

Prevalence of Caprine Sub-Clinical Mastitis, its Etiological Agents and their Sensitivity to Antibiotics in Indigenous Breeds of Kohat, Pakistan

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Abstract

This study was designed to find the prevalence of sub clinical mastitis in goats in Kohat (Pakistan). Based on Surf Field Mastitis Test and California Mastitis Test, it was observed that prevalence of mastitis were higher in goat having age 2-4 or more than 4 years and had completed 3 or more than 3 lactations. On the basis of milking technique, prevalence of mastitis was higher in folded thumb method as compared to whole hand method. Teddy and Desi breeds were found to be more susceptible than Beetle breed. Based on sanitation, mastitis prevalence was higher where sanitary conditions were poor. Similarly, prevalence was high in those cases where teat injury was present in goats. Among the isolated bacteria from the milk samples, *staphylococcus aureus* was in highest percentage while gentamicin was more effective against mastitic bacteria *in vitro* study compared to other antibiotics. It was concluded from this study that age, breed, number of lactation, milking method, sanitary conditions and teat injury played significant role in the prevalence of sub clinical mastitis in goats. Moreover, the etiological agents and their sensitivity to different antibiotics were also revalidated.

Key words: Goats, mastitis, bacteria, antibiotics

Introduction

Mastitis is a general term which refers to inflammation of the mammary glands, regardless of the cause. It is a multiple etiological disease complex, being most prevalent in high yielding dairy cattle, buffaloes, goats and sheep throughout the world (Watts, 1988). Mastitis is accompanied by abnormal alterations in physical, chemical and bacteriological composition of milk or

clots, flakes, or watery milk is the clinical sign most consistently observed (Arshad, 1999).

The importance of goats as a source of meat and dairy products has been well discussed and documented in a recent review by Haenlein (2004). This importance is also reflected by increase in goats population during the last 20 years (FAO, 2001). Goats are the main supplier of dairy and meat products for rural people in Kohat (Pakistan). Therefore, any factor that adversely affects the quantity and quality of milk from the goat is of great financial interest. Milk quality is mainly affected by bacterial infection of the mammary gland which causes mastitis (Boscos *et al.*, 1996).

Mastitis in dairy goats, like mastitis in dairy cows, is a disease of considerable economic importance worldwide. The losses due to mastitis might be greater in Pakistan where disease prevention and reporting are not well established (Javed *et al.*, 2009). According to Samiullah *et al.* (2000), mastitis has caused colossal damage to livestock production in N.W.F.P. (Pakistan) by increasing the culling of morbid animals. Poor management and sanitary conditions, lack of therapeutics and control measures like pre and post milking teat dipping are some major factors which play vital role in the development of this disease in goats.

Owing to immense importance of dairy goat milk, it is imperative to provide disease free wholesome milk to the consumers. Though in developed countries proper strategies of disease monitoring and control have been developed, however, in Pakistan, little information is available regarding prevalence of sub clinical mastitis in goats particularly in the North West Frontier Province of Pakistan. Therefore, the present study was designed to investigate the prevalence of caprine sub clinical mastitis in Kohat (Pakistan), its etiological agents, and their sensitivity to different antibiotics.

Materials and Methods

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In this study 543 adult lactating goats were subjected to sub clinical mastitis test in different areas (rural, urban and peri-urban) of Kohat (Pakistan). Goats are the dominant species of livestock in this region. To find the prevalence of sub clinical mastitis, in term of various epidemiological factors, the experimental animals were categorized according to their age (1-2, 2.1-4 and >4 years), breed (Beetle, Teddy and Desi), number of parity (1, 2 or ≥ 3), milking technique (whole hand method and folded thumb method), sanitation (poor, acceptable and proper) and teat injury (present or absent).

Udder of the goats were properly washed with disinfectant and then dried with clean cloth. First few strikes of milk were discarded. Five ml milk sample from each goat was obtained in a sterile beaker and processed for mastitis test. Milk samples were subjected to Surf field mastitis test (SFMT) and California mastitis test (CMT) simultaneously. CMT was conducted according to the method described by Schalm and Noorlander (1957). SFMT was performed as described by Muhammad *et al.* (1995). Briefly, 3% solution of commercially available detergent was prepared by adding 3g of commercially available detergent (Surf Excell Unilever, Pakistan) to 100 ml distilled water. Milk samples from individual halves and solution were then mixed in equal quantities in petri-dishes separately. CMT was performed by taking small sample of milk from each goat and placed into a plastic paddle. An equal amount of CMT reagent was added to the milk. The paddle was rotated to mix the contents. In approximately 10 seconds, read the score while continuing to rotate paddle. The change in consistency of milk indicates the degree of severity of mastitis. The results were interpreted as negative, trace, P1 (weak positive), P2 (distinct positive) and P3 (strong positive).

