

Prevalence and risk factors of subclinical bovine mastitis in some dairy farms of Sylhet district of Bangladesh

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Abstract

A cross-sectional study was undertaken to report prevalence and to identify risk factors of subclinical mastitis of dairy cattle in Sylhet district of Bangladesh. Among 325 dairy farms of the district 12 farms (3.7%) were selected conveniently for this study. All the dairy cows of the 12 farms were selected for sample collection. Fresh milk samples from each of the selected dairy cows were collected aseptically in separate sterilized test tube as RF, RH, LF and LH quarter of the udder. Rapid modified White Side Test (WST) was used to detect subclinical mastitis (SCM). Results of WST and data derived from filled in questionnaire were entered in Microsoft Excel 2003 and transferred to STATA[®], version 8.0/Intercooled (Stata Corporation, Texas, USA, 2003). The overall prevalence of SCM and its distribution in different categories of variables in cow and their exact binomial 95% confidence intervals were calculated in STATA[®]. Simple bivariable associations among independent variables were investigated by χ^2 test in STATA[®]. Multiple logistic regression analysis with backward elimination method was used to identify risk factors of SCM. To identify significant variation in quarter SCM, linear regression analysis was performed after arcsine transformation of the data. The overall prevalence of SCM found in this study is 54%. Dairy cows with teat lesions had significantly increased SCM (OR=12342, *P* value=0.000, 95% CI=762, 199798) than others without teat lesions. The Holstein Friesian X Jersey X Sahiwal breed has significantly decreased (OR=0.18, *p*=0.03, 95% CI 0.04, 0.85) SCM than other breeds. The prevalence of SCM found in this study is in agreement with others. The injury in the teat

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increases the probability of getting infected with microbes and thereby mastitis. If the prevalence of teat lesion can be decreased the probability of subclinical mastitis will also be decreased. The negatively associated Holstein Friesian X Jersey X Sahiwall breed may help in planning mastitis control program if this finding can be validated by a more powerful case-control or cohort study design.

Key words : Bovine, subclinical mastitis, prevalence, risk factors, multiple logistic regression

Introduction

Among the foods of animal origin milk is most important as it can be consumed by the people of all age classes but milk production is not adequate to meet consumers demand in Bangladesh. There are about 20,582 mini-dairy farms each comprising five or more high yielding crossbred cows in private sectors of Bangladesh¹⁾. One of the most important causes of reduced milk production is mastitis (clinical and subclinical)²⁾. The problem arising from the disease of cows' udder has down the development of dairy industry³⁾. Blowey⁴⁾ reported that financial loss of 40 pound sterling for a case of clinical mastitis in dairy cows. A cow affected with mastitis may not achieve its full milk yield potential in subsequent lactation⁵⁾. The mastitis positive cows seem to have an increased risk of culling⁶⁾.

The prevalence of clinical (13.3%) and subclinical mastitis (15.8-19.5%) in dairy cattle of Bangladesh has been reported by several authors^{2, 7, 8)}. The prevalence of subclinical mastitis and its risk factors were not reported from Sylhet district of Bangladesh. Therefore the present study was conducted in Sylhet district of Bangladesh with the following objectives: 1) to determine the prevalence of Subclinical (SCM) mastitis in dairy cows and 2) to determine the risk factors of SCM.

Materials and Methods

Survey design and sampling

A cross-sectional observational study was conducted in Sylhet district which is situated at southeast part of Bangladesh. Geographically Sylhet district is usually a hilly area with heavy rainfall. The study was performed during April 2006 to June 2006. In Sylhet there are about 325 dairy farms having about 2, 27,786 cows. Among 325 farms, 12 (3.7%) farms were selected conveniently for the study. The selected 12 dairy farms had 456 cattle (average: 38) from where 225 were dairy cow. All of these 225 dairy cows (0.09%) were selected for studying subclinical mastitis (SCM).

Sample collection and detection of SCM

Collection of milk sample: Fresh milk samples from each of the selected dairy cows were collected aseptically in separate sterilized test tube as right front (RF), right hind (RH), left front (LF) and left hind (LH) quarter of the udder and brought out for rapid modified Whiteside test (WST).

Detection of SCM

The WST was performed as per the modified schedule described earlier by Murphy

and Hansen⁹⁾. The reagent used for modified Whiteside test is 4% sodium hydroxide solution. Five drops of well mixed milk are placed on a glass plate with a dark background and two drops of 4 % sodium hydroxide are added to the milk. The mixture of milk and reagent should be stirred rapidly with an applicator stick for 20 to 25 seconds. Milk from normal animals will have no change after the addition of sodium hydroxide. Milk from a cow suffering with acute or sub-acute mastitis will become thick and viscid, but that from an animal with a chronic case may have only a few white flakes. The basis of Whiteside test and CMT is same which reflects the presence of excess leukocytes in milk as an indicator of inflammation. The Whiteside test is quick, simple and inexpensive in comparison to CMT.

