Bangl. J. Vet. Med. (2011). 9 (2): 155 – 159

BACTERIAL PATHOGENS AND RISK FACTORS ASSOCIATED WITH MASTITIS IN BLACK BENGAL GOATS IN BANGLADESH

M. A. Islam, M. A. Samad and A. K. M. Anisur Rahman

Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. e-mail: aminbau14@gmail.com

ABSTRACT

The objectives of this study were to isolate major bacteria responsible for and to identify the potential risk factors associated with clinical and subclinical mastitis in Black Bengal goats in Bangladesh. A cross sectional study was undertaken on 242 lactating does during January to August 2009. Data on probable risk factors was recorded by using questionnaire. Clinical mastitis was detected by gross signs of udder infection during physical examination and abnormal milk whereas subclinical mastitis was recognized California Mastitis test (CMT) Milk samples of all clinical and subclinical mastitis goats were subjected to culture for isolation and identification of responsible bacterial pathogens. Multiple logistic regression model using a backward stepwise method was used for identification of risk factors. The overall prevalence of clinical mastitis and subclinical mastitis were 4.54% and 37.19%, respectively. Bacterial pathogens isolated were coagulase negative *Staphylococcus* (73.73%), *Staphylococcus aureus* (26.67%), *Streptococcus* sp. (20%), *Bacillus* sp. (70%) and *Escherichia coli* (6.67%). Both clinical and subclinical mastitis were significantly associated with age ($p<0.001$), parity ($p<0.001$), lactation stage ($p<0.001$), litter size ($p<0.05$) and teat lesions ($p<0.001$).

Key words: Bacteria, Risk factors, Mastitis, Goat

INTRODUCTION

Milk is an ideal food for human being irrespective of ages and undoubtedly the most important one among the foods of animal origin. Consumption of goat milk is gaining popularity day by day among the rural households in Bangladesh. The goat milk is highly nutritious and has a similar nutritional profile to those of human's breast milk. But milk quality may be affected by bacterial contamination of mammary gland, which causes clinical and subclinical mastitis (Boscos *et al.,* 1996).

Mastitis in dairy goats is a disease of considerable economic importance worldwide like in dairy cows. Clinical mastitis (CM) presents significant clinical features of inflammatory signs in udder tissues and abnormal udder secretion whereas the only indicator of subclinical mastitis is higher somatic cell count in milk without any visible abnormalities in udder tissue and milk. Unlike cow milk, goat milk contains fairly high cell content because of apocrine process of secretion (Wooding *et al.,* 1977). Mastitis in goat is mainly of sub-clinical type (McDougall *et al.,* 2002) which causes reduced milk yield, kid mortality and is responsible for major economic losses (Contreras *et al.,* 2003). However, gangrenous mastitis occurred as common clinical form of mastitis in goats (Samad, 2008). Several causal agents and predisposing factors have been attributed to dairy goat mastitis with *Staphylococcus* sp. as the main etiological agent (Ibrahim *et al.,* 2009). Predisposing factors such as poor management and hygiene, teat injuries and faulty milking machines are known to hasten the entry of infectious agents and the course of the disease (Majic *et al.*, 1993).

Review of literature yielded very limited information on the diseases of goats in Bangladesh (Samad, 2000) particularly mastitis which is universally recognized as one of the most costly diseases in the dairy industry. This study was undertaken isolate major bacterial pathogens responsible for and to identify potential risk factors of clinical and subclinical mastitis in Black Bengal goats.

*Corresponding e-mail address: aminbau14@gmail.com

MATERIALS AND METHODS

The study was conducted on 242 lactating Black Bengal does of three organized goat farms and those brought to Bangladesh Agricultural University Veterinary Clinic during the period from January to August 2009.

Clinical mastitis was detected by gross signs of udder infection during physical examination and abnormal milk whereas subclinical mastitis (SCM) was recognized California Mastitis test (CMT).

A semi-structured questionnaire was used to collect all related information including husbandry system, age, number of parity, litter size, lactation stage, abnormalities in milk and udder tissues.

Ten (10) ml udder half milk samples were collected in labeled sterilized test tubes. Immediately after collection, pH of milk samples was determined using digital pH meter. CMT was performed following the manufacturer's (Leukocytest®, Synbiotic Corporation, France) instruction and scored as negative (-), weak positive (1+), distinct positive (2+) and strong positive (3+) with method described by Asefa *et al*. (2006).

The isolation and identification of bacterial pathogens were performed according to the procedure described by Quinn *et al.* (1994). In Brief, 0.1 ml milk sample was cultured on blood agar and nutrient agar. The inoculated plates were incubated at 37°C aerobically for 24-48 hours. The bacterial pathogens were identified by morphology, haemolysis, and biochemical tests like catalse, coagulase and anaerobic fermentation of mannitol (Hargital *et al.*, 1992).

