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Prevalence, aetiology and risk factors of subclinical mastitis in goats in Bangladesh

Sazeda Akter[a,*], Md. Mizanur Rahman[a], Md. Abu Sayeed[a], Md. Nurul Islam[b], Delower Hossain[c], Md. Ahasanul Hoque[a], Gerrit Koop[d]

[a] Department of Medicine & Surgery, Chattogram Veterinary and Animal Sciences University (CVASU), Zakir Hossain Road, Khulshi, Chattogram, 4225, Bangladesh
[b] One Health Institute, Chattogram Veterinary and Animal Sciences University (CVASU), Zakir Hossain Road, Khulshi, Chattogram, 4225, Bangladesh
[c] Department of Medicine and Public Health, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, 1207, Bangladesh
[d] Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3583CL, Utrecht, the Netherlands

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ABSTRACT

Goat production in Bangladesh has increased substantially during the last decades due to increasing demand for meat and milk. This growth in goat production is, however, reduced by different diseases, among which mastitis has significant adverse effect on milk production and milk quality and is associated with financial losses. Risk factors and aetiology of goat mastitis in Bangladesh are largely unknown, hampering the effective control of the disease. The current study was therefore undertaken to determine the prevalence of subclinical mastitis (SCM) in goats, describe the pathogens causing SCM and identify risk factors. One hundred and six goats from 88 household goat farms were included in the study. The estimated udder half level prevalence of SCM, based on the California Mastitis Test, was 50.9 % (95 % CI: 44.2–57.7 %). The most prevalent group of pathogens associated with SCM was non-aureus *Staphylococcus* species (NAS) and *S. aureus*. Our study depicted the odds of SCM to be significantly higher in poor and fair body conditioned compared to cachectic goats, goats in late lactation, Jamnapari breed and in goats with bottle shaped teats rather than conical or cylindrical teat shapes. This study shows that SCM in goats in Bangladesh is highly prevalent and suggests the importance of breeding for mastitis resistance, by selecting stronger breeds and goats with good udder or teat conformation.

1. Introduction

The current dairy goat population in the world is around 218 million estimated in 2017 (Food and Agriculture Organization of the United Nations (FAO, 2019). About 26 million goats are being reared in Bangladesh, mainly by marginal people as means of their family income (Food and Agriculture Organization of the United Nations (FAO, 2016). The common goat breeds reared in Bangladesh are the Black Bengal Goat, Jamnapari, several cross breeds and local indigenous goats. Despite its vital role in the economy of the poor people, goat farming has multiple challenges such as infectious and non-infectious diseases, insufficient management practices, poor genetic potential due to indiscriminate breeding with low quality bucks and poor access to veterinary care (Hegde, 2019; Islam et al., 2012b). Quality goat milk production is also very crucial for a sustainable production and income of farmers. Mastitis is considered as one of the most important challenges in dairy goat farming as it reduces animal welfare and threatens public health. In addition, depending on the severity of the disease, mastitis results in financial losses (Nazifi et al., 2011; Razi et al., 2012). Clinical mastitis presents gross inflammatory signs in udder and milk, whereas subclinical mastitis (SCM) is characterized by changes in milk composition with no signs of inflammation or milk abnormalities. Changes in milk composition can be detected by special diagnostic tests such as the California Mastitis Test (CMT), where a CMT score of more than 2 is indicative of inflammation. Gangrenous mastitis, a severe form of clinical mastitis, frequently causes mortality in affected goats or in less severe cases results in loss of the affected gland (Contreras et al., 2007; Koop et al., 2016; Ribeiro et al., 2007; Sarker et al., 2015). The prevalence of clinical and SCM in goats has been estimated in different countries of the world (Contreras et al., 2007) and generally the prevalence of SCM is estimated between 5–30 % (Bergonier et al., 2003; Contreras et al., 2007). The most frequent

* Corresponding author at: Department of Medicine and Surgery, Faculty of Veterinary Medicine, Chattogram Veterinary and Animal Sciences University, Zakir Hossain Road, Khulshi, Chattogram, 4225, Bangladesh.
E-mail addresses: sazeda@cvasu.ac.bd (S. Akter), mizanuhcp@cvasu.ac.bd (Md. M. Rahman), sayeed.dvm@gmail.com (Md. A. Sayeed), nislam@globalhealthdev.org (Md. N. Islam), delowervet@sau.edu.bd (D. Hossain), md.hoque@my.jcu.edu.au (Md. A. Hoque), G.koop@uu.nl (G. Koop).

