

## **PREVALENCE, BACTERIOLOGY AND ANTIBIOTIC SENSITIVITY PROFILE OF SUB-CLINICAL MASTITIS IN GOATS IN DISTRICT JHELUM.**

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**ABSTRACT:** In this study the prevalence of sub-clinical mastitis was determined in milk samples collected from goats of different breeds from district Jhelum. Surf field mastitis test and White side test were used for screening. Out of 500 animals tested, 14.2% animals were found positive for sub-clinical mastitis. Out of the positive samples, 12.2% were having infection in single, while, 2.0% were having infection in both halves of the udder. Beetle breed was infected at the highest rate i.e. 22.54% following desi breed i.e. 10.40% and teddy breed i.e. 8.80%, respectively. Prevalence of mastitis increased with increase in number of parity i.e. 8.5% up to 2<sup>nd</sup> parity and 19.76% up to 3<sup>rd</sup> parity. Bacteriological profile showed that *Staphylococcus aureus* was the most common pathogen i.e. 51.43% isolated. Antibiotic sensitivity test revealed that most of the isolated bacteria i.e. 93.20% were sensitive to Gentamicin.

**Keywords:** Sub-clinical mastitis, Goats, Antibiotic sensitivity, Bacteriological profile and Jhelum.

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### **INTRODUCTION**

Mastitis is an inflammatory condition of the mammary glands characterized by chemical, bacteriological and physical changes in milk or udder (Nazifi *et al.*, 2011). There are three main classes of mastitis: clinical mastitis (CM), sub-clinical mastitis (SCM) and chronic mastitis (ChM) (Islam *et al.*, 2017). In clinical mastitis, there are changes in milk quality i.e. change in color and presence of clots in the milk. In clinical cases mammary glands are swollen. Other symptoms like heat, pain and indurations can be detected by visual observation of the udder. No clinical signs can be observed in SCM. However SCM can be detected by the presence of pathogenic bacterial species in the milk, increased somatic cell count in the milk, and a mild inflammatory response only detectable by laboratory tests (Islam *et al.*, 2017). SCM is one of the most important infectious diseases in goats, sheep and other small ruminants. Chronic mastitis (ChM) is an inflammatory response that lasts for months and its span may last till 2-3 lactations (Islam *et al.*, 2012). Bacterial contamination of mammary glands not only affects the milk quality but also leads to develop clinical and SCM (Islam *et al.*, 2012).

The most important bacterial species responsible for goat mastitis are *Staphylococcus (Staph) aureus* and *Pseudomonas (P) aeruginosa* (Jeph *et al.*, 2013). *Staph. aureus* causes both acute and chronic mastitis that

respond poorly to treatment. Coagulase negative *Staphylococci* are big cause of SCM and CM (Vanderhaeghen *et al.*, 2015). These bacteria are believed to be spread from animal to animal by milker's hands during milking. Another reason for the spread is the contamination of the teat canal after milking. Through the teat opening the pathogenic organism enters the mammary gland and resides in the milk. It does not penetrate the tissue (Jeph *et al.*, 2013). Control of CM, SCM and ChM depend on use of proper cleaning of udder before milking, proper milking machine function, use of post milking teat dip and dry-animal therapy (Jeph *et al.*, 2013 and Mavrogianni *et al.*, 2011).

Mastitis in the goats is mainly sub-clinical (Li and Wang, 2012). It causes reduction in milk production and milk quality. It is one of the most important causes of losing kids as well as responsible for major economic losses (Koop *et al.*, 2016). After identification of the causative agents, SCM can be cured by treating with antibiotics. Antibiotic sensitivity tests can be performed to ensure adequate treatment. In the treatment of small ruminants, intra-mammary and /or parenteral antibiotics i.e. lactam, macrolide, flouroquinolones, penicillin, nafcillin, and dihydro-streptomycin have been found to be effective in reducing the load of mastitic pathogens (Koop *et al.*, 2016). Irrational or incomplete treatment of infections may cause an antibiotic resistance (Memon *et al.*, 2013 and Prescott and Dowling, 2013).