Data was entered into Microsoft excels 2007 and transferred to STATA®-12 (Stata corp, USA, 2011) for further analysis. If one quarter milk was CMT positive then that goat was considered as SCM positive. Risk factors were identified using bivariable followed by multivariable logistic regression models (Hosmer and Lemeshow, 1989). Two way interactions among explanatory variables were checked by chi-square test. If there were significant association between two explanatory variables, one of them was excluded from final logistic regression models. Model fitness was assessed by Hosmer-Lemeshow goodness-of-fit test. Significance level s were set as 10% and 5% for bivariable and multivariable models, respectively.

RESULTS AND DISCUSSIONS

Of the 242 does tested, 11(4.55%) were affected with CM and 90 (37.19%) were affected with SCM (Table 1). On bacteriology, milk samples of all CM goats and 83 (92.22%) of SCM milk yield culture positive with different bacterial species and 7 (7.78%) milk samples from sub clinically affected goats were culture negative. The highest frequencies of bacterial isolates from clinical cases were *Staphylococcus aureus,* followed by coagulase negative *Staphylococcus,* coliform and mixed infection of coagulase negative *Staphylococcus* and *Escherichia coli*. The major bacterial isolates from subclinical cases were in decreasing order as coagulase negative *Staphylococcus, Streptococcus* sp. *Bacillus* sp, *Staphylococcus aureus* and other mixed gram negative bacteria.

Table 1. Bacteria isolated from the milk samples of mastitis goats

Name of the isolates	Frequency of isolates	
	Clinical mastitis, no (%)	Subclinical mastitis, no (%)
Coagulase Negative *Staphylococcus*	3 (27.27%)	52 (57.78%)
Staphylococcus aureus	4 (36.36%)	4 (4.44%)
Streptococcus sp.	1 (9.09%)	4 (4.44%)
Bacillus sp,	--	3 (3.33%)
Escherichia coli	2 (18.18%)	5 (5.55%)
Unidentified gram negative bacteria	1 (9.09%)	14 (15.55%)
Total	11 (100%)	83 (92.22%)

Bacteriological examination of this study revealed that *S. aureus* contribute more in clinical cases of mastitis in goat followed by Coagulase Negative *Staphylococcus*, Coliform, *Streptococcus* sp. which is in agreement with reports from other authors (Assefa *et al.*, 2006; Ibrahim *et al.*, 2009). Among the clinical forms of mastitis in goats, gangrenous mastitis caused by *S. aureus* is more common and fatal, which demand special attention because this bacterium is also responsible for SCM. *S. aureus* secretes several toxins contributing to the pathogenesis of mastitis and also plays a role in foodborne disease, even with pasteurized milk because of the thermostable enterotoxins (Contreras *et al.*, 2007).

The Coagulase Negative Staphylococci (CNS) was the most frequently isolated pathogens from subclinical goat mastitis in goat which is in accordance with report of White and Hinckley (1999) and Contreras *et al.* (2003). Although less pathogenic than *S. aureus*, CNS can produce persistent SCM which significantly increase somatic cell content and consequently cause CM (Contreras *et al.*, 2007).

The pH range of milk of does affected with CM and SCM recorded were ranged from 6.95 to 7.34 (average 7.12) and from 5.93 to 7.22 (average 6.69), respectively whereas the milk pH range of apparently healthy does were 6.44 to 6.61 (average 6.58). This finding is in agreement with the observations of Schultz and Chandler (2009) who reported the average pH of fresh goat milk as 6.53.

The prevalence of both clinical (20%) and subclinical (52%) mastitis appeared to be higher at fifth year of age as compared to earlier age (Table 2). The prevalence was found to be higher in farming goat than those are managed under subsistence system. The prevalence CM and SCM was highest in early lactation stage (5.23 and 46.41%) followed by mid (4 and 28%) and late lactation stage (2.56 and 12.82%). Different types of teat lesions were found in all clinical cases and of 84.6% of subclinical mastitis cases. It was also revealed that the prevalence of both CM and SCM increased with increasing age, number of parity and litter size of does. The potential risk factors for both clinical and SCM identified based on multiple logistic regressions were age of goat, number of parity, lactation stage, litter size and teat lesions (Table 3).

