The prevalence and risk factors of subclinical mastitis in water buffalo (*Bubalis bubalis*) in Bangladesh

Shuvo Singha [a,b,c,d,1,*], Gerrit Koop [d,e], Fabrizio Ceciliani [a,d], Marjolein Derks [d,f], Md. Ahasanul Hoque [c,d], Mohammed Kawser Hossain [d,g], Md. Matiar Rahman Howlader [b], Md. Mizanur Rahman [c,d], Momena Khatun [d,h,i], Sofia Boqvist [d,j], Ylva Persson [d,k]

[a] *Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Via dell'Università 6, 26900 Lodi, Italy*
[b] *Department of Physiology, Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Sylhet 3100, Bangladesh*
[c] *Department of Medicine and Surgery, Chattogram Veterinary and Animal Sciences University, Chattogram 4225, Bangladesh*
[d] *Udder Health Bangladesh, Chattogram 4225, Bangladesh, www.uhb.org.bd*
[e] *Sustainable Ruminant Health, Department of Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584, CL, Utrecht, the Netherlands*
[f] *Farm Technology Group, Wageningen University and Research, Droevendaalsesteeg 1, 6708, PB, Wageningen, the Netherlands*
[g] *Department of Surgery and Theriogenology, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Sylhet 3100, Bangladesh*
[h] *Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bremgartenstr. 109a, CH-3001 Bern, Switzerland*
[i] *Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh*
[j] *Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, 750 07 Uppsala, Sweden*
[k] *Department of Animal Health and Antimicrobial Strategies, National Veterinary Institute, 751 89 Uppsala, Sweden*

ARTICLE INFO

ABSTRACT

Subclinical mastitis (SCM) in water buffalo is responsible for reduced milk yield and quality. This cross-sectional study was carried out to a) estimate the prevalence of SCM, b) identify risk factors associated with SCM, and c) identify farm-level risk factors associated with bulk milk somatic cell count (BMSCC).

The buffalo farms included in this study represented five rearing systems: free-range, semi-free-range, household, semi-intensive, and intensive, providing a total of 3491 functional quarters of 880 lactating buffalo on 248 farms. The California mastitis test score was used to identify SCM. Bulk milk samples ($n = 242$) were used for farm-level BMSCC. Quarter and buffalo-level risk factors for SCM were measured using questionnaires and observations.

The overall SCM prevalence was high at 27.9% at the quarter-level (25th and 75th percentiles: 8.3% and 41.7%) and 51.5% at buffalo-level (25th and 75th percentiles: 33.3% and 66.7%). The geometric mean BMSCC was 217,000 cells/mL of milk (ranging from 36,000-1,213,000 cells/mL), which is low on average, but some farms could improve substantially. The buffalo rearing system, udder location (left versus right), teat shape, udder asymmetry, number of milkers, and having a quarantine facility were associated with buffalo udder health. Our findings suggest that mainly using free-range rearing systems may help decrease the prevalence of SCM primarily by employing buffalo breeding and better farm biosecurity, and udder health control strategies can be designed based on our findings.

1. Introduction

Subclinical mastitis (SCM) is the inflammation of mammary tissue in the absence of clinical signs. This disease reduces the milk yield in dairy animals, impairs animal welfare, and is associated with milk quality deterioration, making milk less suitable for consumption and processing

(Costa et al., 2020). A study by Malik and Verma (2017) reported that the average annual economic loss per buffalo due to mastitis was 70 USD, with mastitis intervention accounting for 55% of the loss and a drop in milk production accounting for 16%. Water buffalo (*Bubalus bubalis*) is the second most important dairy animal in Asia, and about 74% of the global buffalo population lives in south Asian countries (i.e., Bangladesh, Bhutan, India, Pakistan, Nepal, and Sri Lanka) (Minervino et al., 2020).

Diagnosis of SCM is of great importance since this disease remains otherwise unnoticed due to the absence of visible changes in the milk or udder and because SCM contributes to the transmission of intra-mammary infection (IMI) (Kirkeby et al., 2019). Currently, multiple screening tests, such as somatic cell count (SCC) or the California mastitis test (CMT) (Hussain et al., 2018; Aldujaily et al., 2019), are used to detect SCM in water buffalo. Bulk milk somatic cell count (BMSCC) is another helpful indicator representing the udder health status on the farm (Pasquini et al., 2018; Costa et al., 2020). The prevalence of buffalo SCM has been studied in many countries, such as India, Pakistan, and Bangladesh, demonstrating a quarter-level prevalence of 10–46% using CMT (Muhammad et al., 2010; Preethirani et al., 2015; Islam et al., 2019). However, the prevalence is widely variable between the studies and regions.

Water buffalo can be classified into two subspecies: the river-type buffalo, which are concentrated in India, Pakistan, Egypt, and Italy, and swamp-type buffalo, which are concentrated in China, Myanmar, and Thailand (Minervino et al., 2020; Zhang et al., 2020). Within these two buffalo subspecies, the river type is widely distributed in Bangladesh, except for some swamp-type buffalo found only in the eastern part of the country (Samad, 2020). About 70% of the river-type buffalo in Bangladesh are indigenous and non-descriptive, meaning they do not belong to any specific breed, such as Murrah or Nilli-Ravi. A few crossbreeds of indigenous buffalo with Murrah, Nili-Ravi, Surti, or Jaf-frabadi can be found on the surrounding Indian border due to migration from India to Bangladesh. These crossbreeds generally produce 5–10 L of milk, about two times more than the indigenous buffalo (Ali et al., 2014; Samad, 2020). High-yield crossbred buffalo generally have an increased risk of SCM (Islam et al., 2019). Buffalo are mainly reared either in a free-range or an intensive system.