The Whiteside test reactions are scored as follows

Negative (N): The mixture is milky and opaque and entirely free of precipitant.

1+: The background is less opaque but still somewhat milky, with larger particles of coagulated materials being present and thickly scattered throughout the area. A slight degree of clumping is observed.

2+: The background is more watery and large clumps of coagulated material are present. If the stirring has been rapid, fine threads or strings may be present.

3+: The background is very watery and whey-like, with large masses of coagulated material forming into strings and shreds.

Questionnaire-based data collection and processing

Data from each animal and herd were collected using a questionnaire. The animal-level variables were age, breed and teat lesion. The herd level variables were number of dairy cows in each herd, grazing, chances of udder injury in the herd, bedding, floor, sanitation, udder disinfection and post-milking lying. Age was determined by asking owner and also from the farm records where available. The data were entered in Microsoft Excel 2003 and transferred to STATA®, version 8.0/Intercooled (Stata Corporation, Texas, USA, 2003).

Prevalence of SCM

The overall prevalence of SCM and its distribution in different categories of variables in cow and their exact binomial 95% confidence intervals were calculated in STATA®.

Association among independent variables

Simple bivariable associations among independent variables were investigated by χ^2 test in STATA®. One of the pairs of significantly associated variables was selected for inclusion in the final regression analysis and the other was ignored¹⁰⁾.

Multiple logistic regression

Multiple logistic regression for SCM was performed in STATA® with all of the independent variables remained after checking for multicollinearity. Backwards elimination method was used for model building. The maximum model was fit and then variables were removed sequentially until all of them remaining in the model had a P -value ≤ 0.05 . The better model was selected using

likelihood ratio test¹¹⁾. Maximum model contained following variables: age, breed, teat lesion, number of dairy cow in herd, udder wash by water and use of antiseptic during milking.

Linear regression

To determine significantly different prevalence of SCM in quarters, the data (prevalence/percentage) was first transformed using Arcsine transformation in Microsoft Excel 2003. The transformed data was then

analyzed using linear regression model in STATA®.

Results

Prevalence of SCM

The overall prevalence of SCM and the distribution of SCM in different categories of variables are shown in Table 1. A relatively higher prevalence of SCM is observed in cows aged between 5.5-8 years. The overall prevalence of SCM found in this study

Table 1. Apparent prevalence and distribution of subclinical bovine mastitis

Variables	Category level	Prevalence (%)	S. E.	95% C.I.
Age (Years)	0 (2.5, 5.0) ^a	45	0.07	0.32, 0.59
	1 (5.5, 8.0)	58	0.05	0.49, 0.67
	2 (8.5, 10.5)	54	0.7	0.39, 0.67
Breed	Local×Friesian	56	0.04	0.47, 0.64
	Local×Shahiwal	60	0.09	0.41, 0.77
	Friesian×Jersey×Shahiwal	18	0.12	0.02, 0.52
	Local×Friesian×Shahiwal	51	0.08	0.35, 0.68
	Local×Sindhi×Shahiwal	83	0.15	0.36, 0.99
	Local×Jersey×Shahiwal	25	0.22	0.01, 0.81
	Local×Friesian×Sindhi	75	0.22	0.95, 0.99
Teat lesion	Present	99	0.008	0.19, 0.99
	Absent	0.9	0.009	0.0002, 0.05
No. of dairy cow	0 (13-17)	58	0.06	0.45, 0.69
	1 (18-23)	49	0.06	0.37, 0.60
	2 (24-29)	57	0.06	0.44, 0.68
Grazing	Zero grazing	54	0.05	0.44, 0.63
	Grazing	55	0.05	0.45, 0.64
Udder wash by water	Wiping	54	0.03	0.47, 0.60
	Dipping	64	0.13	0.35, 0.87
Use of antiseptic	No	53	0.07	0.38, 0.68
	Yes	54	0.04	0.47, 0.62
Overall prevalence		54	0.03	0.47, 0.61

^a Minimum and maximum category value

is 54%. Among different breeds of cattle highest and lowest prevalences are observed in Local X Sindhi X Sahiwal and Friesian X Jersey X Sahiwal breeds respectively. Ninety nine percent of the cows with teat lesions have SCM.

