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Prevalence of sub-clinical mastitis in cattle & goats and antibiotic sensitivity of their isolates in district Kohat-Pakistan

Muhammad Usman Bashir[1*], Akhlaq Hussain[1], Fatima Bibi[1], Benish Jamal[1], Shazia Mansoor[2], Mian Walid Layaq[2], Muhammad Babar Saleem[3]

[1]*Department of Microbiology, Faculty of Biological Sciences, Kohat University of Science and Technology, Kohat-26000, Pakistan*

[2]*Department of Animal Nutrition, The University of Agriculture, Peshawar-25130, Pakistan*

[3]*Souq Waqif Falcon Hospital, Doha-00000, Qatar*

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Abstract

Mastitis is an infectious disease of the udder. This disease is not only responsible for the pain and fever in animals but causes huge economic losses by causing permanent damage the teat and changes in milk. The purpose of this study was to investigate the prevalence of sub-clinical mastitis (SCM) in the cattle and goats of district Kohat-Pakistan by examining milk samples with the help of surf field mastitis test (SFMT). A total of 200 cattle and 200 goats were considered for sample collection for this study. Among which, 34.5% (69/200) were found positive for SCM in cattle and in goats 46.5% (93/200) were found positive for SCM. Later on, milk samples were also cultured to isolate the bacteria responsible for mastitis. Bacteriological examination of milk samples from cattle revealed *Staphylococcus spp.* (24.50%), *Escherichia coli* (17.50%), *Streptococcus spp.* (15.32%), *Bacillus spp.* (12.23%), *Klebsiella spp.* (10.75%), *Pseudomonas* (8.20%) and others (11.50%) were responsible for SCM and *Staphylococcus spp.* (35.50%), *Escherichia coli* (12.40%), *Streptococcus spp.* (10.10%), *Bacillus spp.* (10%), *Klebsiella spp.* (8.40%), *Pseudomonas* (7.90%) and others (15.7%) were responsible for SCM in goats. Several antibiotics including penicillin, amoxicillin, streptomycin, chloramphenicol, erythromycin, trimethoprim-sulfamethoxazole, gentamycin, ciprofloxacin and tetracycline were used for antibiotic sensitivity test of samples isolated from cattle and goats. Results showed penicillin, chloramphenicol, trimethoprim-sulfamethoxazole amoxicillin and ciprofloxacin were found effective while others were not found effective against bacterial isolates *in-vitro*. In conclusion, this study showed true picture of the situation of SCM in the cattle and goats of district Kohat. This study also helped in determining the etiological agents responsible of SCM and the antibiotics effective against these microorganisms to prevent this issue.

* **Corresponding Author:** Muhammad Usman Bashir ✉ khushakhtar99@gmail.com

Introduction

Mastitis is the inflammation of teat, it is a multifactor disease, which not only causes huge economic losses, but is also responsible for changes in milk composition. Mastitis also affects the surrounding tissues (Fig. 1). There are three major types of this disease in dairy animals: clinical mastitis, sub-clinical mastitis (SCM) and chronic mastitis, different conditions of mastitis are recognized by different characteristics (Rizwan *et al.*, 2016). Generally, no visible change occurs in the milk of animal suffering from SCM while all the 5 cardinal signs of inflammation can be seen in clinical mastitis. However, these changes can also be seen in SCM: changes in somatic cell count and the presence of pathogenic organism. Animal suffering from SCM shows negatively affected milk production, which ultimately leads to lower farm production and economic loss (Aqib *et al.*, 2019).

Pakistan is one of the major country in the world in terms of milk production.

This distinction is only possible with contribution of cattle and goats for milk. The milk production of Pakistan not only support the milk demand of its huge population but also has a potential to export milk and milk-products to the rest of the world.

According to the recent economic survey of Pakistan, there are 47.8 million heads of cattle and 76.1 million heads of goats in Pakistan(Economic survey of Pakistan, 2018-19). Several field surveys conducted to know the major diseases of livestock of Pakistan indicated mastitis as one of major disease of dairy animals of Pakistan (Ali *et al.*, 2008).