Moreover, depending on buffalo-rearing systems in sub-tropical countries, there is limited pasture availability with no or minimal supplementation of concentrates (Tiwari et al., 2007; Habib et al., 2017; Rahman et al., 2019). Nutritional status is important because specific macro and micronutrients regulate metabolism and immunological function in animals, and it is known that abnormalities in metabolism or immunity can cause health disorders in dairy cows (Sordillo, 2016). For example, insufficient nutrition in dairy cows has been reported to be associated with a negative energy balance and oxidative stress, predisposing several disease conditions, such as ketosis and mastitis (Esposito et al., 2014; Krishna et al., 2014; Pérez-Báez et al., 2019). Under these circumstances, it is crucial to identify farm, animal, and quarter-related factors that further increase the risk of SCM in water buffalo to improve udder health.

Knowledge of the prevalence and associated risk factors might help identify control strategies for buffalo mastitis. To meet this need, we conducted a nationwide cross-sectional study on water buffalo with the objectives of a) estimating the prevalence of SCM at both an animal and quarter-level, b) identifying the risk factors associated with SCM prevalence, and c) identifying the farm-level risk factors associated with BMSCC.

2. Materials and methods

2.1. Study area

Bangladesh is divided into 64 districts and 495 subdistricts named "Upazilla". The coastal and semi-coastal districts in Bangladesh, which

are surrounded by the Meghna-Ganga and the Jamuna-Brahmaputra river floodplains, are the key areas of buffalo concentration, where 40% of the buffalo population in Bangladesh are kept because of the availability of fallow pasture land and green forages (Faruque et al., 1990; Hamid et al., 2016). Water buffalo farms and individual lactating water buffalo belonging to 9 districts were included in the study to effectively represent the buffalo population in Bangladesh (Fig. 1).

2.2. Production systems

The buffalo farms included in this study belonged to five different rearing systems: free-range, semi-free-range, household, semi-intensive, and intensive. Free-range buffalo depend on grazing on fallow land in coastal or semi-coastal islands with the supplementation of straw or, at times, small amounts of concentrates. In free-range systems, buffalo are moved from one island to another based on the availability of green forages. In the semi-free-range system, buffalo are moved to the inlands during the dry season (October to March) due to feed scarcity on the islands and stay there for 3–6 months, depending on roughage availability. The household rearing system allows 5–7 h of grazing, combining a supply of a minimal amount of straw, grass, and concentrates. In river-basin areas, the semi-intensive system allows buffalo to stay in sheds at night, graze in nearby pasturelands, and return daily. In an intensive system, buffalo are tied in sheds, stall-fed, and are never allowed to graze.

2.3. Sampling design and study period

Presently, the upazilla veterinary hospitals (UVH), a part of the government livestock services, and a non-governmental organization (NGO) named "Palli Karma-Sahayak Foundation" (PKSF) collected buffalo population data at the sub-district level in Bangladesh. Data on the number of farms was collected through a census carried out by the UVH every year. About 1.5 million buffalo heads were reported in Bangladesh during 2019–2020 (DLS, 2021). However, this is likely an underestimation of the actual number of buffalo since this census depends on farmers' active reporting of farm data. Since no sampling frame was available, lists of buffalo farms were collected from the buffalo farmers' database of UVH, or PKSF involved with buffalo farmers' support and researchers who had the best knowledge about the buffalo population. Based on their information, one buffalo-concentrated sub-district was selected from each district for sampling. A total of 200 farms to be sampled was estimated according to an expected 50% prevalence with 5% absolute precision, assuming an intraclass correlation coefficient of 0.25 for buffalo within farms. A list of buffalo farms was created with the help of UVH and PKSF. The listed buffalo farmers were contacted to request information on the number of lactating buffalo and the farm location. Additionally, four known intensive farms were recruited for sampling because of the limited number of intensive buffalo farms in Bangladesh. Two of the four intensive farms were government-owned, and two more were private buffalo farms. Finally, a total of 248 water buffalo farms from free-range (n = 51), semi-free-range (n = 106), household (n = 33), semi-intensive (n = 54), and intensive (n = 4) buffalo farms were recruited for sampling. The numbers of sampled farms from each study location are given in Table 1. About 25% more farms than the required sample size was included in the study due to uncertainty that all farms could be sampled. For example, the free-range buffalo farms were remotely located and, therefore, sometimes difficult to reach during the morning milking time. Sampling was performed on 1 to 5 lactating buffalo on each farm. All the animals were sampled when a farm had ≤5 lactating buffalo, and 5 were randomly sampled when a farm had ≥5 lactating buffalo. However, on 9 farms (5 semi-free range, 3 households, and 1 semi-intensive farm), only one lactating buffalo could be sampled because of non-cooperative animals on those farms. Because of the small number of intensive farms, all the lactating buffalo from these four farms were sampled. Milk sampling and the recording of
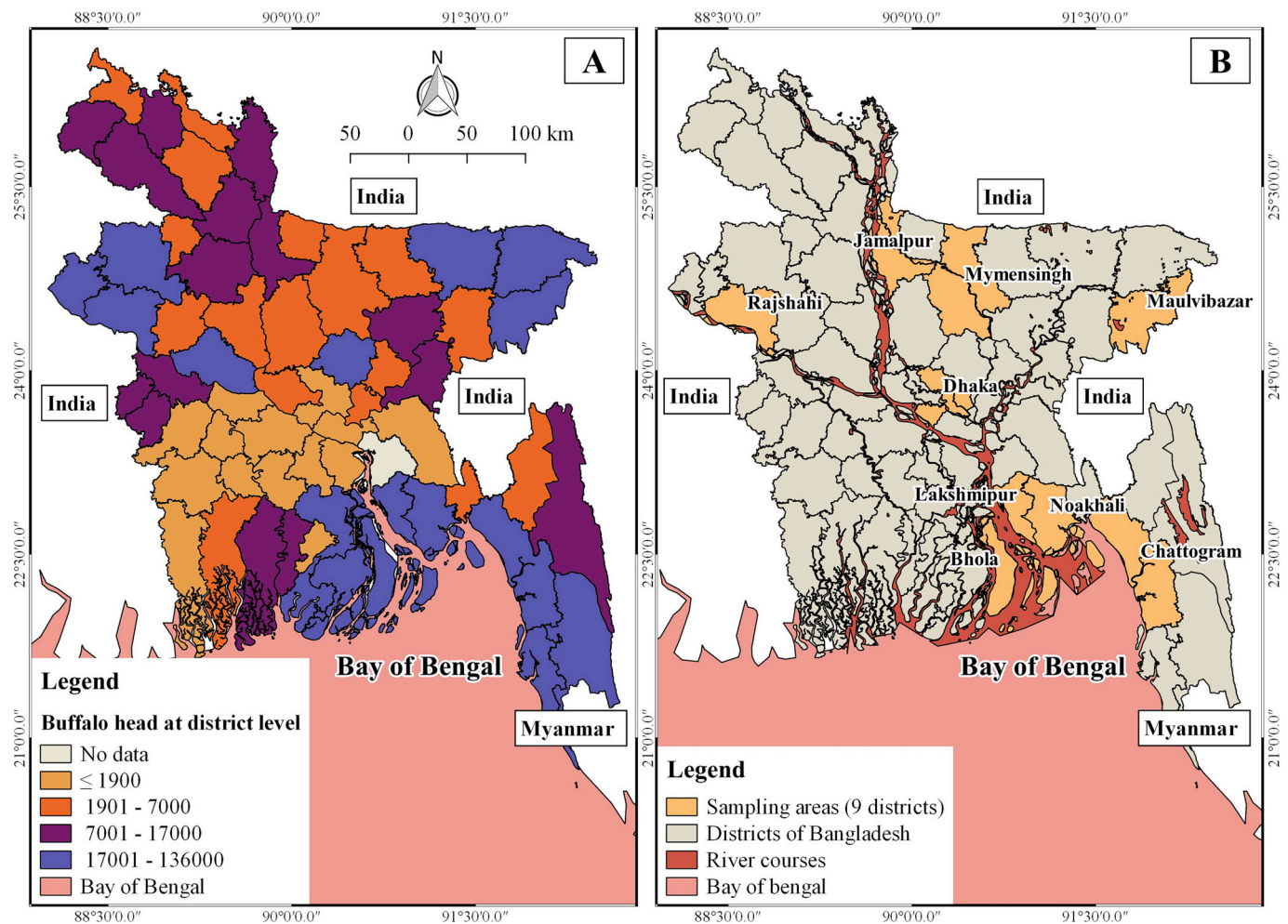
Fig. 1. Location of nine buffalo concentrated districts in Bangladesh where buffalo farms were enrolled in the present study. Fig. 1. A. shows the district-level buffalo heads in Bangladesh (Data source: LDDP (2019)). Fig. 1. B. The orange color indicates the location of 9 districts that were recruited for sampling (Rajshahi, Jamalpur, Mymensingh, Maulvibazar, Dhaka, Lakshmipur, Bhola, Noakhali, and Chattogram) in the study.

