

PREVALENCE, CURRENT ANTIBIOGRAM AND RISK FACTORS ASSOCIATED WITH MASTITIS IN DAIRY GOATS IN PUNJAB

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Abstract: The present study was intended to estimate the prevalence of subclinical mastitis (SCM) in apparently healthy dairy goats, to carry out culture sensitivity of isolated pathogens and to elucidate the associated risk factors of mastitis. A total of 200 goats comprising of 397 udder halves from 10 flocks of Punjab was carried out. Milk samples from were collected and screened for California Mastitis Test (CMT), bacteriological examination, and culture sensitivity pattern of the isolated organism. The potential risk factors associated with the occurrence of SCM were analysed using multiple regression and univariable logistic regression analysis. Prevalence of SCM at goat level was 55.5%, and at udder half level it was 46.0%. There was found no significant ($p>0.05$) difference on the occurrence of SCM between left and right udder halves. Coagulase negative staphylococci were the chief pathogens (75.65%) isolated from infected udder halves. Tetracycline was found to be the most effective drug and penicillin the least. Multiple regression showed that risk factors such as age, parity, milk yield, days in milk and season added significantly ($p < 0.001$) to the prediction. Univariable logistic regression analysis depicted that mastitis was more prevalent in does with increased age, parity and during the season Nov-Dec.

Keywords: Prevalence, antibiogram, risk factors, subclinical mastitis, goats.

Introduction

Goats are significant for subsistence of a large population in the developing world, and especially to weaker and downtrodden sections, who are the most vulnerable members of society in terms of undernourishment and poverty. The dairy goat industry is rapidly gaining importance throughout the world in recent years. The current goat population in India is estimated to be around 135.17 million which is second largest after China (149.38 million) (FAOSTAT, 2013). In India, goats produce 4.00 mMT of milk as per FAO 2013 report. Goat population in India during the last four decades has increased at the fastest rate amongst various livestock species. As far as Punjab is concerned, it has approximately 0.327 million goats, which contributes to 3.67 per cent of total milk (FAOSTAT, 2013). Goat milk's health benefits and nutritional values have given impetus to the consumption of goat milk and its

products (Silanikovea *et al.*, 2010). Among the several problems deterring the livestock development, disease problems pose a great menace to the successful production of livestock and its industry. Therefore, any factor that adversely affects the quantity and quality of milk of dairy animals is of great financial interest. Milk quality is mainly affected by bacterial contamination of the mammary gland, which causes clinical or subclinical mastitis (SCM). This is almost always associated with bacterial infection. Mastitis in the goat is mainly subclinical (McDougall *et al.*, 2002) and results in decreased milk yield in goat in consequence growth retardation and higher mortality rate among suckling kids. The CMT has been found more perfect, efficient and reliable than other field and chemical tests for diagnosis of SCM (Raikwar and Shukla, 2015). The primary etiology of SCM in goats comprises *Staphylococcus spp.*, *Streptococcus spp.* and *Micrococcus spp.* (Persson and Olofsson, 2011). Antibiotic therapy is a key tool in mastitis control. The treatments are more effective when appropriate antibiotic selection can be enhanced using rationale antimicrobial susceptibility test. The indiscriminate and/or overuse of antibiotics can lead to the development of resistance among different bacterial strains. Therefore, regular studies on antibiotic sensitivity of bacterial isolates are mandatory for effective and economical treatment of the disease. Although milk is a very nutritional food that is rich in carbohydrate, proteins, fats, vitamins and minerals but health risk to consumers can be associated with milk, due to the presence of zoonotic pathogens and antimicrobial drug residues (Bradely, 2000). As goats are gaining importance as dairy animals in this part of country, information on mastitis and associated risk factors has vital importance for control interventions. However, information on the occurrence and potential risk factors associated with mastitis occurrence in goats is scarce, which otherwise, is essential to update our knowledge on its epidemiology and control measures. The present study was, therefore, envisaged to estimate the prevalence of subclinical mastitis in apparently healthy dairy goats, to isolate the pathogens causing intramammary infections, and to carry out their culture sensitivity pattern and to elucidate the associated risk factors of mastitis.

