ISSN 1023-1072

Pak. J. Agri., Agril. Engg., Vet. Sci., 2013, 29 (1): 88-95

STUDY ON CLINICAL MASTITIS IN BUFFALOES CAUSED BY *STAPHYLOCOCCAL* SPECIES

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ABSTRACT

An investigation was carried-out on clinical mastitis in buffaloes caused by *Staphylococcal species*. Of the 100 milk samples collected and examined on various culture media, 70 (70%) were found positive for bacterial growth, while 30 (30%) were considered to be free from any bacterial infection. From 70 positive mastitic samples, 29 (41.42%) were purely caused by *Staphylococcus aureus*; while 5 (7.14%) samples were recorded as positive for *Staphylococcus aureus* and other species, the remaining 36 (51.43%) were also found positive for other than *Staphylococcus aureus*. A relatively higher prevalence of bacterial mastitis was noted in front quarters against hind quarters. In front quarters, the higher prevalence of infection was detected in right quarters (28.52%), while lower in left quarters (22.85%). Similarly, a higher prevalence of bacterial infection was recorded in right quarters (35.1%) of hind quarter while lower prevalence was observed in left quarters (12.5%) of hind quarters.

Keywords: Clinical mastitis, buffaloes, prevalence, *Staphylococcal* species.

INTRODUCTION

Clinical mastitis is characterized by sudden onset of swelling and redness of udder, pain and reduced and altered milk secretion from the affected quarters. The milk may contains clots, flakes or of watery in consistency and accompanied by fever, depression and anorexia (Hillerton, 1999). Mastitis, which may be clinical, is an important mammary gland disease that is usually caused by bacterial infection. If not treated properly, it creates a serious problem and resulting economic consequences, mainly reduction in milk production. However, several bacterial pathogens can cause mastitis, *Staphylococcus aureus* is one of the most important etiologic agent in mastitis of cows, buffaloes, goats and sheep (Motta *et al.,* 2001). Moreover, *Staphylococcus aureus* is probably the most infectious agent because it causes a chronic and deep infection in the mammary glands that is extremely difficult to cure (Motta *et al.,* 2001).

The prevalence of clinical bovine mastitis caused by *Staphylococcus aureus* in 53.85% buffaloes in Tehsil Burewala, Pakistan (Hameed *et al.,* 2008). Only a few studies of *Staphylococcal* mastitis concerning buffalo intramammary infections have been reported sub-clinical mastitis in buffalo is not enough to significantly influence milk production as in other domestic ruminants. In Pakistan, the quarter wise and animal wise prevalence of buffalo mastitis from different area of Attock was carried-out. However, the quarter wise prevalence was recorded as 44.75% while animal wise, the prevalence was observed as 44% (Bachaya *et al*., 2005). Whereas in the rural areas of Rio de Janeiro State, Brazil, bovine sub-clinical mastitis was reported to be higher as 40% than in other areas of the country, whereas *Staphylococcus aureus* was identified in up to 37% of isolates from goat with sub-clinical mastitis (Motta *et al.,* 2001).

Knowledge regarding geographical distribution of *Staphylococcus aureus* in animal herds might help to formulate strategies for reducing the spread of infection. During the past decade, the epidemiology of *Staphylococcus aureus* mastitis in dairy animals has been studied using various molecular typing

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methods. Several studies revealed that only a few specialized clones of *Staphylococcus aureus* are responsible for a broad geographic distribution of the disease.

The purpose of the present study was to investigate the prevalence of *Staphylococcal* species in buffaloes suffering from mastitis in and around Tandojam, Sindh, Pakistan.

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 (sprit) 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 the samples, used glassware such as Petri dishes, pipettes, 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 sterilization was carried out in hot air oven at 180°C for one and half hour.

