubaline was significantly associated with mastitis history ($p < 0.001$) at the animal level, pre- and post-teat dipping ($p = 0.041$) and the absence of other dairy animals ($p = 0.048$) at the

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farm level. The findings reflect a high prevalence of SCM in buffaloes and the factors that can be considered in developing effective SCM prevention and control measures.

Keywords: Bubaline mastitis, Malaysia, prevalence, risk factors

INTRODUCTION

Buffalo is the second most important dairy animal after cows. Asia has the largest population of buffaloes and is the leading producer of raw buffalo milk, accounting for 98.8% of the total production (Food and Agriculture Organization of the United Nations, 2022). According to the official data from the Department of Veterinary Services (DVS), Malaysia has 67,959 buffaloes. In Peninsular Malaysia, the buffalo population is estimated at around 50,221, comprising both swamp and Murrah buffaloes (Department of Veterinary Services Malaysia, 2022). Notably, the population of Murrah buffaloes in Peninsular Malaysia has consistently shown growth from 2013 to 2022. However, the specific population of Murrah buffaloes in the western region of Peninsular Malaysia, including Penang and Kedah, are among the lowest, while the population in Selangor is currently unknown. In Malaysia, the buffalo industry primarily focuses on meat rather than milk production. This industry predominantly consists of smallholder operations (Nor & Rosli, 2015). Unfortunately, smallholder farmers often confront various challenges, including limited herd sizes and inadequate husbandry practices. These challenges ultimately hinder productivity and market competitiveness, as Ariff et al. (2015) emphasised.

Mastitis, a condition characterised by mammary gland inflammation, is one of the most prevalent diseases affecting dairy animals worldwide. It causes severe economic losses due to reduced milk yield and quality (Costa et al., 2020). The disease is generally categorised into clinical (CM) and subclinical mastitis (SCM) (Singha et al., 2023). Subclinical mastitis is a disease that is concerning in dairy animals, especially buffaloes. It leads to significant economic losses because it is difficult to detect, with no visible changes in the milk or teat udders (Krishnamoorthy et al., 2021). Managing mastitis and reduced milk yield accounted for 55% and 16% of the loss, respectively (Malik & Verma, 2017). Ali et al. (2021) showed that the prevalence of SCM in buffaloes (66%) is higher compared to cows (53%). It is linked to the high nutrient content in buffalo milk, which promotes bacterial growth post-infection.

Somatic cell counts (SCC), California mastitis test (CMT) and bulk milk somatic cell count (BMSCC) are presently the common methods used in screening for SCM and udder health status at animal and farm levels (Costa et al., 2020; Hussain et al., 2018). Several studies have used CMT to investigate the prevalence of SCM, with the quarter-level prevalence ranging from 10% to 46% and widely variable between countries and regions (Islam et al., 2019; Preethirani et al., 2015; Singha et al., 2023). Given the increasing consumption of buffalo milk and its substantial contribution to milk production in South Asian countries (Ali et al., 2021), it is crucial to understand the prevalence of SCM and associated risk factors to develop effective prevention and control strategies and adopt appropriate therapeutic approaches. Studies conducted in other countries such as Bangladesh, Pakistan, and India have shown that high-yield buffalo, intensively managed, limited availability of pasture, milking practices, farm management, age, parity number, lactation stage, quarters position, and morphology of the teat end increases the risk of SCM in buffalo population (Islam et al., 2019; Salvador et al., 2012; Singha et al., 2023). This information is crucial in developing effective control and prevention measures against mastitis at regional and national levels (Ali et al., 2021; Islam et al., 2019).

In Malaysia, the most common breed of buffalo is the Murrah buffalo, with the capacity to produce an average milk yield of 4.7–5.0 litres per day. This production level remains significantly below the potential of superior buffaloes at an average of 15–20 litres per day (Wahid & Rosnina 2016). Consequently, available data suggests that milk production from buffaloes is yet to meet the demand for milk and dairy products in Malaysia (Mohd Azmi et al., 2021). While diverse factors ranging from genetics to management practices may contribute to the low production level among buffaloes in Malaysia, there is data paucity on the prevalence of SCM and associated factors. Given the evident knowledge gap, it is vital to identify the farm, animal and quarter-related factors that further heighten the risk of SCM in water buffalo in Malaysia. In addition, knowledge of the causative agents and their antimicrobial susceptibility is essential for effective treatment protocols. Therefore, a cross-sectional study was conducted on water buffalo in west Peninsular Malaysia to determine both animal and quarter-level prevalence of SCM bubaline and their associated risk factors.