Materials and Methods

A cross sectional study was carried out from June, 2015 to April, 2016 involving 200 apparently healthy randomly selected lactating beetal crossbred goats from 10 loose dairy goat herds belonging to Ludhiana (Issewaal, Dhaka, Malerkotal), Kapurthala, Patiala (Potapotu), Moga, Bathinda, Faridkot, Hoshiarpur and Ferozpur districts of Punjab. The survey sample size was calculated by the method described by Sergeant (2015). The flocks

were visited during morning hours and the milk samples at quarter level were collected and studied for California mastitis test (CMT), bacteriological examination and culture sensitivity test (CST). Detailed information about total number of goats, goats in milk at the flock, and age, parity, days in milk (DIM), milk yield and previous history of mastitis in the individual animals were recorded. The goats were maintained under loose housing system with grazing in the morning and housed in the evening. The concentrate was offered once or twice daily after milking according to animal's production level by 3 goat farmers. Mineral mixture and liquid calcium were also supplemented regularly by these farmers. Water was provided *adlib*. The goats were hand milked. No pre or post milking teat cleaning procedures were followed by these farmers. Most of the goats were vaccinated for PPR and two goat flocks were vaccinated with brucellosis too. The number of goats per holding varies considerably from 20 to over 100 animals per flock in some instance. Milking is practiced often twice a day (in the morning and evening) by young boys or elderly people.

Study Design and sampling strategy: A cross sectional study was conducted to assess the prevalence of subclinical mastitis in goats. A total of 200 lactating beetal crossbred goats were included in the study. The sample size for the current study was determined according to the method described by Sergeant (2016) for cross-sectional, random sampling method. The sample size calculated was 196 at confidence interval (CI) 7 and 95% level of confidence, and population 3,27,000. However, a total of 200 lactating goats were included in this investigation.

Before collection of milk sample, proper cleanliness and dryness of teats was ensured. The teat orifice was scrubbed with a cotton wool wetted with 70% alcohol (spirit). First few streaks of milk were discarded and the individual udder halves samples (about 15 ml) were collected in sterilized labelled test tubes. Immediately after collection, milk samples were subjected to physical examination with naked eyes to detect any abnormalities in color, odor, consistency, presence of blood and clot, flakes and any other visible abnormalities. The milk samples were then packed in ice and transferred immediately (within 1-2 hours of completion of sampling) to the laboratory for CMT and bacteriological examination.

The CMT was conducted by using CMT kit developed by the university. The leukocyte reagent is composed of Sodium Lauryl Sulfate (3%) and bromocresol purple (0.5%). The reaction was interpreted as per standard method described by Pandit and Mehta (1969). The results were scored as 0, 0.5 (Trace), 1, 2 and 3 depending upon the degree of gel formation. Scores depend on the degree of gellation that was indicated by gelatinous mass in

proportion to severity of infection present (Schuppel and Schwope, 1998). The isolation and identification of microbial organisms from milk samples was done as per standard microbial procedures of National Mastitis Council (1999). Milk samples were cultured onto 7% defibrinated sheep blood agar and MacConkey agar plates. The bacteriologically negative samples were cultured for mycoplasma and fungi onto PPLO agar and Sabroux Dextrose Agar, respectively. The plates were incubated for at least 5 days for mycoplasma and 15 days for fungi. Suspected colonies of bacteria were identified morphologically, microscopically and biochemically according to Hargital *et al.* (1992). The culture sensitivity of isolated organisms was carried out for various antimicrobial agents (Hi-Media) using disc diffusion method (Quinn *et al.*, 1999). The quarter health status was assessed and defined on the basis of bacteriology and SCC estimate (CMT score) of quarter foremilk samples as described below:

