Epidemiological Study of Mastitis in Three Different Strains of Beetal Goat in Selected Districts of Punjab, Pakistan

Muhammad Ijaz Saleem1, Muhammad Saqib1, Muhammad Sajjad Khan2, Ghulam Muhammad1 and Sajjad ur Rehman3

1Department of Clinical Medicine and Surgery; 2Institute of Dairy and Animal Sciences; 3Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan
*Corresponding author: drijazsaleem@gmail.com

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A B S T R A C T

Mastitis in goats, analogous to dairy cattle, is among the momentous diseases of economic value in all parts of the world. Local information of epidemiology of goat mastitis is scanty. In the present study, considering geographical distribution of Beetal goat, 172 flocks were randomly pulled out by “Survey tool box” to reduce biased sampling from peri-urban and urban areas of six districts of Punjab. By following the proportionate sampling strategy and assuming the highest population (70%) of Beetal all over the Punjab, Beetal strains were screened for the prevalence of mastitis from Faisalabad, Jhang, Chiniot, Bahawalpur, Muzzafargarh and Rajanpur districts. Screening of the flocks with surf field mastitis test indicated that overall prevalence of caprine mastitis in Beetal breed was 17.39% while in Beetal Faisalabadi strain 16.53% from 116 flocks, in Beetal Makhicheeni 18.06% from 32 flocks and in Beetal Nuqri 19.33% from 24 flocks. Microbiological examination of milk samples showed various types of mastitic microorganisms. The predominant isolates included Staphylococcus (S). hyicus (45%), S. xylosus (19%), S. simulans (16%), S. aureus (9%) and unidentifiable Staphylococcal species (11%). Antibiotic sensitivity test was conducted on the most prevalent isolates. S. aureus and S. hyicus showed sensitivity to eight antibiotics: amoxicillin, ampicillin, lincomycin, sulfamethoxazole+trimethoprim, novobiocin, enrofloxacin, amoxicillin+ clavulanic acid and oxytetracycline while S. xylosus was sensitive to all antibiotics except novobiocin and S. simulans was sensitive to lincomycin, sulfamethoxazole+trimethoprim, enrofloxacin and resistant to all other antibiotics. The study found over all 16.74% mastitis in different strains of Beetal. The most important parameters as; type of housing, farm hygiene, age, stage of lactation, parity, color, body condition score, teat ends, SFMT score and decreased milk yield were found pertinent risk factors so that prompt attention required for mastitis control in goats.

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INTRODUCTION

The goat is an important animal in many parts of the world, where it is kept as a source of meat, milk and fiber (DaMassa et al., 1992). It is often dubbed as the poor man’s cow in subcontinent. Pakistan is the 3rd largest goat rearing country in the world (Khan et al., 2008). In Pakistan, the economic contribution from goat source is 0.719 million (M) tons of milk, 0.6 M tons of mutton and 22 million hides (Economic Survey of Pakistan 2016-17). Goats, particularly of milk breeds, are worthy source of income for small households and are well recognized for providing nutritional surrogate, particularly for the growing infants in the rural areas of Pakistan.

Goats can bear hard as well as harsh environments in various agro-climatic conditions, predominantly in the tropics and sub-tropics. About 90% goat population is found in the developing countries whereas continent wise, Asia always stand first by producing 80% of total milk mainly from Pakistan, Turkey, China, Bangladesh, Iran and India (Khan et al., 2008).

More than 25 breeds of goat have been reported in Pakistan including the Northern Areas and Azad Jammu and Kashmir. Goats of Beetal, Diara Din Panah (DDP),
MATERIALS AND METHODS

Study locales and settings: For making the present research more demographic and diverse, three areas of Central Punjab (Faisalabad, Chiniot and Jhang) and three areas from Southern Punjab (Rajanpur, Bahawalpur and Muzaffargarh) were selected to conduct this study. Among these districts, three sites were selected based on home tracts of important strains of Beetal goat: Site-I Bahawalpur and Muzaffargarh districts for Beetal Makhichieeni, Site-II Rajanpur district for Beetal Nuqri and Site-III Faisalabad Division for Beetal Faisalabadi or Lyalpuri. The duration of research was about 18 months (January, 2016 to June, 2017).