MATERIALS AND METHODS

Ethics Approval and Consent to Participate

The study was carried out with the approval of the Institutional Animal Care and Use Committee (IACUC) Universiti Putra Malaysia under the animal utilisation protocol (AUP) number UPM/IACUC/AUP-R009/2022. Meanwhile, the survey for collecting farm and animal-based data was approved by the Ethics Committee for Research involving Human Subjects (JKE) Universiti Putra Malaysia with the reference number JKEUPM-2022-054.

Study Design and Study Area

A total of 12 available dairy buffalo farms from the 56 buffalo farms registered and listed by the Department of Veterinary Services (DVS), Malaysia (Figure 1) across three selected states (Selangor, Kedah, and Penang) were recruited for the cross-sectional study from February 2022 to February 2023. These states were selected because they have buffalo farms that are actively producing buffalo milk and are listed under the registered farms in Malaysia. Additional information on the studied farms is presented in Table 1.

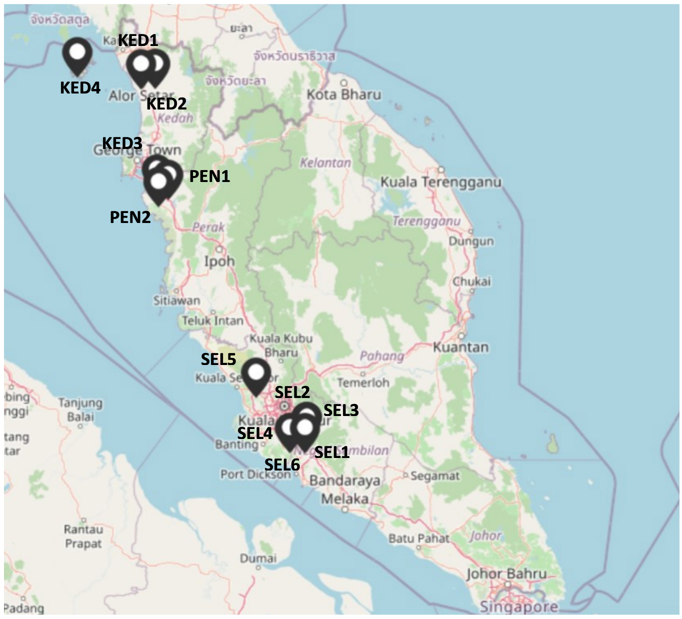

Figure 1. Map showing the distribution of selected farms in Peninsular Malaysia

Table 1
The information on 12 available dairy buffalo farms in Selangor, Kedah and Penang

Farm	District	Rainfall (%, mm)	Temperature (°C)	Animal population	Number of workers on the farm	Breeding system
SEL1	Hulu Langat	60.7, 7.2	27.7	124	5	Semi-intensive
SEL2	Hulu Langat	60.7, 1.5	28.7	5	1	Semi-intensive
SEL3	Hulu Langat	60.7, 1.5	28.9	40	4	Semi-intensive
SEL4	Sepang	60.7, 1.5	29.0	68	2	Semi-intensive
SEL5	Kuala Selangor	75.0, 4.2	29.2	63	5	Semi-intensive
SEL6	Hulu Langat	60.7, 1.5	27.7	650	15	Semi-intensive
KED1	Jitra	42.9, 7.2	28.1	18	2	Semi-intensive
KED2	Jitra	42.9, 7.2	28.2	9	1	Intensive
KED3	Bandar Baharu	42.9, 8.2	26.4	80	3	Semi-intensive
KED4	Langkawi	46.5, 0.0	27.5	49	4	Semi-intensive
PEN1	Seberang Perai	42.9, 0.0	26.6	31	2	Semi-intensive
PEN2	Seberang Perai	42.9, 0.0	26.6	20	5	Intensive

Note. The information about rainfall and temperature was collected from the official website of the Malaysian Meteorological Department (MET) by the Ministry of Natural Resources and Environmental Sustainability (MET, 2022), SEL = Selangor, KED = Kedah, PEN = Penang

