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Bacterial and Antibiotic Profile of Mastitic Milk of Beetal Goat in District Jhelum

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Authors' Contribution
MY and AA designed the study. WA performed experiments and wrote the manuscript. Imran U and Ihsan U helped in sampling and experiments. MAM did analysis. GD provided assistance and research guideline. SUK helped in manuscript finalizing.

Key words
Mastitis, Bacterial isolates, Surf field mastitis test, Biochemical test, Risk factor, Antibiotic sensitivity

ABSTRACT

A total of 514 milk samples collected independently from beetal goats were tested for prevalence of clinical and subclinical mastitis through surf field mastitis test. The bacterial isolates identified through different biochemical test and colony morphology from positive milk sample were *Staphylococcus aureus* (25.4%), *Escherichia coli* (20.3%), Streptococcus (16.9%) in which (6.7%) *Streptococcus agalactiae*, and (10.1%) other streptococcus species, *Pseudomonas aeruginosa* (15.2%), *Salmonella* (13.5%) and *Klebsiella pneumonia* (8.4%). S*taphylococcus aureus* was found most prevalent pathogen that causes mastitis in goats. The overall prevalence of goat mastitis was recorded (11.67%). Risk factor like age, lactation stage, parity number and unhygienic condition showed statistically significant association with the prevalence of mastitis in beetal goats. Antibiotics sensitivity profiles of investigated bacteria showed that these were most sensitive to gentamycin, ciprofloxacin and amoxicillin.

INTRODUCTION

Goats are mainly reared for the purpose of milk and meat all over the world and considered one of the ancient housekeeping species (Hale *et al*., 2011). It has been evaluated that 460 million goats are present in the world, which produce almost 4.50 million tons of milk and 1.20 million tons of meat approximately (Hirpa and Abebe, 2008). In Pakistan, entire population of goats recorded is 63.1 million that produce 0.779 million tons milk and 0.629 million tons meats (Anonymous, 2003). Beetal breed is one of the described breed out of 36 goat breeds bear great significance in milk production via FAO in 2006. Beetal goat has 4.2 million populations in Pakistan, constitute 7.8% of the whole goat population of Pakistan (GOP, 2008). Mastitis is the inflammatory state of the udder, described by chemical and physical changes occur in milk quality (Nazifei *et al*., 2011). Mastitis has been considered the most imperative and costly disease of milk industry, it causes severe damage to dairy industry in the form of decreased milk production, cost of treatment, increasing labor, and early elimination (Miller *et al*., 1993). The most essential modifications occur in milk of animals during mastitis include discoloration, appearance of small clots and existence of large amount of leukocytes. There are three main sub-types of mastitis that is clinical mastitis, sub-clinical mastitis and chronic mastitis. (Anonymous, 2003). In clinical mastitis milk color changes and clots also appears in the milk. Subclinical mastitis can be diagnosed by different laboratory test including microbial investigation, California mastitis test, surf field mastitis test, somatic cell count and white side test (Wakoya *et al*., 2006). In chronic mastitis, blisters and granulomas appear in the tissue of mammary gland that can reduce milk production while such cases are not often diagnosed and only visible at abattoirs (Hulten *et al*., 2004).

Although *Staphylococcus* species are considered to be the most frequent causative agent for mastitis, Streptococcus species, *Pseudomonas aeruginosa*, corynebacteria, and fungi have also been reported to cause mastitis in ruminants (Contreras *et al*., 2007). Mastitis disease cannot be completely removed from animals herd, but their occurrence could be reduced to minute level. Antimicrobial therapy considers to be an important component of mastitis control in dairy production systems (Oliver *et al*., 2011). Antibiotics such as lactam, macrolide, flouroquinolones, penicillin, nafcillin, and dihydro-streptomycin have been found most effective in decreasing

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the liability of mastitis bacteria (Chaffer *et al*., 2003). In Punjab province a total loss of Rs. 240 million per annum have been recorded due to this disease. The aim of this study is to identify the bacterial isolates and to determine their drug sensitivity.