Table 1
Distribution of 880 buffalo sampled from 248 buffalo farms over five different buffalo rearing systems in nine buffalo concentrated districts in Bangladesh.

District	Total buffalo population	Number of buffalo sampled	Number of farms from different buffalo rearing systems				
			Free-range	Semi-free-range	Household	Semi-intensive	Intensive
Noakhali	50,680	182	9	113	20	40	–
Jamalpur	11,955	145	17	84	9	35	–
Chattogram	44,494	135	85	38	12	–	–
Rajshahi	18,550	118	–	–	2	116	–
Moulvibazar	55,363	105	–	103	–	2	–
Bhola	52,190	100	48	13	30	7	2
Laxmipur	29,971	52	–	–	–	–	52
Mymensingh	3087	28	5	15	4	4	–
Dhaka	1621	15	–	–	–	–	15
Overall	**267,911**	**880**	**164**	**366**	**77**	**204**	**69**

potential risk factor data (section 2.5) were conducted between February 2020 and April 2021 (sampling was interrupted for 6 months due to the national lockdown in the SARS-CoV2 pandemic from April to September 2020).

2.4. Detection of subclinical mastitis using the California mastitis test and bulk milk somatic cell count

The SCM status of each buffalo quarter was determined and categorized on a scale from 1 to 5 using CMT, following the process

described by Baloch et al. (2016). The CMT was performed on each functional quarter of a buffalo before milking was started, after discarding the first 1–2 squirts of milk. A quarter was considered positive when the CMT score was ≥2 with no visible signs of CM (any changes in milk or swelling, and/or redness, or painfulness of the mammary gland). A buffalo was considered SCM-positive when one or more of its four functional quarters had SCM. Bulk milk SCC was determined in thoroughly mixed morning bulk milk from all the lactating buffalo on each farm following Singha et al. (2021) using a DeLaval cell counter (DeLaval Group, Stockholm, Sweden) (Adkins et al., 2017).

