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PREVALENCE AND ASSOCIATED RISK FACTORS OF SUBCLINICAL MASTITIS IN DAIRY BUFFALOES IN SELECTED FARMS IN SELANGOR

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PREVALENCE AND ASSOCIATED RISK FACTORS OF SUBCLINICAL MASTITIS IN DAIRY BUFFALOES IN SELECTED FARMS IN SELANGOR

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CERTIFICATION

It is hereby certified that we have read the project paper entitled “Prevalence and Associated Risk factors of Subclinical Mastitis in Dairy Buffaloes in Selected Farms in Selangor” by Nur Farah Afifah binti Zaini and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for course VPD 4999 – Project.

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DEDICATION

This project paper is dedicated to:

To Allah S.W.T., the ultimate source of wisdom, whose guidance has lit my path and granted me the strength to embark on this academic journey.

To my parents, whose endless affection and constant encouragement have been the foundation of my educational pursuits. Your sacrifices and prayers have strengthened my determination.

To my friends, companions in both successes and challenges, your companionship has made the journey more enjoyable and the burdens lighter.

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LIST OF ABBREVIATIONS

Subclinical mastitis	**SCM**
Clinical mastitis	**CM**
California mastitis test	**CMT**
United States Dollar	**USD**
Body condition score	**BCS**

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada kepeluan kursus VPD 4999-Projek.

PREVALENS DAN FAKTOR- FAKTOR RISIKO BERKAITAN MASTITIS SUBKLINIKAL DALAM KALANGAN KERBAU TENUSU DI LADANG TERPILIH DI SELANGOR

Oleh

Nur Farah Afifah binti Zaini

2023

Penyelia: Prof Madya Dr Rozaihan Mansor

Kerbau memainkan peranan penting dalam industri tenusu sebagai sumber bekalan susu kedua terbesar di dunia. Walau bagaimanapun, kesihatan dan produktiviti mereka boleh terjejas oleh penyakit seperti mastitis subklinikal. Mastitis subklinikal menyumbang kepada kerugian kewangan dalam kalangan petani akibat masalah seperti pengurangan kualiti dan kuantiti susu, susu terbuang, dan kos rawatan. Kajian keratan lintang ini dijalankan untuk menganggar prevalens mastitis subklinikal pada peringkat kerbau dan peringkat suku ambing dalam kerbau tenusu dan untuk menentukan faktor risiko yang berkaitan. Kajian ini dilaksanakan di enam ladang kerbau tenusu yang diurus secara separa intensif yang terletak di Selangor dalam tempoh dua minggu. Kerbau menyusu yang tidak menunjukkan tanda sistemik dan tiada keabnormalan pada ambing dan susu telah dipilih untuk menyertai kajian ini. Sebanyak 46 ekor kerbau dari enam ladang telah dijadikan sampel. Sampel susu daripada 184 suku ambing daripada 46 ekor kerbau telah

menjalani Ujian mastitis California (CMT) untuk pengesanan mastitis subklinikal. Faktor risiko peringkat kerbau dan suku ambing diukur menggunakan soal selidik dan melalui pemerhatian di ladang. Kajian itu merekodkan 39.13% dan 26.09% prevalens mastitis subklinikal masing-masing pada peringkat kerbau dan peringkat suku ambing. Ujian khi-kuasa dua bagi faktor risiko peringkat kerbau mendedahkan perbezaan yang signifikan secara statistik ($p < 0.05$) dalam prevalens mastitis subklinikal dan peringkat laktasi kerbau. Kerbau dalam peringkat laktasi hujung (lebih daripada 3 bulan hingga 6 bulan) mempunyai risiko yang lebih tinggi untuk diuji positif untuk mastitis subklinikal berbanding dengan kerbau pada peringkat penyusuan awal (kurang daripada 3 bulan). Sementara itu, faktor lain seperti umur, baka, simetri ambing dan purata hasil susu harian tidak menunjukkan perkaitan yang signifikan dengan mastitis subklinikal. Dalam regresi logistik binari, faktor risiko peringkat suku ambing iaitu kedudukan suku ambing dan bentuk puting, kedua-duanya tidak mempunyai perkaitan yang signifikan dengan mastitis subklinikal. Kesimpulannya, penemuan kajian ini boleh digunakan dalam mengesyorkan dan membangunkan strategi kawalan dan intervensi masa hadapan untuk mengurangkan kejadian mastitis subklinikal dalam kerbau di Malaysia.

Kata kunci: *kerbau, tenusu, mastitis subklinikal, prevalens, faktor r isiko, ujian mastitis california.*

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999- Project.

PREVALENCE AND ASSOCIATED RISK FACTORS OF SUBCLINICAL MASTITIS IN DAIRY BUFFALOES IN SELECTED FARMS IN SELANGOR

by

Nur Farah Afifah binti Zaini

2023

Supervisor: Assoc Prof Dr Rozaihan Mansor

Buffaloes play an important role in the dairy industry as the second largest source of milk supply in the world. However, their health and productivity can be significantly affected by diseases such as subclinical mastitis (SCM). SCM contributes to financial losses among farmers due to problems such as reduced milk quality and quantity, discarded milk, and treatment costs. This cross-sectional study was carried out to estimate the buffalo-level and quarter-level prevalence of SCM in dairy buffaloes and to determine the associated risk factors. The study was performed at six semi-intensively managed dairy buffalo farms located in Selangor over a period of two weeks. Lactating buffaloes that showed no systemic signs and no abnormalities of the udder and milk were selected to participate in the study. A total of 46 buffaloes from six farms were sampled. Milk samples from 184 quarters from 46 buffaloes were subjected to California Mastitis Test (CMT) for the detection of SCM. Buffalo and quarter-level risk factors were measured using questionnaires and through on-farm observations. The study recorded 39.13% and

