SOME EPIDEMIOLOGICAL ASPECTS OF MASTITIS IN COWS AND BIOCHARACTERIZATION OF ISOLATED STAPHYLOCOCCI

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ABSTRACT

Among one thousand cows, 220 animals were positive for mastitis thus the point prevalence being 22 per cent. Of the total milk samples, 486 (12.21%) were positive by White side test and 10.93 per cent by pH indicator paper technique. Incidence was higher in hind quarters as compared to the fore-quarters and slightly higher in right quarters than the left ones. Incidence of mastitis was the maximum(23.19%) in crossbred cows and the minimum (7.69%) in nondescript indigenous breeds. Occurrence of mastitis increased with the increase in age and number of lactations.The disease was more prevalent in cows during the first month of lactation (24.90%). Prevalence of mastitis was higher in farm than in field conditions. The occurrence of mastitis was lower in cows where washing of udders was carried out prior to milking. Suckling of calf showed a nonsignificant effect. A total of 190 milk samples were positive for bacterial isolations. Various isolated pathogenic microorganisms were *Staphylococcus aureus* (32.09%), coagulase negative staphylococci (12.06%), *E. coli* (16.o8), *Streptococcus agalactiae* (3.01%), *Streptococcus uberis* (7%), *Pseudomonas aeruginosa* (7.5%), *Corynebacterium pyogenes* (3.51%), Bacillus (3.01%) and Klebsiella (2.01%). Total fungal isolates were 11, among which 9 were Candida and 2 were Aspergillus. The staphylococci were highly sensitive to oxytetracycline , sulphamethaxazole/ trimethoprim and chloramphenicol, while streptococci were highly sensitive to oxytetracycline, erythromycin and chloramphenicol. E.coli was sensitive to oxytetracycline , gentamicin, chloramphenicol and streptomycin. All DNAse positive *Staphylococcus aureus* strains were coagulase positive. Penicillin resistance was showed by 49.24 per cent *Staph. aureus* cultures and 50 per cent coagulase negative staphylococcus strains. Out of 32 penicillin resistant strains of *Staphylococcus aureus*, 26 (81.25%) were resistant due to penicillinase production.

INTRODUCTION

Mastitis is one of the most important hazards confronting the milk supply and continues to be an enigma for the dairy industry all over the world. In terms of economic losses, it is undoubtedly the most important disease with which the dairy has to contend. Mostly the losses are due to decreased milk production (10-50 %), discarded milk, premature culling of mastitic animals, treatment expenses and additional labour costs. From the veterinary public health viewpoint, there is an additional danger that the contamination of milk may cause spread of diseases like tuberculosis, sore throat, brucellosis etc. The presence of Staphylococcus aureus and its toxins in the marketed milk is considered to present a high degree of risk to the consumers. The antibiotic residues in milk may lead to the development of sensitivity syndromes in human beings.

The infection originates either from the infected udder or the contaminated environment. Various determinants of the disease are the stage of lactation, lactation number, trauma to the udder, teat and teat canal, loose teat sphincters, lesions on the teat skin, immunological status of each mammary gland, load of infection in the environment and managemental conditions (Blood and Radostits, 1989).

Staphylococcal mastitis in cattle is gaining a considerable importance because of high incidence rate, animal to animal spread , their intracellular localization and comparatively poor response to treatment. The strains isolated from different animal sources tend to have different physiological properties and thus can act as a marker for determining the origin of isolated strains.

The indiscriminate use of chemotherapeutic agents at low therapeutic levels over a longer period of time may result in development of drug resistant strains of staphylococci and other bacteria. Regular screening of antibiotic sensitivity of bacterial isolates is, therefore, mandatory for efficient and economical treatment and control of the disease.

Mastitis can only be controlled properly when its epidemiology is thoroughly known. However, the information regarding the epidemiology of cow mastitis, status of infection, treatment patterns etc. are scanty in the country. The present research project, was therefore designed to study the epidemiology of cow mastitis, isolation of the causative microorganisms, antibiogram assay of different bacterial isolates and biocharacterization of the isolated staphylococci.

