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STUDY ON THE INCIDENCE OF CLINICAL MASTITIS IN BUFFALOES CAUSED BY BACTERIAL SPECIES

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ABSTRACT

An investigation on the incidence of different bacterial species in clinical mastitic milk samples of buffaloes was carried-out. The bacterial species identified were: *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Micrococcus luteus, Proteus vulgaris, Pseudomonas aeruginosa, Streptococcus dysgalactiae, Streptococcus uberis* and *Citrobacter* species and their incidence in milk samples was 48.57, 2.85, 10.0, 15.71, 4.28, 1.42, 11.42, 4.28 and 1.42% respectively. Of the 70 positive mastitic milk samples examined, 55 (78.57%) and 15 (21.43%) were determined as having pure and mixed (2-3 bacterial species in individual samples) bacterial infection respectively. The incidence and predominance of bacterial species were also observed. The most predominant species recorded was *Staphylococcus aureus* and its dominancy was noted 34 (48.57%) times in the samples while the second most dominant species observed was *Micrococcus luteus* and its dominancy was recorded 11 (15.71%) times in the samples. The rest of the organisms formed a fraction of these major species.

Keywords: Incidence, clinical mastitis, buffaloes, bacterial species

INTRODUCTION

Mastitis is a multifactorial disease and very difficult to control. It results from injury, chemical irritation and infection caused by different bacterial species. Mastitis is most expensive disease of dairy animals resulting in the reduction of milk production and quality. The estimated annual losses due to mastitis are about $ 184 per animal. These expenses in terms of reduction of production, discarding milk, drug therapy, veterinarian charges, premature culling, and extra use of labour (Anonymous, 1998).

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Bovine mastitis is the inflammation of the parenchyma cells of the mammary glands of cattle, buffalo and other animals (Radostit *et al.,* 1996) associated with microbial infections (Schroeder, 1997) and physiological changes (Shouky *et al*., 1997). Mastitis is caused by a group of infective and potentially pathogenic bacteria (Bezek and Hull, 1995), viruses (Wallenberg *et al.,* 2003), fungi and algae (Radostit *et al*., 1996). The incidence of bacterial species in mastitic milk samples of different animals was studied worldwide. The incidence of coagulase negative *Staphylococc*i (1.50%), coagulase-positive *Staphylococci* (1.59%), *Streptococcus* sp. (*Streptococcus dysgalactiae*, 3.7%; *Streptococcus uberis*, 0.83%), *Enterococcus* sp. (1.04%), *Escherichia coli* (1.56%), *Pseudomonas* sp. (1.59%), and yeast (1.5%) was recorded in Korea (Park *et al*., 2007). The bacterial agents responsible to cause inflammation of udder are classified as either contagious or environmental, based upon their primary reservoir and mode of transmission. *Staphylococcus aureus* and *Streptococcus dysaglactiae* are recognized as contagious bacterial species, commonly transmitted among dairy animals through contact with infected milk. The pathogens reside in environment are of 2 types, one is Coliforms (*Escherichia coli, Klebsiella*) and other is *Streptococcal* species other than *Streptococcus dysgalactiae* entering into the udder between milkings, when teats are exposed to mud, manure, and dirty bedding materials (Anonymous, 1998). The epidemiology of bacterial mastitis in dairy animals has been studied using various molecular typing methods. Several studies reveled that only a few specialized clones are responsible for a broad geographic distribution. However, the purpose of the present study was to investigate relative incidence of various bacterial species in buffaloes suffering from mastitis.

MATERIALS AND METHODS

One hundred milk samples from clinical mastitis of buffaloes were collected during 2010. Before collection of milk samples, the surroundings of teat canals were cleaned with antiseptics (spirit) and then first few drops of milk were discarded. The milk samples were collected in sterilized bijoux bottles and brought to the Laboratory of the Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam. Before processing of the samples, used glassware such as Petri dishes, pipettes and flasks etc were kept in 1% HCL solution overnight and washed well with distilled water for several times then dried in oven at 65◦C. After that, the sterilization was carried-out in hot air oven at 180◦C for one and half an hour. The bacterial culture media were prepared and used for detailed investigation of bacterial organism by Difco (1962). Both, solid and broth media were used. In solid media: nutrient, blood and MacConkey's agars and while in broth medium: nutrient broth was prepared; cultured and colony characteristics were recognized. A pure colony from cultured dishes was picked up and smeared on a cleaned glass slide and stained by Gram's Method of staining and staining characteristics were recorded. Furthermore, a few biochemical tests were also conducted to confirm the identification of bacterial organisms, for this purpose, oxidase, coagulase, indole, Voges Proskauer, urease, methyl red,

gelatin liquefaction, Simmon's citrate, H_2S production, catalase and TSI tests were carried-out (Difco, 1962 and Bergey's, 1992).