Sample size and sampling: Sample size was calculated from the expected prevalence of about 20% (known disease status) with confidence interval of 95% and absolute desire precision 5%. Expected prevalence of 20% was deduced from the average prevalence of goat mastitis in three previous studies (Rashid et al., 2017, Rizwana et al., 2016 and Najeeb et al., 2013). The sample size was estimated using the following equation (Thrusfield, 2007). 

\[ n = 1.96^2 \times P_{exp} \times (1 - P_{exp}) / d^2 \]

Where: 
- \( n \) = required sample size
- \( P_{exp} \) = expected prevalence
- \( d \) = desired absolute precision

The number of samples thus calculated was subjected to the following equation for adjusted number to reach the maximum number of samples (Thrusfield, 2007): 

\[ n_{adj} = \frac{(N \times n)}{(N + n)} \]

Where: 
- \( N \) = total population
- \( n \) = calculated sample size through formula

Following the proportionate sampling strategy and assuming the highest population (70%) of Beetal all over Punjab, 172 flock of Beetal from the selected districts and screened out respectively for the prevalence of mastitis in three strains of Beetal goat.

Epidemiological parameters: Epidemiological parameters including age, stage of lactation, parity, stage of pregnancy, length of lactation period, amount of milk, farming system, housing and management conditions, previous disease, vaccination, deworming, and treatment history were recorded on a predesigned questionnaire. Previous milk production and any mastitis control strategy (post milking teat dipping and dry period antibiotic therapy) were also recorded.

Diagnostic procedures: Field diagnosis of both clinical and sub-clinical mastitis was performed by complete physical examination of udder and Surf Field Mastitis Test (SFMT) in all milking animals of selected goat farms. SFMT score 1 was considered as negative whereas score 2 traces, 3 positive (weak degree of gel formation), 4 distinct positive and 5 for strong positive for sub-clinical mastitis (Schalm et al., 1971). The SFMT score of 2 (traces) was considered healthy milk because goat milk has intra-cytoplasmic particles that resemble with somatic cells (Smith and Roguinsky, 1977).
Surf Field Mastitis Test (SFMT) scores: This test was performed as described previously (Schalm et al., 1971; Muhammad et al., 1995 and Muhammad et al., 2010). The SFMT is a CMT-like test which has been demonstrated to be comparable to CMT in terms of sensitivity and specificity of mastitis detection (Badiuzzaman et al., 2015). The reactions of these tests were interpreted as shown in Table 1 (Schalm et al., 1971).

Microbiological examination of milk: Surf Field Mastitis Test (SFMT) positive milk samples were collected aseptically in sterile vials by recording the udder halves and SFMT score (Muhammad et al., 2010). These milk samples were transferred to the Mastitis Research Laboratory, University of Agriculture, Faisalabad, for further microbiological examination. The microbiological assays were performed as described by National Mastitis Council Inc., USA (Anonymous, 1990). Briefly, for the isolation of mastitic pathogens, milk samples were aseptically cultured at 37°C for 24 hours on blood agar and MacConkey’s agar. Gram-positive, catalase-positive, α and β hemolytic cocci isolates were presumptively identified as Staphylococci and subjected to coagulase test using rabbit plasma (at 4 hours) for grit of coagulation property of organism. For species identification of Staphylococci, biotyping of the isolates was conducted by using a commercial kit (api-STAPH; BioMerieux, France). Latex Slide Aglutination test using Staphytect plus kit (Oxoid, Ltd, Basingstoke, Hampshire, UK) was conducted to check the presence of protein A, clumping factor and certain polysaccharides that are found entirely in S. aureus.

Antibiotic sensitivity test: Disc diffusion method on Mueller-Hinton medium was used for the sake of antibiotic susceptibility profiles of the prevalent mastitic organisms following the guidelines (Anonymous, 2012).

Statistical analysis: The data obtained were analyzed by Chi Square test for the prevalence, incidence and other risk factors associated with mastitis (Steel et al., 1997) in Table 3.

RESULTS AND DISCUSSION

Goat population of selected area: The total reported goat population in selected districts was 4,648,234 heads (Anonymous, 2006). According to Livestock Census, it was observed that Muzaffargarh is the biggest city of keeping goats among selected districts followed by Bahawalpur (1044722 head), Faisalabad (904918 head), Rajanpur (633524 head) and 503496 head for Jhang and Chiniot because after Livestock Census 2006, Chiniot was declared as independent district so that their goat population is divided equally.