The bacterial culture media were prepared and used for detailed investigation of bacterial organisms (Difco, 1962). Both, solid and broth media were used. In solid media: nutrient, blood and MacConkeys agars and while in broth medium: nutrient broth were prepared, the cultural and colony characteristics of the growing causative agent 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. A few biochemical tests were conducted to confirm the identification of bacterial organism, for this purpose, oxidase, coagulase, indole, Vogues-Proskauer, urase, methyl red, gelatin liquefaction, Simmons's citrate, H_2S production, catalase and TSI tests were carried-out (Difco, 1962).

RESULTS AND DISCUSSION

The overall prevalence of bacterial species in 100 mastitic milk samples of buffaloes during present investigation is shown in Table 1. Of the samples collected and examined on various culture media, 70 (70%) were found positive for bacterial growth, while 30 (30%) were shown no growth on culture media and considered to be free from any bacterial infection. It is concluded from the present study that majority of the mastitis cases in buffaloes are caused by bacterial species. However, mastitic milk samples which exhibited no growth on culture media could be attributed to some predisposing factors of non-bacterial etiology or buffaloes may be treated previously with a variety of antibiotics prior to sampling.

During present survey on clinical mastitic milk samples of buffaloes, 70 mastitic milk samples were positive for bacterial infections, of which 29 (41.42%) were purely caused by *Staphylococcus aureus*, whereas 5 (7.14%) samples were recorded as positive for *Staphylococcus aureus* and other species, while remaining 36 (51.43%) were also found positive other than *Staphylococcus aureus* (Table 2). The study reveals that the *Staphylococcus aureus* was the common bacterial species responsible to cause clinical mastitis in buffaloes.

Table 1. The overall number and percentage prevalence of clinical mastitis caused by various bacterial species in buffaloes.

Animal species	Total No. of mastitic milk samples examined	No. of positive milk samples	% of positive samples	No. of negative samples	% of negative samples
Buffalo	100	70	70%	30	30%

Milk samples from both the quarters of buffaloes were collected and examined for bacterial presence (Table 3). In front quarters, 20 right and 16 left quarters were found infected with different bacterial species, the prevalence was observed as 28.52 and 22.85% respectively. While in hind quarters, 25 rights and 9 left were also infected with various bacterial species, the prevalence of bacterial mastitis in hind quarters was recorded as 35.1 and 12.5% respectively. A relatively higher prevalence of bacterial mastitis was noted in front quarters as compared to hind quarters. In front quarters, the higher prevalence of infection was detected in right quarters (28.52%), while lower in left quarters (22.85%) of front quarters. Similarly, a higher prevalence of bacterial infection was recorded in right quarters (35.1%) of hind quarter while lower prevalence was observed in left quarters (12.5%) of hind quarter (Table 3).
Generally, the overall prevalence of bacterial infection was recorded in right (35.1%) of hind quarter whereas second higher prevalence of bacterial infection was also found in the right (28.52%) of the front quarter (Table 3). It is clearly concluded from present survey that the right side quarters of both front and hind quarters are more susceptible and get infected as compared to left side, because we have recorded a large number of quarters were involved in mastitis caused by various bacterial species. The one of the major reason may be the buffaloes who always lay down for longer period on the ground by right side and the quarters of the both, the right side remain in continuous touch with contaminated manure or soil, therefore the chances of prevalence of bacterial infection in right side quarters would be higher as compared to other side.

