Bangladesh Journal of Veterinary and Animal Sciences, Vol. 4, No. 1 & 2, July 2016

ISSN 2227-6416

Bangladesh Journal of Veterinary and Animal Sciences

Journal home page: www.cvasu.ac.bd/journal/userlist

Research article

Prevalence of *S. aureus* and *E. coli* causing subclinical Mastitis in goat with their antibiotic resistance pattern and associated risk factors

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ARTICLE INFO

Article history :
Received: 19/08/2016
Accepted: 23/08/2016

Keywords :
Mastitis, bacteria, Antibiotic sensitivity, Risk factors, goat

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ABSTRACT

An investigation was carried out to evaluate the status of the caprine clinical and subclinical mastitis in Chittagong Metropolitan Area. A total of 61 lactating goats were sampled during the period of January 2015 to June 2015 from Shahidul Alam Quadary Teaching Veterinary Hospital (SAQTVH) of Chittagong Veterinary and Animal Sciences University (CVASU). Clinical diagnosis of mastitis (clinical and subclinical) in goats was based on the California Mastitis Test (CMT). The prevalence of subclinical mastitis was estimated at nearly 64% (39 positive samples). One of the sampled animals showed clinical signs of mastitis. Milk samples were further analyzed for the isolation and identification of bacterial agents that were previously found associated with caprine subclinical mastitis. 28 (71.79% among 39 positive samples) samples were culture positive to *Staphylococcus aureus* and 14 (35.90% among 39 positive samples) were culture positive to *E. coli*. Among all tested samples, the overall prevalence of subclinical mastitis caused by S. aureus was 46% and *E. coli* 23%. 10 antibiotic disks were used to evaluate the antibiotic sensitivity of the isolated bacteria. The most sensitive antibiotics against *Staphylococcus aureus* were amoxicillin (82.15%), trimethoprim (64.29%), doxycycline (100%), ciprofloxacin (67.86%). The most sensitive antibiotics against *E. coli* were colistin sulphate (85.71%), doxycycline (100%), gentamycin (100%), and ciprofloxacin (92.86%). In addition, suspected risk factors such as age of animal, parity, length of lactation period, type of farming system and type of housing floor etc. for subclinical mastitis were also analyzed with advanced statistical tools. The findings of the present study will facilitate accuracy in public service from the Clinical Pathology Laboratory, CVASU through proper diagnosis followed by treatment.

To cite this paper : *Roy, S., Islam, M. S., Barua, S.R., Islam, M.S. and S. Chowdhury. 2016. Prevalence of S. aureus and E. coli causing subclinical Mastitis in goat with their antibiotic resistance pattern and associated risk factors . Bangladesh Journal of Veterinary and Animal Sciences, 4 : 38-42*

1. INTRODUCTION

Recently worldwide, raising goats is the major sector for the national economic development particularly for rural areas. Nowadays, raising goats is generally having an upward trend, both in sectors of the stock and the production level. There are about 50,500 thousand goats in Bangladesh (FAO, 2011) which is 6.7% of total goat population of the world. The domestic goat farming has secured an important source of income for many farmers in this country. According to FAO (2011), goat milk production reached an amount of 15.86 million tons per day, registering a growth rate of over 24%. Currently, almost 2.50 million tons (15.74% of total) of goat milk is produced in Bangladesh. Goat milk production is of major economic importance in many countries including Bangladesh (Haenlein, 1996). Therefore, any factor such as mastitis that adversely affects the quantity and quality of goat milk is of great financial interest in Bangladesh. Majority of infections are caused by gram-positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Escharichia coli*. *Staphylococcus aureus* is the most common etiological agent causing mastitis following Escherichia coli (Unakal and Kaliwal, 2010). Therefore, knowledge of routine physical examination of udder and diagnostic screening tests for early detection (i.e. during subclinical form) of mastitis and proper treatment of affected animal is one of the paramount importance in

order to minimize losses encountered due to subclinical as well as clinical mastitis. Several risk factors such as milking hygiene, management practice, and stage of lactation have influence on the occurrence of mastitis in goats (East et al., 1986 and Boscos et al., 1996). Antimicrobial drugs are the main therapeutic tool used to control bacterial diseases. However, little information is available concerning sensitivity patterns in small ruminant for mastitis pathogens. Therefore, a combination of early detection by field tests followed by identification of pathogens along with their sensitivity to antimicrobial drugs is crucial for prevention of economic losses to this disease. Although many developing countries have proper strategies for this disease monitoring and control, there is little information available regarding the prevalence of subclinical mastitis in goats in Bangladesh. The present study was conducted to estimate the prevalence and associated risk factors of subclinical mastitis caused by *S. aureus* and *E. coli* in lactating goats in Chittagong, Bangladesh.

