Research Article



Prevalence of Subclinical Mastitis in Dairy Goats Caused by Bacterial Species

Murk Pirzada¹, Kanwar Kumar Malhi^{1*}, Asghar Ali Kamboh¹, Rahmatullah Rind¹, Shahid Hussain Abro¹, Shakeel Ahmed Lakho¹, Khalique ur Rehman Bhutto², Noor ul Huda³

¹Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, 70060, Pakistan; ²Vaccine Production Unit Tandojam, Pakistan; ³Department of Veterinary Medicine, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, 70060, Pakistan.

Abstract | Present study was carried out to demonstrate the bacterial organisms responsible for subclinical mastitis in goats. A total of 200 milk samples were collected from two hundred quarters of goats from surroundings of Tandojam and analysed for subclinical mastitis using California Mastitis Test (CMT) and conventional bacterial culture technique. A total of 76 (38%) milk samples were found positive by CMT, those also showed bacterial growth on culture media. Among those 43 (56.58%) were from right quarters whereas remaining 33 (43.42%) were detected with subclinical mastitis from left quarters. Of these positive samples, 60 (78.94%) were recorded as pure (one bacterial specie), whereas 16 (21.05%) samples were found contaminated with mixed bacterial species. A total of nine bacterial species were isolated from the milk samples. The incidence of *Staphylococcus aureus* was found highest (36.84%) in both right and left quarters of subclinical mastitis, followed by *Bacillus subtilis* (18.42%), *Bacillus cereus* (10.52%), *Proteus vulgaris* (9.21%), *Citrobacter* species (6.57%), *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus epidermidis* (5.26% each) and *Streptococcus agalactiae* (2.63%). The incidence of bacterial organisms was found higher in right side quarters (56.58%) as compared to left quarters (43.42%). These results indicated a higher prevalence rate of subclinical mastitis in dairy goats in surroundings of Tandojam, thus suggested the use of effective udder disinfectants, and improved sanitation at farms; and also proposed a regular screening of animals for early detection and management of subclinical mastitis in goats.

Keywords | Mastitis, Goat, Bacterial species, Udder, Tandojam

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*Correspondence | Kanwar Kumar Malhi, Sindh Agriculture University, Tandojam, Pakistan; Email: kanwar103@gmail.com

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INTRODUCTION

oat (*Capra hircus*) is a versatile animal and known as poor man's cow in Pakistan. The goats are main source of meat and dairy products (Haenlein, 2004). Of all the domestic animals, the goat undoubtedly has the greatest range of adaptations. Goat thrives well on poor agricultural lands, where they browse on incidental vegetation. In arid areas where vegetation is scant and is too steep for cultivation, goats roaming more widely than other herbivores and meet their requirements from browsing on weeds neglect-

ed by others, they has higher tolerance to feed deficient in crude protein and certain minerals. Goat milk is cheap, wholesome, easily digestible and nutritious (Panicker et al., 2015). It is recommended for use in dyspepsia, peptic ulcer and pyloric stenosis. Therapeutic potential of goat milk for liver dysfunction, jaundice, biliary disorders, acidosis and insomnia have also been reported (Miranda et al., 2010).

Mastitis is an infection of udder tissue that leads clinical to subclinical inflammation and transitory to permanent blocking of milk ducts. Fungi, yeast, virus and bacteria can



cause this udder infection. Milk quality is mainly affected by infection of the mammary gland which causes mastitis (Boscos et al., 1996). Subclinical mastitis is the inflammation of udder that cannot be detected by clinical methods such as inspection, palpation and organo-leptic examination (Contreras et al., 2007). The subclinical mastitis has also adverse effects on the hygienic quality and physiochemical properties of milk. The subclinical mastitis is characterized by having no visible signs either in the udder or in the milk, but the milk production decreases. The real diagnosis of subclinical mastitis is based on somatic cell count and by bacteriological culturing of milk. Physiologically, goat milk contains a larger number of somatic cells as compared to cows and sheep. Milk from normal quarters generally ranges of 600,000 - 800,000 somatic cells per milliliter (Monsang et al., 2014; Paape et al., 2001). The bacterial pathogens such as Staphylococcus aureus, Streptococcus agalactiae and other environmental pathogens have been reported as the cause of mastitis and subclinical mastitis in dairy goats (Virdis et al., 2010). Byeng et al. (2007) reported the Staphylococcus aureus, Pseudomonas aeruginosa and coagulase negative Staphylococcus as the most prevalent pathogens in subclinical mastitis of goats. It has been estimated that subclinical mastitis is more prevalent in goats as compared to cattle. Moreover, difference in causative agents responsible for subclinical mastitis in different animal species have also been reported (Paape and Capuco, 2001).