26.09% SCM prevalence at buffalo-level and quarter-level respectively. The Chi-square analysis of buffalo-level risk factors revealed statistically significant differences ($p < 0.05$) in the prevalence of SCM and lactation stage. Buffaloes in the later lactation stage (more than 3 months to 6 months) had a higher risk of being tested positive for SCM compared to buffaloes in the early lactation stage (less than 3 months). Meanwhile, other factors such as age, breed, udder symmetry and average daily milk yield showed no significant association with SCM. In binary logistic regression, the quarter-level risk factors which were quarter position and teat shape, both had no significant association with SCM. In conclusion, the findings of this study can be useful in recommending and developing future control and intervention strategies to reduce the occurrence of SCM in buffaloes in Malaysia.

Keywords: *buffaloes, dairy, subclinical mastitis, prevalence, risk factors, california mastitis test*

1.0 INTRODUCTION

According to the Food and Agriculture Organization (FAO, 2023), the estimated total world population of buffaloes is 204 million head and most of them are distributed in Asia. In many regions, dairy buffaloes are a crucial component of the milk supply chain, maintaining a consistent flow of dairy products. In fact, a large portion of the world's milk production is contributed by domestic water buffaloes, and they are major milk producers in many countries such as India and Pakistan. In Malaysia, the water domestic buffaloes are classified into two subspecies which are river buffalo and swamp buffalo. Crossbreeding between the river buffaloes (Murrah breed) and the swamp buffaloes was attempted in many Southeast Asian countries including Malaysia, intended to improve milk production. The swamp type is mainly reared for meat and draught while the Murrah crossbred is mainly reared for milk production (Mohd Azmi et al., 2021).

The health and productivity of this species can be compromised by various illnesses and diseases. Dairy buffaloes are equally susceptible to most diseases affecting dairy cattle (Wahid and Rosnina, 2011) such as mastitis. Mastitis has a devastating impact on the farm profitability and the reputation of the dairy industry due to issues with animal welfare and milk quality (De Vliegher et al., 2012). Subclinical mastitis (SCM) is the most common form of mastitis. Despite its subtle clinical signs, it can have a profound impact on the milk quality and quantity, animal welfare, and the economic sustainability of dairy farming operations.

SCM is characterized by inflammation of the udder with no apparent clinical signs such as swelling, heat upon palpation, or abnormal milk, making it a hidden threat within the dairy

herds. The poor milk quality resulting from this disease not only causes a significant economic loss, but it also poses a potential risk to the health and safety of the consumers due to increased somatic cell counts and decreased milk compositional quality. As it remains undetected, no intervention management is implemented to address several consequences from SCM (Salvador et al., 2011).

Despite the economic and nutritional importance of buffalo milk, there are still limited studies published on SCM in dairy buffaloes compared to that of dairy cattle especially in Malaysia. Furthermore, the prevalence of SCM is higher in buffaloes (46%) than in dairy cattle (42%), and the prevalence of SCM was found to be higher (42%) than the prevalence of clinical mastitis (15%). (Krishnamoorthy et al., 2021). Study of SCM prevalence in buffaloes has been reported in various countries ranging from 10-46% using CMT (Muhammad et al., 2010) and it is widely variable between different regions (Singha et al., 2023).

Understanding the prevalence and risk factors associated with SCM in dairy buffaloes is essential for the sustainable growth of the dairy industry. By exploring the extent of the problem and identifying the factors contributing to its occurrence, better preventative strategies, improved diagnostic techniques, and enhanced treatment protocols can be established to mitigate its impact. Mastitis prevalence was found to be associated with stage of lactation, parity, breed, milk yield, body weight, presence of anatomical abnormality of the udder and other management aspects including nutrition (Almaw et al., 2008). Nutritional status is important as it affects the metabolism and immunological functions in animals. Abnormalities in these physiological functions can predispose to several disease conditions such as ketosis and mastitis (Esposito et al., 2014). This study allows us to estimate the prevalence of SCM in dairy buffaloes at the animal and quarter

level as well as to identify the risk factors so that appropriate intervention and control measures can be implemented.

In the present study, the prevalence of subclinical mastitis was assessed in dairy buffaloes kept under different farms' managemental conditions in selected farms in Selangor. The associated risk factors were also identified.

2.0 LITERATURE REVIEW

2.1 Dairy buffaloes in Malaysia

According to data from the Department of Veterinary Services Malaysia, as of 2021 the total of buffalo population in this country is 47,350 head. 6387 of these buffaloes are of Murrah type and 1766 of them are females in the age of more than 2 years old. However, the exact and accurate number of dairy buffaloes in Malaysia is not available. In Southeast Asia, water buffalo milk has not been produced and consumed traditionally except in parts of Indonesia and Philippines (Tsuji, 2019). In other countries of the region, such as Malaysia, buffaloes are mainly used for work purposes and only a small percentage are fully utilised for milk production (Cruz, 2007). However, the current state of buffalo milk production in Malaysia is not well understood and underdeveloped (Mohd Azmi et al., 2021). Malaysia is primarily dependent on imports to meet its domestic demand for dairy products, and the value of those imports has grown over time (Suntharalingam and Zhong, 2015). Dairy buffaloes in Malaysia can produce milk from 4 to 6 liters per day (Zainuddin, 2017). Local Murrah crossbred buffaloes can only produce 4.7 L of milk per day in one lactation period, yielding a total of 1000 L of milk annually on average (Wahid and Rosnina, 2016). Nonetheless, there are potential to increase the productivity of buffaloes in Malaysia and other parts of Southeast Asia for meat, milk and hide production (Pineda et al., 2021)