CMT score	Microbial pathogen	
	Not detected	Detected
< 1	Healthy	Latent infection
≥1	Non-specific mastitis	Specific mastitis

Data collection and statistical analysis: Structured data handling format was prepared and every important information (variable) associated with the overall objective of the investigation was properly gathered and recorded. Age, parity, days in milk, milk yield, flock size, housing, previous history of mastitis were recorded. Age of the study animals was recorded from the owners' information. Factors that usually affect the prevalence of subclinical mastitis were recorded and were analyzed by using SPSS (Statistical Package for the Social Sciences) version 16.0 software and statistically significant associations were determined by the chi-square test and multiple regression was applied to explain the prediction of various independent variables on dependent variable. univariable logistic regression. Logistic regression analysis and odds ratio (OR) was done to see the strength of association. $P < 0.05$ was taken as statistically significant.

Results and Discussion

Prevalence of subclinical mastitis

A total of 397 quarter foremilk samples from 200 apparently healthy randomly selected lactating beetal crossbred goats from 10 loose dairy goat farms belonging to Ludhiana (Issewaal, Dhaka, Malerkotal), Kapurthala, Patiala (Potapotu), Moga, Bathinda, Faridkot, Hoshiarpur and Ferozpur districts of Punjab and subjected to California Mastitis Test (CMT)

and bacteriological examination to observe the prevalence of disease. The average prevalence of sub-clinical mastitis was found to be 55.50 % at animal basis with 22.50% and 23.50% quarters showing specific and non-specific mastitis respectively (Table 1). Out of total 184 affected udder halves, 17.39% (32/184; Left = 11 and Right = 21) udder halves were unilaterally affected whereas 152 udder halves (76 goats) had bilateral affection. In the present study, Left (47.30%) and right side (52.70%) udder halves did not show any significant difference in affections with mastitis ($\chi^2 = 1.314$; $df = 01$; $p > 0.05$) (Table 2).

The observed prevalence in this study is close to the result obtained by several researchers from different parts of the world (Swai *et al.*, 2008, Sarker and Samad, 2011, Begum *et al.*, 2012, Gebrewahid *et al.*, 2012, Bourabah *et al.*, 2013, Mbindyo *et al.*, 2014, Raikwar and Shukla, 2015 and Pirzada *et al.*, 2016). However, the prevalence of subclinical mastitis is relatively higher than many other workers like Ndegwa *et al.* (2000) as 9.8%, Kostelic *et al.* (2009) as 20% and Zamin *et al.* (2010) recorded as 13%. This variation in prevalence of disease may be attributed to several factors such as breed differences, age and parity of the animals, stage of lactation and different management practices followed on each farm (McDougall *et al.*, 2002). There was found no significant association of left and right udder halves with the occurrence of mastitis in this study which is in corroboration with the findings by Al-Ramahi and Al-Nassrawi (2007).

Antibiogram of isolated bacterial pathogens

Milk samples were collected from 397 udder halves of 200 lactating goats. Of all udder half samples, 115 (28.97%) samples were culture positive and 282 (71.03%) samples yield no bacterial growth (Table 3). Isolates from positive cases were 87 (75.65%) coagulase negative *Staphylococci* (CNS), 22 (19.1%) coagulase positive *Staphylococci* (CNS) and 6 (5.22%) *Streptococcus spp.* Most of the subclinical cases were due to coagulase negative *Staphylococci* (CNS). In the present study, most of the subclinical mastitis infections were due to coagulase negative *Staphylococci* species accounting for 75.65% of the positive quarters. This finding is in agreement with the result of Al-Ramahi and Al-Nassrawi (2007). Other than coagulase negative *Staphylococci* bacteria, *Staphylococcus aureus* (CS) and *Streptococcus spp* were the common pathogens observed in the present study. These results regarding the distribution of pathogens correspond well with other studies on subclinical mastitis infection in dairy goats throughout the world (Leitner *et al.* 2004, Persson and Olofsson, 2011 and Begum *et al.*, 2012). The large proportion of coagulase negative *Staphylococci* infections is consistent with the absence of clinical intramammary infections,

in fact, some authors have proposed coagulase negative *Staphylococci*s etiological agents of subclinical intramammary infections in goats (Leitner *et al.*, 2004). In addition, subclinical infections by *Staphylococcus aureus* are also common in goats (Moroni *et al.*, 2004). Finally, it may be said that in this study a significant number of isolates were contagious pathogens; appropriate milking practice such as milking of affected goats last, cleanliness of udder, milker's hand hygiene and culling could be feasible options in the control program in this region.