Keeping in view the above facts, the present study was therefore designed to investigate the prevalence and incidence of subclinical mastitis caused by bacterial agents in dairy goats in surroundings of Tandojam, Hyderabad. It will be helpful to formulate the effective control and preventive strategies for subclinical mastitis in dairy goats.

MATERIALS AND METHODS

SAMPLE COLLECTION

For present study, quarter milk samples (n= 200) were collected from dairy goats from surroundings of Tandojam, Hyderabad to analyse the subclinical mastitis caused by bacterial species. Samples were collected according to standard procedure described by National Mastitis Council, USA (Oliver et al., 2004). Only those animals were included in the study that not treated with any kind of antimicrobial agent in last 120 hours. Prior to sample collection, teats were cleaned thoroughly with a disposable paper towel and then dipped with a disinfectant. Teat ends were sterilized with a cotton swabs moistened with 70% isopropyl alcohol. After discarding the first few streams, about 10 mL milk was collected in sterilized sample bottles. Immediately after collection, samples were cooled and transferred to the laboratory on ice, where stored at -20°C until analysed.

CALIFORNIA MASTITIS TEST (CMT)

Caprine subclinical mastitis was analyzed by the California mastitis test (CMT) using 10% Teepol (Leucocytest®; Synbiotics Corporation, Paris). The CMT was done as per the instructions of manufacturer. In brief, 2 mL milk was stripped into each well of the test plate from individual teats and an equal amount of test reagent (10% Teepol) was added to the milk. The plate was stirred for 10 seconds to mix the test reagent with milk. The plate was kept at room temperature for 30 seconds, then any visible change (clot) was noted in the wells.

BACTERIOLOGICAL ANALYSIS

Milk samples were examined using conventional culture technique as described previously (Khan et al., 2015). Briefly, 10 μ L milk sample was plated onto nutrient agar and blood agar that was supplemented with 5% defibrinated horse blood (Difco, Michigan, USA) Plates were incubated at 37°C for 24 h and observed for bacterial growth. On primary culture media, bacteria were identified tentatively on the basis of colony morphological features, hemolytic properties on blood agar and catalase test. Isolates identified presumptively as *Staphylococci* were confirmed according to procedure described earlier (Nazia et al., 2015), whereas other bacterial isolates were confirmed according to method of Gillespie and Oliver (2005).

DATA ANALYSIS

The results were analysed by using Microsoft Office Excel 2012, and were presented in percentages that calculated by dividing the number of samples positive for sub-clinical mastitis with total number of samples analysed x100.

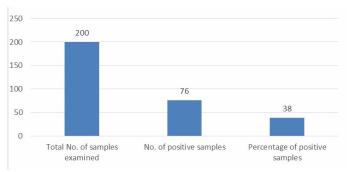


Figure 1: Overall prevalence of subclinical mastitis in dairy goats (revealed by California mastitis test)

RESULTS AND DISCUSSION

PREVALENCE OF SUBCLINICAL MASTITIS IN DAIRY GOATS ANALYSED BY CMT

In present study, CMT was used to analyse the subclinical mastitis in dairy goats in surroundings of Tandojam. The overall number and percentage prevalence of subclinical mastitis recorded by CMT was presented in Figure 1. Of 200-quarter samples, 76 (38%) were found positive,

while 124 (62%) were recorded as negative. In right quarters, 40 samples were found with subclinical mastitis and the prevalence was observed as 52.63%. While in the left quarters, 36 samples were found with subclinical mastitis and the prevalence was calculated in the left halves as 47.36% (Figure 2). These results are in agreement with the results of Islam et al. (2012), those declared a 36% prevalence of subclinical mastitis in goats in Dhaka using CMT test. These results are also in close agreement with another study that reported a 35.5% prevalence of subclinical mastitis by CMT in goats at parturition (McDougall et al., 2002).