2.5. Questionnaire design and data collection

A questionnaire was developed in English and tested on 16 farms in a pilot study (Singha et al., 2021) before starting the main study. We prepared 2 separate datasets, combined quarter-level and buffalo-level (dataset A) and farm-level (dataset B). Dataset A included the quarter-level information on the position of the tested quarters (front left, front right, hind left, or hind right) and teat shape (cylindrical, bottle, or funnel). Buffalo-level information included the age of the buffalo in years (2.5 to 6, > 6 to 8 or > 8 to 20 years), breed (indigenous versus cross breed), stage of lactation (3 months or less, > 3 to 6 months, > 6 to 9 months or > 9 months), average daily milk yield (3 L or less versus 3.1 L to 9 L), udder symmetry (symmetrical or asymmetrical), CM history (yes or no), abortion history (yes or no), and the number of milkers on the farm (single versus multiple). Dataset B included general farm-level information such as type of buffalo rearing system (free-range, semi-free-range, household, semi-intensive or intensive), type of farm area (coastal, semi-coastal, inlands, islands or river basin), season (winter: late December to early February; spring: late February to early April; summer: late April to end of June; rainy: early July to early September or autumn: late September to early December), communication with the employees (good contact with the staff, irregular visits to the farm, rare visits to the farm or managed by farmers themselves), district, buffalo type (river type, swamp type or mixed), wallowing water source (tube-well, deep tube-well, pond, river or rain water), feeding system (stall-feeding, grazing or mixed), supplied feed (no additional food supply, concentrate, roughage and concentrate, locally available straw and roughage, only roughage from grazing), buffalo source (stock, stock or purchased, purchased or contract), history of any buffalo mortality in the last 12 months (yes or no) and restraining during milking (yes or no). Milking was performed by hand, using three traditional techniques: complete hand milking, stripping, or knuckling. Generally, one or more of these techniques is used on a buffalo farm depending on milkers' preference and buffalo teat length or diameter. Complete hand milking involves grasping the teat with five fingers and pressing it against the palm. Stripping involves firmly holding the teat between the thumb and index finger and drawing down the length of the teat while applying pressure. Knuckling involves bending the thumb and pressing the teat against the index finger with the nail and end of the thumb. Information related to the milking technique was collected, such as fore stripping (yes or no) and hand milking (full hand, stripping, or knuckling). Other relevant data was also collected, such as udder hygiene (excellent, good, fair, or poor), a score of milkers' hygiene (excellent, good, or fair), feed offered before milking (yes or no), and whether farms use a quarantine facility for the newly purchased buffalo (yes or no). The corresponding author can provide the full version of the questionnaire upon request. Written consent was obtained from the buffalo farmers before administering the questionnaire. Data collection was performed in face-to-face interviews with the farmers (farm owner or acting manager) and through on-farm observations. All interviews were conducted in Bengali, but the questionnaire was recorded in English. The study was approved and performed in line with the guidelines of the SAU research system (SAU/Ethical committee/AUP/21/06) of Sylhet Agricultural University, Bangladesh.

2.6. Statistical analyses

2.6.1. Descriptive statistics

Data were imported into R (version 4.1.2; R Foundation for Statistical Computing, Vienna, Austria) for statistical analysis. The analysis was carried out separately for datasets A and B. The dependent variables considered were, for dataset A, the SCM status of an individual quarter (i.e., healthy or SCM positive) and the SCM status of an individual buffalo, and for dataset B, the BMSCC (cells per mL of milk) as the indicator of SCM status at the farm-level. The BMSCC distribution was positively skewed, so a log10 transformation was performed to restore a normal distribution, which was checked using a visual inspection of the histogram. Descriptive statistical analysis was carried out to calculate the prevalence of SCM (at the quarter and buffalo levels) and the summary of BMSCC (at the farm-level). To calculate the sampling-corrected prevalence, sampling weights were created based on the total buffalo population in an area, the number of buffalo, and the sampled quarters (Dohoo et al., 2010b). The probability of selecting each buffalo was calculated by dividing the number of buffalo sampled by the total number in an area, as estimated by the UVH/PKSF. Sampling weights were calculated using the inverse of the sampling probability. We then estimated the buffalo and quarter-level prevalence, correcting for weighting and taking clustering by the district into account using STATA SE-17.0 (Stata Corp. College Station, TX, USA).

2.6.2. Quarter and animal-level risk factor models

Two generalized linear mixed-effects logistic regression models were constructed using the R package "lme4" (Bates et al., 2015) to identify factors associated with SCM from dataset A. For generalized linear mixed-effects logistic regression models, the GLMER function was used. Farm and buffalo ID within farms were used as random effects in these models. Subject-specific effects were used to calculate the odds ratio, meaning that fixed effects in a mixed model represent the effects of that factor within the cluster. The significance of the random effect terms was checked with the likelihood ratio test (LRT) using the latent variable approach (Dohoo et al., 2010a). Then, fixed variables that were significant at $P \leq 0.2$ in the univariable logistic regression were selected for the multivariable logistic regression. The model was manually constructed using a forward selection procedure, applying the maximum likelihood estimation procedure (Dohoo et al., 2010a). The statistical significance of the contribution of individual predictors was determined using Wald's test and the LRT. The presence of confounders was assessed by adding one of the variables in order of significance (the variables with the lowest P-value were added first) and, in each step, assessing whether the beta coefficient of other variables in the model changed by >30%, which was deemed confounding. Simultaneously, we assessed the collinearity based on significant changes (> 30%) in the standard error. If a variable was found to be non-significant ($P > 0.05$), was not a confounder, and no indications of collinearity were seen, the variable was removed from the model in the next step. Finally, significant ($P \leq 0.05$) variables at LRT were presented regarding odds ratio and 95% C.I.

2.6.3. Farm-level BMSCC risk factor model

A linear regression model was fitted using dataset B to identify the association of farm-level variables with log10-transformed BMSCC (LBMSCC). The linear regression model used the LM function from the R base. Variables significant in the univariable linear regression at $P \leq 0.2$ were used in multivariable linear regression models. Variance inflation factors (VIF) for the factors were also examined to diagnose collinearity (a VIF value ≥ 10 was deemed to indicate collinearity). Variables with >20% missing values were removed. We assessed the presence of any confounding variable in the model using a similar procedure as discussed for the logistic regression model. If confounding was present, the confounding variable was kept in the model to correct the coefficient estimates for confounding. If two variables showed collinearity, the most biologically meaningful variable was kept in the model. To assess the homoscedasticity of the residuals, a Breusch-Pagan test was performed using the library "skedastic" (Farrar, 2020), and we performed a visual inspection of the residuals plotted against predicted values of the fitted model. Finally, the significant ($P \leq 0.05$) variables were presented as beta values and their corresponding 95% CI.