2.2 Subclinical Mastitis in Dairy Buffaloes

Mastitis is defined as the inflammation of mammary gland parenchyma that can be infectious, traumatic, or toxic in nature which can be classified as clinical mastitis (CM) or

subclinical mastitis (SCM) (Kibebew, 2017). SCM is characterized by an increase in the somatic cell count without visible signs in the udder or in the milk (Ruegg, 2007). It leads to two thirds of the milk production losses compared to CM (Radostits, 2007). Mastitis results in 70 USD of the average annual economic loss per buffalo, with 55% of the losses being from intervention strategies and 16% was from drop in milk output (Malik and Verma, 2017). In comparison to cattle, buffaloes have certain characteristics that can contribute to greater risk of developing mastitis such as more pendulous udder and longer teats. In contrast, they have long and narrow teat canals which may be expected to prevent microbial invasion (Fagiolo and Lai, 2007). Mastitis can lead to changes in the composition and nutritional quality of buffalo milk such as decreased in lactose, increased in chloride, and impaired rennet coagulation time, and these factors can negatively influence the production of food made from buffalo milk (Tripaldi, 2010).

2.3 Prevalence of SCM in dairy buffaloes

The prevalence of SCM in buffaloes has been studied in many countries, resulting in different percentages of prevalence. A review study done by Mohd Amin et al. (2023) found that the animal-based prevalence of SCM in buffaloes across countries in Asia ranges between 36.38 % to 77.98 %. Meanwhile, the quarter-level SCM prevalence ranges between 9.77 % to 64%. A study conducted in East Java, Indonesia found relatively high prevalence of bubaline SCM with a percentage of 68.18% and 66.72% at the buffalo level and quarter level respectively (Khasanah, 2021). Another study done in Chitwan region in Nepal recorded 30% prevalence of SCM in buffaloes, where CMT showed 15 out of 50 buffaloes tested positive for the disease (Bhandari et al., 2021). However, Aliul et al., in 2020 found relatively low prevalence of SCM, where only 21 from 200 (10.50%) buffaloes were detected with SCM. The quarter-wise prevalence of SCM in

Nili Ravi buffaloes in the district of Pakhtunkhwa, Pakistan was 41.8% (Ali et al 2014). A study performed in a larger population of buffaloes in Ethiopia recorded 41.02% (224/546) of SCM prevalence in dairy buffaloes. The most recent study done in selected farms in Selangor, Malaysia by Rosslan et al., in 2022 stated that the prevalence of bubaline SCM was 56.67%. The prevalence of this disease varies among countries depending on the locations and various risk factors that can contribute to the occurrence of SCM.

2.4 Risk factors of SCM

Mastitis is a complex multifactorial disease that can be associated with several factors that can be categorised into pathogens, environment or management, and the animals themselves. Intra-mammary infection by bacterial pathogens is considered to be the primary cause of mastitis (Cheng and Sung, 2020). Many previous studies have identified different types of pathogens to be the most common cause of SCM. Among the most prevalent bacterial pathogens found in SCM infection were Staphylococcus spp (Ali et al., 2011), Streptococcus spp (Harjanti et al., 2018) and *Corynebacterium bovis* (Tenhagen et al., 2006). These pathogens transmit from animal to animal mainly during milking and appear to develop chronic subclinical infections with outbreaks of clinical incidents (Fesseha et al., 2021). Therefore, proper hygiene, good maintenance of the milking equipment, and post-milking teat disinfection are important to reduce the contact between reservoirs and uninfected animals (Smith and Hogan, 1993).

Host factors such as age, lactation stage, parity and milk yield have shown significant association with the prevalence of SCM (Ganie et al., 2021). A study done by Almaw et al., in 2008 also found that SCM was associated with milk yield, breeding, lactation stage

and abnormality of the udder. Other than that, the nutritional status of the animal is another crucial factor. Deficiency in a specific type of micro or macronutrient that regulates the metabolism and immune function of an animal can predispose dairy cows to health problems (Sordillo, 2016). Insufficient nutrients during the lactation stage of dairy cows are associated with negative energy balance which consequently increases the susceptibility to infection of the mammary gland (Shaheen, 2016).

Management factors that can contribute to the occurrence of bubaline mastitis include herd size, type of bedding, cleanliness of the farm, milking method, milking hygiene practices, history of the post parturient disease, as well as dry cow management and antibiotic therapy (Biswas et al., 2020; Kaur et al., 2015; T. Ali et al., 2014). A study conducted by Singha et al., in 2023 found that SCM prevalence was associated with factors such as the buffalo rearing system and number of milkers in the farms.

3.0 MATERIALS AND METHODS

3.1 Procedure

Six (6) dairy buffalo farms located in Selangor were selected to participate in the study based on the database obtained from the Department of Veterinary Services Malaysia. A cross-sectional study was conducted in each selected farm by face-to-face interviews with farmers and through on-farm observation. Farmers were invited via telephone or formal letters to participate in the study and upon agreement, visitation was conducted, and farmers were then interviewed using a set of structured questions. Data collection took place over a period of two weeks (29th August 2023 to 5th September 2023).

3.2 Animal Selection and Assessment of the Mammary Gland System

Lactating buffaloes from various stages of lactation from each farm were selected for the study. These buffaloes were subjected to clinical examination of the mammary gland by observing the symmetry of the mammary gland. Next, the udder and each teat were palpated to detect any signs of inflammation such as swelling, heat and painful response. Lasty, the lymph nodes were palpated and assessed for any enlargement.