Since there is no physical damage to the teat or udder in case of SCM, therefor majority of the illiterate farmers of Pakistan are not much familiar to this underlying condition. These famers are also not very familiar to the technique to detect SCM. Keeping in view the above-mentioned issue and its importance, this study was designed to conduct a prevalence study of SCM in cattle and goats of district Kohat-Pakistan.

Material and methods

Sample collection

Milk samples from 200 cattle and 200 goats (apparently mastitis free) were aseptically collected. Briefly, each teat was washed thoroughly, dried with tissue paper and then disinfected with 70% alcohol before the collection of sample in a falcon tube (15mL). It is worth mentioning the first two streams of milk from each teat were discarded in order to get the true sample and minimize the chance the contamination. These samples were stored and transported to Laboratory in Kohat University of Science and Technology (KUST) in ice box (4°C).

Surf Field Mastitis Test (SFMT)

The confirmation of SCM in cattle and goat milk samples was done by surf field mastitis test (SFMT) as described earlier (Muhammad *et al.*, 1995). Briefly, 1 mL of milk was mixed with equal amount of 3% surf solution (Surf Excel, Unilever, Pakistan) in specially designed plastic plates available for commercial use in market. The milk and surf solution were mixed together for 1 min. Later the mixture was examined for SCM. Milk samples showing agglutination or color change after mixing with surf solution were considered positive for SCM.

Bacterial investigation

All the milk samples positive for SCM were further utilized for bacterial culture to know the etiological agent. Each milk sample was cultured on different agar media (nutrient agar media, blood agar media) in microbiology laboratory of KUST. Milk sample was cultured using spread out technique. Later individual bacterial colonies were further separated by multiple streaking. Identification of pure bacterial colonies was done on the basis of culture characteristics, microscopic morphology of colonies, gram staining techniques (Rizwan *et al.*, 2016; Ferdous *et al.*, 2018).

Antibiotic sensitivity

Bacterial isolates were further used for antibiotic sensitivity test. Disk diffusion method was applied on bacterial colonies to check either these antibiotics are sensitive against isolated bacteria or not. Several

antibiotic discs (Thermo Fisher Scientific), including penicillin, amoxicillin, streptomycin, chloramphenicol, erythromycin, trimethoprim-sulfamethoxazole, gentamycin, ciprofloxacin and tetracycline were used for this purpose. These antibiotic discs were placed on media containing pure bacterial culture and were incubated at 37°C for 24 hours (Islam *et al*., 2012).

Results and discussion

Prevalence of sub-clinical mastitis (SCM)

Sub-clinical mastitis (SCM) is a type of mastitis, which shows no physical characteristics. Therefore, it is difficult for farmers to know if this condition in underlying in their animals (Ali *et al*., 2010). A total of 200 cattle and 200 goats were sampled to know the prevalence of SCM. The results of this study showed 34.5% (69/200) were found positive for SCM in cattle and in goats 46.5% (93/200) were found positive for SCM (Fig. 2).

Several studies have been conducted in Pakistan for the prevalence of SCM in various dairy animals in different cities of country. As far as the cattle and goats are concerned, we have compared few studies results. The prevalence of overall mastitis (clinical + sub-clinical) was 47.58% in cattle as per a previous study (Chishty *et al*., 2007).SCM was 29% in cattle in Bangladesh (Islam *et al*., 2011). Bovine SCM was 36% in Muzaffargarh, Pakistan (Bachaya *et al*., 2011).

Table 1. Number of antibiotics used for antibiotic sensitivity test. Effective/Ineffective against bacterial isolates of SCM in cattle and goats.

Sr. No.	Antibiotics used for antibiotic sensitivity test	Effective/ineffective against bacterial isolates
1	Penicillin	Effective
2	Amoxicillin	Effective
3	Streptomycin	*Ineffective*
4	Chloramphenicol	Effective
5	Erythromycin	*Ineffective*
6	Trimethoprim-sulfamethoxazole	Effective
7	Gentamycin	*Ineffective*
8	Ciprofloxacin	Effective
9	Tetracycline	*Ineffective*

Another study showed a 11.41% prevalence of SCM in goats (Ali *et al*., 2010). This study was conducted in 2010 in the same region. As our results have shown a tremendous increase in the SCM positive goats. So, it can be concluded that the prevalence of SCM in goats in district Kohat has increased over a period of 10 years.