The antibiotic sensitivity patterns of bacterial isolates are presented in Table 5. In this trial, eleven most commonly used antibiotics were used. It is evident from this table that the tetracycline was found to be the most effective and penicillin the least effective against various mastitis pathogens in this study. The *in vitro* antibiogram studies of the bacterial isolates from milk revealed tetracycline (90.43%) to be most effective drug followed by enrofloxacin and Cloxacillin ((83.48% each). These drugs particularly tetracycline and cloxacillin are not being frequently used for treatment in this area might be a possible reason for higher efficacy. Tetracycline proved to be the drug of choice in this study. No similar antibiogram pattern could be found in the literature. However, Zamin *et al.* (2010) and Rola *et al.* (2015) reported 80.72% and 93.7% sensitivity of tetracycline, respectively. In this study, most of the bacterial isolates showed resistance to penicillin and streptomycin. Indiscriminate and frequent use of these antibiotics in animals could be the reason for their ineffectiveness against bacterial isolates. Da Silva *et al.* (2004) and Begum *et al.* (2012) also reported that bacteria in goat mastitis were more resistant to Penicillin G. This may be attributed to indiscriminate and/or overuse of antibiotics because frequent use of the same antibiotics may result into antibiotic resistance (Tras *et al.*, 2007). Plasmids mediated beta-lactamase enzymes production is supposed to be mainly responsible for resistance to penicillin. Different results obtained from antibiotics susceptibility may be largely due to misuse of antibiotics and difference of bacterial strains. Since, streptomycin has been extensively used along with penicillin for treating mastitis; it may have led to the development of high resistance in bacteria against this antibiotic.

Analysis of risk factors associated with mastitis in goats

During the study period, certain potential host and environmental risk factors such as age, parity, lactation stage, milk yield, previous history of mastitis, season, housing and flock size were taken into consideration to see the integration with the occurrence of subclinical mastitis in lactating goats. A multiple regression was run to predict the prevalence of subclinical

mastitis from dependent variable. All these variables significantly predicted the prevalence of subclinical mastitis $F(8, 95) = 21.448, p < 0.001, R^2 = 0.473$. Furthermore, risk factors such as age, parity, milk yield, days in milk and season added significantly ($p < 0.001$) to the prediction. However, other factors did not add significantly. Univariable analysis of potential risk factors showed that prevalence was significantly higher ($P < 0.05$) in does with increased age, parity, and during the season Nov-Dec. Furthermore, mastitis prevalence was significantly higher ($P < 0.05$) in does sampled during the period from November to December than the periods from September to October. There was an increasing trend of mastitis prevalence with increasing age. Conversely, other risk factors did not show significant differences for mastitis prevalence. Mastitis prevalence has progressively increased with age and parity number of the does studied, and in corroboration with other studies (Moroni *et al.*, 2005 and Megersa *et al.*, 2010). The protracted exposure of multiparous animals to pathogens increases the prevalence of disease in comparison to primiparous or with less parity animals. Age is the most significant factor in determining the prevalence of mastitis in goats. In goats increased milk cell count has been reported to be elevated with increasing age and lactation. Since mastitic animals are not immediately culled, and acute cases may become chronic with the passage of time (Bergonier *et al.*, 2003). An increased prevalence related to parity has been reported in goats by Bergonier *et al.* (2003). When the duration of exposure to infection is long and spontaneous cure rate is low, prevalence increases. In the present study, no significant effect of DIM on udder health was observed. This finding is in agreement with Begum *et al.* (2012). However, Megersa *et al.* (2010) observed a significant relationship with lactation stage. The reason of increased rate of prevalence during Nov-Dec months could be higher number of kidding in these months which leads to increased susceptibility to udder infection, and further the udder halves affected with non specific mastitis were considered as mastitis.