Goats are the main supplier of dairy and mutton products for rural people in Pakistan (Anonymous., 2013 and Anonymous., 2015). Therefore, any factor that adversely affects the quantity and quality of goat milk is of great financial interest. In Pakistan, statistics of current losses due to mastitis are not available, although it was estimated that in Punjab province alone, a total loss of Rs.240 million per annum has been reported by this disease (Ashfaq *et al.*, 2015). It is one of the most important reasons for termination of lactation and involuntary culling of dairy animals (Koop *et al.*, 2016). Mastitis is responsible for serious economic losses due to reduction of milk and as a carrier of infection for healthy animals (Tanja and Karen, 2010). Failure to start early treatment and irrational usage of antibiotics has made the condition much complicated. Diagnosis of SCM is not easy and direct bacteriological assay is the recommended method by (Koop *et al.*, 2012). Although, some diagnostic tests (Surf Field Mastitis Test, White Side Test and California mastitis test) are used to diagnose SCM, however, bacteriological culture is considered to be a gold standard in the diagnosis of SCM (Koop *et al.*, 2012).

In district Jhelum, SCM has resulted in serious economic losses due to delayed diagnosis and hectic usage of antibiotics, by the goat breeders. The present study is therefore designed to study the prevalence, bacteriological and antibiotic sensitivity profile of SCM in goats in district Jhelum.

## MATERIALS AND METHODS

**Trial animals:** Five hundred (500) adult lactating goats of different breeds were selected randomly from four tehsils of district Jhelum. Twenty five animals (goats) with apparently healthy and normal udders were selected from each village without any discrimination for urban or rural background / status. These goats were grouped into two groups. Animals up to second parity/lactation were placed in group A while animals with third parity/lactation and onwards were placed in group B. Also, the data regarding breed, parity and number of kids in current litter was collected.

**Milk sampling:** The udders of the selected animals were washed with plain water and soap and dried with clean tissue papers. The teats were cleaned with antiseptic swabs. Separate swab was used for each teat. First few milk streaks were discarded. Five ml of milk sample from each teat was collected in a sterile container and tested for SCM. Milk samples were subjected to surf field mastitis test and white side test for the confirmation of SCM.

**Surf field mastitis test:** Surf field mastitis test (SFMT) was performed by mixing 3 gms of commercially available detergent i.e. Surf in 100 ml of distilled water.

Equal quantities of milk sample from individual halves and the surf solution were mixed in petri dishes separately. The petri dishes were rotated and the change in the consistency of milk was recorded. A gel formation of varying degrees was observed in the milk samples which were positive for SCM. The remaining milk samples were categorized as negative (Muhammad *et al.*, 1995).

**White side test:** White side test (WST) was performed on all milk samples for confirmation of SCM reported by (Sanchez *et al.*, 2004). One drop of 1N Sodium hydroxide solution (NaOH) was mixed with 5 drops of milk on a glass plate and stirred with an applicator for 10 seconds. The gel formation immediately after mixing was considered as positive and no gel formation as negative for SCM.

**Bacteriology and antibiotic sensitivity profile of the milk samples:** Mastitis positive milk samples in labeled disposable plastic syringes were shifted in sealed plastic bags under refrigeration to the University Diagnostic Laboratory at University of Veterinary and Animal Sciences (UVAS), Lahore for bacteriology and antibiotic sensitivity profile.

**Bacteriology:** Mastitis positive milk samples were primarily cultured on blood agar (BA). The inoculated plates were incubated at 37°C, aerobically for 24-48 hours. The bacterial growth was identified by colony morphology, Gram staining, characteristic hemolytic patterns and biochemical tests by following (Murray *et al.*, 1994).

**Antibiotic sensitivity profile:** Each milk sample showing bacterial growth was sub-cultured on Muller-Hinton medium and the sensitivity profile of bacterial isolates was determined by using disc diffusion method according to the guidelines of Clinical Laboratory Standards Institute (CLSI, 2009). Eight antibiotics including Streptomycin (30ug), Gentamicin (30ug), Ampicillin (10ug), Amoxicillin (25ug), Ciprofloxacin (5ug), Enrofloxacin (5ug), Lincomycin (15ug) and Oxytetracycline (30ug) were tested.

**Statistical analysis:** Chi-Square test was applied on the recorded data with level of significance ( $p=0.05$ ) calculated through Statistical Package for Social Sciences (SPSS 2002).