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causes of SCM are primarily non-aureus *Staphylococcus* species (NAS) and to a lesser extent *S. aureus*, gram-negative bacteria and *Streptococci* (Contreras et al., 2007; Leitner et al., 2008). Knowing the distribution of common causal agents of mastitis helps prioritize management measures to control mastitis, but the importance of various pathogens may differ by region, breed and husbandry system (Hogeveen et al., 2011). Many studies have been conducted to identify the risk factors of SCM in goats in different countries including Bangladesh (Megersa et al., 2010; Moroni et al., 2005b; Pirzada et al., 2016; Razi et al., 2012). However, Razi et al. (2012) identified risk factors that can hardly be influenced through management, such as age, parity, stage of lactation and housing; whereas manageable risk factors such as breed and teat shape as well as the role of body condition score (BCS) have not been studied in Bangladesh. Knowledge of such manageable risk factors is needed to provide farmers with advice to prevent mastitis in their animals. The aim of the present study, therefore, was to estimate the prevalence of SCM, to determine the aetiology of SCM and to identify manageable risk factors associated with SCM.

2. Materials and methods

2.1. Description of the study area

The study was conducted in selected backyard and smallholding goat farms in Chattogram Metropolitan city of Bangladesh. The city is geographically located at 22°22′0″N and 91°48′0″E. Livestock rearing is common practice among some inhabitants of this city as major or subsidiary income source. Goats are reared in intensive, semi intensive, free ranging and tethering systems in this area. Google maps (https://www.google.com.bd/maps) were used to get geo-coordinates of the location of the individual farm. ArcGIS-ArcMap version 10.2 (ESRI, USA) was used to produce a map locating farms under the study (Fig. 1)

2.2. Reference population, source population and sample population

All goats in small household farms, having upto 10 goats per farm, within Chattogram metropolitan city were considered as the reference population. Goats of small household farms having at least one lactating goat per farm were treated as a source population. A total of 88 goat farms were chosen, aiming to cover different goat rearing zones of the Chattogram metropolitan city. These farms were then used for sampling under a cross-sectional study design. All clinically healthy lactating goats per farm enrolled were sampled, resulting in N = 106 goats. Clinically healthy was defined as no visible changes in the udder or the milk and normal body temperature and feeding behavior. Distribution of sampled farms among different zones of the city was as follows: Khulshi (27), Bayezid (8), Akbarshah and Firozshah (35), Halishahar, Export processing zone and Bondar (6), Pahartali (7), Kalurghat and Panchlaish (2), Doublemuring and Kotowali (3).

2.3. Data collection

A structured questionnaire was used to collect information related to farmers' and farm demography, farm characteristics and management in a face to face interview. Data on the udder and teats were recorded by physical examination during farm visits. The teat shape was classified according to Samad (2003) while the teat end callosity thickness was classified according to Koop et al. (2013). Two trained interviewers administered the questionnaire together on each farm. Around 45 min were required to complete each interview. The full questionnaire is given in Supplementary material 1.

2.4. Milk sampling

After discarding 1–2 squirts of foremilk, 2 ml milk and 2 ml of CMT reagent was added to the CMT paddle and the test score was assessed and graded from 1 to 5. According to an increase in viscosity the scores