2. MATERIALS AND METHODS

2.1. Selection of study population, area and duration

In this study, a total of 61 lactating goats were sampled during the period of January 2015 to June 2015 from Shahidul Alam Quadery Teaching Veterinary Hospital (SAQTVH) of CVASU covers the Chittagong Metropoliton Area, Chittagong, Bangladesh. A prototype questionnaire was used to collect data regarding information about age, breed, health, body condition score, weight, farm size, lactation number, duration of lactation, previous exposure level, housing system, rearing system, farm hygiene, feeding habit, calving problem and management of farm

2.2. Collection of milk samples

Milk samples were collected by soaking the teat with disinfectant (70% ethyl alcohol) and drying off by cotton. Strict aseptic procedure was maintained when collecting milk samples in order to prevent contamination with microorganisms present on the skin of flanks, udder and teats and in the hospital environment. 5 ml of milk was taken from each udder halves into sterilized test tubes with rubber cap. Each sample was labeled with an identification number immediately after collection and transported to Clinical pathology laboratory at Department of Pathology and Parasitology, CVASU for laboratory analysis.

2.3. Determination of subclinical mastitis

CHEIL BIO Cheil Bio Co. Ltd, South Korea (marketed by ACI, Bangladesh) was used according to manufacturer protocol for the present study.

2.4. Isolation and Identification of S. aureus and E.coli

The milk samples were streaked onto Mannitol Salt Agar (MSA) and McConkey Agar (MaC) and incubated at 37°C for overnight. The isolates were identified as *S. aureus* and *E.coli* on the basis of colony morphology. *S. aureus* produced Golden or Bright yellow coloured colonies and *E. coli* produced large pink colored colonies in Mannitol Salt Agar (MSA) and McConkey Agar respectively. The presumptive colonies of *E. coli* were further cultured onto selective medium Eosin Methylene Blue (EMB) Agar.

2.5. Antibiotic Sensitivity test

The antibiotic sensitivity test indicates if the isolated bacteria were sensitive to different antibiotics. The Kirby-Bauer disk diffusion test was used in this experiment to determine whether the isolated organisms were susceptible or resistant to a range of selected of antimicrobial agents (Uddin, 2011). In the present study the following antibacterial agents (Oxoid) were used: Amoxicillin (AML; 10 µg), Azithromycin (AZM; 15 µg), Ceftriaxone (CRO; 30 µg), Ciprofloxacin (CIP; 5 µg), Colistin sulphate (CT; 10 µg), Doxycycline (DO; 30 µg), Gentamicin (CN; 10 µg), Streptomycin (S; 10 µg), Trimethoprim (SXT; 25 µg), Oxytetracyclin (OT; 30 µg). Interpretation of the test results; sensitive (S), intermediate sensitive (I), and resistant (R) was drew based on CLSI criteria, 2007.

2.6. Statistical Analysis

The collected data (from questionnaire and laboratory) were entered into a spread sheet program (Excel; Microsoft, Redmond, WA, USA) and transferred into Stata 11 (StataCorp, College Station, Texas, USA) for analysis. To estimate the strength and statistical significance of association between a risk factor and the presence of *Staphylococcus aureus* and *E. coli* in goat milk, univariable chi square and logistic regression analysis was done.

Sample size was calculated using the following equation:

To estimate prevalence with 95% confidence limit-

$$N = \frac{1.96^2 \times (p)(1-p)}{d^2}$$

Here, 1.96 = z value for P = 0.05 or 95% confidence level. P = Estimated prevalence (50%) and d = Desired precision (5% error)

3. RESULTS

3.1. Descriptive statistics of the study population

Among 61 samples, 39 (63.93%, 95% CI: 26.76-51.24) showed a positive test in commercially available CMT kit. Among the 39 positive samples, 24 showed slight reaction (+) to the reagent, 7 showed moderate

reaction (++) and 8 showed heavy reaction (+++). Mean number of goats were harbored in the farms included in to the study were 4.22 (SD 4.01) with a minimum animal number 1 and maximum 30. 28 percent farms had ≤2 goats and 56% had 3-5 goats in their farm. Only single farms had 30 and 10 goats. Among 61 sampled goats, 10 (16.39%) were black Bengal and 23 (38%) were jamuna pari. One Patnaiya goat was examined. The rest of 44.26% goats were cross breed. The variable housing system was consisted of 2 categories, intensive and semi-intensive. 39 (64%) sampled goats were from intensive farming system and the rest 22 (36%) from semi-intensive. Variable 'rearing system' had 4 levels, where sampled animals were distributed as tethering 3 (5%), grazing 15 (25%), no grazing 31 (51%) and plain land 12 (20%). Most of the sampled goats 44 (72%) had a moderate body condition score (BCS).