The prevalence of SCM in goats was 36% on a goat farm in Bangladesh (Islam *et al*., 2012). The variation in the percentage of SCM in various studies shows and confirms that mastitis is a result of multifactor.

Bacterial Isolates

Bacterial isolates from SCM positive milk samples from cattle and goats revealed that *Staphylococcus spp*.(24.50%), *Escherichia coli*(17.50%), *Streptococcus spp*.(15.32%), *Bacillus spp*.(12.23%), *Klebsiella spp*. (10.75%), *Pseudomonas*(8.20%) and others (11.50%)were responsible for SCM in cattle (Fig. 3A) and *Staphylococcus spp*. (35.50%), *Escherichia coli* (12.40%), *Streptococcus spp*. (10.10%), *Bacillus spp*. (10%), *Klebsiella spp*. (8.40%), *Pseudomonas* (7.90%) and others (15.7%) were responsible for SCM in goats(see Fig. 3B).

Various researchers have conducted several studies regarding bacterial isolates from mastitis affected milk samples. The percentage of various bacteria contributing in total number of positive samples varies in each study. However, most of the studies have concluded that *Staphylococcus spp*. (especially

Staph aureus) is widely responsible for mastitis in dairy animals (Bachaya *et al.*, 2005; Chishty *et al.*, 2007; Gooraninejad *et al.*, 2007; Ali *et al.*, 2008, 2010; Pirzada *et al.*, 2016; Aqib *et al.*, 2019). Our results regarding *Staphylococcus spp.* are in line with all these studies few of which has been mention above.

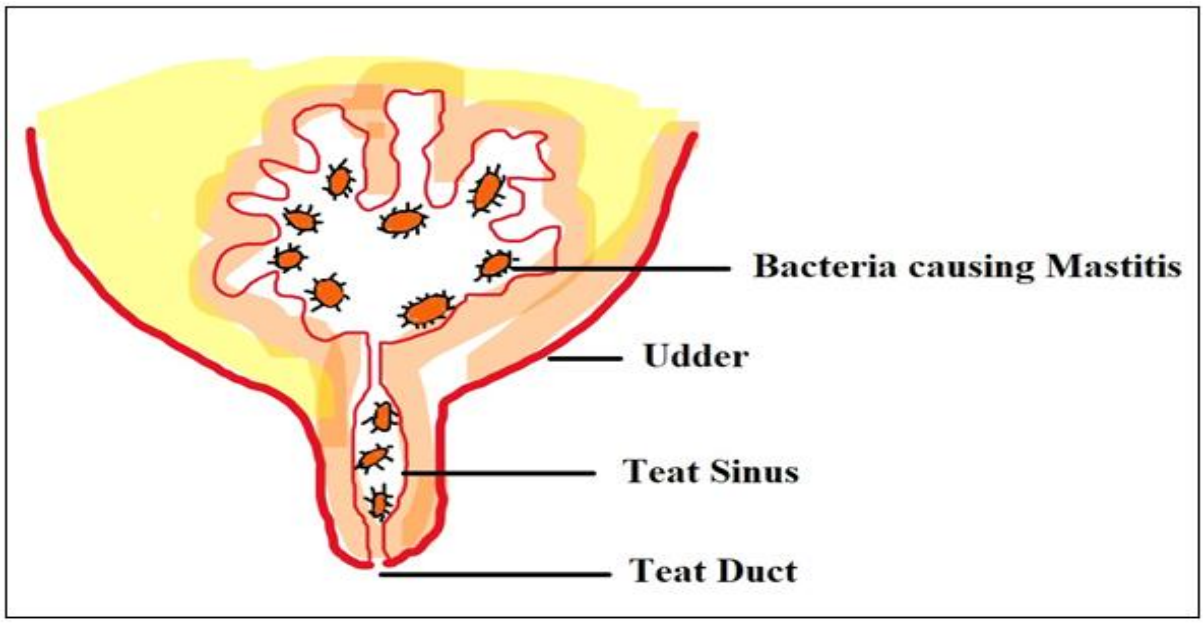

Fig. 1. A schematic diagram of a teat and udder showing mastitis.