3. Results

3.1. Descriptive statistics and farm characteristics

A total of 3520 quarters of 880 lactating buffalo in 248 farms were

enrolled in the study, of which 29 quarters could not be sampled because 17 quarters were blocked due to a previous history of CM, 7 quarters were congenitally absent, and 5 quarters of 4 animals could not be sampled because the animals did not cooperate during the milking. Therefore, 3491 quarters were examined using CMT. The number of sampled buffalo ranged between 1 and 41 per farm. On average, 67% of the lactating and 24% of the total buffalo population per farm were sampled. The average farm size was 22.8 buffalo (ranging from 1 to 278 buffalo per farm) (Supplementary material 1). The studied farms comprised 81.6% crossbreeds and 18.4% indigenous non-descriptive breeds. The crossbreeds mainly crossed indigenous non-descriptive breeds with the Murrah, Nilli-Ravi, Jafarabadi, Mediterranean, and swamp-type water buffalo breeds. The majority of the buffalo farms were comprised of river type (79%), followed by the swamp type (10%), and then a mixed population of both types (11%). Buffalo mostly wallowed in water (83%) but also in muddy places (2%), and about 14% wallowed in both places. Only 1% of the farms didn't allow the buffalo to wallow. Among the solely grazing-dependent farms, 77% allowed grazing for 12–18 h, while the other 23% allowed grazing for 2–6 h daily. In the non-grazing-based farms, buffalo were fed once (36%), twice (53%), and in some farms, 3–4 times (11%) a day. All farms performed hand milking, but no farm applied pre- or post-teat dipping. The milker did pre-milking udder stimulation through calf suckling and hand massage. A single person milked all the lactating buffalo in 55% of the farms, while multiple people performed the milking on 45% of the farms. About 75% of the farmers got veterinary support from the government or private means. Farmers also got support from UVH through regular deworming and vaccination programs. More of the farmers who participated in the study had a primary education level (grades 1–5) (47%) than those who had no formal education (32%), a higher secondary education (18), or a college degree (3%). The farmers' ages ranged between 13 and 85 years, and all were male. Only 8% of the farmers indicated that they knew how to formulate the ration to meet the nutrient requirements of the buffalo.

3.2. Quarter and animal-level prevalence and farm-level BMSCC

The overall prevalence of SCM, corrected for the sampling design, was 27.9% (95% CI 26.3–29.7) at the quarter-level, with the 25th and 75th percentiles at 8.3% and 41.7%, respectively; at the buffalo-level, this was 51.5% (95% CI 47.8–55.2), with the 25th and 75th percentiles at 33.3% and 66.7% respectively (Fig. 2). Among the SCM positive quarters, CMT score 2 was most frequently observed irrespective of the type of buffalo rearing system (Table 2). At the farm-level, 242 buffalo farms were sampled as we failed to perform BMSCC on 6 farms because farmers accidentally mixed the buffalo and cow milk in those farms. The geometrical mean BMSCC was 217,000 cells/mL of milk, which varied between the farms and ranged from 36,000 to 1,213,000 cells/mL of
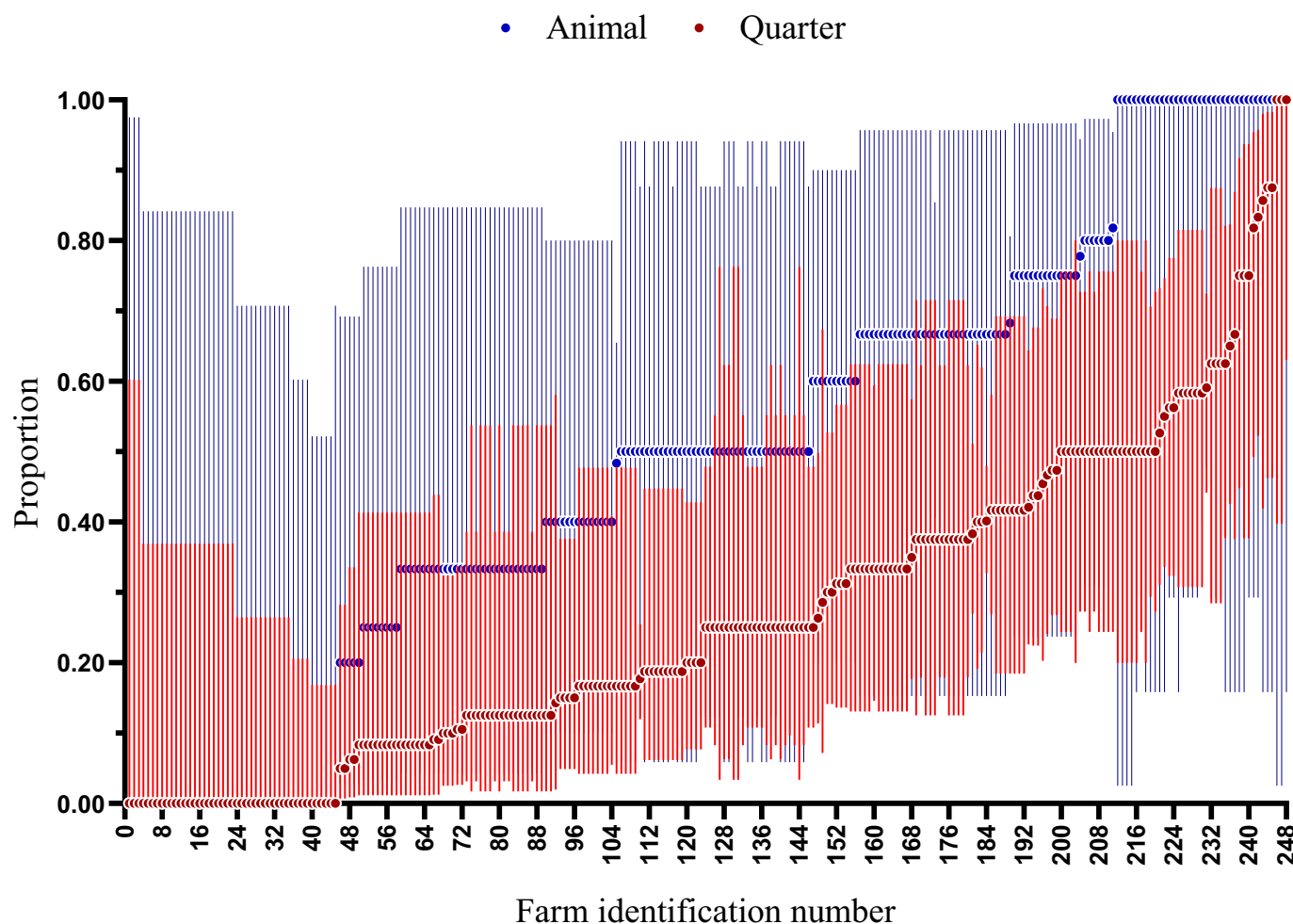