MATERIALS AND METHODS

In this study, 514 milk samples were collected aseptically from beetal goats in four tehsils Jhelum, Sohawa, Dina and Pind dadan khan of Jhelum district. The teats were cleaned with 70% ethyl alcohol cotton swab. First three milk streaks were discarded then 5 to 10ml of milk was collected aseptically in a sterile beaker. The clinical mastitis was recognized by palpation of udder and visual observation of teat lesions. Milk samples were collected according to the national mastitis council (NMC, 1990). Parameters like changes in milk color, presence of grossly visible substances, swelling, pain, redness, udder hardening, and obstruction of the teats were assessed for detection of clinical mastitis. Sub-clinical mastitis was diagnosed by surf field mastitis test (Muhammad *et al*., 1995). From goats teat milk was stripped into well of the paddle plate and an equal amount of surf solution was added. The paddle plate was then rotated for 10 seconds to proper mix the surf solution and milk. After 25 seconds, variations in the milk were detected. Development of clots in milk and gel like flakes were suggestive of the disease. The positive milk samples were cultured on nutrient agar media, blood agar enriched media and selective macConkey agar media for bacterial identification according to standard procedures (Quinn, 1994). For the identification of gram negative bacteria, the triple sugar iron, Indole, Urease, Citrate and Oxidase test were performed, while for gram positive cocci chain and cocci cluster catalase, coagulase, bile test and camp test were conducted (Balows *et al*., 1991). The isolates were tested for antibiotic sensitivity against various antibiotics including gentamycin, ciprofloxacin, oxytetracycline, amoxicillin, sulfa-trimethoprim and kanamycin according to disc diffusion method described by (Bauer *et al*., 1966). The inhibition zone around every antibiotic disc was measured according to national committee for clinical laboratory standards (Watts *et al*., 2018). Several risk factors potentially associated with prevalence of mastitis were recorded. These factors included lactation period, parity, age and rearing area sanitation. First, the data of these factors were arranged for univariate logistic regression analysis to determine the individual risk factor effect on mastitis. The factors that ensured p value <0.05 during the univariate analysis were preferred as possible risk factor for multivariate logistic model analysis. The final logistic model was constructed with a significant level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Table I shows prevalence of mastitis in different tehsils and union councils of district Jhelum. Overall prevalence of mastitis was 11.67%. Union council Sauwal of tehsil Pind Dadan Khan showed 12.32% prevalence. Union council Kala Gujjran of tehsil Jhelum showed 8.88%, union council Khukha of tehsil Dina showed 15.23% prevalence and union council Lehri of tehsil Sohawa showed 10.93% prevalence of mastitis. Our results agreed well with previously reported (Zamin *et al*., 2010) prevalence of 14.5%.

Table I. Mastitis Prevalence in different tehsils and union councils of district Jhelum.

Union councils (Tehsil)	No of positive/No of samples tested	Percentage of positive
Sauwal (Pind dadan khan)	18/146	12.32%
kala gujjran (Jhelum)	12/135	8.88%
Khukha (Dina)	16/105	15.23%
Lehri (Sohawa)	14/128	10.93%
Total	60/514	11.67%

Risk factors

Table II shows the risk factors associated with mastitis in beetal goat. It was observed goats of 2 and 3 years of age had no significant association with disease development. On the other hand, goats in the age of 4, 5 and 6 years had mastitis 22%, 7.3% and 1.9% in comparison with control 24 (9.2%) showing (P<0.05) significant association. It increases the odds of mastitis 18.391 (3.862-87.576), 111.189 (17.047-725.221) and 21.645 (1.936-241.956) times, with 95% confide-nce interval. In combination with other risk factors its effect become more prominent as the odd ratio increased 19.465 (4.264-88.850) times. Mekibib *et al*. (2010) showed that the univariate analysis of possible risk factors was extremely higher (P<0.05) with increasing age and the odds of mastitis increased after 4 year of age. This result is similar to (Amin *et al*., 2011) that the odd ratio of mastitis increased to 52 (33.5, 69.97) in 5 years of age.

Beside that it was observed that first and second month of lactation stage were significantly associated to mastitis. Odd ratio of lactation stage during initial months increases the odds of mastitis 37.074 (5.758-238.697) times with 95% confidence interval. Mastitis recorded in three and four months of lactation stage were 12 (4.6%)

and 5 (1.9%) showing increased in the odds of mastitis 77.033 (12.784-464.181 and 10.019 (1.937-51.812) times. After multiple logistic regression analysis, it was found to have significant association with mastitis, as increased the adjusted odd ratio (AOR) 99.311 (16.902-583.511) and 12.814 (1.971-48.852) times. On the base of this analysis, it is concluded that early stage of lactation is a potential risk factor for mastitis and the prevalence of mastitis was also high while prevalence decreased with increasing lactation period. The finding of current study coincides to the outcomes of (Svensson *et al*., 2006) that 31% of mastitis was recorded in first seven days of lactation, while maximum prevalence reported in period of 7 to 30 days after parturition.

It is evident from statistical analysis that the odds of mastitis increases 16.635 (2.299-120.371) and 25.277 (2.946-216.866) times with 95% confidence interval. The finding of this study support the earlier research work with solid significant association of mastitis with increasing parity (Mekibib *et al*., 2010). Finding of increasing prevalence of CM and SCM with the increasing number of parity are also in agreement with mostly previous reported research work (Amin *et al*., 2011). The prevalence of mastitis has increased gradually with increasing parity number of the studied goats. This is in close agreement to other findings (McDougall *et al*., 2002).

Table II. Univariate and multivariate logistic regression analysis of possible risk factors associated with mastitis in beetal goats.