MATERIALS AND METHODS

During the year 1995-96, one thousand milk samples from different livestock farms and privately owned animals were collected randomly from cows at different stages of lactation (Table 1). Complete history of the individual animals was recorded through a questionnaire for epidemiological studies. Individual quarter milk samples were collected in sterilized test tubes after discarding first few streams of milk.

All the milk samples were tested for pH using indicator paper technique (TAD Pharmazeutisches, Werk GmbH) and subjected to Whiteside test (Schalm *et al.*, 1971). The samples were processed for microbiological examination. The positive milk samples were spread over the nutrient agar plates. Slides were prepared for bacterial morphological studies. All the positive milk samples were subjected to cultural examination for proteolytic organisms on milk agar, nutrient gelatin medium, Staph 110 medium and blood agar.

For mycological studies, the positive milk samples were subjected to isolation of fungi on Sabouraud's agar plates (Cruickshank, 1975). After 24 hours, the isolates were separated according to their colony morphology and pure cultures were preserved for morphological and cultural characteristics.

Pure cultures isolated on different general purpose and selective media were processed for morphological and biochemical tests for identification and characterization of the organisms. The bacterial isolates were tested for their sensitivity to various antibacterials including sulphamethoxazole/trimethoprim, gentamicin, streptomycin, kanamycin, erythromycin, chloramphenicol, ampicillin, penicillin, oxytetracycline, novobiocin and bacitracin (Collee *et al.*, 1989).

All the staphylococcal isolates were tested for haemolysis, coagulase production, biochemical activity, sugar fermentation, DNAse activity and B-lactamase production. Deoxyribonuclease (DNAse) test was used to differentiate the DNAse producing Staph. aureus from other staphylococci. Beta-lactamase activity of penicillin resistant organisms was detected by rapid acidometric filter paper test (Monica, 1989). For rapid acidometric filter paper test, strips of Whatmann No.1 filter paper were placed in the bottom of a petri dish, two to three drops of buffered crystalline penicillin bromocresol purple solution were added until saturation of the paper. Using a sterile wire loop, few colonies were transferred from the culture plate to the filter paper to cover approximately 5 mm area with a distance of about 10-20 mm. Petri dish lid was replaced and the plates were incubated at 35-37 °C for 30 minutes. Change of colour from purple to yellow was considered as evidence of B-lactamase production.

Table 1: Sources of cow samples for epidemiologic investigations and isolation of mastitis pathogens.

Sources	No.of Animals	No. of Samples
Dairy Farm Livestock Management Deptt., U.A.F.	30	120
Cross Breeding Research Project, ABG Deptt. U.A.F.	40	159
Livestock Production and Research Institute, Bahadarnagar.	180	718
Livestock Farm, Qadirabad.	190	756
Private Dairy Farm, Rawalpindi.	110	438
Field survey (Faisalabad,Jhang, Okara, Sahiwal)	450	1,789
Total:	1,000	3,980

RESULTS AND DISCUSSION

Mastitis is a major problem at livestock farms in Punjab. The prevalence of mastitis was 22.0 per cent in lactating cows with a quarter infection rate of 12.21 per cent. It was different in various breeds of cows (Table 2). Previously clinical and subclinical mastitis has been reported to be 25 to 50 per cent on our various livestock farms(Hashmi *et al*, 1980; Manzoor *et al*, 1984; Rasool *et al*, 1985). Variations in the prevalence of mastitis may be due to different managemental factors.

Crossbred cows had a higher prevalence (26.77) of mastitis than any other breed and the indigenous desi breed. Dutta *et al.*, (1990) concluded that the risk ratio for development of mastitis was 1.21 to 1.89 times greater in Jersey cows than in the crossbred cows. This difference may be due to inheritance as well as due to more developed udders.