Mean age of the sampled lactating goats was 2.40 years (minimum 1 and maximum 7 years). 30 goats (49%) were aged between 1 to 2 years and the rest 31 (51%) were more than 2 years old. Among the study population, mean parity number was 2.14 (SD 1.04). 18 (30%) were belongs to first parity, 34 (56%) were in 2nd to 3rd number parity and the rest 9 (15%) were in 4th to 5th parity. Mean lactation period of the study population was 61.11 days with a maximum period calculated as 180 days. Mean body weight of the sampled goats was 28.29 Kg (minimum 18 and maximum 40 Kg). 75% goats (n= 46) among the sampled population visited the hospital without any prior mastitis problem. The rest 25% (n= 15) had prior history of mastitis. Among the farmers of the sampled goats, 34% said that they knew about mastitis; however majority farmers (66%) had no idea about the disease. 91% (obs. 56) farmers said that they call veterinary doctor for correction of health problems in their farms. The rest either calls veterinary field assistant (VFA) or tries to solve the problem with own experience.

3.2. Isolation of microbial agents and their resistant pattern

Among 39 CMT positive sample, 28 (71.79%) and 14 (35.90%) were confirmed as *Staphylococcus aureus* and E. coli respectively. From 10 examined antibiotics, the most sensitive antibiotics against *Staphylococcus aureus* are amoxicillin (82.15%), trimethoprim (64.29%), doxycycline (100%), gentamycin (46.43%), ciprofloxacin (67.86%), azithromycin (53.57%), oxytetracyclin (46.43%). Intermediate sensitive (I) antibiotics are gentamycin (7.14%), streptomycin (7.14%), ciprofloxacin (3.57%), azithromycin (7.14%), oxytetracyclin (3.57%). High percentage of resistant antibiotics are cefriaxone (100%), colistin sulphate (96.43%), trimethoprim (35.71%), gentamycin (46.42%), streptomycin (92.85%), azithromycin (39.28%), oxytetracyclin (50%). The sensitive antibiotics against *Escherichia coli* are colistin sulphate (85.71%), doxycycline (100%), gentamycin (100%), streptomycin (85.71%), ciprofloxacin (92.86%). Intermediate sensitive (I) antibiotics are cefriaxone (7.14%), amoxicillin (7.14 %), doxycycline (7.14%). The most resistant antibiotics are cefriaxone (92.86%), amoxicillin (92.86%), trimethoprim (78.57%), streptomycin (14.29%), azithromycin (100%), oxytetracyclin (100%).

3.3. Risk factor analysis (univariable)

In univariable logistic regression analysis it was observed that goats from farms having 3-5 goats are in significantly 2.27 times higher risk of having mastitis compared to farms having 2 or less goats (baseline group). However, farms with more than 5 goats showed less risk (OR=0.3) of having mastitis in their goats (Table 1).

Table 1. Evaluation of risk factors for the occurrence of mastitis in lactating goats tested with Chi square test and univariable logistic regression analysis

Variable	Category	Observation	Number positive (%)	OR	P-value
Farm size	≤2	17	10 (59)	1	0.02
	3-5	34	26 (76)	2.27	
	>5	10	3 (30)	0.3	
Housing system	Intensive	39	24 (62)	1	0.60
	Semi-intensive	22	15 (68)	1.33	
Breed type	Pure breed	34	23 (68)	1	0.49
	Cross	27	16 (59)	0.69	
BCS	2	9	7 (78)	1	0.16
	3	44	25 (57)	0.37	
	4	8	7 (88)	2	
Age (years)	≤2	30	17 (58)	1	0.24
	>2	31	22 (71)	1.86	
Lactation stage	1	18	12 (67)	1	0.92
	2-3	34	21 (62)	0.80	
	>3	9	6 (67)	1	
History of disorder during calving	No	46	28 (61)	1	0.38
	Yes	15	11 (73)	1.76	
Knowledge about mastitis	No	40	26 (65)	1	0.81
	Yes	21	13 (62)	0.87	
Body weight (Kg)	≤25	29	17 (59)	1	0.41
	>25	32	22 (69)	1.55	
Duration of lactation (days)	≤30	34	23 (68)	1	0.49
	>30	27	16 (59)	0.69	