The milk samples from mastitic goats were maintained at Department of Clinical Medicine and Surgery, Ununiversity of Agriculture, Faisalabad, Pakistan. These milk samples were cultured on different media like blood agar, McConkey's agar and nutrient agar. The inoculated plates were incubated at 37°C aerobically for 24-48 hours. The bacterial isolates

were identified by morphology, haemolysis, and test for catalase, clumping factor, coagulase, DNase and anaerobic fermentation of mannitol (Hargital *et al.*, 1992).

In vitro antibiotic sensitivity profiles of isolates to 9 antibiotics (gentamicin, enrofloxacin, trimethoprim + sulphamethoxazole, amoxicillin, tetracycline, tylosin, linomycin, ampicillin and pencillin G), were determined by disc diffusion method on Muller-Hinton agar following the guidelines of National Committee for Clinical Laboratory Standards Anonymous, 1990), and now called Clinical Laboratory Standards Institute (Anonymous, 2005). *Staphylococcus aureus* ATCC 25923 (American Type Culture Collection, Rockville, Maryland, USA) was used as the sensitive quality control organism. The results were interpreted by measurement of inhibition zone diameter according to the interpretive standards of National Committee for Clinical Laboratory Standards. Data obtained was analyzed on simple percentage basis.

Results

In this study, out of 543 goats, 62 (11.41%) and 71 (13%) were found positive for mastitis on SFMT and CMT basis respectively. Age, breed and parity showed influence on prevalence of mastitis as shown in Table 1. Goats above four years of age showed higher prevalence of mastitis i.e., 51.6% and 53.5% as shown by SFMT and CMT respectively. Goats in the category of 2-4 years showed prevalence of mastitis as 30.6 and 26.7% by SFMT and CMT respectively. In 1-2 years of age goats, it was further decreased to 17.1% and 19.7% as shown by both SFMT and CMT respectively. Goat of Beetle breed showed prevalence of 14.5% and 12.6% as shown by SFMT and CMT respectively, while it was 29.0% and 29.5% in teddy breed on the basis of SFMT and CMT respectively. Prevalence was 56.4% and 57.7% in the case of Desi goats on the basis of SFMT and CMT respectively. On the basis parity, highest prevalence of 46.7% and 53.5% was recorded by SFMT and CMT respectively in goats having 3 or more than 3 parities.

Table1. Prevalence of sub clinical mastitis on the basis of age, breed and parity.

Test	Age (years)			Breeds			Parity		
	1-2	2-4	>4	Beetle	Teddy	Desi	1	2	≥ 3
SFMT	11(17.1)	19(30.6)	32(51.6)	9(14.5)	18(29.0)	35(56.4)	11(17.7)	22(35.4)	29(46.7)
CMT	14(19.7)	19(26.7)	38(53.5)	9(12.6)	21(29.5)	41(57.7)	11(15.4)	22(30.9)	38(53.5)

Values given in parentheses are percentage base prevalence

SFMT: Surf Field Mastitis Test; CMT: California Mastitis Test

Prevalence of mastitis as affected by milking technique, sanitation and teat injury is given in table 2. Milking technique was further divided into whole hand method and folded thumb method. SFMT based prevalence was 43.5 and 56.4% in whole hand and folded thumb method respectively. While CMT based prevalence was 43.6 and 56.3% in whole hand and folded thumb

method respectively. Prevalence on the basis of sanitation was highest i.e., 54.8 and 57.4% in goats reared in poor condition as determined by SFMT and CMT respectively. Prevalence on the basis of presence of teat injury was 53.2 and 57.7% in goats as shown by SFMT and CMT tests respectively.

Table 2. Prevalence of sub clinical mastitis as affected by milking technique, sanitation and teat injury

Test	Milking technique			Sanitation		Teat injury		
	Whole hand method	Folded thumb method	thumb	Poor	Acceptable	Proper	Present	Absent
SFMT	27(43.5)	35(56.4)		34(54.8)	16(25.8)	12(19.3)	33(53.2)	29(46.7)
CMT	31(43.6)	40(56.3)		41(57.4)	17(23.9)	13(18.3)	41(57.7)	30(42.2)

Values given in parentheses are percentage base prevalence

SFMT: Surf Field Mastitis Test; CMT: California Mastitis Test

The results of different isolated obtained in mastitic milk sample are given in table 3. *Staphylococcus aureus* was exclusively in highest percentage (45.34%) in the samples followed by streptococcus species with 22.74%. *E. coli* was 11.55%, while, *Klebsiella* species were lowest of all the species i.e. 3.65%.

Table 3. Percent prevalence of bacterial isolates from the milk samples from mastitis goats.