3.3 Detection of Buffaloes with Subclinical Mastitis by California Mastitis Test (CMT)

The buffaloes that showed no systemic signs and abnormalities of the mammary glands and milk were selected and subjected to CMT to detect SCM. Two ml of milk was collected from each quarter of an udder and added into an equal volume of CMT reagent, then the test plate was swirled gently for 10 seconds to mix thoroughly. Result is considered negative when there are no changes in the appearance or consistency of the mixture, whereas the result is positive when there is presence of jelly-like consistency or thread-like streaks in the mixture (Baloch et al., 2016). The score of CMT was determined based on Table 1 and recorded for further analysis. An individual buffalo was considered positive if one or more out of four quarters scored +1 and above (+2 and +3). A quarter was considered positive if it was scored trace and above (+1, +2 and +3).

Table 1: Scoring of California Mastitis Test

CMT SCORE	DESCRIPTION	TEST APPEARANCE
N	Negative	Mixture remains liquid with no evidence of formation of precipitate
T	Trace	A slight precipitate which tends to disappear with continued movement of the paddle
+1	Weak positive	A distinct precipitate but no tendency toward gel formation
+2	Distinct positive	The mixture thickens immediately with some gel formation, and the mixtures tend to move in toward the center with motion
+3	Strong positive	A distinct gel forms which tends to adhere to the bottom of the paddle and a distinct central peak form during swirling

(Sahar Kandeel et al., 2018)

3.4 Questionnaire Design and Data Collection

Questionnaire was adapted from Singha et al., (2023) and constructed in dual languages (English and Malay). Only one person from each farm will be given the questionnaire. Participants must be either the farm owners or farm workers of any race and gender and are able to read and understand English/ Malay for the purpose of answering the questionnaire. The questions only required respondents to answer the questionnaire using simple responses (e.g., 'yes' or 'no') or by choosing the most appropriate answer from multiple choice answers. It consists of three sections which are Section A, Section B and

Section C. Section A questions were pertaining to the demographic characteristics of the respondents including farm's name and location, age, gender, education level, position held in the farms and years of experience in the farming industry. Section B were on general farm-level information such as type of rearing-system, buffalo type, feeding system, supplied feed, and number of milkers on the farm, milking practices, and milking techniques such as machine or hand milking. In Section C the questions were on buffaloes and quarter level information of each buffalo tested with CMT. Details such as position of positive quarters and teat shape were obtained. Buffalo-level information includes age of buffaloes, breed, stage of lactation, average daily milk yield, udder symmetry, and history of mastitis.

3.5. Statistical analysis

Data collected from the questionnaires were filled into Google forms to assist in accurate and efficient data tabulation. The data was later tabulated into Microsoft Excel sheet. Descriptive statistical analysis was carried out to calculate SCM prevalence. The buffalo-level and quarter-level prevalence of SCM were calculated as the proportion of affected buffaloes and quarters in the herd expressed in percentage.

IBM SPSS Statistics Version 29 was used to carry out statistical analysis to find out the association between variables and their odds ratio. The association between SCM prevalence and buffalo-level and quarter-level risk factors were calculated using Pearson chi-square and logistic regression respectively. A significant value of $P<0.05$ was assumed in both tests.

4.0 RESULTS

4.1 Demographic characteristics

A total of 6 respondents from six dairy buffalo farms in Selangor participated in this study.

As shown in Table 2, all except one respondent was the farm owner. All of them were male and mostly were Indians (50%). Out of these six respondents, 33.33% of them were in the age group of 41-50 years old and 51-60 years old. The education level for half of the respondents was secondary education while the rest continued their studies in tertiary education such as diploma, degree, and masters. Most of the respondents (83.33%) have more than 20 years of experience in the farming industry.

Table 2: Descriptive statistics of demographic characteristics of 6 respondents

Demographic characteristics (N=6)		**n**	**(%)**
Position	Farm owner	5	83.33
	Farm manager	1	16.67
	Farm worker	0	0.00
Gender	Male	6	100.00
	Female	0	0.00
Race	Malay	2	33.33
	Indian	3	50.00
	Chinese	0	0.00
	Others	1	16.67
Age	20-30 years	1	16.67

	31-40 years	1	16.67
	41-50 years	2	33.33
	51-60 years	2	33.33
	61-70 years	0	0.00
Education level	Primary education	0	0.00
	Secondary education	3	50.00
	Tertiary education	3	50.00
Experience in the farming industry	Less than 10 years	1	16.67
	10- 20 years	0	0.00
	More than 20 years	5	83.33

4.2 Farm characteristics

In this study, a total of 6 dairy buffalo farms were visited. As shown in Table 3, all farms were managed semi-intensively. The majority of the farms were comprised of river type (83.33%) then followed by mixed type (16.67%). All six farms raised their animals in groups with a mixed feeding system (grazing and feedlot) and consist of multiple milkers. Fifty percent (50%) of the farms practiced hand milking technique while the rest used machines for milking. Only one farm (16.67%) milks the buffaloes at a separate and dry place while most of them milk at the same place (83.33%). Two times a day milking frequency was practiced at all six farms. For milk letdown practice, all six farms allow the calves to suckle method while three farms (50%) used this method together with oxytocin injection. Fifty percent (50%) of the farms offered feed before milking and other farms did

not. Only one out of six farms washed and dried the teat of the udder before milking (16.67%) as part of the milking practice. None of the farms performed pre-dipping and post-dipping milking practices. All except one farm did not wipe and dry each teat with a separate paper towel or cloth. All of the farmers (100%) cleaned their milking machines or utensils regularly and cleaned their farms twice a day. Four out of six farms (66.67%) had a history of mastitis and used antibiotics as the treatment. Most of the farms (83.33%) practiced dry therapy and (83.33%) did not train their workers for inspection, washing and disinfection of teats. Milkers from 50% of the farms washed and dried their hands before milking while the other half did not.

