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BACTERIOLOGY OF MASTITIS IN BUFFALOES IN TEHSIL SAMUNDRI OF DISTRICT FAISALABAD, PAKISTAN

L. ALI, G. MUHAMMAD, M. ARSHAD[1], M. SAQIB AND I. J. HASSAN[2]

Department of Clinical Medicine and Surgery; [1]Department of Microbiology; [2]Department of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan

ABSTRACT

Two hundred fore-milk samples collected from 200 mastitis quarters of buffaloes (clinically mastitis quarters n = 17, sub-clinically mastitis quarters n = 183) were subjected to microbiological examination. The diagnosis of sub-clinical mastitis was based on the results of Surf Field Mastitis Test (SFMT). A total of 214 isolates of 13 different microbial species were recovered. *Staphylococcus aureus* was the most frequently recovered bacterial species accounting for 49.53% of all the isolates, followed by *Streptococcus agalactiae* (23.83%), *Staphylococcus hyicus* (8.88%), *Staphylococcus epidermidis* (6.54%), Bacillus spp. (3.74%), *Staphylococcus hominis* (1.40%), *Escherichia coli* (1.40%), *Staphylococcus xylosus* (0.93%), *Streptococcus dysgalactiae* (0.93%) and Corynebacterial spp. (0.93%). Yeast and prototheca each accounted for 0.47 percent of isolates. Two (0.93%) isolates were identified as coagulase negative staphylococci species. In view of preponderance of the contagious pathogens (*S. aureus, Str. agalactiae*), it is recommended that mastitis control in the area of study should be based on contagious mastitis control practice.

Key words: Mastitis, buffalo, milk samples, associated microorganisms

INTRODUCTION

The productive efficiency of dairy animals is adversely affected by suboptimal management, poor nutrition and various diseases. Mastitis is one of the most important impediments confronting the economics of milk production in Pakistan. It is a multifactorial and the most costly disease of the dairy industry throughout the world (DeGraves and Fetrow, 1991) that affects both quality and quantity of milk (Arshad *et al*., 1995). Field survey of major livestock diseases in Pakistan has indicated that mastitis is one of the most important diseases of dairy animals in the country (Cady *et al*., 1983; Ajmal, 1990; Hussain *et al*., 2005). Owing to transmissibility of animal diseases such as tuberculosis, brucellosis and leptospirosis through milk to human beings, this disease is also important from zoonotic view.

Mastitis is the outcome of interaction of various factors associated with the host, pathogen(s) and the environment. Infectious agents, in particular various species of bacteria, are the most important etiologic agents of mastitis. The objective of this study was the isolation of different types of microorganisms associated with mastitis in buffaloes kept under field conditions in Samundri area of district Faisalabad, Pakistan.

MATERIALS AND METHODS

Research period and area

All 28 Union Councils of Tehsil Samundri, District Faisalabad of Punjab province of Pakistan comprising 133 villages constituted the universe of the study population. The study was conducted over a 3-month period (September-November, 2005). Mastitis control practices (i.e. post milking antiseptic teat dipping, dry period antibiotic therapy and culling the affected animals) were not applied in the study area.

Collection of milk samples and diagnosis of mastitis

Two hundred fore-milk samples collected from 200 mastitis quarters of buffaloes (clinically mastitis quarters n = 17, sub-clinically mastitis quarters n = 183) were subjected to microbiological examination. Milk samples were collected at the time of afternoon milking. Milk samples were not collected from animals treated with antibiotics by any route till 96 hours post treatment. Procedure described by National Mastitis Council Inc., USA (Anonymous, 1990) was followed for the collection of samples. Sterile glass vials of 15 ml capacity, labeled as LF (left front), LR (left rear), RF (right front), and RR (right rear) were used. Each teat was scrubbed with a pledget of cotton moistened with 70% ethyl alcohol. A separate pledget was used

for each teat. After discarding the first few streams, about 10 ml of milk was collected aseptically. Collected samples were immediately cooled and transferred to the laboratory in an ice box for microbiological examination. The diagnosis of sub-clinical mastitis was based on the results of Surf Field Mastitis Test (Muhammad *et al*., 1995).

Isolation and identification of microorganisms

Procedures described by National Mastitis Council Inc., USA (Anonymous, 1987) were followed for culturing the milk samples and identification of mastitis pathogens. Briefly, the samples were shaken eight times to get a uniform dispersion of the pathogens. Using a platinum loop, 0.01 ml of each milk sample was streaked onto Esculin-blood agar and MacConkey's agar plates. Four quarter milk samples were cultured on a 100 mm plate by plating individual quarter sample on one quadrant of plate and incubated at 37°C for 48 hours. A quarter was considered to be infected if 5 or more similar colonies were present on the plate (Robinson *et al*., 1988). The representative colonies of the microorganisms were isolated and purified by streaking onto fresh Esculin-blood agar plates. Catalase positive, Gram positive coccal isolates were presumptively identified as Staphylococci or Microococci and subjected to the tube coagulase test and a commercial identification kit (STAPH-Trace system, BioMerieux-France). Organisms other than Staphylococci were identified as per criteria recommended by National Mastitis Council, Inc. USA (Anonymous, 1990).