Fig. 2. Within farm quarter and animal level prevalence of subclinical mastitis in 248 water buffalo farms in Bangladesh. Farm identification numbers of each buffalo farm were represented in X axis (data shorted by the animal level prevalence per farm) and the proportion of quarter and animal levels SCM were presented in the Y axis. The red circles represent the point estimate of the quarter level prevalence of SCM, and the red vertical lines represent the 95% confidence intervals. The blue circles represent the point estimate of the animal level prevalence of SCM, and the grey vertical lines represent the 95% confidence intervals in each corresponding buffalo farm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2

Quarter level prevalence of subclinical mastitis based on California mastitis test score (subclinical mastitis at CMT score ≥ 2) on 3491 buffalo quarters of 880 buffalo from 248 buffalo farms of five different buffalo rearing systems in Bangladesh.

Types of rearing systems	Number of buffalo quarters tested	CMT = 1 n (%)	CMT = 2 n (%)	CMT = 3 n (%)	CMT = 4 n (%)	CMT = 5 n (%)
Free-range	641	484 (75.5)	96 (15.0)	32 (5.0)	21 (3.3)	8 (1.2)
Semi-intensive	814	618 (75.9)	112 (13.8)	57 (7.0)	25 (3.1)	2 (0.2)
Semi-free-range	1461	1092 (74.7)	206 (14.1)	101 (6.9)	54 (3.7)	8 (0.5)
Household	308	212 (68.8)	59 (19.2)	19 (6.2)	16 (5.2)	2 (0.6)
Intensive	267	149 (55.8)	47 (17.6)	22 (8.2)	23 (8.6)	26 (9.7)
Overall	**3491**	**2555 (73.2)**	**520 (14.9)**	**231 (6.6)**	**139 (4.0)**	**46 (1.3)**

milk with the 25% and 75% percentiles at 124,000 and 355,000 cells/mL of milk, respectively.

3.3. Quarter-level risk factor model

The univariable analysis of the association between SCM and 4 quarter-level variables is shown in Supplementary material 2. No confounding or collinearity was observed in the multivariable model. In the final model, SCM prevalence was significantly associated with the intensive buffalo-rearing systems, quarters located on the left, and funnel-shaped teat (Table 3).

3.4. Buffalo-level risk factor model

The univariable analysis of the association between SCM and 9 buffalo-level variables is shown in Supplementary material 2. The rearing system confounded the association of SCM with a previous history of CM and contained similar information; therefore, the previous record of CM was removed from the final model. No collinearity was observed in the stepwise forward selection procedure. In the final model (Table 4), buffalo with asymmetrical udders, milked by a single milker,

Table 3

Multivariable mixed-effects (farm and animal) logistic regression analysis of subclinical mastitis (defined as CMT score ≥ 2) regressed against the quarter level risk factors of 3455 quarters of 872 buffalo in 248 farms expressed as odds ratio (OR), 95% confidence interval (95% CI), and P value.

Variable name	Categories	Number of observations	OR (95% CI)	P
Quarter position[a]	Right quarters	1728	Reference	0.006
	Left quarters	1727	1.3 (1.1 to 1.6)	
Rearing system[a]	Free-range	633	Reference	0.004
	Semi-intensive	810	1.1 (0.5 to 2.1)	
	Semi-free-range	1441	1.1 (0.6 to 1.9)	
	Household	304	2.0 (0.9 to 4.7)	
	Intensive	267	6.6 (2.2 to 19.5)	
Teat shape[a]	Cylindrical	2684	Reference	0.003
	Bottle	445	1.3 (0.9 to 1.9)	
	Funnel	326	2.1 (1.4 to 3.3)	

[a] Odds ratio was estimated based on subject-specific beta-estimates.

Table 4

Multivariable random-effect (farm identification number) logistic regression analysis of the association between subclinical mastitis (defined as at least one of the quarters has a CMT ≥2) and various risk factors of 853 buffalo from 246 buffalo farms expressed as odds ratio (OR), 95% confidence interval (95% CI), and P values.

Variable name	Categories	Number of observations	OR (95% CI)	P
Udder symmetry[a]	Symmetrical	573	Reference	<0.001
	Asymmetrical	278	1.8 (1.3 to 2.4)	
Number of milkers in the farm[a]	> 1	407	Reference	0.008
	1	444	1.5 (1.2 to 2.1)	
Rearing system[a]	Free-range	155	Reference	0.016
	Semi-intensive	200	1.0 (0.6 to 1.6)	
	Semi-free-range	358	1.1 (0.7 to 1.6)	
	Household	72	1.3 (0.7 to 2.3)	
	Intensive	68	2.7 (1.5 to 5.0)	

[a] Odds ratio based on subject-specific beta-estimates.

and intensive buffalo rearing systems had higher odds of being SCM positive.

3.5. Bulk milk somatic cell count risk factor model

The univariable association between LBMSCC and 18 farm-level variables is shown in Supplementary material 2. Several confounding variables were identified during the model-building process. Adding districts to the model affected the association between farm area type and LBMSCC. The farm area and district contained similar information, but the farm area was biologically more meaningful in association with BMSCC. Therefore, we removed the district from the final model. No collinearity was observed. The type of wallowing area was not included in the final model because of >20% of missing values. In the final model (Table 5), the winter season was significantly associated with a lower level of LBMSCC than the autumn season. Farms that provide a quarantine facility to the buffalo were also significantly associated with a lower LBMSCC than farms that did not provide any quarantine facility. Farms with a history of buffalo mortality in the previous 12 months were significantly associated with a higher LBMSCC than farms with no

Table 5

Multivariable linear regression analysis of the association between the log10-transformed bulk milk somatic cell count with farm level risk factors in 237 buffalo farms in Bangladesh expressed as co-efficient (β), 95% confidence interval (95% CI), and P values.