Name of isolates	% of total isolates
<i>Staphylococcus aureus</i>	45.34
Streptococcus spp.	22.74
<i>E. coli</i>	11.55
<i>Klebsiella</i> spp.	3.65

The percent antibiotic sensitivity of the isolated species of bacteria has been presented in table 4. It is evident from the results that the given bacteria were highly sensitive to gentamicin (96.15%) while least sensitive to penicillin G (42.13%). The sensitivity of the other bacteria falls in between these two antibiotics.

Table 4. Percent antibiotic sensitivity of isolated bacteria

Name of antibiotic	% sensitivity
Gentamicin	96.15
Enrofloxacin	92.31
Trimethoprin + Sulfamethaxozole	88.46
Amoxicillin	84.62
Tetracycline	80.72
Tylosin	80.77
Lincomycin	76.92
Ampicillin	46.15
Penicillin G	42.31

Discussion

In our study, prevalence of sub clinical mastitis in goats was more in aged and having highest number of parity. Similarly, prevalence was high in those cases where milking was done with thumb fold method, sanitary conditions were poor and teat injury was present in goats. Numerous factors such as farm, breed, age, stage of lactation, milk production, management conditions, and intramammary infections have been mentioned to affect the severity of mastitis in goat (Poutrel *et al.*, 1997). Bergonier *et al.* (2003) reported that incidence of sub clinical mastitis is more in multiparous than primiparous French goats. High incidence may be observed in dairy ewes during suckling, suckling-milking periods (Las Heras *et al.*, 1999; Leitner *et al.*, 2001) or during first third of lactation in goats (Bergonier *et al.*, 2003).

Age is the most significant factor in determining the prevalence of mastitis in goats. In goats increased milk cell count has been reported to be increased with increasing age and lactation (Zeng *et al.*, 1999). Since mastitic animals are not immediately culled, and acute cases may become chronic with the passage of time (Bergonier *et al.*, 2003). It is also note worthy that the disease is chronic in nature and it may take a long time before being detected.

An increased prevalence related to parity has been reported in ewes and goats (Boscos *et al.*, 1996; Leitner *et al.*, 2001; Bergonier *et al.*, 2003). In ewes and goats, association between teat injuries and mastitis has been reported (Ameh and Tari, 1999; Bergonier *et al.*, 2003). Teat infection caused by trauma or cutaneous ecthyma house staphylococcus bacteria (Bergonier *et al.*, 2003). Teat injury is one of the major clinical sign in mastitic goats in our study. Since thorny plants are abundantly available in Kohat which is the

major source of feed for dairy goats. Therefore, there is likelihood that thorns of these plants may cause teat injury to the goats and hence mastitis. In this study it was also observed that Teddy and Desi breeds are more susceptible to the mastitis attack. The reasons are still unknown and more research studies are needed to give final conclusion.

High prevalence has been reported at drying-off or at parturition in relation with environmental contamination such as poor hygiene practices in dairy goats (Bergonier *et al.*, 2003). Further, Bergonier *et al.* (2003) narrated that environmental factors like humidity, litter, wet bedding, forage, mixed reservoirs (infected animals, litter and the environment), housing, feedstuffs, clusters, equipments, human and other animals are the major determinant of mammary pathology in dairy goats. A conclusion can be easily drawn from this and the previous studies that old age, the added burden of high milk production for longer period and multiple numbers of parturitions produce stress on their body. As a result such animals are easily become the host of infectious agents due to low immunity level.

Subclinical mastitis in goats is mainly of bacterial origin (Bergonier *et al.* 2003). Highest presence of staphylococcus in milk sample in this study. Similar results were also reported from other countries like USA (38.2%), Spain (70.0%) and Kenya (60.3%) by some authors (White and Hinckley, 1999; Sanchez *et al.*, 1999; Ndegwa *et al.*, 2001). In the matter of antibiotic sensitivity, Castro *et al.* (2001) reported that gentamicin shows high *in vitro* sensitivity to bacteria. On the other hand, the isolates were more resistance to Pencillin G. Da Silva *et al.* (2004) reported that bacteria in caprine mastitis are more resistant to Pencillin G. This may be due to misuse of antibiotics because frequently use of the same antibiotics may lead to antibiotic resistance (Tras *et al.* 2007). Different results obtained from antibiotics susceptibility may be mainly due to misuse of antibiotics and difference of bacterial strains.

It is concluded from this study that the incidence of mastitis is high in older, having completed greater number of lactation and being milked by folded thumb method. Moreover, prevalence was high in those goats where sanitation conditions were poor and teat injury was present in goats. Further, Teddy and Desi goats were found to be more vulnerable to mastitis infection. It was also revealed that *staphylococcus aureus* bacteria are more in number as causative agents while gentamicin is most effective antibiotic in controlling caprine mastitis.

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