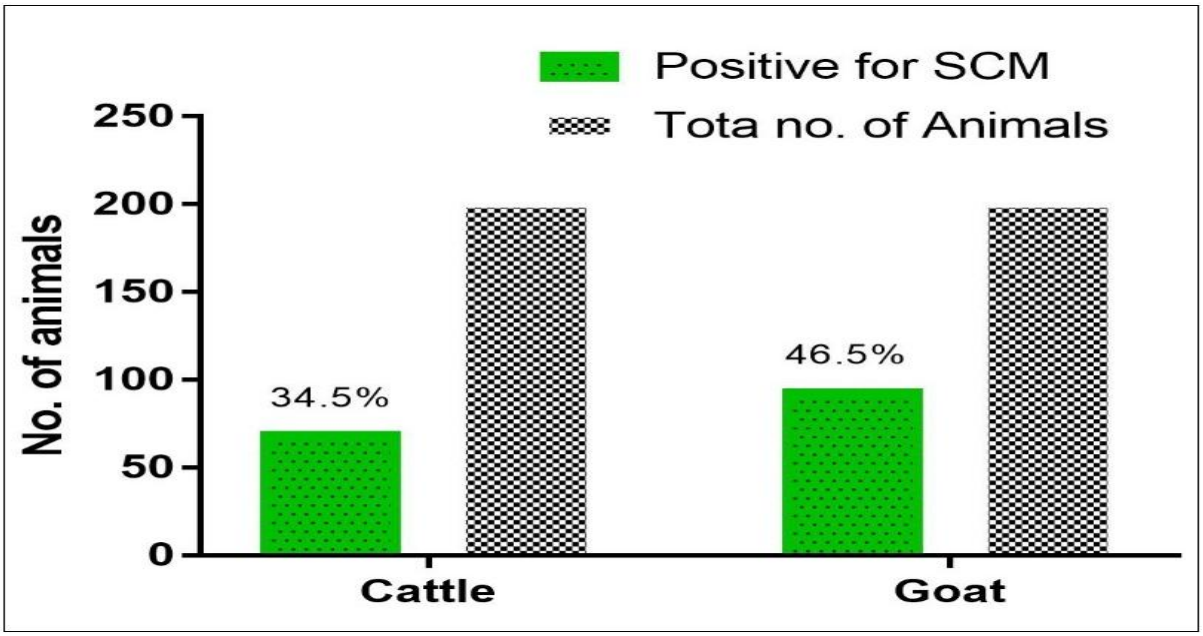

Fig. 2. Prevalence of sub-clinical mastitis (SCM) in cattle and goat in district Kohat of Pakistan.

Antibiotic sensitivity by disk diffusion method

A sensitivity analysis determines the sensitivity of a specific bacteria to a specific antibiotic. In other words, it evaluates the ability of a drug to kill the bacteria. Results from this sensitivity test support the effective treatment of disease(Islam *et al.*, 2012). Keeping in the view the importance of this parameter, we evaluated a number of antibiotics on bacterial isolates of SCM in cattle and goat.

Several antibiotics including penicillin, amoxicillin, streptomycin, chloramphenicol, erythromycin,

trimethoprim-sulfamethoxazole, gentamycin, ciprofloxacin and tetracycline were used for antibiotic sensitivity test of samples isolated from cattle and goats. Results showed penicillin, chloramphenicol, trimethoprim-sulfamethoxazole, amoxicillin and ciprofloxacin were found effective while others were not found effective against bacterial isolates *in-vitro* (Table 1).