Factors	Control (%)	Mastitis (%)	Univariate analysis		Multivariate analysis	
			OR (95%)	P-value	OR (95%)	P-value
Age						
2 years	75 (28.8%)	5 (1.9%)	.00		.00	
3 years	82 (31.5%)	9 (3.5%)	1.570 (.319-7.732)	.579	1.969 (.426-9.113)	.386
4 years	24 (9.2%)	22 (8.5%)	18.391 (3.862-87.576)	.000	19.465 (4.264-88.850)	.000
5 years	11 (4.2%)	19 (7.3%)	111.189 (17.047- 725.221)	.000	91.745 (14.491-580.867)	.000
6 years	8 (3.1%)	5 (1.9%)	21.645 (1.936-241.956)	.013	21.140 (1.813-246.450)	.015
Lactation stage						
1 month	10 (3.8%)	14 (5.4%)	.00		.00	
2 months	14 (5.4%)	26 (10.0%)	37.074 (5.758-238.697)	.000	23.823 (4.257-133.313)	.000
3 months	30 (11.5%)	12 (4.6%)	77.033 (12.784-464.181	.000	99.311 (16.902-58.511)	.000
4 months	71 (27.3%)	5 (1.9%)	10.019 (1.937-51.812)	.006	12.814 (1.971-48.852)	.005
5 months	75 (28.8%)	3 (1.2%)	1.346 (.238-7.624)	.737	1.159 (.207-6.472)	.867
Parity						
1	43 (16.5%)	3 (1.2%)	.00		.00	
2	58 (22.3%)	8 (3.1%)	3.007 (.409-22.086)	.279	2.307 (.348-15.280)	.386
3	64 (24.6%)	12 (4.6%)	2.228 (.323-15.383)	.416	1.666 (.266-10.417)	.585
4	24 (9.2%)	23 (8.8%)	16.635 (2.299-120.371)	.005	12.868 (2.016-82.116)	.007
5	11 (4.2%)	14 (5.4%)	25.277 (2.946-216.866)	.003	20.295 (2.665-154.542)	.004
Rearing area						
Hygienic	108 (41.5%)	24 (9.2%)	.00			
Unhygienic	92 (35.4%)	36 (13.8%)	2.989 (0.965-9.262)	.058		

Table III. Antibiotic sensitivity pattern of isolates against antibiotics.

Antibiotics sensitivity	Bacterial isolates					
	S. aureus	***E. coli***	***Streptococcus***	***P. aeruginosa***	***Salmonella***	***K. pneumonia***
Gentamycin	92%	87%	86%	80%	78%	77%
Ciprofloxacin	88%	74%	82%	76%	74%	72%
Amoxicillin	82%	74%	75%	72%	70%	68%
Oxytetracycline	80%	70%	68%	70%	66%	64%
Sulpha-trimethoprim	74%	70%	68%	65%	60%	54%
Kanamycin	70%	66%	60%	58%	54%	50%

It was further found that mastitis in goats that were living in unhygienic area were 36 (13.8%) among healthy control of same group showing (P<0.05) significant association. It increases the odds of mastitis 2.989 (0.965-9.262) times. However same result was described by (Bergonier *et al*., 2003) that environmental factors like humidity, litter, wet bedding, forage, mixed reservoirs, housing, clusters, and feed stuffs are the major determinant of mammary pathology in dairy goats. Similar results also concluded by (Amin *et al*., 2011) and found the prevalence higher in a goats rearing in unhygienic area and farming goats than those are managed under subsistence system.

Antibiotic sensitivity

Highest prevalence of *Staphylococcus aureus* was 25.4% followed by *Escherichia coli* 20.3%, Streptococcus 16.9%, and *Streptococcus agalactiae* 6.8% other streptococcus 10.1%, *Pseudomonas aeruginosa* 15.2%, *Salmonella* 13.5% and *Klebsiella pneumonia* 8.4% in lowest prevalence. The similar prevalence has also been recorded by (Contreras *et al*., 2007). However, relative difference in prevalence and bacterial isolates of goat mastitis may be due to difference in geographic regions. The main bacterial species involved in bacteriology of both clinical and sub clinical mastitis of dairy goat are *Staphylococcus aureus, Escherichia coli, Streptococcus, Salmonella*, *Pseudomonas species* and *Klebsiella pneumonia*. Similarly, the study conducted by Wakoya *et al*. (2006) concluded that staphylococcus species was the major pathogen identified in Ethiopia, while *Escherichia coli*, Streptococcus species, Pseudomonas, *Klebsiella pneumonia*, corynebacterium, bacillus and salmonella species were also identified.

Table III shows the antimicrobial sensitivity of the identified bacteria isolated from goat's milk samples against antibiotics like gentamycin, ciprofloxacin, oxytetracycline, amoxicillin, sulfa-trimethoprim and kanamycin. These finding are similar to those of (Anjum *et al*., 2010) who reported that gentamicin, ciprofloxacin, amoxicillin norfloxacin, and kanamycin were most effective among the 13 antibiotics tested *in vitro*. However, (Islam *et al*., 2012) reported that gentamicin treatment of goats and sheep with sub-clinical mastitis increased milk production significantly. The response of host immune system was a significant factor to the causative bacteria for the austerity and regulation of mastitis (Bannerman *et al*., 2009). The probable justification for resistance of bacterial organisms to the existing antibiotics could be due unselective and prolonged use of antibiotics by individual farmers without prescription of professionals.

CONCLUSION

This study will be useful for veterinarians and researcher for the medication of mastitis by using drug of choice according to the occurrence of pathogens in these areas.

Statement of conflict of interest

The authors have declared no conflict of interest.

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