Table 2. Risk factors of associated with mastitis in goats based on bivariable logistic regression model (N=484)

Risk factors	**Sample tested**	**Positive**		**Prevalence (95 % CI)**	
		CM	**SCM**	**CM**	**SCM**
Age (years) *					
2	21	0	0	00	00
3	72	1	16	1.39(0.25, 7.47)	22.22(14.17, 33.09)
4	124	5	61	4.03(1.73, 9.09)	49.19(40.55, 57.88)
5	25	5	13	20(8.86, 39.13)	52 (33.5, 69.97)
Rearing System					
Farming	216	10	83	4.60(2.53, 8.31)	38.43(32.2, 45.06)
Subsistence	26	1	7	3.85(0.68, 18.89)	26.92(13.70, 46.08)
Parity*					
One	20	0	0	00	00
Two	36	0	2	00	5.56(1.54, 18.14)
Three	47	1	17	2.13(0.38, 11.11)	36.17(23.97,50.46)
Four	62	3	33	4.84(1.66, 13.29)	53.22(40.98, 65.09)
Five	61	4	33	6.56(2.58, 15.68)	54.09(41.72, 65.99)
Six	11	3	9	27.27(9.75, 56.56)	81.82(52.3, 94.86)
Litter Size*					
One	12	0	2	00	16.67(4.7, 44.48)
Two	196	7	68	3.57(1.74, 7.19)	34.69(28.38, 41.59)
Three	26	3	15	11.54(4, 28.98)	57.69(38.95, 74.46)
Four	8	1	5	12.5(2.24, 47.09)	62.5(30.57, 86.32)
Lactation Stage*					
Early (<2 months)	153	8	71	5.23(2.67, 9.98)	46.41(38.69, 54.3)
Mid (2-3 months)	50	2	14	4 (1.1, 13.46)	28(17.47, 41.67)
Late (>3 months)	39	1	5	2.56(0.45, 13.18)	12.82(5.6, 26.71)
Teat Lesions: [a] *					
Present	78	11	66	100 (74.12, 100)	84.62(75.01, 90.97)
Absent	164	0	24	00	14.63(10.04, 20.85)

a=wound, cracks, warts etc. *= potential risk factors at the level of 5% significance

Table 3. Risk factors of clinical and subclinical mastitis in Black Bengal goats based on multivariable logistic regression model

Factors	*P*-value	Odds ratio (OR)	95% CI
Age	0.001	2.6	1.97, 3.93
Parity	0.001	1.6	1.4, 1.8
Lactation stage	0.001	0.58	0.46, 0.73
Litter size	0.003	7.6	2.9, 19.6
Teat lesions	0.000	104.3	37.4, 291.29

The prevalence of CM in this study was 4.55%. Contreras *et al.* (2007) reviewed that prevalence of CM in goat is not more that 5%. The prevalence of SCM in this study is consistence with the findings of Islam *et al.* (2011) who reported the subclinical caprine mastitis in Bangladesh as 36% based on CMT kit. Prevalence of SCM in dairy goats ranged between 19.4% and 47% (Contreras *et al.*, 2003) and from 20 to 50% (Bergonier and Berthelot, 2003). Contreras *et al.* (2007) summarized the results from various research groups and noticed a prevalence of SCM in goats of 5 to 30%. The variability in the prevalence of caprine mastitis between reports could be attributed to the difference in management of the farms, milking management practices, breed considered or technical knowledge of the investigators. Besides, mastitic animals are not often immediately culled, and acute cases may become chronic for several months or more (Bergonier *et al.*, 2003).

The indicator of SCM is high somatic cell count in even though the milk and udder appeared normal. In this study we used a threshold of somatic cell count of 268000/ mL of milk (CMT score 1+) for differentiate between normal udder and udder with SCM. A somatic cell count less than 1,000 means the goat's glands are healthy; 2,000 to 500,000 indicates an infection by weak pathogens (easy to treat), over a million, consider a problem, and over 1,500,000 count definitely have an infection (Haskell, 2005). In goats increased milk cell count has been reported to be increased with increasing age and lactation (Zeng *et al.*, 1999). Bergonier *et al*. (2003) reported that incidence of SCM is more in multiparous than primiparious French goats. An increased prevalence related to number of parity has also been reported in ewes and goats (Boscos *et al*., 1996).

The prevalence CM and SCM was highest in early lactation stage (5.23 and 46.41%) followed by mid (4 and 28%) and late lactation stage (2.56 and 12.82%) which is supported by Zeng *et al.* (1999). Prevalence was also significantly ($p<0.001$) higher in does with teat lesions (100 and 84.62%) than that of having no teat lesions (0.00 and 14.63%). Teat injury is one of the major clinical sign in mastitic goats observed in this study. Association between teat injuries and mastitis has also been reported in ewes and goats by Ameh and Tari, (1999) and Bergonier *et al*. (2003).