Table 3: Descriptive statistics of farm characteristics of 6 farms

Farm characteristics (N=6)		**n**	**(%)**
Rearing system	Extensive system	0	0.00
	Intensive system	0	0.00
	Semi-intensive system	6	100.00
Types of buffaloes	River buffaloes	5	83.33
	Swamp buffaloes	0	0.00
	Mixed	1	16.67
Buffaloes' placement	Individually	0	0.00
	In group	6	100.00
Type of feeding system	Feedlot	0	0.00
	Grazing	0	0.00
	Mixed	6	100.00

Number of milkers	Single	0	0.00
	Multiple	6	100.00
Method of milking	Hand milking	3	50.00
	Machine	3	50.00
Place of milking	Milking at the same place	5	83.33
	Separate and dry place	1	16.67
Frequency of milking	One time	0	0.00
	Two times	6	100.00
	Three times	0	0.00
Practice for milk letdown	Allow calf to suckle	3	50.00
	Feeding concentrates and massaging the udder	0	0.00
	Allow calf to suckle + Oxytoxin injection	3	50.00
Is there any feed offered before milking	Yes	3	50.00
	No	3	50.00
Are the buffaloes' teats washed and dried before milking?	Yes	1	16.67
	No	5	83.33

Do the buffaloes' teats undergo cleaning and dipping before milking?	Yes	0	0.00
	No	6	100.00
Are the buffaloes' teats cleaned using chlorhexidine gluconate and 70% alcohol after milking?	Yes	0	0.00
	No	6	100.00
Are the buffaloes' teats dipped with iodine after milking?	Yes	0	0.00
	No	6	100.00
Is each of the teat of the buffaloes cleaned using separate paper towels or individual cloth towels?	Yes	1	16.67
	No	5	83.33
Are the milk utensils or machines regularly cleaned before milking?	Yes	6	100.00
	No	0	0.00
How often is the buffalo pen cleaned?	Once a day	0	0.00
	Twice a day	6	100.00
	Never	0	0.00
Does the farm have any case of mastitis?	Yes	2	33.33
	No	4	66.67
Is antibiotics used to treat mastitis?	Yes	2	33.33
	No	4	66.67
Do the buffaloes undergo dry therapy at the end of lactation?	Yes	1	16.67
	No	5	83.33

Does the milker undergo training for inspection, washing, and disinfection of teat?	Yes	1	16.67
	No	5	83.33
Does the milker wash and dry his hands before milking?	Yes	3	50.00
	No	3	50.00

4.3 Prevalence of subclinical mastitis

Table 4: Buffalo- level prevalence of subclinical mastitis

	Farms	Total no of lactating buffaloes	Animals with positive SCM	Prevalence percentage (%)
1	FARM A	20	6	30
2	FARM B	11	5	45.45
3	FARM C	3	2	66.67
4	FARM D	6	2	33.33
5	FARM E	1	0	0.00
6	FARM F	5	3	60.00
	Total	46	18	39.13

Table 5: Quarter- level prevalence of subclinical mastitis

	Farms	Total no of quarters	Total no of positive quarters	Prevalence percentage (%)
1	FARM A	80	23	28.75
2	FARM B	44	7	15.91
3	FARM C	12	6	50.00
4	FARM D	24	3	12.50
5	FARM E	4	0	0.00
6	FARM F	20	9	45.00
	Total	184	48	26.09

The overall buffalo-level prevalence was 39.13% where 18 out of 46 buffaloes were tested positive with SCM. Meanwhile, the overall quarter-level prevalence was 26.09% with 48 from 184 quarters tested positive for SCM. Among the six farms, the highest animal level prevalence was recorded at Farm C (66.67%) followed by Farm F (60%) and Farm B (45.45%). Farm D recorded 33.33% buffalo level prevalence of SCM. Farm A, however, showed the lowest prevalence (30%) and no positive case was recorded in Farm E.

4.4 Statistical analysis

From Table 6, Pearson's chi square revealed that there was a significant association between lactation stage and the prevalence of SCM. Meanwhile other risk factors such as udder symmetry, age, breed, and average daily milk yield had no significant influence on the prevalence of SCM in dairy buffaloes in the selected farms.

Table 6: Association between buffalo-level risk factors and SCM

		Animal level SCM		X^2 (df)	Pearson Chi-Square	P-value (Asymptotic significance (2-sided))	Fisher's Exact test (2-sided)	95% Confidence level interval	OR value
		Negative	Positive						
1.	**Udder symmetry**								
	Asymmetry	20 (43.5%)	13 (28.3%)	1	0.003[a]	0.953	1	0.257-3.591	
	Symmetry	8 (17.4%)	5 (10.9%)						
2.	**Age**								
	2.5 years - 6 years	22 (47.8%)	14 (30.4%)	1	0.004[a]	0.949	1	0.25-4.385	
	> 6 years - 8 years	6 (13%)	4 (8.7%)						
3.	**Breed**								
	Murrah	24 (52.2%)	14 (30.4%)	1	0.480[a]	0.488	0.693	0.369-7.954	
	Nili-ravi	4 (8.7%)	4 (8.7%)						
4.	**Lactating stage**								
	3 months/ less	17 (37%)	5 (10.9%)	1	4.763[a]	0.029	0.38	1.117-4.455	4.018
	> 3 months - 6 months	11 (23.9%)	13 (28.3%)						
5.	**Average daily milk yield**								
	3L/ less	9 (19.6%)	2 (4.3%)	1	2.664[a]	0.103	0.16	0.713-20.136	
	> 3L - 9L	19 (41.3%)	16 (34.8%)						

Note: df = degree of freedom, OR = odds ratio

P-value <0.05 are significantly different.