Selection of Animals

The sample size for this study was determined using EpiTools Epidemiological Calculators (Ausvet). Based on a population size of 534 adult buffaloes in 12 farms across three selected states and an expected prevalence of 24.2% (Badua et al., 2020), with a 95% confidence interval (CI) and a precision level of 5% (Thrusfield, 2005), the minimum required sample size was computed as 185. The sample size was determined based on the population of 534 adult female buffaloes on the farms. The total population of Murrah buffaloes in the western region of Peninsular Malaysia, including Penang and Kedah, is among the lowest, while the population in Selangor is currently unknown. Since there were 217 dairy buffaloes available on the 12 farms from the three states, all of them were selected for the study. Specifically, 172 dairy buffaloes were selected from Selangor (six farms), 21 from Kedah (four farms), and 24 from Penang (two farms). During a single visit to each farm, all lactating buffaloes underwent the CMT test to diagnose SCM before milk sampling.

Animal and Farm Characteristics

Data on individual animals and farm management were obtained through interviews, farm records, on-farm assessment, and cow assessment. Based on the information gleaned from previous studies (Costa et al., 2020; Singha et al., 2023), nine animal-based and 18 farm-based variables were investigated as potential risk factors for SCM bubaline. As shown in Table 2, the research variables were categorised as described by Demil et al. (2022).

Table 2
Categories of animal-based and farm-based data and assessment methods used in the studied farms

	Animal-based data			
No.	**Variables**	**Categories**	**Methods**	**References**
1	Udder quarters position	Left front, right front, left hind, right hind	CA	Kashyap et al. (2019); Singh et al. (2023)
2	Lactation stage	Early lactation (≤90 days), mid-lactation (≥90-180 days), late lactation (≥180 days)	Interview, FR	Hameed et al. (2012); Srinivasan et al. (2013); Swami et al. (2017)
3	Age	≤6 years, ≥6–9 years, ≥9 years	Interview, FR	Hameed et al. (2012); Salvador et al. (2012); Swami et al. (2017)
4	Calving cycle	≤4 cycles, 4–7 cycles, ≥7 cycles	Interview, FR	Hameed et al. (2012); Vishwakarma et al. (2010)
5	Breed	Murrah, Nilli-ravi, Crossbreed Sawah	Interview, CA	Javed et al. (2022); Kashyap et al. (2019); Tiwari et al. (2022)
6	Calving interval	<1 year, >1-year	Interview	Chishty et al. (2007)

Table 2 *(continue)*

	Animal-based data			
No.	**Variables**	**Categories**	**Methods**	**References**
7	History of mastitis	Yes, no	Interview	Badua et al. (2020); Tiwari et al. (2022)
8	Milk yield	≤8L, 8–12L, ≥12L	Interview	Jaglan et al. (2022)
9	Milk letdown practice	Allowing the calf to suckle, oxytocin injection	CA	Hameed et al. (2012)
	Farm-based data			
No.	**Variables**	**Categories**	**Methods**	**References**
1	Farm size	<4 acres, 4–20 acres, >20 acres	Interview	Aliul et al. (2020)
2	Paddock size	<1000ft^2, 1000–10000ft^2, >10000ft^2	Interview	Badua et al. (2020)
3	Breeding system	Semi-intensive, intensive	Interview	Singh et al. (2023); Tiwari et al. (2022)
4	Milking method	Hand milking, machine milking	Interview, OA	Badua et al. (2020); Hameed et al. (2012); Ottalwar et al. (2018)
5	Frequency of milking	Once daily, twice daily	Interview, OA	Singha et al. (2024)
6	Milking place	Milking inside the paddock, milking outside the paddock or in a milking parlour	Interview, OA	García-Acevedo et al. (2023)
7	Practices of hand and teat hygiene before milking	Yes, no	Interview, OA	Badua et al. (2020); García-Acevedo et al. (2023); Malik and Verma (2017)
8	Towel drying of teats	Yes, no	Interview, OA	
9	Pre- and post-teat dipping	Yes, no	Interview, OA	Aliul et al. (2020)
10	Use of antibiotics for mastitis treatment and dry therapy	Yes, no	Interview	Badua et al. (2020); Bhandari et al. (2021)
11	Presence of other dairy animals	Yes, no	OA	Badua et al. (2020)
12	Type of milking utensils	Mix (using plastic and stainless steel) plastic, stainless steel	Interview, OA	Bomfim et al. (2023)
13	Frequency of cleaning milking utensils	Once daily, twice daily	Interview	
14	Frequency of paddock cleaning	Once daily, twice daily, three times daily	Interview	Bhandari et al. (2021)
15	Number of milkers	<3 milkers, >3 milkers	Interview	Singh et al. (2023)
16	Water source on the farm	Underground water, wastewater	Interview, OA	Zaki et al. (2010)

