PREVALENCE, ANTIBIOTICS SUSCEPTIBILITY PROFILING, AND ASSOCIATED RISK FACTORS OF COLIFORM MASTITIS IN DAIRY GOATS

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ABSTRACT: This study aimed to determine the prevalence of coliform sub-clinical mastitis in dairy goats, their associated risk factors, and antimicrobial susceptibility patterns of *E. coli*. A total of 150 pure and local breed dairy goats were included in this study. Milk samples (n=150) were screened by the Surf Field Mastitis Test (SFMT). The *E. coli* isolated from mastitic milk were cultured on MacConkey's agar and confirmed through PCR. Antibiotic susceptibility profiling was done through the disc diffusion method. The associated risk factors and antibiotic susceptibility profile were analyzed by chi-square, logistic regression, and ANOVA. Out of 150 screened animals, 26% pure breed and 32% local breed were positive for SCM. The higher prevalence of SCM with significant association was noted in local breeds (32%), early lactation stages (57.14%). The different risk factors showed statistically significant associations such as cylindrical teat shape, daily manure removal, pipeline water source, use of oxytocin for milk let-down and post milk teat dipping. Amoxicillin, cloxacillin, gentamicin, colistin sulphate, procaine-penicillin, enrofloxacin, ampicillin, oxytetracycline, florfenicol, and streptomycin were used for anti-bio-gram profiling against *E. coli* isolates. Enrofloxacin, gentamicin, and ampicillin were found to be most effective *in vitro*. The study emphasizes breed variations, age, and management practices as influential factors for the prevalence of SCM. The effective control strategies, early detection, and prudent antibiotic use are recommended to alleviate SCM.

Keywords: Molecular epidemiology; Risk factors; ampicillin; Antibiotic profiling, antibiogram profiling.

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INTRODUCTION

Mastitis is the inflammation of the parenchyma of the mammary gland. It not only reduces milk production but also leads to detrimental changes in the milk composition (Grispoldi *et al*., 2019). The most prevalent managemental illness of dairy animals is SCM (Bobbo *et al*., 2017; Viguier *et al*., 2009). During SCM, milk production decreases by 10-26% (Dhakal *et al*., 2007). *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Escherichia Coli*, and *Corynebacterium pyogenes* are repeatedly isolated from the infected milk of SCM lactating dairy animals. 80% subclinical mastitis in bovines is due to coliform microbes (Fahim *et al*., 2019). Coliform mastitis is more prevalent in early-stage lactation animals than the animals that are in mid-lactation stage animals (Abegewi *et al*., 2022). *E. coli* type mastitis is usually a commensal type (Momtaz *et al*., 2012; Suojala *et al*., 2011).

S. aureus and *E. coli* are the leading causes of SCM (Hinthong *et al*., 2017). SCM in dairy animals is commonly detected after laboratory examination of the milk, as there is no gross swelling of the udder or apparent changes in the milk (Baloch H *et al*., 2016). In Pakistan, SCM is detected by CMT and SFMT under field conditions (Ahmad *et al*., 2012; Rizwan *et al*., 2021). To treat SCM, most antibiotics are used (Aqib *et al*., 2017). These antibiotic residues are transferred to humans through milk consumption (Rizwan *et al*., 2016). Tylosin, amoxicillin, enrofloxacin, and penicillin are most commonly used to treat SCM in the field. Amoxicillin shows lower efficacy against coliform bacteria than norfloxacin (Abegewi *et al*., 2022). Procaine penicillin is the most commonly used against SCM (Khan *et al*., 2018). Few studies have reported the risk factors associated with SCM on Pakistani dairy farms. These studies have generally focused on larger farms and mainly cattle. This study aimed to provide data on prevalence, associated risk factors, and antibiotic resistance for coliform SCM on dairy farms in Multan, Pakistan.