Binary logistic regression was performed to measure the association between quarter level risk factors and the occurrence of SCM. Table 7 showed that the teat shape and quarter position had no significant association (p >0.05) with SCM.

Table 7: Association between quarter-level risk factors and SCM

							95% Confidence Interval	
	B	**S.E.**	**Wald**	**df**	**Sig.**	**Exp(B)**	**Lower**	**Upper**
Quarter Position								
Position			0.674	3	0.879			
Position (1)	-0.331	0.471	0.492	1	0.483	0.718	0.285	1.809
Position (2)	-0.331	0.471	0.492	1	0.483	0.718	0.285	1.809
Position (3)	-0.215	0.464	0.214	1	0.644	0.807	0.325	2.004
Constant	-0.827	0.320	6.656	1	0.010	0.437		
Teat shape								
Shape			0.582	2	0.748			
Shape (1)	0.103	0.456	0.050	1	0.822	1.108	0.453	2.709
Shape (2)	0.390	0.556	0.493	1	0.483	1.477	0.497	4.391
Constant	-1.179	0.404	8.499	1	0.004	0.308		

Note: S.E. = standard error, df = degree of freedom,

P-value <0.05 are significantly different.

5.0 Discussion

This cross-sectional study estimated the prevalence and identified the risk factors of SCM in dairy buffaloes in six farms located in the state of Selangor. This study recorded 39.13% and 26.09% buffalo and quarter-level prevalence respectively. SCM is known to be a prevalent disease in buffaloes with high buffalo (51-82%) and quarter-wise prevalence (28-43%) (Sharif and Ahmad, 2007; Islam et al., 2019; Singha et al., 2021). However, the buffalo-level prevalence was lower than what was demonstrated in a study conducted by Rosslan et al., in 2022 in six dairy buffalo farms in Selangor (56.67%). This could be attributed to the much smaller sample size in this study. During the sampling period, many of the buffaloes were dry and non-lactating, so only a small number of lactating buffaloes were available to be sampled.

Generally, all six farms practiced similar management systems and milking practices, making it difficult to make any comparison and to measure any association. All six farms did not apply teat-dipping before or after milking as part of their practice and generally had poor milking and udder hygiene practices. This might increase the exposure to pathogens and the likelihood of transmission of bacteria, contributing to the occurrence of SCM. Good milking management involves pre-dipping and post-dipping in a sanitizing solution (Bewley et al. 2012). Teat dipping applied before and after milking is important to reduce contamination of the udder or milk by contagious and environmental pathogens during milking practices (Nickerson et al., 2019). Furthermore, pre-dipping encourages milk letdown, which speeds up the milking process, and helps to ensure that the maximum amount of available milk is harvested without causing injury to the sensitive teat tissues (Gooder, 2014). However, most farmers were reluctant to include this step in the milking practice due to issues with cost, time, and workers training as well as compliance.

Nonetheless, teat dipping is a good mastitis control and prevention strategy that has been proven to reduce intramammary infection. Other than that, most farms did not wipe the udder dry after washing them with water which could also be a contributing factor to SCM of buffaloes in the farms. Water can play a major role in promoting the growth of mastitis pathogens on the teats and udder. Water dripping from the udder can also act as a freeway for pathogens to enter the teat orifice, causing infection.

This study focuses on host factors that could potentially contribute to SCM occurrence. The only risk factor that showed significant association with the prevalence of SCM was the lactation stage. The result revealed that buffaloes in the later lactation stage (3 months to 6 months) had higher likelihood of developing SCM compared to those in the early lactation stage (less than 3 months) with the OR value of 4.018. This finding agrees with previous studies that also found that there was a higher likelihood of SCM in the later lactation stage (Silva *et al.*, 2021; Bhandari *et al.,* 2021; Almaw *et al*., 2008). Almaw et al., 2008 explained that this could be related to repeated exposure to infectious microorganisms during the milking practices. Other than that, it could be attributed to the accumulation of chronic infections which have not been detected during early lactation as discussed by Silva et al., in 2021. In addition to that, buffaloes in later lactation stages had low milk yield. As milk production decreases, the somatic cell count increases because a similar number of cells are diluted in a lesser volume of milk, giving a higher CMT score (Pugh, 2002). However, the finding of Ali *et al*., (2014) and Salvador *et al.*, (2012) is in direct contrast with the result of the current study, where they found that higher odds of developing SCM exists among cows in the early lactation stage.

This study demonstrates that SCM prevalence is higher in buffaloes aged between 2.5 years to 6 years. This could be because most of the buffaloes in the farms were in that

age group, and only a small percentage of them were between the age of more than 6 years to 8 years. However, age factor had no significant influence on the disease prevalence. Other studies have demonstrated that age had a significant influence on the occurrence of SCM in buffaloes (Ganie et al., 2021; Silva et al., 2021). Despite no significant association, this current study showed that SCM was more prevalent in buffaloes with asymmetric udder. Singha et al. in 2023 explained that this could be attributed to the presence of persistent pathogens from previous infection as udder symmetry could be the result of scar tissue formation from past infection, causing shrinkage of the udder gland.