Of the 100 samples examined through their growth on different cultural media, 70 (70%) samples were found positive and showed bacterial growth while 30 (30%) were found free from any bacterial growth and considered to be free from any bacterial infections. Bachaya *et al.* (2005) conducted an investigation to determine the prevalence of bacterial species in clinical/sub- clinical mastitis of udder quarters and individual buffalo in Attock district of Punjab. The milk samples were subjected to surf field mastitis test (SFMT) considered to be reliable test, the overall prevalence in udder quarters and individual buffalo was recorded as 58.75 and 77.98% respectively. They further observed that the maximum prevalence of bacterial species in mastitis in individual buffalo was observed 82.61% in Tehsil Pindighaib, followed by 73.33, 80.0 and 76.0% in Tehsil Attock, Jand and Fateh Jang respectively. Whereas Sabry and Salama (2007) also carried-out a study on clinical, bacteriological and therapeutic aspects with acute mastitis in buffaloes. On bacteriological examination out of 80 mastitic milk samples, 56 were found with acute mastitis, the bacterial prevalence in mastitis was recorded as 70%. The overall prevalence of clinical mastitis in buffaloes caused by bacterial species observed by above authors is very similar to the findings of present study regarding the prevalence, because they also recorded in Pakistani buffaloes. Furthermore, that the environmental and managemental conditions are very similar in both the provinces, they recorded in Punjab while we observed in the province of Sindh. The prevalence of clinical mastitis always depends on managemental and environmental and milking conditions but it is least concerned over the breed and species of the animals. Therefore, the present results regarding the overall occurrence of clinical mastitis buffaloes caused by bacterial species are in agreement with the results of the above authors. However, the data presented in this text for the overall prevalence of clinical mastitis in buffaloes are also differed from Arshad *et al.* (2006), Marta *et al.* (2007), Hameed *et al.* (2008), Yousaf *et al.* (2009) and Oliveria *et al.* (2000) who recorded some what lower prevalence in buffaloes and other animals. Further that, they recorded only species-wise individually but not general prevalence caused by pathogenic bacterial species, therefore, these results could not be compared with the results of the above authors recorded for individual bacterial species in their studies. However, the results could be compared and interpreted later on, with the results of above authors for the individual bacterial incidence because we have also recorded in this survey for individual species.
During investigation, a particular emphasis was given to record the prevalence of *Staphylococcus aureus* in clinical mastitic milk samples of buffaloes. For the purpose, 100 samples were collected and examined, 70 samples were found positive for bacterial infection. Of the 70 positive samples, 29 (41.42%) were purely caused by *Staphylococcus aureus* (Table 2). One can expect from these results that how much this pathogenic species was so active that caused 41.42% clinical mastitis in buffaloes in the province Sindh, Pakistan.

Similar results regarding the prevalence of *Staphylococcus aureus* in mastitic milk samples of buffaloes were recorded by Arshad *et al*. (2006), who observed the prevalence of *Staphylococcus aureus* in 23 out of 33 mastitic milk samples. Whereas Hameed *et al.* (2008) recorded the prevalence of *Staphylococcus*

aureus in 53.85% mastitic milk samples of buffaloes while 50% in cattle samples. Khan and Muhammad (2005) also observed similar prevalence of *Staphylococcus aureus* in mastitic samples of buffaloes. They measured the highest (45%) frequency of *Staphylococcus aureus* among the isolates in the samples of the buffaloes during their study. However, a lower prevalence was recorded by Chah *et al.* (2003) and Nordhaug *et al.* (1994) both recorded 26.1 and 8.6% in humans and cattle samples respectively.

The results about the prevalence of Staphylococcus *aureus* in clinical mastitic milk samples of buffaloes recorded in the present study are close in all respects to that of Arshad *et al.* (2006) and Hameed *et al.* (2008). The reasons for the similarity in the results are that both authors conducted a study in conditions where managemental and environmental conditions are prevailing with the same pattern, even the same species of the buffaloes because there is no clear description of animals mentioned by the authors, this might be due to mixed breed, therefore the bacterial species showed similar prevalence of infection in the area where both authors conducted investigation as the present study where we carried-out under the same conditions and got very similar results. Unfortunately the results of present survey do not agree with the findings of Chah *et al.* (2003) and Nordhaug *et al.* (1994), who recorded some variable results, this variation might be due to difference in the managemental and environmental systems, further that the conditions might not favouring the pathogenic organism for more prevalence, secondly the animal species which might play role in decreasing the prevalence due to strong immunity as compared to the animals of the present study that made animals to resist the organisms for less prevalence in causing clinical mastitis in buffaloes or may be due to some other reasons which are not clear to be brought in discussion in detail.