## RESULTS AND DISCUSSION

In this Study, 500 lactating goats were tested for SCM. Out of the tested animals, 71 animals (14.2%) were found positive for SCM. Sixty one animals (12.2%) were having the SCM in single teat while 10 animals (2.0%) were having both halves of the infected udder. Regarding prevalence of SCM in goats, our results were in close

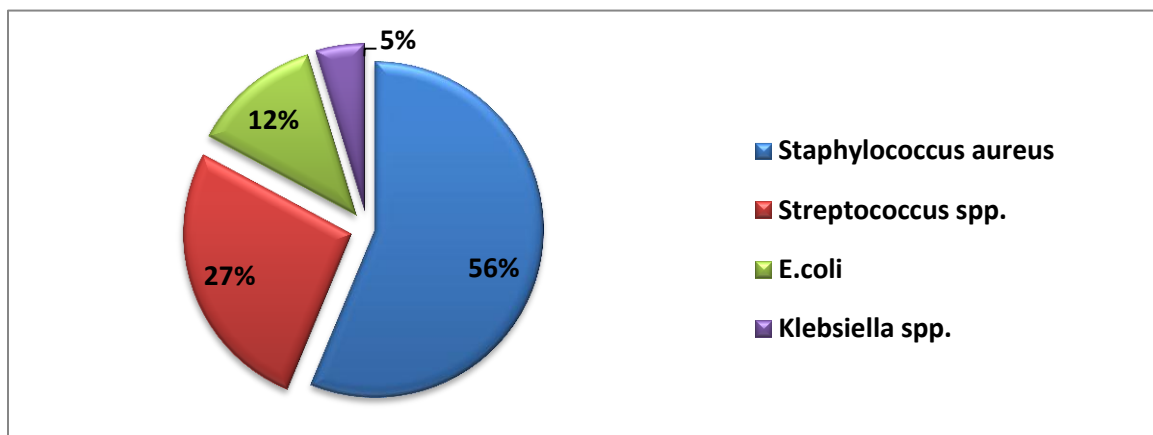
comparison to other related studies carried out by (Gebrewahid *et al.*, 2012 and Kostelic *et al.* 2009). In another study conducted by Ameh and Tari (1999) on goats in Maiduguri, Nigeria, 17% prevalence of SCM was found. Similar results were also reported by Gebrewahid *et al.* (2012) who concluded 18.03% and 28.14 % prevalence of SCM in goats and sheep, respectively. Kostelic *et al.* (2009) also reported similar results i.e. 20% prevalence of SCM while working on French Alpine goats. In Pakistan data regarding prevalence of SCM in goats and sheep is scanty as reported by (Najeeb *et al.*, 2013 and Ali *et al.*, 2010). In these studies the prevalence and antibiotic profile of SCM in goats was also studied with varying results.

In the present study, the effect of breed was also found on the prevalence of SCM. The occurrence of SCM in beetle breed was recorded highest i.e. 22.54% when compared with the rest of the local breeds. Out of 202 animals of desi breed, 21 animals (10.40%) were found infected. While, in teddy breed, 11 animals (8.80%) out of 125 tested animals were positive for SCM. These results were in agreement with the findings of Ali *et al.* (2010) who found that desi goat breeds in district Kohat of Khyber Pakhtunkhawa were affected badly by SCM.

It was also observed that the incidence of SCM increased with age (parity). Among the tested animals, 247 animals were up to second parity. Out of these, 21 animals (8.50%) were found infected with SCM. While 253 animals were in third lactation and onwards. Out of those 253 animals, 50 animals (19.76%) were infected (P-value = 0.0001). The increasing trend for incidence of SCM with age is compatible with those narrated by Islam *et al.* (2012), who recorded that the goats within 2-4 years of age or above or those having completed 3 or more lactations were mostly affected (Islam *et al.*, 2012). Similar facts were derived in another study conducted in black Bengal goats, where CM and SCM were significantly associated with age (P<0.001) and parity (P<0.001) reported by (Islam *et al.*, 2011).

Effect of the number of kids in previous lactations on prevalence of SCM was also studied. It was reported that the prevalence of SCM in animals with single kid, twins and triplets in previous kidding was 13.40%, 14.67% and 16.67%, respectively (P-value = 0.8893). No data could be found to strengthen or contradict the findings regarding the effect of number of kids in previous kidding on prevalence of SCM. Results regarding the most common pathogen involved in SCM in goats have shown that *Staph. aureus* was the most prevalent (51.43%) followed by *strept. spp.* (24.29%), *E.coli* (11.42%) and *Kl. spp.* (04.29%) (Fig-1). The results regarding isolated organisms were consistent with the results reported by Ali *et al.* (2010) as *staph. aureus* (45.34%), *Strept. spp.* (22.74%), *E.coli* (11.55%) and *Kl. spp.* (3.65%). Similar findings were also reported by Islam *et al.* (2012) as 20.8% to 46.6% prevalence of *Staph. aureus* as pathogen of SCM. In another study Al-Ramahi and Al-Nassrawi (2007) reported that most of sub-clinical intra mammary infections were caused by coagulase negative *Staph. spp.*