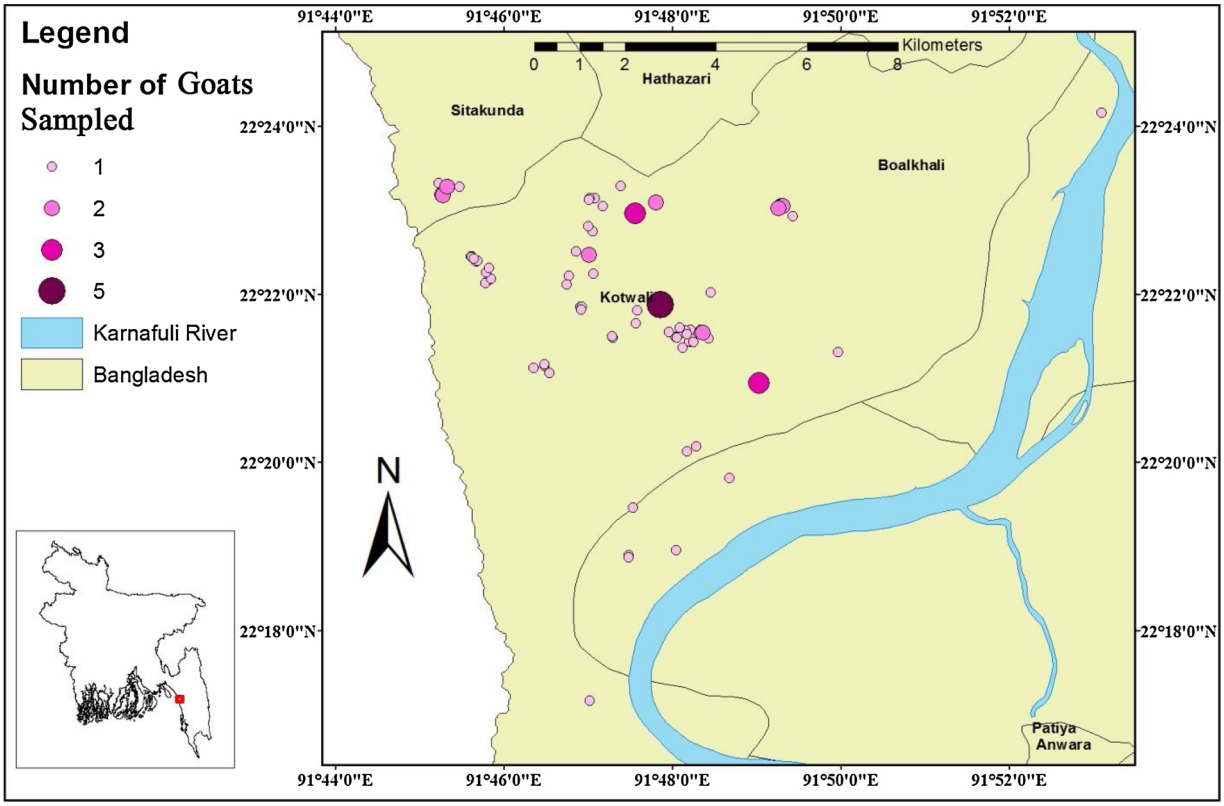

Fig. 1. Location and number of goats sampled from 88 goat farms visited in Chattogram metropolitan city (circles of some farms are overlapping).

were ranked, where the highest viscosity (CMT 5) is more or less correlated to the highest SCC; modified from Schalm et al. (1971) and a score 2 or more was considered as SCM positive. If a sample of a goat in a farm scored 2 or more on CMT evaluation, then the corresponding farm was defined as positive to SCM. In order to collect milk samples for bacteriological examination, teat end of goats with or without SCM were swabbed with cotton balls soaked with 70 % ethyl alcohol (Stuhr et al., 2013). A total of about 10 ml milk per udder half was collected aseptically in 15 ml sterile falcon tubes with a unique identification number. All samples collected were immediately transported through ice box to the laboratory at Chattogram Veterinary and Animal Sciences University, Bangladesh. Samples were stored at −20 °C before further bacteriological analysis.

2.5. Bacteriological examination

Bacteriological analysis was performed on 212 milk samples. Culturing was performed according to previous work by Persson and Olofsson (2011), excluding 12 biochemical reactions and not examined for beta-lactamase production. Culture of *Pseudomonas* species and *Bacillus* species were considered laboratory contamination and these isolates were therefore ignored. Samples in which more than three morphologically different colony types were seen were recorded as contaminated. Mixed cultures of various NAS species were recorded as NAS. A combination of NAS and an *Enterobacter* species was also recorded as NAS.