Note. CA = Cow assessment, FR = Farm records, OA = On-farms assessment

Detection of SCM-affected Animals

CMT was conducted to detect SCM at an early stage, following the procedures outlined by Schalm et al., 1971. Briefly, 2 mL of milk sample from clean teats were mixed with 2 mL of CMT reagent (Kruuse Bovivet CMT liquid, Kruuse, Denmark) on a CMT paddle. The paddle was gently rotated for 10 seconds, and any colour changes or formation of viscous gel were promptly observed. The visible reaction disappears after approximately 20 seconds, particularly for the weak reaction. The CMT results were recorded as follows: negative for no reaction (indicating uninfected or healthy), trace or 1+ score (indicating weak infection with SCM), and 2+ and 3+ scores (indicating strong infection with SCM). Buffaloes that tested negative and showed traces of the CMT were classified as healthy. Conversely, buffaloes that scored +1, +2, or +3 on the CMT in at least one quarter were classified as SCM.

Statistical Data Analysis

Descriptive analysis was performed to summarise the farm characteristics, prevalence of SCM, and farm and animal-based data. The association between the position of udder quarters and the prevalence of SCM bubaline was analysed using the Chi-square test. Logistic regression models were built to determine the factors associated with the prevalence of SCM bubaline. Parameter estimates were computed at a 95% confidence interval with crude odds ratio (COR) and adjusted odds ratio (AOR) at the univariable and multivariable levels, respectively. Model fit was assessed using the Hosmer-Lemeshow test. All the analyses were conducted using IBM SPSS Statistics version 27.0 (IBM Corp., USA).

RESULTS

Farm Characteristics and Screened Buffaloes

The study encompassed a range of lactating buffaloes, with a minimum of 2 animals per farm on the SEL5 farm and a maximum of 97 animals per farm on the SEL6 farm. The Murrah buffalo breeds constituted 83.9% of the sampled animals. Farms with a few animals were deliberately included to augment the sample size and gain a comprehensive understanding of the variations in animal and farm management data. The overall farm characteristics are presented in Table 3.

Prevalence of SCM Bubaline

The overall animal-level prevalence of SCM bubaline was 40.1% (87/217, 95% Confidence Interval (CI): 0.0%–66.7%). It included buffaloes with positive CMT results (+1, +2, and +3) in at least one quarter (Table 4). The mean prevalence of bubaline at the animal level on farms in Selangor, Kedah, and Penang was 41.3% (71/172, 95% CI: 0.0-53.6), 38.1% (8/21, 95% CI: 0.0–66.7), and 33.3% (8/24, 95% CI: 18.2–46.2), respectively.

Table 3
Characteristics of the 12 buffalo farms recruited in the study from three states in Peninsular Malaysia

Categories	**Selangor (*n* = 172 buffaloes, 6 farms)**	**Kedah (*n* =21 buffaloes, 4 farms)**	**Penang (*n* =24 buffaloes, 2 farms)**	**Total (%)**
Farm size (acres)				
<4	3	1	1	5 (41.7)
4–20	2	1	1	4 (33.3)
>20	1	2	0	3 (25.0)
Paddock size (ft^2)				
<1,000	2	2	2	6 (50.0)
1,000–10,000	3	2	0	5 (41.7)
>10,000	1	0	0	1 (8.3)
Breeding systems				
Semi-intensive	6	3	1	10 (83.3)
Intensive	0	1	1	2 (16.7)
Milking method				
Hand	4	3	2	9 (75.0)
Machine	2	1	0	3 (25.0)
Milking frequency				
One time	0	2	0	2 (16.7)
Two times	6	2	2	10 (83.3)
Milking place				
Milking inside the paddock	4	3	2	9 (75.0)
Milking outside the paddock	2	1	0	3 (25.0)
Hand hygiene before milking				
No	0	0	0	0 (0.0)
Yes	6	4	2	12 (100.0)
Teat hygiene before milking				
No	0	0	0	0 (0.0)
Yes	6	4	2	12 (100.0)
Pre and post-teat dipping				
No	5	3	1	9 (75.0)
Yes	1	1	1	3 (25.0)
Towel drying of teats				
Yes	0	0	0	0 (0.0)
No	6	4	2	12 (100.0)
Type of milking utensils				
Mix	6	4	2	12 (100.0)
Plastic	0	0	0	0 (0.0)
Stainless steel	0	0	0	0 (0.0)