It may be concluded from this study that the trend of both clinical and SCM increased with increasing age, number of parity and litter size of does. Prevalence was also higher in early lactation stage.

REFERENCES

1. Ameh JA and Tari IS (1999). Observations on the prevalence of caprine mastitis in relation to predisposing factors in Maiduguri. *Small Ruminant Research* 35: 1–5.
2. Assefa W, Molla B, Belihu K, Kleer J and Hildebrandt G (2006). A Cross-Sectional Study on the Prevalence, Antimicrobial Susceptibility Patterns, and Associated Bacterial Pathogens of Goat Mastitis. *International Journal Applied Research Veterinary Medicine* 4: 169-176.
3. Bergonier D and Berthelot X (2003). New advances in Epizootiology and control of ewe mastitis. *Livestock Production Science* 79: 1–16.
4. Bergoniera D, Crémouxb RD, Rupp R, Lagriffould G and Berthelota X (2003). Mastitis of dairy small ruminants. *Veterinary Research* 34: 689–716
5. Boscos C, Stefanakis A, Alexopoulos C and Samartzi F (1996). Prevalence of subclinical mastitis and influence of breed, parity, stage of lactation and mammary bacteriological status on Coulter Counter Counts and California Mastitis Test in the milk of Saanen and autochthonous Greek goats. *Small Ruminant Research* 21:139–147.

6. Contreras A, Luengo C, Sanchez A and Corrales JC (2003). The role of intramammary pathogens in dairy goats. *Livestock Production Science* 79: 273-283.
7. Contreras A, Sierra D, Sanchez A, Corrales J, Marcoc J, Paape M and Gonzalo C (2007). Mastitis in small ruminants. *Small Ruminant Research* 68: 145–153.
8. Hargital C, Egyhaze K and Markus G (1992). Trends of changes in the antibiotic sensitivity of udder pathogenic bacteria. Allatorvosok Lapja, 47: 429-432
9. Haskell S (2005). Caprine milk quality and mastitis. A speech at WDGAs caprine field day, Arlington Field station, Arlington, WI, 11-12-2005.
10. Hosmer DW and Lemeshow S (1989). Applied Logistic Regression. New York: Wiley, xiii: 307.
11. Ibrahim A, Kursat K and Haci AC (2009). Identification and Antimicrobial Susceptibility of subclinical mastitis pathogens isolated from hair goats' milk. *Journal of Animal and Veterinary Advances* 8: 4086-90.
12. Islam MR, Ahamed MS, Alam MS, Rahman MM, Sultana T, Roh YS and Kim B (2011). Identification and Antibiotic Sensitivity of the Causative Organisms of Sub-clinical Mastitis in Sheep and Goats. *Pakistan veterinary Journal* 32(2): 179-182.
13. Majic B, Jovanovic BV, Ljubic Z and Kukovics S (1993). Typical problems encountered in Croatia in the operation of goats milking machines. Proceedings of the 5th Internacional symposium on machine milking of small ruminants. Budapest, Hungary. pp. 377-379.
14. McDougall S, Pankey W, Delaney C, Barlow J, Murdough PA and Scruton D (2002). Prevalence and incidence of subclinical mastitis in goats and dairy ewes in Vermont, USA. *Small Ruminant Research* 46: 115– 121.
15. Quinn PJ, Carter ME, Markey B and Carter GR (1994). Clinical Veterinary Microbiology. Wolfe publisher, England.
16. Samad MA (2000). An overview of livestock research reports published during the twentieth century in Bangladesh. *Bangladesh Veterinary Journal* 34: 53 -149.
17. Samad MA (2008). Animal Husbanry and Veterinary Science. Volume II, LEP pub no.11, Bangladesh Agricultural University campus, Mymensingh.
18. Schultz EW and Chandler R (2009). The acidity of goat's milk in terms of hydrogen ion concentration, with comparisons to that of cow's and human milk. http//www.jbc.org by on March 28, 2009
19. StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP.
20. White EC and Hinckley LS (1999). Prevalence of mastitis pathogens in goat milk. *Small Ruminant Research* 33: 117-121.
21. Wooding FB, Morgan G and Craig H (1977). "Sunbursts" and "christiesomes": cellular fragments in normal cow and goat milk. *Cell Tissue Research* 185: 535–545.
22. Zeng SS, Escober EN, Hart SP, Hinclley L, Baulthaus M, Robinson GT and Jahane G (1999). Comprehensive study of the effect of testing laboratory, counting method, storage and shipment on somatic cell count in goat milk. *Small Ruminant Research* 31: 253–260.