Variable name	Categories	Number of observations	β	95% CI	P
	Intercept		5.59	5.29 to 6.08	<0.001
Season	Winter	75	Reference		<0.001
	Rainy	3	0.06	−0.27 to 0.40	
	Summer	70	0.14	0.04 to 0.23	
	Spring	87	0.26	0.17 to 0.35	
	Autumn	2	0.50	0.10 to 0.91	
History of buffalo mortality	No	119	Reference		0.026
	Yes	118	0.23	0.11 to 0.35	
Having a quarantine facility	Yes	29	Reference		<0.001
	No	208	0.09	0.009 to 0.16	

history of buffalo mortality.

4. Discussion

This cross-sectional study estimated the prevalence and identified the risk factors of buffalo SCM in nine major buffalo-concentrated districts in Bangladesh. Subclinical mastitis is known to be a prevalent disease in water buffalo, with a high buffalo (51–82%) and quarter-level prevalence (28–43%) (Sharif and Ahmad, 2007; Islam et al., 2019; Singha et al., 2021). Our investigation demonstrates the high prevalence of buffalo SCM at buffalo (52%) and quarter (28%) levels. A higher prevalence of SCM at the buffalo-level is more probable than at the quarter-level because a single SCM-positive quarter results in a buffalo positive for SCM. In our study, the average herd size was medium (22.8 buffalo/ herd), and sampled farms did not use teat dipping or antiseptics and generally had poor milking and udder hygiene practices. Therefore, indirect contact between buffalo on the farm may increase the likelihood of transmission of the disease, leading to a higher prevalence of SCM. Alternatively, a large stock density in buffalo farms in Bangladesh (Habib et al., 2017) and the high temperature and humid climate in subtropical countries may cause stress to the buffalo and further increase their susceptibility to SCM (El Nahas et al., 2017; Ahmad et al., 2019). Although a prior small-scale investigation in Bangladesh (Singha et al., 2021) showed a greater prevalence than our study, the SCM prevalence estimate in our study was corrected for the multistage sampling approach for district-level stratification, thus is more reliable for extrapolation at the population level.

The mean BMSCC in this study was low overall (217,000 cells/mL of milk) and is in line with another study conducted in Bangladesh (Singha et al., 2021). It also falls within the range of BMSCC values considered acceptable in neighboring countries, such as India (200,000 cells/mL of milk) and Nepal (500,000 cells/mL of milk) (Dhakal, 2006; Alhussien and Dang, 2018). However, the mean BMSCC in this study exceeds the recently proposed BMSCC level of 100,000 cells/mL in Murrah buffalo by the National Dairy Research Institute, India. Bulk milk SCC may fluctuate due to seasonal influences; for example, BMSCC is often expected to be low in winter since the temperature humidity index remains below the critical threshold that poses reduced thermal stress to the buffalo during this season (Choudhary and Sirohi, 2019). The BMSCC in the winter season in our study (172,390 cells/mL) was considerably low and might also be influenced by an overall decrease in mean BMSCC (Supplementary material 2). Our study observed a discrepancy where, although BMSCC was low, a high animal-level SCM prevalence (52%) was observed. A previous study suggested that, in specific NAS-associated IMI, BMSCC may remain low. For example, *Staphylococcus (Staph.). xylosus*, *Staph. cohnii*, and *Staph. equorum* were more frequently isolated from quarters of dairy cows with a low SCC, while a high SCC was revealed from IMI associated with *Staph. capitis*, *Staph. gallinarum*, *Staph. hyicus*, *Staph. agnetis,* or *Staph. simulans* (Condas et al., 2017). This speculation may partly explain the link between BMSCC and SCM prevalence in our study. Our study included five buffalo-rearing systems to represent the nationwide buffalo population in Bangladesh. Therefore, this study's findings can help establish an initial benchmark since BMSCC data in water buffalo is currently limited. If buffalo farmers could access a regular BMSCC testing program, there is scope to improve the farm's udder health by recreating the management practices in farms with low BMSCC.

The left quarter position was associated with a higher prevalence of buffalo SCM in this study. Several other studies have also shown this trend (Moroni et al., 2006; Srinivasan et al., 2013; Ali et al., 2014). The association between quarter position and SCM can be partly explained by the fact that on the studied farms, milking was performed by hand from the left side, increasing the exposure and exerting more pressure on the left quarters (Ali et al., 2014). When milking the right teat, the left teat orifice will be open, allowing IMI pathogens to enter the mammary glands and cause mastitis. Therefore, the left-side milking practice may

also allow IMI pathogen contamination from the right teat to the left teat. However, no previous study has identified whether the left teats are more often infected by different pathogens, such as gram negatives, because of environmental contamination or the contagious transmission between udder glands. The type of hand milking could also play a significant role in teat injury and increase the prevalence of SCM (Hameed et al., 2012), but it was not significant in our study.

A strong association was observed between an intensive buffalo-rearing system and the prevalence of SCM. A previous study (Khan et al., 2019) in Pakistan supported the idea that prevalence was much higher on intensive farms (77%) than on household farms (23%). In intensive farms in our study, buffalo lived in stressful conditions due to a lack of access to grazing and wallowing, which can compromise the immunity of the animals. Intensive buffalo farms in our study comprised crossbreed buffalo (e.g., Indigenous x Nili-Ravi and Indigenous x Murrah) that had a higher milk yield compared to the indigenous buffalo, and these crossbreeds are generally known to be susceptible to mastitis (Ali et al., 2014; Charaya et al., 2014). In intensive farms in our study, the herd size was large, ranging from 50 to 278 animals per herd, and animal density was high in intensive farms, which may also increase the chances of spreading IMI pathogens from infected to healthy buffalo on the farm (Ali et al., 2014).