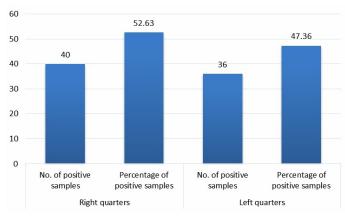


Figure 2: Prevalence of subclinical mastitis (analysed through California mastitis test) in right and left quarter of dairy goats

Our present results regarding the prevalence of subclinical mastitis in dairy goats were also similar to that of Manser (2000) who examined 170 milk samples from five herds of goats and declared 85 goats with subclinical mastitis. The prevalence of subclinical mastitis in different herds was recorded between 15- 79%. Sarker and Samad (2011) recorded the prevalence of subclinical mastitis in black Bengal goats and reported a significantly higher incidence in left udder halves (79.66 %) as compared to the right udder halves (20.34%). Whereas in present study the prevalence was recorded higher (52.63%) in right quarters as compared to left side quarters (47.36%). This inconsistency in results could be due to the breed differences (Mangi et al., 2015), as in present study dairy goats of different breeds were examined for prevalence of subclinical mastitis.

PREVALENCE AND INCIDENCE OF BACTERIAL SPECIES IN SUBCLINICAL MASTITIS MILK SAMPLES

The number and percentage incidence of each bacterial species isolated and recognized from subclinical mastitis milk samples of dairy goats were presented in Table 1 and Figure 3. All CMT positive samples were further confirmed as positive by bacterial culture method. From 76 positive mastitis milk samples, 60 (78.94%) were recorded as pure (one bacterial specie), whereas 16 (21.05%) samples were found with mixed bacterial infection (Figure 3). A total of nine bacterial species were recognized from sub-

clinical mastitis milk samples of goats. It includes *Staphylococcus aureus* (36.84%), *Bacillus subtilis* (18.42%), *Bacillus cereus* (10.52%), *Proteus vulgaris* (9.21%), *Citrobacter* species (6.57%), *Escherichia coli* (5.26%), *Micrococcus luteus* (5.26%), *Staphylococcus epidermidis* (5.26%) and *Streptococcus agalactiae* (2.63%). The incidence of bacterial organisms was found higher in right quarters (56.58%) as compared to left quarters (43.42%) (Table 1).

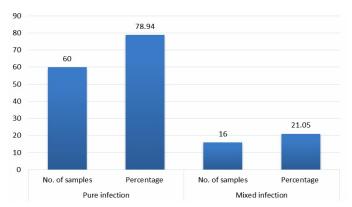


Figure 3: Incidence of pure and mixed bacterial species in subclinical mastitis milk samples of dairy goats

Table 1: Incidence of individual bacterial species in subclinical mastitis milk samples of dairy goats recovered through culture technique

Bacterial species	Occurrence in Right Quarters No. (%)	Occurrence in Left Quarters No. (%)	Total occurrence No. (%)
Staphylococcus aureus	15 (19.74)	13 (17.11)	28 (36.84)
Bacillus cereus	3 (3.95)	5 (6.58)	8 (10.52)
Citrobacter species	4 (5.26)	1 (1.32)	5 (6.57)
Escherichia coli	1 (1.32)	3 (3.95)	4 (5.26)
Micrococcus luteus	3 (3.95)	1 (1.32)	4 (5.26)
Proteus vulgaris	4 (5.26)	3 (3.95)	7 (9.21)
Staphylococcus epidermidis	3 (3.95)	1 (1.32)	4 (5.26)
Streptococcus agalactiae	2 (2.63)	0 (0.00)	2 (2.63)
Bacillus subtilis	8 (10.53)	6 (7.89)	14 (18.42)
Total	43 (56.58)	33 (43.42)	76 (100)

Batavani et al. (2003) examined periodic incidence of individual bacterial species in subclinical mastitis milk samples of goats. The most common bacterial isolates found from subclinical mastitis cases were coagulase negative *Staphylococci* (41%), *Bacillus cereus* (33%), *Staphylococcus aureus* (22%) and *Streptococcus* spp. (4%). Similarly, Zamin et al. (2010) recorded the higher incidence of *Staphylococcus aureus* in milk samples, while Manser (2000) recorded

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the incidence of coagulase-negative Staphylococci (80%), coagulate-positive Staphylococci (16%), alpha-haemolytica Streptococci (2%) and Pasteurella haemolytica (2%). Sarker and Samad (2011) isolated major bacterial pathogens from subclinical mastitis of goats. The bacterial pathogens isolated were: Staphylococcus spp., followed by Escherichia coli and Bacillus spp. and their incidence was recorded as. 38.98, 27.12 and 10.17%, respectively. The results regarding percentage incidence of each bacterial species in subclinical mastitis of goats recorded in this study were similar to the findings of the above workers. The higher incidence of Staphylococcus aureus in milk samples of subclinical mastitis of goats was recorded in this survey was also demonstrated by above workers. Escherichia coli are known as the most common environmental contaminants because these are found as a normal flora in the intestine of almost all farm animals (Ansari et al., 2014; Begum et al., 2014). From above workers, Sarker and Samad (2011) reported the incidence of Escherichia coli as a second most in subclinical mastitis milk samples, however in the present study we observed the Bacillus subtilis as second most prevalent organism. These variations might be due to differences in geographical location and/or management conditions at the farm (Abel et al., 2014).