Table 3 *(continue)*

Categories	Selangor (*n* = 172 buffaloes, 6 farms)	Kedah (*n* =21 buffaloes, 4 farms)	Penang (*n* =24 buffaloes, 2 farms)	Total (%)
Utensils cleaning frequency				
One time	0	2	0	2 (16.7)
Two times	6	2	2	10 (83.3)
Antibiotics used to treat mastitis				
No	3	3	2	8 (66.7)
Yes	3	1	0	4 (33.3)
Antibiotics used for dry therapy				
No	5	3	1	9 (75.0)
Yes	1	1	1	3 (25.0)
Cleaning farm frequency				
One time	1	3	0	4 (33.3)
Two times	5	1	0	6 (50.0)
Three times	0	0	2	2 (16.7)
Presence of other dairy animals				
No	3	3	0	6 (50.0)
Yes	3	1	2	6 (50.0)
Number of milkers on the farm				
<3	2	4	1	7 (58.3)
>3	4	0	1	5 (41.7)
Water source in the farm				
Underground water (pipe)	5	4	2	11 (91.7)
Wastewater (lake)	1	0	0	1 (8.3)

Table 4

The animal-level and quarter-level prevalence of SCM bubaline in selected states in Peninsular Malaysia

Prevalence		Selangor	Kedah	Penang	Total
Animal-level	Number examined	172	21	24	217
	Number of positive CMT	71	8	8	87
	Prevalence (%)	41.3	38.1	33.3	40.1
	95% confidence interval (%)	0.0–53.6	0.0–66.7	18.2–46.2	0.0–66.7
Quarter-level	Number examined	650	77	95	822
	Number of positive CMT	183	17	44	244
	Prevalence (%)	28.2	22.1	46.3	29.7
	95% confidence interval (%)	9.9–40.1	6.3–33.3	43.2–49.0	6.3–49.0

Meanwhile, the quarter-level prevalence of SCM bubaline was 29.7% (244/822, 95% CI: 6.3%–49.0%), including quarters with positive CMT results (trace, +1, +2, and +3). A total of 46 quarters that were blind were excluded from the examined quarters. The mean quarter-level prevalence of SCM bubaline on farms in Selangor, Kedah, and Penang was 28.2% (183/650, 95% CI: 9.9–40.1), 22.1% (17/77, 95% CI: 6.3–33.3), and 46.3% (44/95, 95% CI: 43.2–49.0), respectively. In addition, the CMT scores of SCM bubaline at the quarter level on farms in Selangor, Kedah, and Penang were presented in Table 5.

Table 5
The CMT scores of SCM bubaline at quarter level in selected states in Peninsular Malaysia

State	Number examined	CMT score (n) (proportion positive [%])				
		Negative	Trace	+1	+2	+3
Selangor	650	470 (72.3)	45 (6.9)	79 (12.2)	44 (6.8)	12 (1.8)
Kedah	77	60 (77.9)	6 (7.8)	11 (14.3)	0 (0)	0 (0)
Penang	95	51 (53.7)	27 (28.4)	10 (10.5)	3 (3.2)	4 (4.2)
Total	822	581 (70.7)	78 (9.5)	100 (12.2)	47 (5.7)	16 (1.9)

Risk Factors of SCM Bubaline

Udder Quarter Position Level

Table 6 shows results regarding the relationship between the investigated factors and the quarter-level prevalence of SCM. No significant association was detected between quarter position and the prevalence of SCM bubaline.

Table 6
Relationship analysis between the prevalence of SCM bubaline with udder quarters position

Quarters	Sample (*n*)	Positive SCM (*n*) (proportion positive (%)	Chi-square		
			X^2	*df*	*p*-value
Quarters position					
LF	206	63 (30.6)	0.795	3	0.851
RF	208	62 (29.8)			
LH	210	65 (31.0)			
RH	198	54 (27.3)			

Note. LF= left front, RF= right front, LH= left hind, RH= right hind

Animal Level

The association between the animal-based factors and the prevalence of SCM bubaline are presented in Table 7. In the univariable model, significant factors at the *p*-value of 0.10 include mastitis history (p <0.001) and the daily milk yield (p = 0.009) at the animal level. Buffaloes without a mastitis history were less likely (OR: 0.64, 95% CI: 0.002-0.019) to have SCM compared to those with a mastitis history. Buffaloes that produced more than 12L of milk were more likely (OR: 1.91, 95% CI: 1.01-3.64) relative to those producing less than 12L. However, only mastitis history was significantly associated with the prevalence of SCM ($p < 0.001$) at the multivariable level. Buffaloes without a mastitis history were less likely (OR: 0.005, 95% CI: 0.001-0.017) to have SCM than buffaloes with a mastitis history.