2.6. Statistical analysis

Data were cleaned and checked for integrity in MS Excel 2007 before exporting to STATA-IC-13 (Stata Corp., Texas, USA). Several variables were recorded to facilitate analyses as categorical variables. Descriptive statistics were computed on the data of farmers' demography (frequency distribution and percentages), farm characteristics (summary statistics), characteristics of sampled goats, management features of farms and the frequency distribution of organisms cultured from milk samples. The prevalence of SCM based on CMT scores higher than a certain threshold was estimated at the farm level as well as the individual goat level and udder half level, and 95 % confidence intervals were calculated using the normal approximation. Risk factor analysis for SCM based on CMT was performed at farm, goat and quarter level as follows: on farm level, Chi-squared tests were performed to evaluate the association between the binary response variable of CMT positive farm (yes/no) and the six independent farm level variables (farm size, rearing system, feeding system, flooring material, muddiness of floor and amount of faeces on the floor). As none of the factors was significant at $p \leq 0.25$, no multivariable model was built. Similarly, univariable screening was performed to assess the association between SCM and categories of each of the selected potential risk factors at goat level (floor materials, body condition score, parity, stage of lactation, breed and age in months). Factors associated with SCM at $p \leq 0.25$ were considered for inclusion in the multivariable analysis. The multivariable logistic regression model was constructed as previously described by Belgrad et al. (2018). Briefly, backward stepwise logistic regression model was done and variables having $p \leq 0.05$ in Wald's test were primarily retained in the model. The variables retained were evaluated for confounding and biologically plausible interaction. Interactions were assessed between factors by constructing two-way interaction product terms for the significant main effect factors in the model, forcing them into the model and examining changes in the coefficients and p values of the main effects. Confounding was checked by re-adding one by one, the variables removed in the stepwise backward procedure. A variable was considered as confounder if its removal changed the regression coefficients of one or more of the remaining variables by ≥15 % (Dohoo et al., 2003). Collinearity between categorical factors was tested using the two-tailed p value using the Fisher's exact test (Hoque et al., 2015). Two factors were considered collinear if the p value was ≤0.05. The sensitivity of the final model was then assessed for goodness-of-fit using the Hosmer–Lemeshow test described by Dohoo et al. (2003). Output of the model was presented as OR and 95 % confidence interval.

The same procedure was followed to test associations at udder half level, for the factors of teat shape, teat end callosity thickness, teat size and teat end shape. Factors significant at $p \leq 0.2$ in the univariable screening were tested in a multivariate analysis. A Generalized Estimating Equation (GEE) model was performed as previously described by Sayeed et al. (2017). Briefly, the GEE model was built including significant variables ($p < 0.05$). A backward-elimination procedure was used by fitting the full model and reducing the model based on significance of the variables one by one. The confounding was checked by adding or removing a variable from the model and the colinearity was checked using Chi-square test between independent variables. Variables that were significant ($p \leq 0.05$) based on Wald test were considered as risk factors for SCM. The model output was again expressed as OR and 95 % confidence interval.

3. Results

3.1. Demographics of farmers, farm and farm management

Most goat farmers in the Chattogram metropolitan area keep goats as a side business. Their main professions often were to have another business of their own (34 %), job (13 %) or work at other agricultural farming business (10 %). About 25 % of the goat farmers were housewives and the remaining 18 % had other professions. Of the goat farmers, 33 % were illiterate, but 25 % had secondary level of education. Cross-breed goats (72 %) were the dominant breed of goats reared. The farm floor was made of concrete and bricks (17 %), plastic and jute bags (23 %), but most frequently of wood (60 %). Goats were generally fed with grass (26 %), concentrates (15 %), grass and concentrate combined (46 %) and other types (12 %) of non-conventional feed stuffs. Farm floor cleanliness was variable, but the majority of floors were clean and dry. The majority of farmers responded that they cleaned their farms daily (75 %). More detailed descriptions of the farmer and farm characteristics are given in Supplementary Table 1.

3.2. Prevalence and aetiology of subclinical mastitis

The prevalence of SCM was over 50 %, using CMT ≥ 2 as threshold (Table 1). Among the 212 udder half samples subjected to bacteriological culture, the proportion of culture positive samples was approximately 70 %. The most prevalent species were NAS and *S. aureus* (Table 2). Samples with CMT score1 and 2 were mostly culture negative or positive for NAS, whereas *S. aureus* was also cultured from milk with these CMT scores, but was more frequently than other pathogens isolated from milk with CMT score 3 and 4

3.3. Risk factors for SCM

On farm level, none of the potential risk factors was associated with SCM at $p \leq 0.25$. At goat level, several variables were univariably

Table 1
Prevalence of subclinical mastitis according to the California mastitis test (CMT) at different levels in goats of Chattogram metropolitan city, Bangladesh.