RESULTS AND DISCUSSION

The incidence of each bacterial species in clinical mastitic milk samples of buffaloes.

The incidence of each bacterial species isolated and recognized from clinical mastitic milk samples of buffaloes are presented in Table 1. Of the 100 samples studied, 70 (70%) were found positive for bacterial growth. Nine bacterial species were recognized from clinical mastitic milk samples of buffaloes. The bacterial species identified from samples were *Bacillus cereus* (2), *Citrobacter* species (1), *Escherichia coli* (7), *Micrococcus luteus* (11), *Proteus vulgaris* (3), *Pseudomonas aeruginosa* (1), *Streptococcus dysgalactiae* (7), *Streptococcus uberis* (3) and *Staphylococcus aureus* (34), and their incidence in samples was 2.85, 1.42, 10.0, 15.71, 4.28, 1.42, 10.0, 4.28 and 48.57%, respectively. However, the higher incidence of bacterial species in clinical mastitic milk samples of buffaloes was recorded for *Staphylococcus aureus, Micrococcus luteus, Escherichia coli* and *Streptococcus dysgalactiae*, showed incidence of 48.57, 15.71, 10.0 and 10.0% respectively, while somewhat lower *Bacillus cereus*, *Citrobacter* species, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Streptococcus uberis*, which had their presence in the order of 4.28, 1.42, 4.28, 1.42 and 4.28% respectively.

The incidence of different bacterial species in mastitic milk samples of various quarters of buffaloes.

The incidence of different bacterial species in mastitic milk samples of various quarters of buffaloes was also studied. For the purpose, 100 mastitic milk samples were analyzed and the incidence of different pathogenic organisms was determined from different quarters of udders of buffaloes. The data about the incidence of each bacterial species in different quarters were gathered in Table 2. The most common bacterial pathogen detected in both the quarters (front and hind quarters) of the buffaloes was *Staphylococcus aureus* and its incidence was noted as 6 and 8 in the right and left of front quarter respectively while 15 and 5 in the right and left quarters of hind quarter respectively. However, its higher incidence was observed to be 15 in right quarters of hind quarter. The second most pathogenic species encountered during current investigation were *Micrococcus luteus* and *Streptococcus dysgalactiae* and their incidence in different quarters was also observed (Table 2).

The incidence of pure and mixed bacterial species in the clinical mastitic milk samples of buffaloes

The incidence of mixed and pure infections in clinical mastitic milk samples recorded is given in Table 3. Of the 70 positive mastitic milk samples examined, 55 (78.57%) and 15 (21.43%) were determined having pure and mixed bacterial species respectively. It is clear from the present investigation on clinical mastitis

in buffaloes that pure infection was common as compared to mixed infections. Only a small number of samples were recorded with mixed infections whereas a huge number of samples (55) were detected with pure infection or caused by individual bacterial species (Table 3).

The incidence and predominance of bacterial species in the mastitic milk samples of buffaloes.

The incidence and predominance of the bacterial species found during present study are shown in Table 4. The most predominant species recorded in clinical mastitic milk samples of buffaloes was *Staphylococcus aureus* and its dominancy was recorded 34 (48.57%) times in the samples while the second most dominant species observed was *Micrococcus luteus* and its dominancy was recorded 11(15.(71%) times in the samples. Whereas the third most dominant species noted in the mastic samples was *Streptococcus dysgalactiae* and its dominancy detected 8 (11.42%) times in samples. The rest of the organisms recorded formed a fraction of these major species. It was concluded that the species were most infective and are responsible to cause mastitis in buffaloes. Further concluded that the most dominant species are considered to be pathogenic and show their dominance in causing various kinds of diseases in various subjects and cells of the organs. This might be due to nature of their toxins they posses and number of receptors present on their cell wall to adhere and infect various types of cells of individual or common subjects.

The incidence of bacterial species in various conditions of mastitic milk samples of buffaloes.