In most of the animals, only one (6.7%) or two quarters (7.9%) were affected, while four quarters prevalence was the minimum (Table 3). Kubra (1983) recorded that in most of the animals, only one quarter was affected. It could be due to the fact that pathogenic organisms might not have entered in all the quarters at the same time as the predisposing factors like injury, defective sphincter etc, could vary from quarter to quarter.

The prevalence was higher in hind quarters as compared to fore-quarters and higher in right quarters than in the left ones (Table 4). Kubra (1983), Al-Shawabkeh and Aziz (1988), Pluvinage *et al.* (1990) also reported similar findings, while according to Rasool *et al.* (1985), mastitis is more frequent in fore-quarters of Sahiwal cows. The reasons for higher prevalence in hind quarters could be more development of hind quarters than the front ones, relatively more exposure to urine, dung and injuries and while milking they are pulled forwards and sideways which may lead to undue stress.

The prevalence of mastitis increased as the age advanced and consequently lactation number (Table 5,6). Kubra (1983), Naghmana (1984), Rasool *et al.* (1985) and Pluvinage *et al.* (1990) also made similar observations. This may probably be due to the damage caused to the glandular tissue during previous lactations, the increased sensitization of the glands to the subsequent invasion of organisms, lower resistance of the animals in old age, chronicity of the disease and development of antibiotic resistance.

The very high prevalence (27.27%) during the first month of lactation is an indication of infection, probably prior to freshening (Table-5) as has also been reported by Bakken (1981) and Pluvinage *et al.* (1990).

The prevalence of mastitis was higher in farms than individually owned animals. This difference may be due to close confinement of cows in farms in poor hygienic environment thus favoring the transmission of infectious microorganisms (Sharma, 1986). Prevalence of disease was greater in herds and individual animals with known disease history. This may be due to the damage caused to the glandular tissue during previous infection.

Out of 220 mastitic cows, 8.63 per cent were suffering from endometritis. Morcos *et al.* (1988) reported that out of 50 cows with endometritis following parturition, 45 (90%) developed acute mastitis while in 50 cows without endometritis, 11 (22%) developed mastitis.

It was observed that the prevalence of mastitis in general was lower in animals maintained in good hygienic environment with proper management. Oz *et al* (1986), Osteras and Lund (1988) and pluvinage *et al.* (1990) all concluded that housing, management and feeding parameters play an important role in the control of bovine mastitis.

As for as the microflora of cows milk collected from healthy and clinically mastitic animals is concerned, on cultural examination the maximum number (67.82%) of bacterial microflora belonged to proteolytic category including the maximum number of staphylococcus species (20.60%), Corynebacterium (3.50%) and Bacillus (3.01%). Sudharma *et al.* (1986) observed that 32% of clinical and subclinical mastitis in cows was caused by staphylococcal species. It was maximum percentage of the proteolytic bacteria isolated from the milk samples. Kotowski (1988) isolated 30.1 per cent staphylococcus spp. from the bovine mastitis. Jung *et al.* (1990) isolated 39.3 per cent Staphylococcus aureus strains from normal and mastitis affected cows. Naghmana (1984) isolated 55.26 per cent Staphylococcus aureus from the milk samples. In the present study, 32.66 per cent of Staphylococcus aureus were isolated and identified whereas 12.06 per cent species were identified as coagulase negative staphylococci.

Biocharacterization of Staphylococcus aureus spp. may provide a comprehensive information on interrelated qualities and hence the most reliable method to distinguish Staphylococcus aureus from other species. Coagulase and DNAse test were also conducted for the detection of Staphylococcus aureus which were positive for this particular species. Noel and Holt (1989) mentioned the differential characters of Staphylococcus aureus from the other species of staphylococci with respect to coagulase and DNAse activity.