Locales and Beetal goat strains distribution: A total of 3195 goats from 172 flocks were collected for screening of mastitis from 6 goat populated districts of Punjab (3 from central and 3 from southern Punjab) to make this study more diverse in form of climate and geography. Depending upon the population of goats in each district, random flocks were pulled out by using ‘survey tool box’ to remove biased sampling. By considering the reported goat population of selected districts (Anonymous, 2006), Beetal Faisalabadi 1673 goats were screened out for mastitis from 85 flocks from district Faisalabad. From Jhang, Beetal Faisalabadi 201 goats from 14 flocks were added in this study, from Chiniot, Beetal Faisalabadi 359 goats from 17 flocks, from Bahawalpur, 631 Beetal Makhi Cheeni goats from 32 flocks and from Rajanpur, 331 Beetal Nuqri goats from 24 flocks were selected.

Goat strains related distribution of samples: Proportionate sampling was conducted according to strains population in six selected districts. Out of 3195 goats sampled, 2233 (69.89%) were selected for Beetal Faisalabadi goats from the districts of Faisalabad, Chiniot, Jhang, Bahawalpur and Rajanpur. For Beetal Makhi Cheeni 631 goats (19.74%) were selected from districts Bahawalpur and Muzaffargarh while for Beetal Nuqri 331 goats (10.35%) from Rajanpur were screened out for mastitis (Table 2).

### Table 1: Guidelines used for interpretation (scoring) of Surf Field Mastitis Test (SFMT)

| Symbol (score) | Suggested meaning | Description of visible reaction |
|---------------|-------------------|--------------------------------|
| 1             | Negative          | Mixture remains liquid.         |
| 2             | Traces            | A slight slime forms and was seen by tipping the paddle back and forth. |
| 3             | Week Positive     | A distinct slime but with no tendency towards gel formation. |
| 4             | Distinct Positive | The mixture thickens immediately with the gel formation. |
| 5             | Strong Positive   | A gel forms with convex surface of mixture, which is so viscous that it adheres to the bottom of the receptacle of the test paddle. |

### Table 2: Prevalence of mastitis according to geographical area

| Area          | No. of goats | Mastitic goats | Prevalence (%) | p-value | Confidence interval (95%) |
|---------------|--------------|----------------|----------------|---------|--------------------------|
| Faisalabad    | 1673         | 238            | 15.42          | 0.000   | 13.77-17.23              |
| Jhang         | 201          | 28             | 13.93          |         | 09.82-19.40              |
| Chiniot       | 359          | 71             | 19.78          |         | 15.99-24.21              |
| Bahawalpur    | 425          | 80             | 18.82          |         | 15.39-22.81              |
| Rajanpur      | 331          | 64             | 19.33          |         | 15.45-23.94              |
| Muzaffargarh  | 206          | 34             | 16.50          |         | 12.05-22.17              |
| Total         | 3195         | 535            | 15.20          |         | 15.49-18.07              |

| Strain (Beetal) | No. of goats | Mastitic goats | Prevalence (%) | p-value | Confidence interval (95%) |
|-----------------|--------------|----------------|----------------|---------|--------------------------|
| Faisalabadi     | 2233         | 357            | 15.99          | 0.192   | 15.30-18.40              |
| Makhi Cheeni    | 631          | 114            | 18.07          |         | 15.26-21.26              |
| Nuqri           | 331          | 64             | 19.34          |         | 15.45-23.94              |
| Total           | 3195         | 535            | 16.74          |         | 15.49-18.07              |
Epidemiology of mastitis in different strains of Beetal: 
The study inferred overall prevalence of 16.74% caprine mastitis in Beetal goat while strains of Beetal Faisalabadi, Beetal Makhicheeni, and Beetal Nuqri suffered 15.99%, 18.07%, and 19.34%, respectively. The overall strain-wise prevalence in Beetal goat was observed as highest 19.34% in Beetal Nuqri followed by 18.06% in Beetal Makhicheeni and 15.99% in Beetal Faisaladadi (Table 2). The major species of Staphylococcus genus found in caprine mastitis were S. hyicus, S. xylosus, S. simulans, S. aureus. Some unidentifiable Staphylococcal species were also observed.