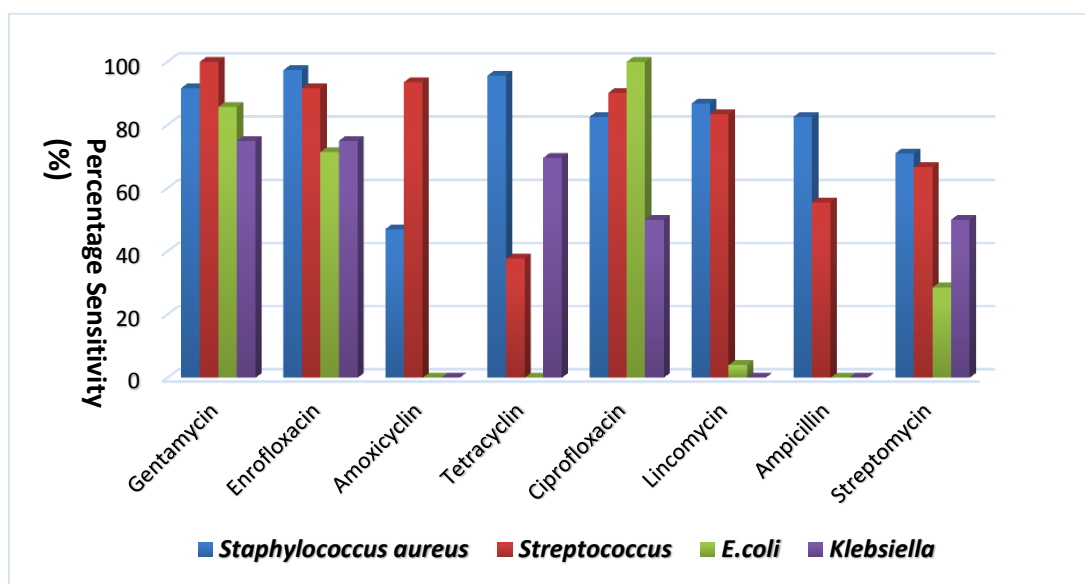
Gentamicin was found to be the most effective antibiotic (93.20%) in the antibiotic sensitivity profile of the isolated bacteria (Table-1 and Fig-2). Among the other antibiotics, Enrofloxacin (89.27%), Tetracycline (78.85%), Ciprofloxacin (74.73%) and Lincomycine (69.39%) were found to be effective against mastitis causing pathogens. Comparable results were found in the study conducted by Ali *et al.* (2010). According to their study, Gentamicin was (96.15%), enrofloxacin (92.31%), Trimethoprim-sulpha (88.46%), Amoxicillin (84.62%), Tetracycline (80.72%), Tylosin (80.77%), Lincomycin (76.92%), Ampicillin (46.15%) and Pencillin G (42.31%) effective against SCM in goats. However, these results are not in agreement with those reported by Najeeb *et al.* (2013) who stated that Amoxicillin and clavulanic acid combination was the most effective (83.33%) treatment. This difference may be due to the fact that they used amoxicillin in combination with clavulanic acid while only amoxicillin was used in the present study.



**Figure-1: Percentage of bacterial isolates in the cases of sub-clinical mastitis in goats**

**Table-1: Antibiotic sensitivity profile percentages of individual bacteria isolated from the cases of sub-clinical mastitis in goats.**

Bacteria /Antibiotic	Genta (%)	Enro (%)	Amoxi (%)	Tetra (%)	Cipro (%)	Linco (%)	Ampi (%)	Strepto (%)
<i>Staphylococcus aureus</i>	91.7	97.37	47.06	95.65	82.6	86.8	82.6	71.05
<i>Streptococcus</i>	100	91.67	93.6	37.7	90.15	83.33	55.5	66.67
<i>E.coli</i>	85.71	71.43	0	0	100	4	0	28.57
<i>Klebsiella</i>	75	75	0	69.6	50	0	0	50



**Figure-2: Antibiotic sensitivity profile of the bacteria isolated from the cases of sub-clinical mastitis in goats**

**Conclusion:** The present study indicated considerable prevalence of subclinical mastitis in goats in District Jhelum with the isolation of major pathogenic microorganisms from subclinical mastitis. It is concluded from this study that the incidence of mastitis was high in older animals who had completed maximum number of lactations. Moreover, Beetle goats were found to be more vulnerable to mastitis infection. It was also revealed that *staph. aureus* were more in number as causative agents while gentamicin was most effective antibiotic in controlling caprine mastitis. The reported prevalence rate could be very important from the economic loss and public health point of view and initiation of a mastitis control program in goats would be beneficial for controlling subclinical mastitis.

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