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FREQUENCY DISTRIBUTION OF MASTITOGENS AS AFFECTED BY POST MILKING TEAT DIPPING AND *Staphylococcus aureus* VACCINATION IN SAHIWAL COWS

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This study was undertaken at Livestock Experiment Station, Dept. of Livestock Management, University of Agriculture, Faisalabad, Pakistan with an aim to determine the effect of post milking teat dipping + *Staphylococcus aureus* vaccination on the frequency distribution of mastitogens in Sahiwal cows. For this purpose, 20 lactating Sahiwal cows apparently free from mastitis were selected from livestock Experiment Station herd where no mastitis control program was in practice. Treatments were C=control; D=Post milking teat dipping only; V=*Staphylococcus aureus* vaccination only and DV=Teat dipping plus *Staphylococcus* vaccination. An iodophore (Germ IOD™, Cenavisa S. A., Laboratories Distributed in Pakistan by Fair International Trading Co.,Karachi, Pakistan) was used as a teat dip @150ml /litre of water for a study period of 12 weeks. *Staphylococcus aureus* vaccine prepared by Dept. of Clinical Medicine & Surgery University of Agriculture Faisalabad. Pakistan was administrated I/m @ 5 ml/animal. Study results indicated that *Staphylococcus aureus* (70%) was most prevalent followed by *Streptococcus agalactiae* (13.33%) and mixed infection (3.33%). However, among minor pathogen, coagulase negative *staphylococci* (10%) were the most prevalent pathogens followed by bacillus Spp. (3.33%) but none of the micrococci, diphtheroids and yeast were found. On overall basis, the number of quarters affected by mastitogens decreased from 9 to 2 (77.7% reduction), 8 to 6 (25% reduction) and 6 to 2 (66.6% reduction) due to teat dipping only, vaccination only and teat dipping plus vaccination, respectively. However in control cows, number of quarter positive for intra mammary infection increased from 7 to 12 (71.10% increase).

Keywords: Mastitogens, teat dipping, S. aureus vaccination, point prevalence of mastitis pathogens, Sahiwal cows

INTRODUCTION

The dairy industry of Pakistan is comprised of both cattle and buffaloes contributing 95% of the total milk production. Both of the species are susceptible to mastitis. However, their susceptibility may differ (Allore, 1993). Mastitis is one of the limiting factors in the development of dairy industry in Pakistan. Mastitis is recognized worldwide as the most prevalent and costly disease of dairy animals. In addition to causing colossal economic losses to the farmers, the disease is important from consumer's and milk processor's point of view. This is because the milk from affected animals may harbour the organisms potentially pathogenic for humans (zoonosis) and processing of such milk results in sub-optimal output of substandard finished fermented products like yogurt, cheese, etc. (Muhammad *et al.,* 1995).

The economic losses of mastitis due to mortality rate are negligible but the production losses due to lowered milk quality/quantity, destruction of affected quarters, increased charges of treatment and culling processes are tremendous. There is an additional danger that the bacterial contamination of the milk from the affected cows may render it unfit for human consumption and in rare cases provide a mechanism of spread of diseases like tuberculosis, sore throat, brucellosis, leptospirosis etc. and has got zoonotic importance. The organism involved in mastitis may vary from community to community. Mastitis is the outcome of interaction of various factors associated with the host, pathogen (s) and environment. The etiology of mastitis is very complex because a large number of microorganisms are known to cause inflammation of udder (Radostitic *et al.,* 2000). With the use of antibiotics and improved herd hygiene, the incidence of streptococcal mastitis has been greatly reduced through out the world but the incidence of streptococcus mastitis has increased greatly. In most countries staphylococcus is the most predominant cause of sub-clinical (Singh and Buxi, 1982) mastitis and is also isolated from the clinical cases (Kapur *at al.,* 1992).These spread from infected to clean udders during the milking process through contaminated milker's hand and cloth towels used to wash or dry udder of more than one animal and may be by flies. Transmission of the pathogens may occur during milking but primarily between milking. Coliform infections are usually associated with unsanitary environment, while Klebsiella are found in saw dust that contains bark or soil. Approximately 70-80 % of

coliform infections are manifested by abnormal milk, udder swollen quarters, watery milk and depressed appetite. Environmental pathogens are most often responsible for clinical cases.