The most common bacterial pathogen detected in both udder halves of the goat was *Staphylococcus aureus* and its incidence was noted 15 and 13 in the right and left quarters respectively. In consistent to our study, some other workers also reported the *Staphylococcus aureus* as a most common etiological agent in subclinical mastitis milk samples of both udder halves (Tyagi et al., 2013; Zamin et al., 2010).

CONCLUSIONS

From the present study, it was concluded that the subclinical mastitis in dairy goats caused by bacterial organisms is very high (38%) in the area. *Staphylococcus aureus* was found as the most dominant species that could be associated with other bacterial species that caused infection in the udders of goats. The higher infection of bacterial species was recorded in the right quarters in subclinical mastitis as compared to left side quarters. Present results suggested that improved sanitation at caprine farming and regular screening of animals should be carried out for early detection and management of subclinical mastitis in goats.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTION

Murk Pirzada and Noor ul Huda conducted the research work, whereas, Asghar Ali Kamboh, Shahid Hussian Abro and Rahmatullah Rind were the advisors. Kanwar Kumar Malhi wrote the manuscript, and Shakeel Ahmed Lakho, Shahid Hussain Abro and Khalique ur Rehman Bhutto done the proof reading of manuscript.

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REFERENCES

- •Abel FAS, Adeyemi OA, Eruvbetine D, Sogunle OM, Oluwole OB, Elemo GN (2014). Effect of stocking density and quantitative feed restriction on growth performance, digestibility, haematological characteristics and cost of starting broiler chicks. J. Anim. Health Prod. 2(4): 60–64. http://dx.doi.org/10.14737/journal.jahp/2014/2.4.60.64
- Ansari ARMIH, Rahman MM, Islam MZ, Das BC, Habib A, Belal SMSH, Islam K (2014). Prevalence and antimicrobial resistance profile of *Escherichia coli* and *Salmonella* isolated from diarrheic calves. J. Anim. Health Prod. 2(1): 12 – 15. http://dx.doi.org/10.14737/journal.jahp/2014/2.1.12.15
- Begum S, Hazarika GC, Rajkhowa S (2014). Prevalence of *Escherichia coli* from pigs and cattle. J. Anim. Health Prod. 2(3): 38 – 39. http://dx.doi.org/10.14737/journal. jahp/2014/2.3.38.39
- Batavani RA, Mortaz E, Falahian K, Dawoodi MA (2003). Study on frequency, etiology and some enzymatic activities of subclinical ovine mastitis in Urmia, Iran. Small Ruminant Res. 50(1-2): 145-150. http://dx.doi.org/10.1016/S0921-4488(03)00122-6
- •Boscos CA, Stefanakis, Alexopoulos Samartzi (1996).Prevalence of subclinical of breed, mastitis and influence parity, mammary lactation and bacteriological status on Coulter Counter Counts and California Mastitis Test in the milk of Saanen and autochthonous Greek goats. Small Ruminant Res. 21(2): 139-147. http://dx.doi.org/10.1016/0921-4488(95)00824-
- Byeng RM, Grant T, Steve PH (2007). Effect of subclinical intramammary infection on somatic cell counts and chemical composition of goats' milk. J. Dairy Res. 74: 204-210. http:// dx.doi.org/10.1017/S0022029906002378
- Contreras A, D Sierra, A Sanchez, JC Corrales, JC Marcoc, MJ Paape, C Gonzalo (2007). Mastitis in small ruminants. Small Rum. Res., 68: 145-153.
- •Gillespie BE, Oliver SP (2005). Simultaneous detection of mastitis pathogens, *Staphylococcus aureus*, *Streptococcus uberis*, and *Streptococcus agalactiae* by multiplex real-time polymerase chain reaction. J. Dairy Sci. 88: 3510–3518. http://dx.doi.org/10.3168/jds.S0022-0302(05)73036-8
- Haenlein GFW (2004). Nutritional value of dairy products of ewe and goat milk. J. Dairy Sci. 4: 159-178.
- •Islam MR, Ahmad MS, Alam MS, Rahman MS, Sultan T, Roh Y-S, Kim B (2012). Identification and antibiotic sensitivity of the causative organisms of sub-clinical mastitis in sheep and goats. Pak. Vet. J. 32(2): 179-182.
- •Khan A, Rind R, Shoaib M, Kamboh AA, Mughal GA, Lakho