Funnel-shaped teats were associated with a higher prevalence of SCM than cylindrical teats. A study (Kaur et al., 2018) has suggested that in Murrah buffalo, the cylindrical teat is the most common and has a higher prevalence of SCM, which is contradictory to the results of this study. To our knowledge, no previous studies have discussed the association between teat shape and the prevalence of SCM in water buffalo. In funnel-shaped teats, the skin is loose, generally wrinkled, and rough, making them difficult to clean and may increase exposure to IMI-causing pathogens. Due to the roughness of the teat skin, the funnel-shaped teats also need increased pressure during hand milking, which could increase the chance of teat injury. Moreover, in our study for pre-milking stimulation, calf suckling was the most common, and it was common practice to drag the calf away from the teat. At the same time, any suckling might cause teat injury and make the quarter more susceptible to SCM (Bhandari et al., 2021).

In this study, buffalo with an asymmetrical udder had a higher SCM prevalence. A previous study found that in CM-affected buffalo, udder fibrosis followed by the teat canal and cistern disappearance was observed (Abu-seida et al., 2014). We observed that a significantly higher proportion of buffalo with an asymmetrical udder had a previous CM history than those with a symmetrical udder. Therefore, it can be speculated that in our study, udder asymmetry may result from a previous infection causing scar tissue, leading to shrinkage of the udder gland. This is attributable to the presence of persistent IMI-causing pathogens from previous clinical cases and the transfer of those pathogens resulting in the development of new cases of IMI.

A single milker was associated with a higher prevalence of SCM in the buffalo than multiple milkers. This is surprising, and it is challenging to hypothesize a causal mechanism behind this. One explanation could be that the milking hygiene was inadequate when a single worker was involved, concerning multiple workers. For example, no teat dipping or hygienic practices were observed between milking one buffalo and the next. Therefore, when a single milker was responsible for milking all the buffalo on the farm, this was more likely to contaminate a contagious pathogen to all the lactating animals in a herd than with multiple milkers. A previous study in Poland demonstrated the transmission of methicillin-resistant *Staphylococcus aureus* among agricultural animals, farm workers, and their household members. Multiple-locus variable number tandem repeat analysis, multilocus sequence typing (ST398), and spa (t034) typing have revealed the same drug-susceptibility profiles in bovine and human isolates (Krukowski et al., 2020). On the buffalo farms in this study, the single milker stayed close to the buffalo while they grazed and did other day-to-day farm management tasks nearby. Usually, during the milking time in the morning, a single person

performs the farm environment cleaning (e.g., cleaning the manure) and then starts the milking immediately, without hand washing or disinfectant. Therefore, IMI pathogens are more likely to spread from the milker to the other buffalo in the herd.

On the other hand, in herds where multiple milkers were involved, the farm environment cleaning was performed by other workers who were sometimes not involved in the milking process. Therefore, it is possible that the milkers had less exposure to the lactating buffalo, reducing the risk of SCM in those animals. However, a higher risk of SCM may also be linked to misclassification bias, resulting from the failure to report the true prevalence, or sampling bias, resulting from underestimating the actual prevalence of SCM. Finally, although it is unclear, it could be speculated that, compared to the multiple milkers, the greater exposure of the single milkers may be the most crucial reason for the higher prevalence of SCM in water buffalo.

Our study evidenced that the winter season was associated with low BMSCC. These findings agreed with earlier studies in India (Bhutia et al., 2019) and Pakistan (Baloch et al., 2018), which showed that there was a lower prevalence of SCM in winter (20–28%). The association of low BMSCC in buffalo could be speculated to be due to the lower heat stress in winter. Water buffalo generally tolerate cold weather better than hot or humid climatic conditions (Marai and Haeeb, 2010). However, the association may also have to do with the buffalo-rearing systems, and we must be cautious about interpreting the high BMSCC in Autumn. Only intensive farms were studied during the autumn (September to early December), where BMSCC was higher than any other buffalo-rearing system in this study. This shows how the high BMSCC may have been strongly associated with the Autumn season because of the effect of the intensive rearing system over any seasonal influences.

The absence of a quarantine facility was associated with a high BMSCC in this study. The buffalo farmers in this study often expanded the herd by introducing newly purchased buffalo. If quarantine is not followed in these situations, there is a higher likelihood of introducing disease from the new animal to other animals in the herd. Buffalo farmers who are concerned with the provision of quarantine facilities are also expected to be more concerned with the overall farm management than farmers who do not have a quarantine facility.

Buffalo farms with a previous history of buffalo mortality were associated with a high BMSCC. In our study, farmers reported that buffalo mostly died during outbreaks of hemorrhagic septicemia and parasitic diseases, which agrees with a previous study in Bangladesh (Islam et al., 2017). However, neither vaccination nor deworming substantially impacted buffalo mortality. On the other hand, the buffalo-rearing system was strongly linked to farms where animals had previously died. Interestingly, the highest number of animal deaths happened in free-range systems, where 80% of farmers had no formal education or only a primary educational qualification. They were unaware of buffalo nutrition, disease prevention, or biosecurity science. Free-range farms are also remotely located, making it hard for the farmers to talk to each other and be updated about vital management improvements.

5. Conclusion

In conclusion, there is a high animal and quarter-level SCM prevalence in water buffalo. Mean bulk milk somatic cell count is satisfactory but varies significantly between farms. The non-manageable risk factors in our study were the buffalo rearing system, season, and quarter position. The controllable risk factors were the number of milkers, teat shape, and udder symmetry. Teat shape and udder symmetry are manageable through selective breeding. Our findings indicate that the education of farmers regarding milking technique, hygiene, and rearing system may help further improve udder health on buffalo farms in Bangladesh.

Appendix A. Supplementary data

Supplementary data (Supplementary material 1 and 2) to this article can be found online at https://doi.org/10.1016/j.rvsc.2023.03.004.

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