Level		≥ 2 CMT	
	N	%	95 % CI
Farm	88	53.4	46.97–63.7
Animal	106	50.9	41.4–60.5
Udder half	212	50.9	44.2–57.7

Table 2
Frequency distribution of pathogens isolated from milk with CMT scores 1, 2, 3 or 4 from 212 udder halves of 106 goats in 88 farms in Chattogram metropolitan city of Bangladesh.

	CMT score				
Culture result	CMT1 (%)	CMT2 (%)	CMT3 (%)	CMT4 (%)	All (%)
NAS[1]	44 (56)	23 (29)	11 (14)	0 (0)	78 (37)
S. aureus	7 (32)	7 (32)	7 (32)	1 (5)	22 (10)
Streptococcus spp.	0 (0)	1 (100)	0 (0)	0 (0)	1 (0)
Contaminated	6 (67)	1 (11)	2 (22)	0 (0)	9 (4)
No growth	57 (56)	31 (30)	14 (14)	0 (0)	102 (48)
Total	114 (54)	63 (30)	34 (16)	1 (0)	212 (100)

[1]NAS = non-aureus *Staphylococcus* species.

linked to SCM. Breed, BCS and stage of lactation also acted as confounders in the multivariable model. The final multivariable logistic regression model revealed lower BCS, late lactation and the Jamnapari breed to be associated with higher odds of SCM (Table 3). At udder half level, teat shape and teat end callosity thickness were unconditionally associated with SCM at $p \leq 0.25$, but only teat shape was retained as significant factor in the GEE model correcting for clustering within goat. The odds of being positive for SCM were 6.9 (95 % CI: 2.7–17.5) for the combined category of bottle shaped and collapsed shaped teats compared with conical and cylindrical teat shapes (Table 4).

4. Discussion

The present study was conducted to explore prevalence, causative pathogens and risk factors for SCM in goats. The overall prevalence of SCM (CMT score ≥ 2) was high, which is a major concern for profitable goat farming in the study area. This finding is in line with previous studies which also reported high prevalence (Begum et al., 2016; Pirzada et al., 2016) although many other studies show that lower prevalence are possible in goats (Ali et al., 2010; Islam et al., 2012b; McDougall et al., 2002; Moroni et al., 2005b; Razi et al., 2012). The differences in prevalence between studies are likely due to differences in prevalence of the associated risk factors. The variables that were associated with SCM in our study were BCS, stage of lactation, breed and teat shape.

Does with better BCS had higher odds of SCM compared to cachectic goats. This is somewhat counter intuitive. However, studies in cattle have shown that cows with BCS more than 3 is more likely to have IMI compared to those less than 3 (Tolosa et al., 2015) and Treacher et al. (1986) stated that fatter cows during calving have impaired milk production resulting from a concert effect of mastitis with other diseases in contrast to thinner ones, although the mechanism behind this finding was not clearly explained. Our finding is not in line with previous studies which identified low BCS as a risk factor for mastitis (Megersa et al., 2010; Mungube et al., 2004). In our study, goats were not at all too fat, but had low to moderate BCS. Possibly, the lower milk yield in cachectic goats made these animals less susceptible to SCM, or the association may have been confounded by other unknown factors. The increased odds of SCM in mid and late lactation period compared to early lactation (OR = 5.1) corresponds to multiple earlier studies (Haenlein, 2004; Koop et al., 2010b; Persson et al., 2014; Stuhr et al., 2013). The higher odds of SCM in late-lactation are likely the result of a low self-cure rate resulting in a higher prevalence of chronic cases with increasing lactation length. This is in line with our finding that NAS and *S. aureus* were the most prevalent pathogens responsible for SCM, which are known to be persistent during lactation (Koop et al., 2012).

The Jamnapari breed had higher odds of SCM than the Black Bengal goats and cross breeds. Jamnapari goats are being reared for high milk yield and longer lactations and have a more pendulous udder. The higher milk yield may cause the animals to be less resistant to intramammary infections, specifically under climatic conditions that are suboptimal for higher yielding breeds. This is in line with a previous study in cows, which showed that cows with a higher Holstein-Friesian blood level are at more risk for SCM than indigenous cows (Mekonnen et al., 2017).