This current study found that the quarter-level SCM was more prevalent in the front left quarter position. However, quarter position showed no significant association with SCM in binary logistic regression. This agrees with Hammer et al. 2012 who also found no association between quarter position and SCM prevalence. However, other previous studies have shown that left quarter position was associated with a higher prevalence of SCM in buffaloes (Singha et al., 2023; Ali et al., 2014; Moroni et al., 2006). Ali et al., in 2014 explained that this could be related to the milking practice. In most farms, including the farms involved in this current study, milking was performed from the left side of the animals, increasing exposure, and exerting more pressure on the left quarter position. Singha et al., 2023 explained that, when milkers proceed to milk the right teat, the teat orifices of the left quarter remain open, allowing pathogens to enter and causes infection. This however contradicts the finding from Hayat (2020) which revealed that the right-side quarters were at higher risk of developing mastitis. More studies should be done to evaluate which quarter position had actual influence on the occurrence of mastitis and to be compared with the milking practice.

In addition, this study showed SCM was not influenced by the teat shape. However, Singha et al (2023) suggested that funnel shaped teats were associated with a higher prevalence of SCM than cylindrical teat. This could be attributed to the looser, more wrinkled, and rougher skin of funnel-shaped teats, making it difficult to clean, leading to higher exposure to IMI-causing pathogens. A study from Kaur et al. (2018) however found that 'others' teat shape contributed to a greater prevalence of SCM. This current study categorised teat shape into three types only (cylindrical, funnel, bottle), which may not be adequate for various teat shapes observed in the farms. As a result, unidentifiable shapes were categorized into the most similar shapes in that category. This, coupled with subjective observation of the researcher could affect the accuracy of the data collection. It is suggested that more specific teat shapes be included in future studies. The non-significant results from this study could be attributed to the small sample size. Small sample size reduces statistical power, and a statistically non-significant result could be due to inadequate sample size (Andrade, 2020). This limitation can be improved by increasing the sample size by including more farms to participate in the study for more accurate and reliable results. There should be better time arrangements with the farmers to obtain the most suitable time when a high number of buffaloes are in lactating season as well as to avoid sampling during dry season. Other than that, future studies should include farms with different management systems and practices so that factors such as management and environment could be assessed for their contribution to SCM occurrence in buffaloes.

6.0 CONCLUSION

In conclusion, 39.13% buffalo-level and 26.09% quarter-level prevalence of SCM was recorded in dairy buffaloes in six selected farms in Selangor. Lactation stage had shown significant association with SCM suggesting that buffaloes in the later-lactation stage were four times more likely to be infected with SCM. Based on this, farmers should pay more attention to buffaloes in this stage such as maintaining a good BCS and providing optimal nutrition so that the buffaloes are in excellent health condition to fight the infection. Nevertheless, more studies should be done to estimate the prevalence and to identify the risk factors contributing to bubaline SCM involving other states in Malaysia so that the status of the disease in this country can be assessed and improved strategies could be developed and implemented to mitigate its impact to the dairy industry.

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8.0 APPENDICES

APPENDIX 1

SECTION A: FARMER'S INFORMATION AND BACKGROUND:
BAHAGIAN A: MAKLUMAT DAN LATAR BELAKANG PEMILIK LADANG:

Farmer's name:
Nama penternak:

_____

Farm name and location:
Nama ladang dan lokasi:

_____

Position in the farm:
Jawatan di ladang:

_____

Gender:
Jantina:

	Male Lelaki		Female Perempuan

Race:
Bangsa:

	Malay Melayu		Chinese Cina		Indian India		Others (Please state) Lain-lain (Sila nyatakan)

Age:
Umur:

	20-30 20-30		31-40 31-40		41-50 41-50		51-60 51-60		61-70 61-70

Educational level:
Tahap pendidikan:

	Primary school Sekolah rendah		Highschool Sekolah menengah		University/ College Universiti/ Kolej

Experience in the farming industries:
Pengalaman dalam bidang penternakan:

	Less than 10 years Kurang daripada 10 tahun		10 - 20 years 10 - 20 tahun		More than 20 years Lebih daripada 20 tahun

SECTION B: (FARM-LEVEL INFORMATION)
BAHAGIAN B: (MAKLUMAT PERINGKAT LADANG)

1. What is the rearing system used on this farm?
   Apakah sistem penternakan yang digunakan di ladang ini?

	Extensive system Sistem terbuka		Semi-intensive system Sistem separa tertutup		Intensive system Sistem tertutup

2. What type of buffaloes used?
   Apakah jenis kerbau yang digunakan?

	Swamp buffaloes Kerbau paya		River buffaloes Kerbau sungai		Mixed Campur

3. What is the size of the buffaloes pen?
   Apakah saiz kandang kerbau tenusu?

   _____

4. How are the buffaloes placed?
   Apakah cara penempatan kerbau?

	Individually Secara individu		In group Secara berkumpulan

5. What type of feeding system used at this farm?
   Apakah sistem pemberian makanan yang digunakan di ladang ini?

	Feedlot Fidlot		Grazing Ragutan		Mixed Campur

6. How many milkers are there at the farm?
   Berapa ramaikah pemerah susu yang terdapat di ladang?

	Single Seorang		Multiple Lebih daripada seorang

7. What is the method used for milking?
   Apakah kaedah yang digunakan untuk memerah susu?

	Full hand Menggunakan semua jari		Knuckling Bengkokkan ibu jari ke atas puting kerbau		Stripping Menggunakan ibu jari dan jari telunjuk sahaja		Machine Mesin

8. Where is the place for milking?
   Dimanakah tempat untuk memerah susu?

	Milking at the same place Memerah di dalam kandang		Separate and dry place Memerah diluar kandang dan tempat yang kering

9. What is the frequency of milking?
   Berapakah kekerapan memerah susu?

	One time Sekali		Two times Dua kali		Three times Tiga kali

10. What is the practice used for milk letdown?
    Apakah amalan penggunaan untuk mengeluarkan susu?

	Allow calf to suckle Membenarkan anak kerbau menyusu		Feeding concentrates and massaging the udder Memberikan makanan dan mengurut ambing		Oxytoxin injection Memberikan suntikan oksitosin		Other (Please state) Lain-lain (Sila nyatakan)

11. Is there any feed offered before milking?
Adakah sebarang makanan diberikan sebelum memerah susu?

	Yes Ya		No Tidak

12. Are the buffaloes' teats washed and dried before milking?
Adakah puting kerbau dicuci dan dikeringkan sebelum memerah susu?