- SA, Malhi KK, Nizamani AR, Yousaf A (2015). Isolation, identification and antibiogram of Escherichia coli from table eggs. J. Anim. Health Prod. 3(4): 99-103. http://dx.doi.org/10.14737/journal.jahp/2016/4.1.1.5
- •Mangi MH, Kamboh AA, Rind R, Dewani P, Nizamani ZA, Mangi AR, Nizamani AR, Vistro WA (2015). Seroprevalence of brucellosis in Holstein-Friesian and indigenous cattle breeds of Sindh Province, Pakistan. J. Anim. Health Prod. 3(4): 82-87. http://dx.doi.org/10.14737/journal. jahp/2015/3.4.82.87
- Manser PA (2000). Prevalence, causes and laboratory diagnosis of subclinical mastitis in the goat. Vet. Record. 118(20): 552-554. http://dx.doi.org/10.1136/vr.118.20.552
- McDougall S, Supré K, De Vliegher S, Haesebrouck F, Hussein H, Clausen L, Prosser C (2010). Diagnosis and treatment of subclinical mastitis in early lactation in dairy goats. J. Dairy Sci. 93(10): 4710-21. http://dx.doi.org/10.3168/jds.2010-3324
- •Miranda GC, Lama DL, Mattiello S (2010). The importance of social behaviour for goat welfare in livestock farming. Small Ruminant Res. 90(1-2): 1-10. http://dx.doi.org/10.1016/j. smallrumres.2010.01.006
- Monsang SW, Pal SK, Kumar M, Roy J, Sharma CS, Norjit SMN (2014). Bilateral mastectomy for successful management of chronic suppurative mastitis in a black bengal doe (*Capra hircus*). J. Anim. Health Prod. 2(2): 28 30. http://dx.doi.org/10.14737/journal.jahp/2014/2.2.28.30
- •Nazia, Malhi KK, Durrani NU, Kamboh AA, Lakho SA, Rind R, Abro SH, Soomro NM (2015). Prevalence of septic arthritis caused by *Staphylococcus aureus* in poultry birds at Tandojam, Pakistan. J. Anim. Health Prod. 3(3): 73-77.
- Oliver SP, Gonzalez RN, Hogan JS, Jayarao BM, Owens WE,

- ed. (2004). Microbiological procedures for the diagnosis of bovine udder infection and determination of milk quality. 4th edition. National Mastitis Council, Inc., Verona, WI.
- Paape MJ, Poutrel B, Contreras A, Marco JC, Capuco AVM (2001). Milk somatic cells and lactation in small ruminants.
 J. Dairy Sci. 84(1): 237-244. http://dx.doi.org/10.3168/jds. S0022-0302(01)70223-8
- Sarker H, Samad MA (2011). Udder- Halves-wise comparative prevalence of clinical and sub- clinical mastitis in lactating goats with their bacterial pathogens and antibiotics sensitivity pattern in Bangladesh. Bangladesh J. Vet. Med. 9(2): 71–78.
- Panicker SS, Kanjirakuzhiyil P, Koodathil R, Kanakkaparambil R (2015). Oestrous response and conception rate in Malabari cross-bred goats following two different oestrus synchronization protocols. J. Anim. Health Prod. 3(2): 39-42. http://dx.doi.org/10.14737/journal.jahp/2015/3.2.39.42
- •Tyagi SP, Joshi RK, Joshi N (2013). Characterization and antimicrobial sensitivity of *Staphylococcus aureus* isolates from subclinical bovine mastitis. J. Anim. Health Prod. 1(2): 20–23.
- •Virdis S, Christian Scarano, Francesca Cossu, Vincenzo Spanu, Carlo Spanu, Enrico Pietro Luigi De Santis (2010). Antibiotic Resistance in *Staphylococcus aureus* and coagulase negative staphylococci isolated from sar goats with subclinical mastitis. Vet. Med. Int. 111(6): 404-412.
- •Zamin A, Muhammad G, Ahmad T, Khan R, Naz S, Anwar H, Farooqi FA, Manzoor MN, AR Usama (2010). Prevalence of caprine sub-clinical mastitis its etiological agents and their sensitivity to antibiotics in indigenous breeds of Kohat, Pakistan. Pak. J. Life Soc. Sci. 8(1): 63-67.