In this current study, NAS was the most frequently isolated bacteria encountered (39.6 %), which confirms the importance of this group of species in goats as has been shown in several other countries and goat rearing systems (Bergonier et al., 2003; Hall and Rycroft, 2007; McDougall et al., 2002; Min et al., 2007). The prevalence of *S. aureus* was 11.3 % in our study which is lower than the study conducted previously in Bangladesh (29.4 %) (Razi et al., 2012) and in Bulgaria (19.8 %) (Bochev and Russenova, 2005), but substantially higher than another study (4 %) conducted by (Persson and Olofsson, 2011). This pathogen can comprise up to 40 % of all isolated bacterial species (Ameh and Tari, 1999; Leitner et al., 2008), and in one report, it was found to be the most frequently occurring species among all isolates (da Silva et al., 2004). Differences in prevalence between studies may be the result of misclassification bias because of differences in culture protocol, for instance whether or not pre culture freezing or pre incubation was done or variations in inoculum volume (Koop et al., 2011), but may also reflect true differences in the importance of this pathogen species in goats. Because of its disease causing potential and because of its zoonotic significance, *S. aureus* control strategies are important to reduce the incidence of gangrenous mastitis (Sarker et al., 2015), and of food borne Staphylococcal disease (Zecconi and Hahn, 2000).

Table 3
Univariable associations between potential risk factors and a positive California Mastitis Test (CMT) and odds ratio (OR) and 95 % confidence interval (CI) of a multiple logistic regression model based on 106 goats of 88 farms in Chattogram metropolitan city of Bangladesh.

Variable	Category	N	CMT positive (%)	P (Chi^{2}test)	OR	95 % CI
BCS[1]	Cachectic	50	20 (40)	0.033	Ref.[2]	
	Poor and fair	56	34 (61)		3.2	1.3-7.8
Parity	1 or 2	55	25 (45)	0.240		
	3 to 15	51	29 (57)			
Lactation stage (days)	Up to 60	78	33 (42)	0.003	Ref.	
	61 to 180	28	21 (75)		5.1	1.8-14.6
Rearing system	Intensive or semi-intensive	44	19 (43)	0.178		
	Free ranging or tethered	62	35 (56)			
Breed	BBG[3] and cross	81	44 (54)	0.211	Ref.	
	Jamnapari	25	10 (40)		3.6	1.2- 10.6

[1]BCS = body condition score.
[2]Ref. = reference category.
[3]BBG = Black Bengal Goat.

Table 4
Univariable association between udder half level potential risk factors and California mastitis test (CMT) status and odds ratio (OR) and 95 % confidence interval (CI) of a Generalized Estimating Equation (GEE) model based on 212 udder halves of 106 goats on 88 farms in Chattogram metropolitan city of Bangladesh.

Factor	Category	N	CMT	P (Chi^2test)	GEE		
			Positive (%)		OR	95 % CI	P
Teat shape	Conical and cylindrical	144	62(43)	< 0.001	1.0		
	Bottle and collapsed	38	32 (84)		6.9	2.7-17.5	< 0.001
	Pencil and short	30	14 (47)		1.2	0.5-2.6	0.694
Teat end callosity thickness	None and thin	190	100(53)	0.148			
	Moderate	22	8(36)				

5. Farmers' and farms' demography and management traits of goat farms

Regardless of farm types, most of the goat farmers had some level of education (67 %) whilst 33 % of the farmers were illiterate in the present study. These results suggest that most of them can understand and read Bengali literature. This makes it possible to educate goat farmers on goat farming by providing leaflets or manuals which may help reduce the risk of SCM as well as clinical mastitis. The fact that many farmers keep goats as a side job, however, makes it challenging to educate them. The low number of goats per farm, and consequently the large number of farms in Bangladesh creates a logistic challenge for reaching out to goat farmers. Still, the fact that goats make an important contribution to a family income and food security makes it important to help improve their health status. A comprehensive program to support goat udder health but also goat husbandry in general, tailored to the situation in Bangladesh would be highly valuable.

6. Conclusion

Goats in Chattogram metropolitan city, Bangladesh, have a high prevalence of SCM, mainly caused by Staphylococci. Higher body condition score, late-lactation, Jamnapari breed and teat shape were associated with the occurrence of SCM. Control programs to improve the udder health of goats in Bangladesh are needed and should focus on educating farmers on which breed should be selected for better udder and teat traits.

Declaration of Competing Interest

No conflict of interest exists for any of the authors.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.smallrumres.2020.106046.

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