MATERIALS AND METHODS

This study was conducted in dairy goats of the district Multan of Pakistan. A total of 150 lactating goats, irrespective of breed, age, number of lactations, and feeding management, were included. A standardized collection method was employed to obtain milk samples from each lactating animal. After careful physical examination of the teats by proper palpation, the teat ends were thoroughly washed using 70% ethanol and then dried off. Then a total of 10ml milk sample was collected from each teat. The SFMT was employed as a diagnostic tool for the detection of SCM. The test is based on the principle of detecting an increased number of leukocytes and SCC through the observation of viscous mass formation at different levels. The positive milk samples were inoculated onto MacConkey's agar and incubated at 37°C for a period of 24 hours. After noticing microbial growth on the culture plate single isolated colony was picked and sub-cultured again on MacConkey's agar for further purification of the isolate by observing the colony morphology. Different tests, including *in-vitro* antibiogram profiling of ten *E. coli* isolates against ten commonly used antibiotics, were performed. The following antibiotics were used: amoxicillin, cloxacillin, gentamicin, colistin sulphate, procaine-penicillin, enrofloxacin, ampicillin, oxytetracycline, florfenicol, and streptomycin.

Risk factors associated with SCM: Data for the associated risk factors with SCM were collected through a predesigned questionnaire through observation and owners' interviews.

Statistical analysis: The data collected were analyzed by chi-square using SPSS. Values were calculated for the association of different risk factors by logistic regression, while the zone of inhibition in the antibiogram study was analyzed by repeated one-way ANOVA. The significance will be checked at 5% probability ($p<0.05$).

RESULTS

Prevalence of SCM: The prevalence of SCM was analyzed and compared between purebred and local animals. We tested purebred and locally bred animals, of which 26% and 32% were positive for SCM, respectively. The age-wise prevalence of SCM was observed and compared between purebred and locally bred animals. In pure and local breed for ages 2-4 years, 4-6 years, and 7-10 years, the prevalence of SCM was 38%, 38% and 39%, respectively. Whereas, SCM prevalence was high for a group of animals that were in 25 to 55 days of lactation in case of pure breed animals, and the same prevalence ratio for local breed animals.

Floor, manure, and teat shape associated risk factors: Risk factors associated with SCM in lactating dairy goats were studied. Samples in case of separate brick-block floor and soil floor were tested. Of the total samples from separate brick-block floors, 30.10% tested positive. Of the samples with soil floor were also tested, which 23.0% were found positive. We observed samples with manure removal once a day, of which there were 34 positive samples. In the case of manure removal 2 times a day, 74 samples were observed, of which 19 tested positive. Lastly, we observed 79 samples with manure removal multiple times a day, with 20 positive samples. Of the 80 samples observed whose teats were round-shaped, 34 tested positive.

Milk letdown methods and water source associated risk factors: Out of the samples in which the source of water was a pipeline, 31.24% were positive for SCM. 26.36% were positive from samples in which the source of water was a well. The positivity ratio was 21.53% in the case of calves and 33.00% in the case of oxytocin used for the removal of milk.

Farm cleanliness, post-milking teat dip, and dry period associated risk factors: We observed samples with farm cleaning once a day, out of which 19.66% tested positive for SCM. 55 samples were tested with cleaning twice a day, out of which 19.65% were positive for SCM. In case of post-milking teat dip, we observed 66 samples in which post-milking teat dip was done, out of which, there were 7.98% tested positive for SCM. We observed samples in which post-milking teat dip was not done, out of which 36.23% tested positive for SCM. This study also involved.

Microbiological investigation: The mastitis-positive milk samples were transferred to the laboratory for further analysis. The samples were primarily cultured on MacConkey agar to identify the causative agents. In SCM, 16 pure isolates of *E. coli* were cultivated during

culturing. The extraction of genomic DNA was done using by DNA extraction kit.

Antibiogram profiling: In antibiogram profiling, we used 16 isolates of *E. coli*, of which 70% were resistant and 30% were sensitive against amoxicillin. In the same way, out of 6 isolates of *E. coli*, 78.87% were resistant and 15.3% were sensitive against cloxacillin. Out of 6 isolates, 10% were resistant, 31% were intermediate, and 26% were sensitive against gentamicin. Out of 8 isolates, 18.0% isolates were resistant, 45.90% isolates were intermediate, and 19.0% were sensitive against oxytetracycline.

DISCUSSION

Mastitis is a highly prevalent disease in lactating cattle, buffalo, sheep, and goats, causing significant economic losses due to veterinary expenses, treatment costs, labor requirements, reduced milk production, repeated mastitis episodes, premature culling of animals, and poor milk quality (Maalik et al., 2019). Moreover, mastitic milk often contains bacteria and their toxins at levels above critical thresholds, posing a serious risk to human health (Ahmad et al., 2021).