Another group of bacterial microflora isolated included streptococcus species. Majority (16.58%) of the species belonged to Streptococcus agalactiae while streptococcus uberis and Streptococcus dysagalactiae were found to be 3.51 and 3.01 per cent, respectively. Streptococci were isolated by Kubra (1983) from bovine milk samples next to the Staphylococcus species and were found to be 33.56 per cent. Naghmana (1984) isolated 18.42 per cent *Streptococcus agalactiae* from cow milk and 10.60 per cent from buffalo milk, whereas *Streptococcus dysagalactiae* was isolated from the cows milk with 5.26 per cent infection rate. *Streptococcus uberis* and *Streptococcus agalactiae* were isolated by Kotowski (1988) from the bovine mastitis. Corynebacterium species have been related to the chronic mastitis of buffaloes, however these organisms have also been isolated from the normal udder of buffaloes(Kubra, 1983). Sudharma *et al.* (1986) observed 7 per cent corynebacterium isolates from the clinically or subclinically mastitic cows. John and Meaney (1987) reported that *Corynebacterium pyogenes* is responsible for mastitis in cows. Bacillus species isolated from milk samples, may be regarded as the normal microflora of the milk as reported by Kubra (1983). Therefore direct involvement of Bacillus species in causing mastitis is questionable.

Table 2: Breed-wise prevalence of mastitis in cows based on White side test.

Breed	Total No. of animals	No. of infected animals	%age of infected animals	
			B*	T**
Sahiwal	427	99	23.19	45.0
Crossbred	396	106	16.77	18.18
Non-discript	52	4	7.69	1.82
Cholistani	63	5	7.94	2.27
Dhani	62	6	9.68	2.73

* Among various breeds; ** Among total infected animals; Chi-square=17.09084**; Degree of Freedom= 4

Table 3: Distribution of animals with No of affected quarters through White side test with relative and overall percentage

No.of White side test +ve quarters	No.of Animals	Overall prevalence	Relative percentage
1	67	30.45	6.7
2	79	35.91	7.9
3	39	17.73	3.9
4	35	15.90	3.5
Total	220		

Table 4: Relative and overall percentages of different affected quarters through White side test

Position of infected Quarters	No. of Quarters	Relative percentage	Overall percentage
Right Hind	174	35.80	4.37
Left Hind	125	25.72	3.14
Right Fore	100	20.57	2.51
Left Fore	87	17.90	2.18
Toal	486		12.21

Table 5: Age-wise prevalence of mastitis in cows

Age in year	Total No of Animals	Infected Animals of each age group	Percentage infected animals from age groups	Percentage infected out of 220
3	77	10	12.98	4.55
4	79	11	13.92	2.00
5	130	18	13.85	8.18
6	138	20	14.49	9.09
7	143	24	16.78	10.90
8	127	31	04.41	14.09
9	122	36	29.51	16.36
10	114	39	34.21	17.73
More than 10	70	31	44.28	14.09

Total Chi-square=32.0176; Degree of Freedom=8

Table 6: Lactation-wise prevalence of mastitis in cows

Lactation No.	Total No. of animals	Infected Animals	Percentage infected animals	Percentage of infected animals out of 220
1	118	15	12.71	6.82
2	161	22	13.66	10.00
3	180	25	13.89	11.36
4	167	32	19.16	14.55
5	141	40	28.37	18.18
6	126	41	32.54	18.64
7 & above	107	46	42.99	20.91

Chi-square=35.52344; Degree of Freedom = 6

Table 7: Stage of lactation in relation to mastitis in cows

Month lactation	Total No. of animals	No. of infected animals	Percentage infected animals	Percentage infected animals out of 220
1	129	60	46.51	27.27
2	104	34	32.69	15.45
3	99	29	29.29	13.18
4	97	27	27.83	12.27
5	101	20	19.80	9.09
6	107	13	12.14	5.90
7	111	12	10.81	5.45
8	107	11	10.28	5.00
9	91	8	8.79	3.63
10 & above	54	6	9.00	2.72