The data regarding the physiological changes caused by bacterial species in the mastitic milk samples of buffaloes are presented in Table 5. From 100 mastitic milk samples, only 70 samples were detected as positive for different bacterial species. The bacterial species changed the milk physiology and produced different characteristics in milk collected from mastitis in buffaloes. The characteristics recorded were the mastitic milk with flakes, watery milk, milk with pus, milk with blood tinge and milk with white colour and odour. Nine bacterial species were identified from the samples. The natures of the organisms were recognized according to the changes produced in the milk samples with pure or mixed infections. All bacterial species except *Citrobacter* species and *Pseudomonas aeruginosa* produced some small and large flakes (36 samples) in samples. From these, the species *Staphylococcus aureus* produced smaller and some larger and greenish flakes in 17 milk samples. Similarly, it also changed milk into watery (2), milk with pus (3), milk with blood tinge (3) and milk samples with unpleasant odour (9). Generally, all the bacterial species were found responsible to produce flakes in milk samples. Furthermore, all except *Bacillus cereus* generated odour in mastitic milk samples. Whereas *Escherichia coli* produced similar changes as seen changes produced by *Staphylococcus aureus* in the mastic milk samples. While *Micrococcus luteus* developed some flakes in the mastitic milk samples but no any physiological changes were recorded in the milk samples as the species was detected from the samples.

The incidence of pure and mixed bacterial species in the mastitic milk samples of buffaloes.

The incidence of the bacterial species in clinical mastitic milk samples of buffaloes are presented in Table 6. However, from 100 samples, 30 were found to be organism free while pure infections were found in only 55 samples. However, mixed infections 2-3 species were recorded in individual samples. While no any 4 different species were detected in a single sample. The present investigation reveals that mixed infections were not common in clinical mastitic milk samples of buffaloes. However, a few samples were detected with mixed infections. A major portion of samples were recorded with pure infection.

Table 1. The number and percentage incidence of individual bacterial species in clinical mastitic milk samples of buffaloes

Bacterial species	Number of samples occurring in	%
Staphylococcus aureus	34	48.57
Bacillus cereus	2	2.85
Citrobacter species	1	1.42
Escherichia coli	7	10.0
Micrococcus luteus	11	15.71
Proteus vulgaris	3	4.28
Pseudomonas aeruginosa	1	1.42
Streptococcus dysgalactiae	8	11.42
Streptococcus uberis	3	4.28
Total	70	

Table 2. The number and percentage incidence of each bacterial species in different quarters of udders of buffaloes.

Bacterial species	Front quarters		Hind quarters		Total Number
	Right	Left	Right	Left	
Staphylococcus aureus	6	8	15	5	34
Bacillus cereus	-	-	-	2	2
Citrobacter species	-	-	1	-	1
Escherichia coli	7	-	-	-	7
Micrococcus luteus	3	1	7	-	11
Proteus vulgaris	2	1	-	-	3
Pseudomonas aeruginosa	-	1	-	-	1
Streptococcus dysgalactiae	-	4	2	2	8
Streptococcus uberis	2	1	-	-	3
Total	20	16	25	9	70
%	28.57	22.85	35.71	12.85	100

Table 3. The number and percentage incidence of pure and mixed bacterial species identified from clinical mastitic milk samples of buffaloes.

Animal species	Total Number of mastitic milk samples	Number of positive samples	% of positive samples	Number of pure samples	% of pure samples	No. mixed samples
Buffalo	100	70	70.0	55	78.57	15

Table 4. The number and percentage predominance of bacterial species in clinical mastitic milk samples of buffaloes

Bacterial species	Number of samples occurring in	%
Staphylococcus aureus	34	48.57
Micrococcus luteus	11	15.71
Streptococcus dysgalactiae	8	11.42
Escherichia coli	7	10.0
Proteus vulgaris	3	4.28
Streptococcus uberis	3	4.28
Bacillus cereus	2	2.85
Pseudomonas aeruginosa	1	1.42
Citrobacter species	1	1.42

Table 5. The number and percentage incidence of bacterial species isolated from different conditions of clinical mastitic milk samples of buffaloes.

Bacterial species	Samples with flakes	Watery samples	Samples with pus	Samples with blood tinge	Samples with white colour and odour
Staphylococcus aureus	17	2	3	3	9
Bacillus cereus	1	1	-	-	-
Citrobacter species	-	-	-	-	1
Escherichia coli	2	1	1	-	3
Micrococcus luteus	8	-	-	-	3
Proteus vulgaris	1	-	-	-	2
Pseudomonas aeruginosa	-	-	-	-	1
Streptococcus dysgalactiae	5	-	-	-	2
Streptococcus uberis	1	-	-	-	2
Total	36	4	4	3	23

Table 6. The number of bacterial species occurred in individual clinical mastitic milk samples of buffaloes.

Number of species present in samples	0	1	2	3	4
Number of samples occurring in	30	55	10	5	0
% of total samples	30.0	55.0	10.0	5.0	0

The incidence of bacterial species recorded in milk samples during study was *Staphylococcus aureus* (34), *Bacillus cereus* (2), *Citrobacter* species (1), *Escherichia coli* (7), *Micrococcus luteus* (11), *Proteus vulgaris* (3), *Pseudomonas aeruginosa* (1), *Streptococcus dysgalactiae* (7) and *Streptococcus uberis* (3) and their incidence in milk samples were 48.57, 2.85, 1.42, 10.0, 15.71, 4.28, 1.42,11.42 and 4.8% respectively.