Conclusions

The average prevalence of sub-clinical mastitis in dairy goats was found to be 55.50 % at animal basis. Left and right side udder halves did not show any significant difference in affections with mastitis. In this study a significant number of isolates were CNS; appropriate milking practice such as milking of affected goats last, cleanliness of udder etc. could be feasible options in the control program in this region. Tetracycline proved to be the drug of choice in this study. The risk factors such as age, parity, milk yield, days in milk and season added significantly ($p < 0.001$) to the prediction by multiple regression analysis. However,

univariable analysis showed that prevalence was significantly higher ($P < 0.05$) in does with increased age, parity, and during the season Nov-Dec.

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Table1. Prevalence of subclinical mastitis

Quarter level			Animal level		
Health status	N	Percent	Health status	N	Percent
Healthy	188	47.00	Healthy	89	44.5
Latent mastitis	25	6.25	#Diseased	111	55.5
Non specific mastitis	94	23.5			
Specific mastitis	90	22.5			
Blind	3	0.75			
Total	400			200	

Animal having at least one quarter with specific or non-specific infection

Table 2. Association of udder halves with the occurrence of mastitis

Udder halves	Healthy	Mastitis	Total	χ^2	df	p
Left	113(53.1%)	87(47.3%)	200(50.4%)	1.314	1	0.269
Right	100(46.9%)	97(52.7%)	197(49.6%)			
Total	213	184	397			

Table 3. Distribution of the different bacterial isolates from the milk samples of goats

Bacterial isolate	Total N (%)	Specific	mastitis	Latent	infection
		quarters No.	(%)	quarters No.	(%)
Coagulase-negative staphylococci (CNS)	87 (75.65%)	67	74.44	20	80.00
Coagulase-positive staphylococci (CS)	22 (19.13%)	17	18.89	5	20.00
Streptococcus spp.	6 (5.22%)	6	6.67	0	0
Total	115	90	100.00	25	100.00

Table 4: Cultural Sensitivity pattern of organisms isolated from milk samples of goats

Organism	N	AMP	AMC	AMS	P	CO	CIT	CIS	G	S	TE	Ex
Overall	115	47 (40.87%)	44 (38.26%)	69 (60%)	28 (24.4%)	96 (83.48%)	91 (79.13%)	91 (79.13%)	68 (59.13%)	54 (46.96%)	104 (90.43%)	96 (83.48%)
CNS	87	34 (39.08%)	28 (32.18%)	49 (56.3%)	17 (19.5%)	75 (86.21%)	68 (78.16%)	70 (80.46%)	48 (55.17%)	41 (47.13%)	83 (95.40%)	76 (87.36%)
CS	22	11 (50%)	13 (59.09%)	16 (72.7%)	18 (81.8%)	18 (81.82%)	19 (86.36%)	18 (81.82%)	16 (72.73%)	12 (54.54%)	19 (86.36%)	16 (72.73%)
Strept	6	2 (33.33%)	3 (50%)	4 (66.7%)	0	3 (50%)	4 (66.67%)	3 (50%)	4 (66.67%)	1 (16.67%)	2 (33.33%)	4 (66.67%)
Specific SCM	90	34 (37.8%)	33 (36.7%)	54 (60%)	20 (22.2%)	75 (83.3%)	72 (80%)	68 (75.6%)	58 (64.4%)	39 (43.3%)	79 (87.8%)	75 (83.3%)
CNS	67	24 (35.8%)	22 (32.83%)	38 (56.7%)	13 (19.5%)	59 (88.05%)	54 (80.59%)	52 (77.6%)	40 (59.7%)	30 (44.77%)	63 (94.02%)	58 (86.56%)
CS	17	8 (47.05%)	8 (47.05%)	12 (70.6%)	7 (41.2%)	13 (76.47%)	14 (82.35%)	13 (76.47%)	14 (82.35%)	8 (47.05%)	14 (82.35%)	13 (76.47%)
Strept	6	2 (33.3%)	3 (50%)	4 (66.7%)	0	3 (50%)	4 (66.7%)	3 (50%)	4 (66.7%)	1 (16.7%)	2 (33.3%)	4 (66.7%)