Table 7
Univariable and multivariable binary logistic regression analysis between animal-level factors and prevalence of SCM bubaline in selected states in Peninsular Malaysia

Categories	Sample (n) (sample frequency [%])	Positive SCM (n) (proportion positive [%])	Univariable level			Multivariable level		
			OR	95% CI	*p*-value	OR	95% CI	*p*-value
Lactation stage				*p*=0.659				
Early	105 (48.4)	42 (40.0)	Ref					
Mid	80 (36.9)	30 (37.5)	0.900	0.495–1.636	0.730			
Late	32 (14.7)	15 (46.9)	1.324	0.597–2.935	0.490			
Age				*p*=0.158				
<6 years	103 (47.5)	36 (35.0)	Ref					
6-9 years	95 (43.8)	40 (42.1)	1.354	0.762–2.404	0.302			
>9 years	19 (8.7)	11 (57.9)	2.559	0.945–6.933	0.065			
Mastitis history								
No	137 (63.1)	12 (8.8)	0.006	0.002–0.019	<0.001	0.005	0.001–0.017	<0.001
Yes	80 (36.9)	75 (93.8)	Ref			Ref		
The volume of milk produced				*p*=0.009			*p*=0.527	
<8L	61 (28.1)	21 (34.4)	Ref			Ref		
8-12L	48 (22.1)	12 (25.0)	0.635	0.274–1.471	0.289	1.812	0.467–7.025	
>12L	108 (49.8)	54 (50.0)	1.905	1.011–3.645	0.052	0.889	0.250–3.162	
Milk letdown practices								
Allow calf to suckle	192 (88.5)	74 (38.5)	Ref					
Oxytocin injection	25 (11.5)	13 (52.0)	1.727	0.748–3.988	0.200			

Farm Level

Table 8 depicts the relationship between the farm-level factors and the prevalence of SCM bubaline. At the univariable level, the prevalence of SCM was significantly associated ($p<0.1$) with paddock size (p=0.001), breeding system ($p=0.045$), milking method

Table 8
Univariable and multivariable binary logistic regression analysis between farm-level factors and the prevalence of SCM bubaline in selected states in Peninsular Malaysia

Categories	Sample (*n*)	Positive SCM (*n*) (proportion positive [%])	Univariate model			Multivariate model		
			OR	95% CI	*p*-value	OR	95% CI	*p*-value
Paddock size (ft²)				*p*=0.001			*p*=0.420	
<1,000	65	18 (27.7)	Ref			Ref		
1,000–10,000	55	17 (30.9)	1.168	0.531–2.571	0.699	0.577	0.130–2.556	0.469
>1,0000	97	52 (53.6)	3.017	1.538–5.921	0.001	0.078	0.002–3.456	0.188
Breeding system								
Semi-intensive	202	85 (42.1)	Ref			Ref		
Intensive	15	2 (13.3)	0.212	0.047–0.963	0.045	0.000	-	0.999
Milking method								
Hand	111	32 (28.8)	Ref			Ref		
Machine	106	55 (51.9)	2.662	1.520–4.662	<0.001	0.000	-	0.999
Milking place								
Milking inside the paddock	111	32 (28.8)	Ref					
Milking outside the paddock	106	55 (51.9)	2.662	1.520–4.662	<0.001			
Hand hygiene before milking								
No	0	(0.0)	Ref					
Yes	217	87 (40.1)	0.669		0.004			
Teat hygiene before milking								
No	0	0 (0.0)	Ref					
Yes	217	87 (40.1)	0.669		0.004			
Pre and post-teat dipping								
No	102	30 (29.4)	0.424	0.242–0.743	0.003	0.033	0.001–0.870	0.041
Yes	115	57 (49.6)	Ref			Ref		