Similar results regarding the incidence of individual bacterial species were recorded by Hameed *et al.* (2008). They recorded relatively higher incidence of *Staphylococcus aureus* in buffaloes (53.85%), followed by *Escherichia coli* (15.8%), *Streptococcus dysaglactiae* (3.85%) respectively. Whereas Park *et al.* (2007) determined the incidence of various bacterial species in bovine mastitis during their study. They recorded the incidence of *Staphylococcus aureus* in 1.59%, *Streptococcus uberis* in 0.83%, *Escherichia coli* in 1.56% and *Pseudomonas aeruginosa* in 1.59% samples. An individual incidence of bacterial species was also observed by Hameed *et al.* (2008) in mastitic milk samples of buffaloes are very close to the incidence of individual bacterial species noted in the present investigation. One can see from the results how the findings are much closed, further that this similarity in the incidence might be due to animals are living in the same conditions as our animals from which the samples were collected and examined. However, the results regarding the incidence of each bacterial species observed from clinical mastitic samples for the present study do not in accordance to the findings of Park *et al.* (2007), who recorded lower incidence of individual species in bovine mastitis as compared to the present results. This lower incidence indicates that the animals were provided highly hygienic conditions, those conditions might be helped in contributing lower incidence of the species to cause mastitis in the animals. Furthermore, this lower incidence may be due to animals are frequently provided treatment to check bacterial infections in udder etc. While such practices of the treatment are very rears on some animal farms as a tool for prevention of bacterial infections of udders in province of Sindh.

During present investigation 100 mastitic milk samples were analyzed and the incidence of different pathogenic organisms was determined in different quarters of udders of buffaloes. The most common bacterial pathogen detected in both the quarters of the buffaloes was *Staphylococcus aureus* and its incidence was noted 6 and 8 in the right and left of front quarter respectively while 15 and 5 in the right and left of hind quarters respectively. However, its higher incidence was observed to be 15 in right of hind quarters. The second most pathogenic species

encountered during current investigation were *Micrococcus luteus* and *Streptococcus dysgalactiae* and their incidence in both quarters was also observed. Similar results were obtained with quarter–wise by Khan and Muhammad (2005); they recorded the higher incidence of bacterial infections in hind quarters of buffaloes. Further reported that, among the isolates, *Staphylococcus aureus* was found the most frequent organism in the mastitic milk samples of both the quarters of buffaloes and its frequency was observed as 45%. Meanwhile they also recorded the incidence of other species indifferent quarters of udders of buffaloes. The pattern and frequency of occurrence of bacterial species in different quarters of udder of buffaloes recorded in this survey are very similar obtained by the above workers. They recorded *Staphylococcus aureus* as the most frequent with higher incidence in various quarters of udders of buffaloes is also observed in the present study. However, the present results regarding the incidence of bacterial species in different quarters of buffaloes do agree with the findings of the above workers, they also recorded similar pattern of incidence and frequency in different quarters of udders of buffaloes.

In this survey, 55 (78.57%) were found with pure infection while 15 (21.42%) were determined with mixed infections. The data regarding the incidence of pure and mixed infections in clinical mastitic milk samples were recorded as 78.57% and 21.42% respectively are in agreement to that of Fazlani (2005), who recorded 56 (83.6%) milk samples with pure infection while 16.4% samples with mixed infections from mastitic milk samples of camel. Unfortunately, it is very difficult to compare the results of the incidence of pure and mixed infections in clinical mastitis of buffaloes of the present study because no any authors presented such findings separately for pure and mixed infections in mastitis of buffaloes caused by various bacterial species. Therefore, the results of the present investigation are very similar to that of Fazlani (2005), who obtained the same results for clinical mastitis in camels as recorded in the present study for buffaloes.