Prevalence of SCM in different quarters

The prevalence of SCM in different quarters is shown in Table 2. The highest prevalence of slight (13.3%) and severe (6.2 %) SCM reactions are observed in right hind (RH) and right front (RF) quarters respectively.

Table 2. Subclinical mastitis (%) in different quarters of udder

Reaction WST*	RF	LF	RH	LH
Negative	74.2	79.6	74.7	75.6
Slight (+)	10.2	7.1	13.3	10.7
Moderate (++)	9.3	9.8	8.0	10.7
Severe (+++)	6.2	3.6	4.0	3.1

* WST: Rapid modified White side test, RF: Right front, LF:= Left front, RH: Right hind, LH: Left hind

Table 4. Variables significantly associated with SCM

Variables	Odds Ratio	SE	P-value	95% CI ^a
Teat lesion (Present)	12342	17532	0.000	762, 199798
Breed (FXJXSAHI)	0.18	0.14	0.03	0.04, 0.85

^a Confidence Interval

Discussion

A cross-sectional survey was conducted to report prevalence and to identify risk factors of SCM in dairy cattle. Due to non probability sampling design the prevalence will not be extrapolated to dairy cattle of

Association among independent variables

Significant 2 x 2 χ^2 test results are presented in Table 3. Only two pairs of the combinations showed significant associations. Age and breed of the animals are significantly associated with grazing.

Risk factors of SCM

Risk factors significantly associated with SCM in cow is shown in Table 4. Only two animal-level variables are significantly associated with SCM. Cows with teat lesions have 12342 times more probability to develop SCM than those without teat lesions. The Holstein Friesian X Jersey X Sahiwal breed has significantly decreased (OR=0.18, p=0.03, 95% CI 0.04, 0.85) SCM than other breeds.

Table 3. Significant association among independent variables (Collinearity)

Pair variables	X2	p value
Age	Grazing	0.000
Breed	Grazing	0.03

Bangladesh. However the risk factors identified from this survey can be extrapolated for the dairy cattle with similar management systems in Bangladesh.

Prevalence of SCM

To make inference about the frequency of outcome or prevalence of exposure in a target population the study subjects should be obtained from a formal random sampling procedure¹¹. Due to lack of adequate knowledge and also lack of resources, a formal random sampling procedure could not be followed strictly.

However the prevalence of SCM is in agreement with others^{12, 13}. A higher prevalence of SCM was reported by some authors^{14, 15} whereas some authors¹⁶⁻¹⁸ reported a lower prevalence than this finding. The possible reasons for this variations may be small size, composition of samples (cross breeds only) and methods applied to detect SCM. Except WST here are a lot of indirect tests to detect subclinical mastitis like California mastitis test (CMT), MDST (Mastrip) and bromthymol blue card test (BTB). Most of the authors reported a relatively lower sensitivity and specificity of WST than other tests^{19, 20}. Some authors reported WST as a superior test than CMT^{7, 21}. As WST is cheaper and easy to perform the farmer may accept this test to monitor mastitis status of their herd.

Prevalence of SCM in different quarters

Subclinical mastitis detected in different quarters does not vary greatly. The distribution of SCM in different quarters was not found in literature.

Association among independent variables

For an association study specially using parametric methods, the important pre-requisite is that the independent variables should be statistically independent. When the indepen-

dent variables are related significantly with each other they are called collinear variables and this phenomenon is called multicollinearity. Multicollinearity results in unstable estimates of regression coefficients, incorrect variance estimates for the coefficients of those parameters in regression models leading to inflated standard error and loss of statistical power¹⁰. When the numbers of independent variables are large the probability of having collinear variables also increases. Solving the problem of multicollinearity with 2x2 χ^2 test has some limitations. The choice of which independent variable is to be removed for final analysis is arbitrary. However, there are other techniques like principal component analysis and non parametric methods (e.g. classification tree) which can handle this problem efficiently during analysis.

Risk factors of SCM

The most important variables found to be significantly associated with SCM is teat lesion. It may explained by the fact that the injury in the teat increases the probability of getting infected with microbes. This is valuable information can be utilized for mastitis control. If the prevalence of teat lesions can be decreased, the probability of subclinical mastitis will also be decreased.

Friesian X Jersey X Sahiwal breed was negatively associated with mastitis. This is a new finding. If this finding can be established using a case-control or cohort studies it will also help in planning mastitis control program. Other established risk factors reported by several authors like lactation period, parity, age and season were not included in this study due to lack of time

and in appropriate design of the questionnaire.

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