AMP: Ampicillin (10 mcg), AMC: Amoycillin+clavulanate (30 mcg), AMS: Amoxycillin+sulbactum (30/15 mcg), P: Penicillin (2 units), CO: Cloxacillin (10 mcg), CIT: Ceftriaxone+tazobactum (30/10 mcg), CIS: Ceftriaxone+sulbactum (30/15 mcg), G: Gentamicin (10 mcg), S: Streptomycin (25 mcg), T: tetracycline (10 mcg) EX: Enrofloxacin (10 mcg)

Table 5. Multiple Regression Analysis of risk factors for the occurrence of subclinical mastitis in dairy goats

Variables	B	t	P	R²	F value	p
Constant	1.424	8.111	0.000	0.473	21.448	0.000
Age	0.185	2.236	0.027			
Parity	0.494	6.325	0.000			
DIM	-0.558	-6.072	0.000			
Milk yield	0.300	2.582	0.011			
Flock size	0.101	0.766	0.445			
Housing	0.155	1.077	0.283			
Season	-0.612	-8.625	0.000			
Previous History	0.121	0.891	0.374			

Table 6. Univariable logistic regression analyses of risk factors for the occurrence of subclinical mastitis in dairy goats

Risk Factors	Category Level	Tested	Positive	Prevalence N (%)	Univariable logistic regression analyses results				
					B	OR	p	95% CI of OR	
								Lower	Upper
Age (Reference <2)	<2 yrs	57	21	36.84					
	<3 yrs	61	33	54.10	-0.571	0.563	0.144	0.263	1.214
	<4 yrs	26	19	73.08	-0.583	0.558	0.130	0.263	1.186
	>4 yrs	56	37	66.07	-1.383	0.251	0.006	0.094	0.671
Parity (Reference parity 1)	1	54	24	44.44					
	2	77	41	53.25	-1.476	0.229	0.006	0.080	0.650
	3	42	25	59.52	-1.123	0.325	0.030	0.118	0.895
	>4	27	21	77.78	-0.867	0.420	0.121	0.140	1.258
DIM (Reference <60d)	<60 d	120	67	55.83					
	<90 d	34	21	61.76	0.234	1.264	0.500	0.640	2.498
	<180 d	46	23	50.00	0.480	1.615	0.297	0.656	3.979
Milk yield (Reference <1 lt)	<1 lt	80	43	53.75					
	<2 lt	83	46	55.42	-0.233	0.792	0.564	0.360	1.746
	<3 lt	37	22	59.46	-0.165	0.848	0.680	0.386	1.861
Flock size (Reference <40)	<40	119	45	37.82					
	>40	81	66	81.48	0.004	0.569	0.990	0.569	1.772
Housing (Reference Open enclosure)	Open enclosure	134	85	63.43					
	Housed	66	26	39.39	-0.311	0.733	0.309	0.402	1.334
Season (Reference Sept-Oct)	Sept-Oct	44	33	75.00					
	Nov-Dec	43	18	41.86	1.306	3.692	0.011	1.357	10.043
	Feb	84	47	55.95	-0.121	0.886	0.803	0.343	2.292
	April-May	29	13	44.83	0.442	1.563	0.302	0.669	3.655
Previous History (Reference value No)	No	89	4	04.49					
	Yes	111	8	07.21	-0.501	0.606	0.426	0.176	2.081