Sub-clinical form of mastitis is more dangerous because it remains hidden from the eyes of farmers, usually precedes the clinical mastitis, has a long duration, drastically reduces milk yield and adversely affects milk quality (NMC, 1990) and is 15 to 40 times more common than the clinical form. In United States, economic losses attributed to mastitis approaches $ 2 billion each year. Out of this, 30 % is due to clinical and 70% due to sub clinical mastitis. It is surmised that losses associated with mastitis in Pakistan may even be proportionately higher than in United State because our dairy farmers are not adopting the preventing measures to that extent (Bilal *et al.,* 2004). It is the need of hour to control this problem through management as is being done in developed countries. Many managemental practices such as teat dipping and vaccination have been applied under modern dairying. Teat dipping is one of the most important practice to reduce the incidence of mastitis. Most commercially available teat dips reduce the new infections up to 50% (Nickerson, 1994). An effective teat dip will reduce the new intramammary infections (IMI) up to 90%, if correctly used (Pankey *et al.,* 1985; Boddie and Nickerson, 2002). The role of monovalent vaccine in the control of mastitis has been reviewed (Smith *et al.,* 1999 and Tomita *et al.,* 2000). Preventing the establishment of an infection and development of an inflammatory response to get rid of infection quickly are ideal achievements of a mastitis vaccine. However, because of the high prevalence and huge economic losses associated with mastitis, even the lesser achievement of reducing the severity of disease and obtaining more rapid clearance of established infection with vaccine would be of great value (Nordhaug *et al.,* 1994). Mastitis vaccination reduced the prevalence of mastitis and improved the quality of milk by reducing somatic cell count (Leitner *et al.,* 2003). In Pakistan, very limited work was done on above mentioned management tools to control mastitis. This study was therefore planned to evaluate the iodophore as teat dip and *Staphylococcus aureus* mastitis vaccine in Sahiwal cows.

MATERIALS AND METHODS

Selection of animals

The study was conducted at Livestock Experiment Station (LES), Department of Livestock Management, University of Agriculture, Faisalabad, on 20 lactating Sahiwal cows apparently free of mastitis. All animals were hand milked and no mastitis control program was in practice at that farm. Animals with one or more blind non functional quarters were not included in the panel of study subjects. Similarly, animals which have had an episode of mastitis from calving to start of trial were excluded. The cows of same parity and stage of lactation were divided randomly into following four groups, each comprising of five cows, C=Control; D=Post-milking teat dipping only; V=*Staphylococcus aureus* vaccination only; DV=Teat dipping plus *staphylococcus aureus* vaccination. An iodophore (Germ IOD, Cenavisa S. A., Laboratories, Fair International Trading Co., Karachi, Pakistan) was used as a teat dip. Teat dipping was done after each milking for a study period of three months. The dip solution was prepared @ 150ml/L of water immediately before use, providing 0.27% available iodine. Each teat was dipped separately in a dip cup, especially made for this purpose, for a contact time of 30 seconds (Nickerson, 1994). Staphylococcus aureus mastitis vaccine (DXS+ Al (OH_3) adjuvant) prepared by Department of Clinical Medicine and Surgery was administrated intramuscularly @ 5ml/animal in the neck region twice at four weeks interval and data was recorded at day 0 (Pre-trial) and then on monthly basis up to 90 days.

Collection of milk samples

Milk samples were collected from all 20 cows following the procedure described by (NMC, 1990). Sterile vials of 15 ml capacity were used. Each teat end was scrubbed vigorously with a separate pledget of cotton moistened with 70 % ethyl alcohol. While holding the vials as horizontal as possible, the cap was removed without touching the inner surface and held with the inner surface facing downwards. After discarding the first few streams, about 5 ml milk was collected aseptically. Immediately after collection, all samples were placed on crushed ice and brought to the Mastitis Research Lab., Department of Clinical Medicine and Surgery, University of Agriculture Faisalabad where bacteriological examination commenced within two hours of sample collection. Procedure described by (NMC, 1990) was followed for culturing the milk samples and identification of mastitis pathogens.

Data collected were subjected to analysis and presented in terms of percent change.

RESULTS AND DISCUSSION

Among the major pathogens, *Staphylococcus aureus* was found in 21 (70%) and *Streptococcus aglactiae* in 4 (13.33%) and mixed infection of *Staphylococcus aureus* and *Streptococci* in 1 (3.33%) quarter, whereas none of the milk sample showed the presence of E.