Bacteriological study of caprine mastitic pathogens: A total of 535 mastitis positive milk samples out of 3195 goats were screened out by using SFMT, with recording goat identity no., udder half marking and score of SFMT (Muhammad et al., 2010). In this study, 357 mastitic samples from Beetal Faisalabadi, 114 from Beetal Makhicheeni and 64 from Beetal Nuqri were studied for microbiological assays. Biotyping of isolates showed that coagulase negative Staphylococci are the main class of bacteria frequently observed. Furthermore, different types of bacteria were found in mastitic samples. E. coli from Enterobacteriaceae, Klebsiella, Proteus, Enterobacter from Micrococcaceae, S. aureus, Micrococcus spp and various spp. of Streptococcus, Bacillus, Corynebacterium and Pseudomonas spp. were also found.

Mastitis prevalence in small ruminants has been reported by Fox and Gay, 1993; Bergonier et al., 2003; Contreras et al. 2003; Contreras et al. 2007; Ali et al. 2010; Megersa et al., 2010; Islam et al., 2011; Islam et al., 2102; Bourabah et al., 2013 and Rashid et al., 2017. The current study’s findings agree with outcomes of study reported by Megersa et al. (2010). Contradiction in prevalence outcome was found in studies conducted by
Contreras et al., 2003 who revealed 9.4-47%, Rashid et al., 2017 showed 21.68% in winter and 25.70% in summer respectively and Rizwan et al., 2016 who reported average 18.29% prevalence of mastitis in different breeds of goat in Pakistan. The findings of this study is also in line with the results of Contreras et al. 2007 and Fox and Gay, 1993 who showed the range of prevalence from 5-30% and 7-40% respectively. The current finding is strongly differing with the results of Hall and Rycroft, 2007 showed 33%, Ali et al. 2010 revealed 30.6%, Islam et al., 2011 reported 6%, Islam et al., 2011 reported 4%, Aqib et al., 2018 showed more than 50%, Bergronier et al., 2003 showed 20-50% and Bourabah et al., 2013 reported 33.9% prevalence. This difference could be due to environmental differences and differences in animal breeds and strains, fluctuation of immune response, housing and management system, all other farmers’ and host’s related determinants, with different diagnostic methods and different level of expertise for diagnosis as well as result interpretations.

Farm management and housing related determinants for mastitis: Current study revealed that type of housing, farm hygiene, age, stage of lactation, parity, color, body condition score, teat ends, SFMT score, milk taste, milk consistency, milk color and milk yield have significantly (p<0.005) associated with mastitis. But it was observed that floor type, condition of floor, source of drinking water, feeding practices, use of mineral, general vaccination, deworming, milking practices, teat shape, teat symmetry, teat injury and supernumerary teat has non-significant (P>0.05) effect on mastitis. This difference of non-significance could be due to environmental differences, differences in strains, fluctuation of immune response, housing and management system, all other farmers’ as well as host’s related determinants. Table 3 depicts that there is significant relation of color with mastitis prevalence in all three strains of Beetal. It was observed that color Black & White 31.9% followed by Black 20.6%, Blackish brown 16.3%, Brown Splashed 11.2%, Brown & White 8.5% while 0.8% in Brown Black. This information is reported for the first time in Pakistan and the reference availability for comparison might be difficult but its significant effect has been observed.

Varying level of education has a significant effect (P<0.05) on mastitis as it was observed that as the level of education increases, marked decline was seen in mastitis prevalence that is due to better awareness about farm hygiene, sanitation, disinfectant, social as well as farmer meetings, watching TV for news in educated farmers community. The flock size has significant effect (P<0.05), as the flock size of 21-40 goats has 46.5% followed by 1-20 goat head 28.8% and more than 40 head 23.7%. This finding is very close to the results about flock size of Megersa et al. 2010 and Rashid et al., 2017. Body condition score has shown that there was highly significant (P<0.001) difference presenting poor and good having 42.4% and 15.2%, respectively while excellent to be 0.1%. The findings were again in agreement with Megersa et al. 2010 and Rashid et al., 2017. The calculated housing system has shown highly significant (P<0.05) effect on mastitis as open area showed 69.0% followed by backyard shed 29.4% and street presenting 0.5% mastitis prevalence. Such variation speaks of cleaning as difficult or impossible for open area as there is no restriction for microbial contamination. The results were concordat with findings of Megersa et al. 2010 and Rashid et al., 2017.