Generally, an overall prevalence of bacterial infection was recoded in right quarters (35.1%) of the hind quarter whereas second higher prevalence of bacterial infection was also found in the right quarters (28.52%) of the front quarter. It is clearly concluded from the present survey that the right side teats of both quarters were more susceptible and were infected as compared to left side, because a larger number of quarters were involved in mastitis caused by various bacterial species. The one of the major reason may be the buffaloes who always lay down for longer period on the ground by right side and the teats of both the quarters' right side remain in continuous touch with contaminated manure or soil, therefore the chances of prevalence of bacterial infection inside teats would be higher as compared to other side. Khan and Muhammad (2005) conducted the quarter-wise comparative study on the prevalence of mastitis caused by bacterial species in buffaloes. They recorded a higher prevalence (29%) of mastitis in the hind quarters of Pakistani buffaloes. While Bachaya *et al.* (2005) also carried-out similar investigation on quarter-wise prevalence of mastitis in buffaloes to record the quarter-wise and animal-wise prevalence in cattle and buffaloes in four different areas of Attock. The overall quarter-wise prevalence of mastitis was recorded as 44.75%, while animal-wise the prevalence was observed as 44%. However, the findings regarding the prevalence of mastitis in hind quarters (35. 1% in right quarters) observed in this study are very close to the values recorded by Khan and Muhammad (2005) who observed 29% prevalence of mastitis in the hind quarters of buffaloes whereas the findings about the prevalence of mastitis in hind or front quarters of buffaloes recorded during present survey are also coincide to the findings of Bachaya *et al.* (2005) who noted somewhat higher prevalence (44.75%) as compared to the present study. It is clear from the study, that we have carried-out proper work and adopted correct procedure that made the prevalence of mastitis in hind quarters very close to the above workers.

Table 2. Number and percentage prevalence of *Staphylococcus aureus* in clinical mastitic milk samples of buffaloes collected and investigated.

Animal species	Total No. of positive mastitic milk samples examined	No. of positive samples with *Staph. aureus*	% of positive samples with *Staph. aureus*	No. of samples with pure *Staph. aureus*	% of samples with pure *Staph. aureus*	No. of samples with *Staph. aureus* and other bacterial species	% of samples with *Staph. aureus and* other bacterial species	No. of positive samples for other bacterial species	% of positive samples for other bacterial species
Buffalo	70	34	48.57%	29	41.42	5	7.14	36	51.43

Table 3. The number and percentage prevalence of clinical mastitis caused by bacterial species in different quarters of udders of buffaloes.

Animal species	Front quarters						Hind quarters					
	No. of un-infected right quarters	No. of infected right t quarters	% of infected right quarters	No. of uninfected left quarters	No. of infected left quarters	% of infected left quarters	No. of uninfected right quarters	No. of infected right quarters	% of infected right quarters	No. of uninfected left quarters	No. of infected left quarters	% of infected left quarters
Buffalo	13	20	28.57	8	16	22.85	7	25	35.71	6	9	12.5

CONCLUSION

It is concluded that *Staphylococcus aureus* is capable to cause up to 42% mastitis in buffaloes alone. Further observed that the species also caused clinical mastitis in buffaloes in association with other bacterial organisms. Right side quarters of both the front and hind quarters are more susceptible and get infected as compared to left side, because a large number of quarters were involved in mastitis caused by various bacterial species. However, relatively higher prevalence of bacterial mastitis was noted in front quarters as compared to hind quarters. In front quarters, the higher prevalence of infection was detected in right quarters (28.52%), while lower in left quarters (22.85%) of front quarters. Similarly, a higher prevalence of bacterial infection was recorded in right quarters (35.1%) of hind quarters while lower prevalence was observed in left quarters (12.5%) of hind quarters.

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(Received January 18, 2011; Accepted March 28, 2013)