Coli, C. Pyogenes or other streptococci. Of the minor pathogens coagulase negative staph (CNS) were the most frequent with frequency of 10% (n=3) followed by Bacillus spp.with a frequency of 3.33% (n=1) but none of micrococci diptheriods and yeast were found (Table 1).

positive for intramammary infections increased from 7 to 12(71.10%).

The results of the present study are in line with those of Allore, 1993; Qamar, 1992; and Khan, 2002 who reported that *Staphylococcus aureus* and

Table 1. Frequency distribution of major and minor mastitis pathogen in 80 quarters of Sahiwal cows

Class and Species	No. of quarters	Frequency %
Major pathogens	26	86.66
Staphylococcus aureus	21	70.00
Streptococcus aglactiae	4	13.33
Mixed infection of *S. aureus and Streptococci*	1	3.33
Minor pathogens	4	13.33
Coagulate negative staph. (CNS)	3	10.00
Bacillus spp.	1	3.33

Major pathogens = Those microorganisms which cause very high rise in milk somatic cell count
Minor pathogens = Those microorganism which cause mild to moderate rise in milk somatic cell count

The frequency distribution of *Staphylococcus aureus* in cows of group C at day 0 was 71.42% (n=5) followed by 57.14% (n=4), 54.54% (n=6) and 58.33% (n=7) at day 30, 60 and 90, respectively but the respective values for *Streptococcus aglactiae* was 28.57 (n=2), 28.57 (n=2), 18.18 (n=2) and 25 % (n=3), respectively. The number of quarters affected by both major and minor mastitogens increased from 7 to 12 (71.1%); at the end of trial in cows of control group (Table 2).

The teat dipping was found the best in reducing the frequency distribution of mastitogens(Table 3). At the start of trial, 9 quarters were affected which decreased to 2 at the end of trial (decrease was 77.7%).The quarters affected by *Staphylococcus aureus, Streptococcus aglactiae* and CNS were decreased from 7 to 2 (71.11 %), 1 to 0 (100%) and 1to 0 (100 %), respectively.

Vaccine respond well against *Staphylococcus aureus* as the number of quarters affected by *Staphylococcus aureus* decreased from 5 to 1 (80 %).However, the quarters affected by *Streptococcus aglactiae* increased from 0 to 4(4 times) as is clear from table 4.

In case of DV group the frequency of *Staphylococcus aureus & Streptococcus aglactiae* at day 0 was 66.66% (n=4) and 16.66% (n=1), respectively which was reduced to 75% (n=1) and 100% (n=0). However, one quarter was affected by mixed infection of *Staphylococcus aureus* and *Streptococcus aglactiae* at the end of trial.

On overall basis, the number of quarters affected by mastitogens decreased from 9 to 2 (77.7%), 8 to 6 (25%) and 6 to 2(66.6%) in groups D, V and DV, respectively. In control cows, number of quarter

Streptococcus aglactiae are the most important mastitogens in dairy animals. The reduction of infected quarters following teat dipping/ vaccination is in line with Pankey *et el.* 1985 and Leitner *et al.* 2003, who reported a reduction of 98% in *Staphylococcus aureus* infection in cows vaccinated with *Staphylococcus aureus* vaccine. Boddie et al. (2002) reported that IMI due to *Staphylococcus aureus* and *Streptococcus aglactiae* reduced by 92.9% and 43.4%, respectively following teat dipping in 0.5% Iodophor.

The main purpose of teat dip is to destroy pathogens at the teat skin particularly at the teat apex and thus prevent infection of teat canal, by preventing multiplication and further colonization of causative organisms in the teat canal. Teat dipping kills almost all organisms left on teat skin after milking and provide a germicidal residue on teats between milking. In addition, teat dips reduce teat canal colonization and help to heal teat end lesions. Teat dipping is one of the most important practices to reduce the number of new mastitis infections. The extent to which teat dipping reduces the incidence of new udder infections depend upon the anti-microbial activity of the teat dip. Different commercial teat dips employ different disinfectants which vary in their efficacy as regard the reduction in the bacterial populations on the teat skin and hence their ability to prevent mastitis infections (Bilal and Abdullah, 2003)