Farm hygiene has shown significant (P<0.05) effect on mastitis as normal 47.1% followed by very poor 33.6%, poor 16.1% and good 2.1% was observed. Table 3 depicts that as the level of farm hygiene is attaining better score, marked decline was seen in mastitis. This finding is also strongly supported by Ameh et al. 2000 and Rashid et al., 2017. It was observed that parity of 3-4 kidding has 53.0% followed by 1-2 kidding 29.9% while 5 and above kidding 16.4%. The chance of mastitis is directly proportional to parity as animal to face more numbers of infections depending upon the environmental conditions, milking practices and sanitations. The results are in line with findings of Ndegwa et al., 2000, Moroni et al., 2005 and McDougall et al., 2002 showing strong correlation between occurrence of mastitis and the passage of time. As the age increases, there is greater risk of diseases likewise in case of mastitis. Age group 4-5 year showed 52.3% followed by 1-3 year 33.6% and more than 5 year 13.0%. Prevalence of mastitis has increased with increasing age in goats as reported by Ameh and Tari, 2000, McDougall et al., 2002 and Moroni et al., 2005 as observed in our study that occurrence of disease was more in 3-5 years of age in all strains of Beetal.

Ease of milking, milk leakage, blood in milk, SFMT score, milk taste and milk yield have very strong connection with the occurrence of mastitis and showing significant (P<0.05) effect. Occurrence of mastitis was accompanied with marked hardess of teat during milking, milk leakage, blood in milk in very severe attack of mastitis, severity of SFMT score and decreased milk yield up to 20%. These findings are very close to the results presented by Ameh and Tari, 2000; McDougall et al., 2002; Ndegwa et al., 2000; Moroni et al. 2005; Athar et al., 2007; Ahmad et al. 2008, Ali et al., 2010; Megersa et al. 2010; Islam et al., 2011 and Rashid et al., 2017.

Antibiotic sensitivity test of prevalent isolates: In the present study, among the selected isolates, S. aureus and S. hyicus were sensitive to amoxicillin, ampicillin, lincomycin, sulfamethoxazole+Trimethoprim, novobiocin, enrofloxacin, amoxicillin+clavulanic acid and oxytetracycline while S. xylosus was sensitive to all except novobiocin. S. simulans was sensitive to lincomycin, sulfamethoxazole+Trimethoprim and enrofloxacin (Table 4). These study findings are comparable with that of Athar et al., 2007; Ali et al., 2010; Islam et al., 2011 and Najaee et al., 2013 who also reported similar kind of trend in antibiotic susceptibility of S. aureus and Coagulase Negative Staphylococcal Spp. Contrarily, Chaudhry and Azam (1995) reported gentamicin to be the most effective in vitro antibiotic followed by chloramphenicol, kanamycin, oxytetracycline, cotrimoxazole, penicillin, doxycycline, ampicillin, and nystatin. Haphazard use of certain antibiotics might have conferred resistance in the present microorganisms and might lead to therapeutic failure. Therefore, use of antibiotics based on antibiotic susceptibility profile is highly warranted to achieving a better cure rate.
Conclusions: The study found over all 16.74% mastitis in different strains of Beetal goat with significantly variable mastitis distribution in studied areas. Type of housing, farm hygiene, age, stage of lactation, parity, color, body condition score, teat ends, SFMT score, milk taste, milk consistency, milk color and milk yield were found pertinent risk factors. The major staphylococcal species with decreasing order were S. hyicus, S. xylosus, S. simulans, S. aureus and unidentified staphylococcal species. The epidemiology of goat mastitis is fulfilling with pathogenic involvement of bacteria requiring immediate attention.

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Authors contribution: MIS and MS designed and conducted the study. MS helped in microbiological assay. MSK performed statistical analysis. GM and SUR observed and guided in research work. All authors contributed in drafting the manuscript.

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