Table 8 *(continue)*

Categories	Sample (*n*)	Positive SCM (*n*) (proportion positive [%])	Univariate model			Multivariate model		
			OR	95% CI	*p*-value	OR	95% CI	*p*-value
Cleaning and drying teat using a towel								
No	217	87 (40.1)	0.669	-	0.004			
Yes	0	0 (0.0)	Ref					
Milking utensils type								
Mix	217	87 (40.1)	0.669		0.004			
Plastic	0	0 (0.0)	-		-			
Stainless steel	0	0 (0.0)	Ref					
Antibiotics used to treat mastitis								
No	78	22 (28.2)	Ref			Ref		
Yes	139	65 (46.8)	2.236	1.233–4.054	0.008	0.201	0.026–1.578	0.127
Antibiotics used for dry therapy								
No	102	30 (29.4)	0.424	0.242–0.743	0.003	0.000	-	0.999
Yes	115	57 (49.6)	Ref			Ref		
Presence of other dairy animals								
No	151	70 (46.4)	2.491	1.316–4.713	0.005	3.181	0.710–30.854	0.048
Yes	66	17 (25.8)	Ref			Ref		
Number of milkers								
<3 milkers	62	17 (27.4)	Ref			Ref		
>3 milkers	155	70 (45.2)	2.180	1.148–4.140	0.017	3.181	0.710–14.259	0.131

(p<0.001), milking place (*p*<0.001), pre-and post-teat dipping (*p*=0.003), antibiotics used to treat mastitis (*p*=0.008) and for dry therapy (*p*=0.003), and the presence of other dairy animals (*p*=0.005). The final factors associated with SCM prevalence at the multivariable level (*p*<0.05) were (pre- and post-teat dipping (*p*=0.041) and the presence of other dairy animals (*p*=0.048). Farms that kept only buffaloes were more likely to have SCM (OR: 3.18, 95%CI: 0.71–30.85) compared to farms with other dairy animals.

DISCUSSION

Mastitis in dairy animals can cause huge economic losses and pose serious public health concerns (Chakraborty et al., 2019). This study is the first attempt to investigate SCM bubaline's prevalence and risk factors in Peninsular Malaysia's selected states. The prevalence of SCM at the animal level and quarter level was 40.1% and 29.7%, respectively. No previous studies have been conducted on SCM bubaline in Peninsular Malaysia. Therefore, the only comparable research available is a recent study on bovine mastitis conducted in dairy farms across Selangor, Perak, Pahang, Negeri Sembilan, and Johor, which reported a higher prevalence of SCM in bovine populations at 67.6%, which is higher than the prevalence found in the current study on bubaline populations from various states (Ali et al., 2020). The use of different detection methods (bacterial culture vs CMT) and study locations might contribute to these discrepancies. Nevertheless, two previous studies conducted in Egypt on both buffaloes and cows reported a prevalence of SCM in buffaloes (44.3% and 44%), which was comparable to that in cows (49.9% and 52.1%) (Ahmed et al., 2018; Algammal et al., 2020). This finding suggests that both buffaloes and cows are equally susceptible to SCM. It is important to note that the prevalence of SCM in buffaloes can have significant economic implications, as it can lead to increased somatic cell counts, decreased milk production, and a significant decline in milk quality, leading to low demand. These implications are similar to those of bovine mastitis (Pizauro et al., 2014).

Numerous reports on the prevalence of SCM in buffaloes have been conducted in different countries. The prevalences reported in this study were higher compared to reports in Brazil (8.35%) (Pizauro et al., 2014), the Philippines (24.22%) (Badua et al., 2020), Bangladesh (27.9%) (Singha et al., 2023), and China (29.44%) (Zhang et al., 2023), but comparable to reports in India (41.51%) (Ottalwar et al., 2018), and Egypt (44.3%) (Ahmed et al., 2018). Additionally, our findings were lower compared to studies conducted in India (68.33%) (Kashyap et al., 2019), Nepal (70%) (Tiwari et al., 2022), and Pakistan (75.31%) (Javed et al., 2022). The variation in the prevalence of mastitis can be attributed to geographic location, climate, animal-level risk factors such as breed, lactation stage, age, parity, quarters, and teat shape, as well as farm-level risk factors such as breeding system, husbandry practices, and farm hygiene (Salvador et al., 2012; Tiwari et al., 2022).