In this study, teat dipping was found more effective than vaccination. The probable reason might be that teat dipping covered the infection both due to *Staphylococcus* and *Streptococcus* whereas vaccination against *Staphylococcus aureus* covered

Table 2. Frequency distribution of mastitis pathogens isolated from quarter at each sampling interval (control group)

Sampling interval	Major pathogens		Staph. aureus		Strep. Agalactia		Mixed infection		Minor pathogens		Coagulase negative staph		Bacillus species	
	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %
0	7	100	5	71.42	2	28.57	-	-	-	-	-	-	-	-
30	7	100	4	57.14	2	28.57	1	14.28	-	-	-	-	-	-
60	9	81.81	6	54.55	2	18.18	1	9.0	2	18.18	-	-	2	18
90	10	83.33	7	58.33	3	25	-	-	2	16.66	1	8.33	1	8.33

Major pathogens = Those microorganisms which cause very high rise in milk somatic cell count
Minor pathogens = Those microorganism which cause mild to moderate rise in milk somatic cell count.

Table 3. Effect of teat dipping on Frequency distribution of mastitis pathogens isolated from quarter at each sampling interval

Sampling interval	Major pathogens		Staph. aureus		Strep. agalactia		Mixed infection		Minor pathogens		Coagulase negative staph		Bacillus species	
	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %
0	8	88.88	7	77.77	1	11.11	-	-	1	11.11	1	11.11	-	-
30	7	87.5	5	62.5	2	25.00	-	-	1	12.5	-	-	1	12.5
60	5	83.33	4	66.66	1	16.66	-	-	1	16.66	-	-	1	16.66
90	2	100	2	100	-	-	-	-	-	-	-	-	-	-

Major pathogens = Those microorganisms which cause very high rise in milk somatic cell count
Minor pathogens = Those microorganism which cause mild to moderate rise in milk somatic cell count

Table 4. Effect of *Staphylococcus aureus* vaccination on frequency distribution of mastitis pathogens isolated from quarter at each sampling interval

Sampling interval	Major pathogens		Staph. aureus		Strep. Agalactia		Mixed infection		Minor pathogens		Coagulase negative staph		Bacillus species	
	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %
0	6	75	5	62.5	-	-	1	12.5	2	25	1	12.5	1	12.5
30	8	100	5	62.5	2	25.00	1	12.5	-	-	-	-	-	-
60	9	100	3	33.33	4	44.44	2	22.22	-	-	-	-	-	-
90	6	100	1	16.66	4	66.66	1	16.66	-	-	-	-	-	-

Major pathogens = Those microorganisms which cause very high rise in milk somatic cell count
Minor pathogens = Those microorganism which cause mild to moderate rise in milk somatic cell count

Table 5. Effect of teat dipping plus *Staphylococcus aureus* vaccination on frequency distribution of mastitis pathogens isolated from quarter at each sampling interval

Sampling interval	Major pathogens		Staph. aureus		Strep. Agalactia		Mixed infection		Minor pathogens		Coagulase negative staph		Bacillus species	
	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %	No. of quarters	Frequency %
0	5	83.33	4	66.66	1	16.66	-	-	1	16.66	-	-	1	16.66
30	6	100	4	66.66	1	16.66	1	16.66	-	-	-	-	-	-
60	4	100	3	75	1	25	-	-	-	-	-	-	-	-
90	4	100	1	50	-	-	1	50	-	-	-	-	-	-

Major pathogens = Those microorganisms which cause very high rise in milk somatic cell count
Minor pathogens = Those microorganism which cause mild to moderate rise in milk somatic cell count

this bacteria only. Secondly, teat dipping prevents the entry of new bacteria which is not true in case of vaccination. Thirdly, dipping of full teat was done after each milking for a contact time of 30 seconds and dip solution may suck by the teat canal and remain adhere with the teat skin for some time post milking, Thus, the bacteria present in teat canal and skin flora opportunists both are killed due to quality dip used in this study. Ultimately, bacterial population decreased which lead to reduction in mastitis.

CONCLUSION

Teat dipping and vaccination are the best management tools and must be included in mastitis control programme with an aim to produce quality milk in more quantity. *Staphylococcus aureus* is the major mastitogens under our conditions .As mastitis vaccine is not available commercially, post milking teat dipping after each milking with any iodophor will be beneficial to reduce the frequency distribution of the mastitogens and improve the milk quality.

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