*OR = Odds Ratio

Semi-intensive farming system had a slightly higher risk (OR=1.33) of mastitis compared to intensive farming, relationship was not statistically significant though. 68% of the pure breed (Black Bengal, Jamuna Pari and Patnyia) goats sampled for the present study was affected with mastitis and 59% cross breed was found positive. Goats with more than 2 years of age had a higher risk (OR=1.86) of the disease compared to the animals with less than or equal 2 years of age.

'Lactation stage' of the goat (1st lactation, 2nd lactation and so on) and 'duration of days the goat is in lactation' did not show any significant relationship with the occurrence of mastitis (Table 3). Animals with higher body weight (>25 kg) had a slightly higher risk (OR=1.55) of having the disease compared to animals with less than or equal 25 kg weight. Goats with the history of disorder during calving had a higher chance (OR=1.76) of having the disease, however was not statistically significant. There was found no relationship whether the owner had knowledge about mastitis or not with the mastitis status of the sampled animals (Table 1).

4. DISCUSSION

In our study, the overall prevalence of subclinical mastitis was 63.93% by CMT in the goat population in Chittagong Metropolitan area (CMA), Bangladesh. However, in Bangladesh, CMT is used widely for diagnosing mastitis (both clinical and subclinical) in large ruminants both in research and field diagnosis purpose (Rahman et al., 2010).

S. aureus was isolated commonly from the subclinical mastitis samples including other bacterial agents (*Escherichia coli, Clostridium perfringens, Streptococcus*, *Pseudomonas*) (Bergonier et al., 2003 and Amin et al., 2011); S. aureus was the most dominant species. About 29.41% of the milk samples were positive for S. aureus (Khandkar et al., 2012). Beheshti et al. (2010) reported that the agents commonly responsible for the subclinical mastitis are *S. aureus* (19.2 %) and *E. coli* (8 %). In our study we observed 71.79% samples harboring *Staphylococcus* aureus and 35.90% had *E. coli* infection among the positive samples in CMT. Different studies concluded that S. aureus is the dominant bacterial species frequently isolated from milk samples collected from subclinical caprine mastitis and some antibiotic resistant strains were also isolated frequently (Mhase et al., 2007 and Gebrewahid et al., 2012). We have evaluated the sensitivity against 10 antibiotics in our study. The most sensitive antibiotics against *Staphylococcus aureus* are amoxicillin (82.15%), trimethoprim (64.29%), doxycycline (100%), gentamycin (46.43%), ciprofloxacin (67.86%),

azithromycin (53.57%), oxytetracyclin (46.43%) and sensitive antibiotics against *Escherichia coli* are colistin sulphate (85.71%), doxycycline (100%), gentamycin (100%), streptomycin (85.71%), ciprofloxacin (92.86%). So, it is suggested that broad spectrum antibiotics such as doxycycline, ciprofloxacin, azithromycin, gentamycin, streptomycin could be the first choice of drug for the treatment purpose for subclinical mastitis in goats in the study region. Smaller sample size in this study might have led to type II error in the statistical analysis. The samples size of the present study was 61 goats. Considering 50% prevalence and 10% allowable error, the calculated sample size for the present study was 92. Due to limited time frame (6 months) and resources for the present study, it was not possible to study 92 samples. Therefore, an extended study with a larger sample size is recommended to evaluate the risk factors of subclinical mastitis in goats.

Our study revealed that higher age (above 2 years) is responsible for an increased prevalence rate (OR=1.86) of subclinical mastitis in caprine in the study area. This finding is supported by the earlier observations where goats with more than 3 years of age had a higher risk of the disease compared to the animals with less than or equal 3 years of age (Sharma et al., 2007 and Ali et al., 2010). Increased age is correlated with increased parity and therefore increased milk production. High yielding cows were found vulnerable to mastitis in different studies (Rahman et al., 2010). However, in the present study parity did not show any effect on the occurrence of subclinical mastitis. Khandkar et al. (2012) showed that farm management system such as type of farming system and type of floor influenced the prevalence of subclinical mastitis. Goats which were raised under traditional conditions and had an earthen floor (soil surface) often got mastitis (Ndegwa et al., 2000). In the present study we observed that Semi-intensive farming system have a slightly higher risk (OR=1.33) of mastitis compared to intensive farming. When comparing indoors farms with semi-intensive ones, levels of SCC were found lower in the former (Rocio Jimenez-Granado et al., 2014).