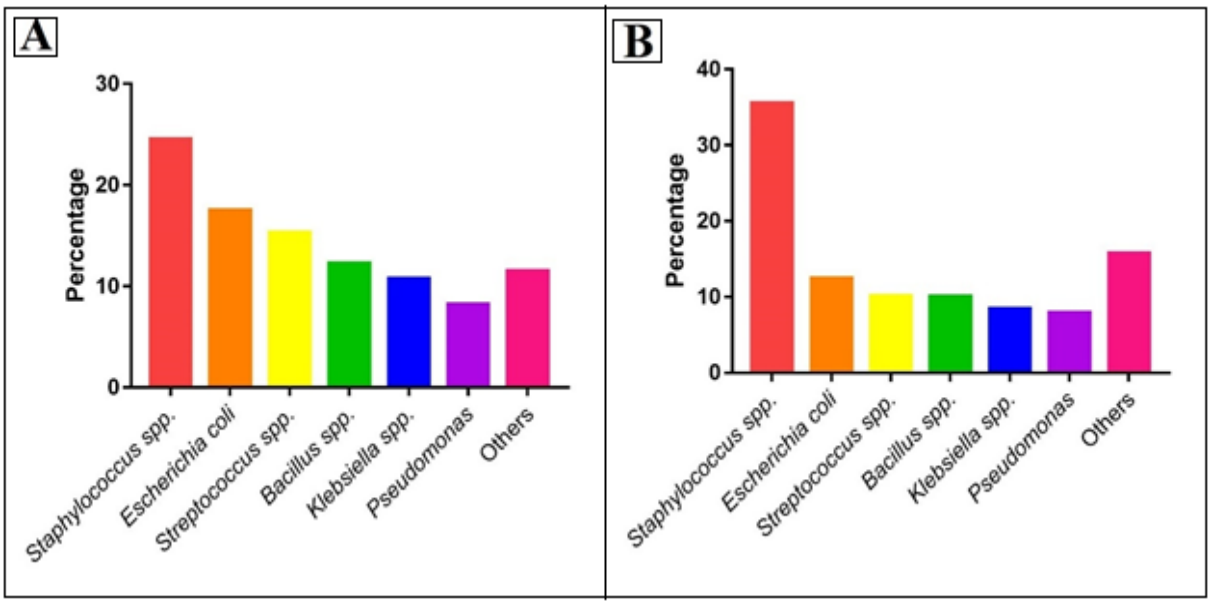

Fig. 3. (A) Percentage of various bacterial isolates from SCM positive milk samples of cattle. (B) Percentage of various bacterial isolates from SCM positive milk samples of goats.

Other researchers found somewhat similar results regarding antibiotic sensitivity test (Gooraninejad *et al.*, 2007; Ali *et al.*, 2010; Islam *et al.*, 2012; Memon *et al.*, 2013; Aqib *et al.*, 2019).However, the increasing number of ineffective antibiotics day-by-day shows the irregular and misuse of antibiotics in animals.

Conclusion

In conclusion, this study showed certainly a high percentage of dairy animals (cattle and goats) suffering from SCM in district Kohat of Pakistan. Bacterial isolates from their milk samples showed a number of bacteria were responsible SCM. Furthermore, antibiotic sensitivity test revealed these bacteria were resistant against streptomycin, erythromycin, gentamycin and tetracycline. In short, this study showed a true picture of underlying subclinical mastitis in cattle and goats of district Kohat and potential antibiotics to cope this situation. Our results showed an alarming situation in the area under study. This situation in not only causing economic losses to farmers but ultimately affecting the economics of state on a larger scale. We recommend to extensively educate the farmers especially small farmers regarding mastitis. Farmers should also be trained to conduct the surf field mastitis test (SFMT) on their own and interpretation of results. Government must take action against quacks, which are responsible for the extensive use of antibiotics in animals, resulting in antibiotic resistance.

There should be a check and balance on pharmacies to avoid selling veterinary antibiotics without prescription. In case of antibiotics use in animals, it is recommended to follow proper withdrawal period before consuming milk of treated animal.

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