The association between the prevalence of SCM bubaline and the position of the quarter was not significant in the present study. Some studies found that the left quarters had a higher prevalence of SCM than the right quarters (Hoque et al., 2022; Kaur et al., 2015; Singha et al., 2023). On the contrary, other studies conducted in Pakistan and Nepal reported that the left front quarter had a higher prevalence of SCM than the right hind quarter, left hind quarter, and right front quarter. The association between quarter position and SCM depends on the position of the farmers during milking, which may increase exposure to pathogens and pressure on the teats (Bhandari et al., 2021; Chishty et al., 2007). The position of the farmers during milking was related to the sequencing of teat milking. Milking the right quarter after the left quarter leaves the teat canal open, allowing pathogens to enter the mammary glands and lead to SCM (Singha et al., 2023). In addition, hindquarters are more susceptible to SCM due to their increased exposure to contamination from urine, faeces, and the tail (Kashyap et al., 2019; Kisku & Samad, 2013). As Kashyap et al. (2019) mentioned, the hindquarters are larger and more pendulous than the front quarters, increasing the risk of contamination from the milking area or barn floor. The lack of a significant association in our study is not fully understood. However, the similarity in the milking practices may annul the relative risk of greater exposure to SCM between left and right quarters and hind and front quarters.

In this study, the prevalence of SCM bubaline was higher in buffaloes with a history of mastitis compared to those without the history. This finding is consistent with previous studies conducted in Nepal (Bhandari et al., 2021; Tiwari et al., 2022) and the Philippines (Badua et al., 2020). Bhandari et al. (2021) suggested that buffaloes with a history of mastitis linked to a high prevalence of SCM due to the overuse or misuse of antibiotics in the treatment of previous mastitis cases, selection pressure, and poor farm hygiene (Ali et al., 2014). Incomplete or ineffective mastitis treatment in buffaloes may also lead to inflammation and damage to the mammary tissue, allowing residual bacteria to persist (Cheng & Han, 2020). It indicates that previous episodes of mastitis may contribute to persistent or recurrent infections, resulting in a higher prevalence of SCM in buffaloes with a history of mastitis.

This study also found significant differences in the prevalence of SCM in farms that practice teat dipping and those not practising the procedure. This finding may stem from several factors, such as the type of disinfectant used, the technique of application, and overall farm hygiene practices (Sharif & Ahmad, 2007; Singha et al., 2023). Improper teat dipping practices, including the use of ineffective disinfectants or inadequate application of the disinfectant, as well as the timing of teat dipping (preferably within 30 seconds after milking), could be the possible causes for the high prevalence of SCM in farms that do not practice teat dipping (Nickerson et al., 2019).

The prevalence of SCM bubaline was significantly higher in farms that kept only buffaloes compared to other dairy animals, such as cows and goats. This result is consistent with the report from the Philippines (Badua et al., 2020), which focused on the association between the presence of other animals on the farm and the prevalence of *methicillin-resistant S. aureus (MRSA)* in bubaline mastitis. On the other hand, the increased risk of SCM in farms that solely keep only one species may be due to factors like limited farm size, poor hygiene practices, and the role of milkers interacting with animals, which increase exposure to environmental and contagious pathogens (Abebe et al., 2023; Gantner et al., 2023; Pletinckx et al., 2011). Diversified livestock farms tend to have more comprehensive biosecurity measures. These measures effectively prevent the transmission of diseases between species (Msimang et al., 2022; Pozo et al., 2024; Renault et al., 2021). Biosecurity measures include separate fencing, control of animal movement, segregated equipment usage, proper manure management, isolation of sick animals, regular veterinary involvement, vaccination protocols, and rigorous testing of bulk tank milk. However, the relationship between the presence of other dairy animals and the prevalence of SCM bubaline requires further investigation.

Despite the strengths of this study in bridging the research gap towards identifying control and prevention measures for SCM bubaline, the research limitations are well-acknowledged. Samples were collected from farms located in three states in Peninsular Malaysia. Hence, the findings may not be generalisable to all buffalo farms in the country. Regarding the investigated factors, information on the biosecurity measures or specific teat dipping procedures implemented by the farms was not collected, including the timing and type of disinfection used. As a result, further investigation is required to elucidate the relationship between teat dipping, biosecurity measures, and the prevalence of SCM bubaline.

CONCLUSION

The current study found a high prevalence of SCM bubaline, associated with a history of mastitis at the individual animal level, pre- and post-teat dipping, and the absence of other dairy animals at the farm level. These findings provide valuable information for developing effective prevention and control measures against SCM, particularly in buffaloes in Peninsular Malaysia. Additionally, educating farmers on good farm management and proper milking practices could assist in reducing the occurrence of SCM in the farms.

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