	Yes Ya		No Tidak

13. Do the buffaloes' teats undergo cleaning and dipping before milking?
Adakah puting kerbau dibersihkan dan dicelupkan dalam larutan khas sebelum memerah susu?

	Yes Ya		No Tidak

14. Are the buffaloes' teats cleaned using chlorhexidine gluconate and 70% alcohol after milking?
Adakah puting kerbau dibersihkan dengan menggunakan chlorhexidine gluconate dan 70% alkohol selepas pemerahan?

	Yes Ya		No Tidak

15. Are the buffaloes' teats dipped with iodine after milking?
Adakah puting kerbau dicelupkan dengan menggunakan iodine selepas pemerahan?

	Yes Ya		No Tidak

16. Is each of the teat of the buffaloes cleaned using separate paper towels or individual cloth towels?
Adakah puting kerbau dibersihkan menggunakan tisu kertas atau tuala berasingan?

	Yes Ya		No Tidak

17. Are the milk utensils or machines regularly cleaned before milking?
Adakah peralatan memerah susu atau mesin kerap dibasuh sebelum memerah susu?

	Yes Ya		No Tidak

18. How often is the buffalo pen cleaned?
Berapakah kekerapan kandang kerbau tenusu dibersihkan?

	Once a day Sekali sehari		Twice a day Dua kali sehari		Never Tidak dibersihkan sama sekali

19. Does the farm have any case of mastitis?
    Adakah ladang mempunyai kes mastitis?

	Yes Ya		No Tidak

20. Are antibiotics used to treat mastitis?
    Adakah antibiotik digunakan untuk merawat mastitis?

	Yes Ya		No Tidak

21. Do the buffaloes undergo dry therapy?
    Adakah kerbau menjalani terapi kering pada akhir laktasi?

	Yes Ya		No Tidak

22. Does the milker undergo training for inspection, washing, and disinfection of teat?
    Adakah pekerja menjalani latihan untuk pemeriksaan, mencuci, dan pembasmian kuman pada puting kerbau?

	Yes Ya		No Tidak

23. Does the milker wash and dry his hands before milking?
    Adakah pekerja mencuci dan mengeringkan tangan sebelum memerah susu?

	Yes Ya		No Tidak

SECTION B: (QUARTER-LEVEL AND BUFFALO-LEVEL INFORMATION)

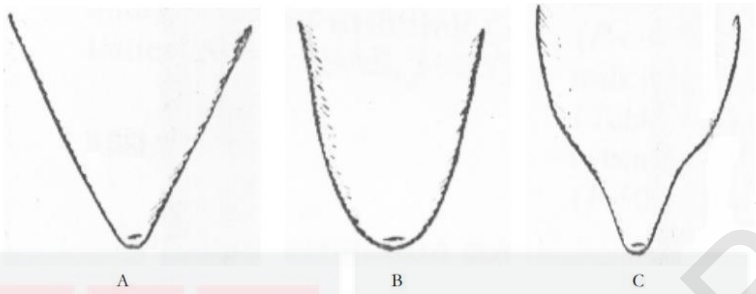
BAHAGIAN B: (MAKLUMAT PERINGKAT KUARTER DAN KERBAU)

24. Buffalo ID
    No kerbau: _____

25. Which quarter is tested positive?
    Belah ambing yang manakah diuji positif?

	Front left Depan kiri		Front right Depan kanan		Hind left Belakang kiri		Hind right Belakang kanan

26. What is the shape of the teat?
Apakah bentuk puting kerbau?



Adapted from James et al (2009)
Diadaptasi daripada James et al (2009)

Teat position Kedudukan puting	Teat shape Bentuk puting		
	Funnel (A) Corong	Cylindrical (B) Berbentuk silinder	Bottle (C) Berbentuk botol
Front left Depan kiri			
Front right Depan kanan			
Hind left Belakang kiri			
Hind right Belakang kanan			

27. Symmetry of the udder:
Simetri udder:

	Symmetrical Simetri		Asymmetrical Tidak simetri

28. What is the age of the buffaloes in years?
Berapakah umur kerbau di dalam tahun?

	2.5 to 6 years 2.5 ke 6 tahun		More than 6 years to 8 years Lebih daripada 6 tahun ke 8 tahun		More than 8 years to 20 years Lebih daripada 8 tahun ke 20 tahun

29. What is the breed of the buffaloes?
Apakah jenis baka kerbau?

	Indigeneous Baka tempatan		Crossbreed Baka campuran

30. What is the stage of lactation of the buffaloes?
Apakah peringkat laktasi kerbau?

	3 months or less 3 bulan atau kurang		More than 3 months to 6 months Lebih daripada 3 bulan ke 6 bulan		More than 6 months to 9 months Lebih daripada 6 bulan ke 9 bulan		More than 9 months Lebih daripada 9 bulan

31. In average, what is the daily milk yield?
Secara purata, berapakah hasil susu harian?

	3L or less 3L atau kurang		More than 3L to 9L Lebih daripada 3L ke 9L

32. Do the buffaloes have any history of clinical mastitis?
Adakah kerbau mempunyai sejarah mastitis klinikal?

	Yes Ya		No Tidak

APPENDIX 2

Funnel-shaped teats

Cylindrical-shaped teats

Bottle-shaped teats