RESULTS AND DISCUSSION

A total of 214 isolates of 13 different microbial species were recovered (Table 1). *Staphylococcus aureus* was the most frequently recovered bacterial species accounting for 49.53% of all isolates, followed by *Streptococcus agalactiae* (23.83%), *Staphylococcus hyicus* (8.88%), *Staphylococcus epidermidis* (6.54%), Bacillus species (3.74%), *Staphylococcus hominis* (1.40%), *Escherichia coli* (1.40%), *Staphylococcus xylosus* (0.93%), *Streptococcus dysgalactiae* (0.93%) and Corynebacterial species (0.93%). Yeast and prototheca each accounted for 0.47 percent of isolates. Two (0.93%) isolates were identified as coagulase negative staphylococci species.

Mastitis is the inflammation of mammary glands. It is the outcome of various factors associated with the host, environment and the pathogens. Among pathogens, bacteria are by far the most frequently associated etiologic agents of this disease. In the present study, *Staphylococcus aureus* was the frequently encountered pathogen. *Streptococcus agalactiae* was the second most frequently isolated mastitis pathogen. These two contagious mastitis pathogens collectively accounted for 73.36% of the isolates. Similar findings have been reported by Ahmad *et al.* (1991) and Allore (1993). The previous studies conducted in India (Joshi and Gokhale, 2006), Pakistan (Arshad *et. al.*, 2006), Indonesia (Estuningsih *et. al*., 2002) and Italy (Moroni *et. al*., 2006) endowed that clinical as well as sub-clinical mastitis in dairy animals is predominantly contagious in nature.

Table 1: Frequency distribution of isolates recovered from clinical (n = 17) and sub-clinical (n = 183) mastitic quarters of buffaloes

S. No.	Species	No. of isolates	Frequency (%)
1.	*Staphylococcus aureus*	106	49.53
2.	*Streptococcus agalactia*	51	23.83
3.	*Staphylococcus hyicus hyicus*	19	8.88
4.	*Staphylococcus epidermidis*	14	6.54
5.	Bacillus species	8	3.74
6.	*Staphylococcus hominis*	3	1.40
7.	*Escherichia coli*	3	1.40
8.	*Staphylococcus xylosus*	2	0.93
9.	*Streptococcus dysgalactiae*	2	0.93
10.	Corynebacterial species	2	0.93
11.	Undifferentiable (nontypable) coagulse negative Staphylococcus species	2	0.93
12.	Yeast	1	0.47
13.	Prototheca	1	0.47
	Total	214	100

The preponderance of contagious mastitis in buffaloes of the study area (Tehsil Samundri) may be ascribed to lack of control practices such as post-milking antiseptic teat dipping, dry period antibiotic therapy, culling of chronically infected animals from the herd as well as the rife proclivity of using milk foam from the milking pail to lubricate the teat during milking. Keeping the finding of the present study in perspective, it may be recommended that the mastitis control in the area of the present study (Tehsil Samundri, District Faisalabad) should be based on control practices aimed at controlling contagious mastitis pathogens (e.g., post-milking antiseptic teat dipping, dry period antibiotic therapy, segregation of infected and non-infected animals, fly control *etc*.). In view of the preponderance of contagious pathogens as the etiologic agents of mastitis in dairy buffaloes and cows, workers at the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad,

Pakistan (Shakoor, 2005; Butt, 2005; Athar, 2006) evaluated mono (*S. aureus* only) and polyvalent (containing *S. aureus*, *Str. agalactiae* and *E. coli*) mastitis vaccines for the control of mastitis in buffaloes. These vaccines were found to be effective as preventative as well as curative against these important mastitis pathogens.

Staphylococcal species other than *S. aureus* (*S. hyicus*, *S. epidermidis*, *S. hominis*, *S. xylosus* and nontypable coagulase negative staphylococcus species) accounted for 18.68% of mastitis isolates. Coagulase negative staphylococci are the most frequently isolated pathogens in dairy herds practicing the mastitis control recommended by National Mastitis Council, Inc. (USA) and mastitis monitoring bodies e.g., Milk Marketing Board, UK (Timms and Schultz, 1987; Barlett, 1992).

In view of preponderance of the contagious pathogens (*S. aureus*, *Str. agalactiae*), it is recommended that mastitis control in the area of study should be based on contagious mastitis control practice.

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