The most dominant species detected from the mastitic milk samples was *Staphylococcus aureus* and its dominancy was recorded 34 (48.57%) times in samples whereas the second most dominant species observed was *Micrococcus luteus* and its dominancy was recorded 11 (15.71%) times in mastitic milk samples of buffaloes. In scientific literature no such kind of investigation was carried out by any authors before on bacterial species of mastitic milk samples of buffaloes. It is very difficult to compare the results regarding predominance of the species in milk samples. However, the results about predominant species in the milk samples of buffaloes could be compared to the results obtained by Deverajani (2005) for open and unopened wounds of camels. She recorded the most predominant species in the wound sample of camels were *Escherichia coli* and *Staphylococcus intermedius,* their dominancy was recorded as 12 (17.91%) times in the samples. Whereas the second most dominant species observed during her survey on wounds of camels was *Staphylococcus aureus* and its dominancy was noted 10 (14.92%) times in wound samples. Nevertheless, there is no doubt that these are the bacterial organisms can cause any kind of

infections in any type of animal species, even organs and also various kinds of cells, therefore the results of the present study can be compared with the findings of Deverajani (2005) observed for the camel wounds. It is very sorry that, these results could not be compared to exact nature of the species regarding their dominancy in mastitic milk samples of buffaloes. However, the incidence and predominance of bacterial species encountered during investigation on clinical mastitis in buffaloes are very close to the findings of above worker. The difference is that, we recorded from clinical mastitis in buffaloes while she recorded in camel wounds. Otherwise, no host specific difference was noted during the study. The same species produced wounds in camels also caused mastitis in buffaloes.

During present study, except *Citrobacter* and *Pseudomonas aeruginosa*, all other bacterial species recognized from samples were produced some smaller and larger flakes/clots (36 samples) in milk samples (Table 5). Sabry and Salama (2007) conducted a study to determine the clinical, bacteriological, and therapeutic role against mastitis in buffaloes. A total of 80 quarter milk samples were obtained aseptically from 56 buffaloes. The bacterial species, *Coliforms* were found the most common organisms (46 cases), followed by *Staphylococcus aureus* (7 cases), *Streptococcus uberis* (3 cases) and *Streptococcus aglactiae* (1 case). They recorded swelling, hotness, painful reaction with serious containing clots in buffaloes suffering from the udder infections of the Coliform organisms. The results obtained regarding the physiological changes caused by various bacterial species in milk samples of buffaloes are in accordance with results of the above workers. They encountered the same bacterial species as recorded in the present study. In this investigation, a number of changes in the milk samples were determined while Sabry and Salama (2007) recorded and mentioned in their study only the clots in the milk samples of buffaloes with clinical mastitis. Further that, in scientific literature, no such related information was available to discuss and compare the results of the present survey on clinical mastitis in buffaloes to other workers. Only single related information was obtained from available literature and the results of the present study were discussed and compared.

The mixed bacterial organisms' 2-3 bacterial species were detected in a single sample. The samples contained single bacterial organism was noted in 55 (55%) samples, 2 bacterial species in 10 (10.0%) whereas 3 bacterial species in 5 (5.0%) samples but none of the samples contained 4 different bacterial species. Kumar (2009) who counted more than single bacterial species in a single mastitic milk sample of buffaloes. The author further stated in the light of his study that mixed infections in the udder was common. On the other hand Rind and Khan (2001) carried-out an investigation on various kinds of wounds located on the skins and hides of domestic animals. They recorded 2-4 different bacterial species in a single wound specimen. However, in a single sample 4 different bacterial species were detected by Rind and Khan (2001). Similar findings regarding bacterial species in a single wound sample was investigated by Talan *et al*. (1989) who counted 2-4 different species in a single sample. Cheema and Mahmmod (1980) recorded 29.06% mixed infections that contained 2-4 different

species. They further stated that mixed infections are common in wound samples. Keeping in view the results of the above authors and the results of the present study indicate that mixed infections are common whether it would be wound or mastitis, in both the conditions, the opportunities of the mixed infections are more common because the wounds on skins/hides and the opening of the teats are directly involved to the ground, therefore there are a lot chances for the bacterial organisms to invade any breach in skins or injuries in the teats or udders.

CONCLUSION

It is concluded from present investigation that these bacterial species *Staphylococcus aureus, Bacillus cereus, Citrobacter species, Escherichia coli, Micrococcus luteus, Proteus vulgaris, Pseudomonas aeruginosa, Streptococcus dysgalactiae and Streptococcus uberis* are considered to be the major causal agents of clinical mastitis in buffaloes. Further concluded that the *Staphylococcus aureus*, *Micrococcus luteus* and *Streptococcus dysgalactiae* are responsible to cause clinical mastitis in buffaloes. It is also concluded that hind quarters are more susceptible to the pathogens as compared to front quarters. All bacterial species might have capability to change milk molecules into flakes, secondly that all species can produce unpleasant odour in milk, thirdly that only two bacterial species: *Staphylococcus aureus* and *Escherichia coli* are the bacterial species responsible to destroy/digest the cells and cause pus in milk samples. It is further concluded that pure infection was common in mastitic milk samples of buffaloes as compared to mixed infections.

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