In the present study, the Surf Field Mastitis Test (SFMT) was used as a cost-effective and field-applicable diagnostic tool for detecting intra-mammary infections (IMIs). Similar to findings by Hameed et al. (2012) and Hussain et al. (2013), this study confirmed that subclinical mastitis (SCM) was more prevalent in non-descript or local breeds compared to purebred animals. Our results demonstrated that SCM prevalence was notably higher in local breeds, confirming earlier observations. Age and high milk yield were significantly associated with increased SCM risk. Prevalence was highest during early lactation compared to late lactation, consistent with recent studies. However, Rahman et al. (2009) reported slightly different trends in breed susceptibility.

WHO has also identified advanced age and high milk yield as key risk factors for SCM. Rahman et al. (2009) documented prevalence rates of 3.3%, 18.7%, 21.8%, and 29.2% in animals aged 3–5, 5–8, and ≥12 years, respectively. In our study, prevalence rates were substantially higher at 47.8%, 52.1%, and 49.0%, respectively. These findings reinforce the role of breed, genetic predisposition, management practices, milk yield, and age in influencing SCM prevalence. Implementation of effective mastitis control strategies—including regular monitoring, improved management, early detection, and timely treatment—is essential.

Environmental and management risk factors also contributed significantly. Animals housed on brick-block flooring had a higher risk of SCM, consistent with Rahman et al. (2009). Teat dipping showed strong protective effects, with a positive ratio of only 8.05% when practiced, compared to 41.65% when neglected (Mbindyo et al., 2020). Dry period length influenced prevalence, with a higher risk of SCM during the 1.5–3-month stage of the dry period. Similarly, calving intervals longer than one year increased the likelihood of SCM, supporting the findings of Demil et al. (2022).

Manure removal frequency was another important factor. Prevalence rates were 18.4% and 26.1% for once- and twice-daily cleaning, respectively. While this supports Bari et al. (2022), our findings also suggest that frequent cleaning under suboptimal hygiene conditions may paradoxically increase infection risk. Water source played a role as well, with prevalence of 27.0% in animals using pipeline water versus 20.4% for those using well water, aligning with Rizwan et al. (2016). SCM prevalence was also higher in animals where oxytocin was used for milk letdown (39.6%) compared to calf suckling (35.8%), consistent with Napolitano et al. (2022).

Teat shape also influenced SCM risk to a minor extent. Positive rates were 28.1% in round teats, 30.2% in flat teats, and 33.0% in cylindrical teats, findings that were slightly different from Hussain et al. (2013).Antibiotic therapy remains the cornerstone of SCM management (Rizwan et al., 2022). However, indiscriminate use has led to the emergence of antibiotic-resistant strains. Culture and antibiotic susceptibility testing are thus essential (Grispoldi et al., 2019). In our study, enrofloxacin and gentamicin were the most effective antibiotics against E. coli, followed by cloxacillin, ampicillin, amoxicillin, and florfenicol. Oxytetracycline showed moderate efficacy. Procaine penicillin was the least effective, with no susceptibility detected. Streptomycin exhibited the highest resistance. These results are consistent with reports by Dhakal et al. (2007), Kaliwal et al. (2011), and Awadkar & Kulkarni (2012). Moderate resistance to oxytetracycline was also observed, corroborating findings from Awadkar & Kulkarni (2012), Alekish et al. (2013), and Mahami et al. (2011). Penicillin resistance was 100%, exceeding levels previously reported by Pitkala et al. (2004), Rajala-Schultz et al. (2009), and Kenar et al. (2012).

In Pakistan, SCM prevalence in dairy animals remains alarmingly high, compromising milk production efficiency. In this study, E. coli was the most commonly isolated pathogen, emphasizing poor management and unhygienic milking practices as major contributors. To enhance milk productivity, farmers should adopt

improved milking hygiene, use dry cow therapy for early mastitis cases, and conduct antibiotic sensitivity testing before treatment. Further research into other etiological agents is essential to reduce the economic burden of this disease.

Conflict of Interest

The authors declare no conflict of interest

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