Total Chi-square=56.54826; Degree of Freedom=9

About 25.62 per cent of the total isolates belonged to the coliform group. It was confirmed that 16.08 per cent of the coliform isolates were *E.coli*, 7.53 per cent of the cultures were Pseudomonas species and 2.01 per cent the klebsiella species

Regarding the antibacterial susceptibility of staphylococci, oxytetracycline was the most effective drug as 73.03 per cent cultures were sensitive to oxytetracycline, followed by sulphamethaxazole/ trimethoprim, chloramphenicol, erythromycin, streptomycin, gentamicin and penicillin. Novobiocin and bacitracin were highly effective against *Staphylococcus aureus* showing 90.76 per cent and 87.69 per cent sensitivity, respectively. These were also highly effective against coagulase negative staphylococci. Rahman and Baxi (1985) found that staphylococci are highly sensitive to neomycin and chloramphenicol, while Sudharma *et al* (1986) observed that majority (84%) of Staphylococcus isolates were sensitive to gentamicin. Costa *et al* (1986) reported that 75 to 90 per cent Staphylococcal strains were sensitive to novobiocin and vancomycin. Prabharkar *et al*. (1988) found that staphylococcus aureus, the most commonly isolated organism from mastitis was sensitive to ampicillin, erythromycin, chloramphenicol, penicillin, oxytetracycline and streptomycin. The lower sensitivity to penicillin, streptomycin and oxytetracycline is thought to be due to their prolonged use for the treatment of mastitis, while Kotowski (1988) reported that the percentage of staphylococci sensitive to oxytetracycline increased from 38.6 to 56 per cent. David *et al*,(1989) in a field survey on the bacteriology of bovine mastitis noted that Staph. aureus was particularly sensitive to cloxacillin and gentamicin.

Incidence of penicillin resistant staphylococci is also emerging as a new problem in the therapy of mastitis. Iqbal *et al*. (1984) isolated 16 strains of Staphylococcus aureus from cow milk to investigate the penicillin resistance. Out of 16 isolates, 13 were resistant to penicillin of which 11 (84.62%) were resistant due to penicillinase production. Jurca (1986) demonstrated penicillinase activity in 31 per cent of coagulase positive and 22 per cent of coagulase negative staphylococcal isolates from bovine mastitis. Crave *et al* (1986) studied 106 strains of staphylococci from bovine mastitis, 69.8 per cent were producing B-lactamase. However, Binde and Gjul (1989) reported that occurrence of penicillin resistant staphylococci was 5.8 and 12.5 per cent in chronic and acute mastitis, respectively.

Streptococcal isolates were highly sensitive to erythromycin and oxytetracycline (91.48%), followed by chloramphenicol (82.97%), gentamicin (80.85%) and sulphamethaxazole/trimethoprim (65.95%). According to Rahman and Baxi (1985), streptococci are uniformly sensitive to neomycin, penicillin, tetracycline, erythromycin, streptomycin and chloramphenicol.

E.coli was sensitive in descending order to oxytetracycline, gentamicin, chloramphenicol, streptomycin and erythromycin. Rahman and Baxi (1985) reported that E.coli was most sensitive to neomycin, followed by chloramphenicol and Streptomycin. Prabhakar *et al*. (1988) recorded that E.coli is sensitive in descending order to chloramphenicol, nitrofurantoin, furazolidone, tetracycline, ampicillin, erythromycin, streptomycin, oxytetracycline and doxycycline. Pseudomonas was sensitive to ampicillin, oxytetracycline and chloramphenicol. Prabhakar *et al* (1988) recorded that Pseudomonas species were sensitive to ampicillin and chloramphenicol.

As regards the mycotic microflora isolated from the milk samples of cows, it was recorded that 5.24 per cent cases showed the presence of Aspergillus and Candida species. The involvement of a broad variety of fungal microflora in the production of bovine mastitis has been reported by Sharma (1986). Involvement of Aspergillus fumigatus and Candida albicans in causing mastitis has been recorded by Naghmana (1984).

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