5. CONCLUSION

Our study also showed some risk factors that associated with subclinical mastitis such as age of the animal, parity, length of lactation period and housing system influenced the prevalence of subclinical mastitis as well as clinical mastitis status in goat population. So, For controlling mastitis, management and hygiene should be maintained and application of sensitive antibiotic could be an effective way to control the mastitis.

ACKNOWLEDGEMENT

The author likes to thank HEQEP CP: 3220: Establishment of clinicopathological lab at CVASU for giving fund to accomplish the research work.

REFERENCES

Amin, A.S. Hamouda, R.H. and Abdel-All, A.A.A. 2011. PCR Assays for detecting major pathogens of mastitis in milk simples. World Journal of Dairy and Food Sciences,6: 199-206.

Beheshti, R. Shaieghi, J. Eshratkhah, B. Ghalehkandi, J.G. and Maheri-Sis,N. 2010. Prevalence and etiology of subclinical mastitis in ewes of the Tabriz region, Iran. Global Veterinaria, 4: 99-302.

Bergonier, D. Cremoux, D.R. Rupp, R. Lagriffoul, G. and Berthelot, X. 2003. Mastitis of dairy small ruminants. Veterinary Research, 34:689-716.

Boscos, C. Stefanakis, A. Alexopoulos, C. and Samartzi F., 1996. Prevalence of subclinical mastitis and influe-nce of breed, parity, stage of lactation and mammary bacteriological status of Counter Counts and California Mastitis Test in the milk of Saanen and auto-chthonous Greek goats. Small Ruminant Research, 21: 139-147.

East, N.E. Birnie, E.F.and Farver T.B. 1986. Risk factors associated with mastitis in dairy goats. American Journal of Veterinary Research, 67: 63-67.

FAO, 2011. FAOSTAT. (http://faostat.fao.org/site/569/Desktop Default.aspx? Page ID=569#ancor).

Gebrewahid, T.T. Abera, B.H. Menghistu, H.T. 2012. Prevalence and Etiology of Subclinical Mastitis in Small Ruminants of Tigray Regional State, North Ethiopia. Veterinary World, 5(2): 103-109.

Haenlein, G.F.W. 1996. Goat milk in human nutrition. Small Ruminant Research, 51: 155-163.

Khandkar, M.A.R. Rahman, M.B. Flores-Gutierrez1, G.H. and Rahman, M.T. 2012. Prevalence of caprine subclinical mastitis in mymensingh area, Bangladesh and characterization of associated bacterial agents and the risk factors. Microbes and Health, 1(1): 1-5.

Mhase, P.P. Harne, S.D. and Suryawanshi P.R. 2007. Aerobic bacterial flora of subclinical goat mastitis in Vidarbha region of Maharashtra. Journal of Bombay Veterinary College,15: 50-51.

Ndegwa, E.N. Mulei, C.M. Munya, S.J. 2000. Risk factors associated with subclinical mastitis in Kenyan dairy goats. Israel Journal of Veterinary Medicine, 56: 1-6.

Rahman, M.M. Islam, M.R. Uddin, M.B. and Aktaruzzaman M. 2010. Prevalence of subclinical mastitis in dairy cows reared in sylhet district of Bangladesh. International Journal of Biological Research, 1(2): 23-28.

Rocio, J.G. Rodriguez, S. Cristina, A. and Estevez, R.. 2014. Factors affecting somatic cell count in dairy goats: a review. Spanish journal of agriculture research.

Uddin, M.A. Hasan, M. Haque, M. and Noor, R. 2011. Isolation and identification of pathogenic Escherichia coli, klebsiella spp. and staphylococcus spp. in raw milk samples collected from different areas of dhaka city, Bangladesh. Stamford Journal of Microbiology, 1(1): 2074-5346.

Unakal, C.G. and Kaliwal, B.B. 2010. Prevalence and antibiotic susceptibility of Staphylococcus aureus from bovine mastitis. Veterinary World, 3(2): 65-67.