The Pharma Innovation Journal 2021; SP-10(7): 846-848

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(7): 846-848

www.thepharmajournal.com
Received: 01-05-2021
Accepted: 22-05-2021

T Surya
ICAR-National Dairy Research Institute, Karnal, Haryana, India

R Selvarani
Veterinary College and Research Institute, Orathanadu, Tamil Nadu, India

Narendra P Singh
ICAR-National Dairy Research Institute, Karnal, Haryana, India

Subrata Koloi
ICAR-National Dairy Research Institute, Karnal, Haryana, India

M Mohan
ICAR-National Dairy Research Institute, Karnal, Haryana, India

Sunil Kumar Mohapatra
ICAR-National Dairy Research Institute, Karnal, Haryana, India

Corresponding Author
T Surya
ICAR-National Dairy Research Institute, Karnal, Haryana, India

A pilot study on identification of infectious mastitogen responsible for mastitis in Sahiwal and Karan Fries cows

T Surya, R Selvarani, Narendra P Singh, Subrata Koloi, M Mohan and Sunil Kumar Mohapatra

Abstract
The majority of mastitis pathogens are bacterial in origin and just a few species of bacteria such as *E. coli*, *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae* and *Streptococcus agalactiae* account for most cases. Season is the major factor which influences the animal health and makes them susceptible to the infection. In summer coliform mastitis is more prevalent because of summer temperature and humidity which increase the coliform counts. In this study, Sahiwal and Karan Fries cows were screened for mastitis from the herd maintained at Livestock Research Centre (LRC) of NDRI, Karnal, Haryana. The milk samples of these mastitic cows were mostly found positive for S. aureus (in Sahiwal 83.33%, in Karan Fries 91.66%) compared to *E. coli* (in Sahiwal 16.67%, in Karan Fries 8.33%) which significantly could be used in treating the animals and further study can be carried to analyse the mode of infection.

Keywords: mastitis, mastitogen, Sahiwal, Karan Fries, *E. coli*, *S. aureus*

Introduction
Mastitis is an inflammation of mammary gland which may be infectious or noninfectious. Organisms such as bacteria, mycoplasma, yeasts and algae have been implicated as causes of the disease. Watts (1988) [1], identified that there are 137 different organisms as a cause of mastitis. The majority of mastitis pathogens are bacterial in origin and just a few species of bacteria such as *E. coli*, *Staphylococcus* aureus, *Streptococcus uberis*, *Streptococcus dysgalactiae* and *Streptococcus agalactiae* (Kuang *et al.*, 2009) [2] account for most cases. Mastitis pathogens have been classified as contagious or environmental pathogen (Blowey & Edmondson, 1995) [3]. Contagious pathogens are adapted to survive within the host (particularly within the mammary gland). They are establishing sub-clinical mastitis, and elevate the somatic cell count (leukocytes predominantly neutrophils and epithelial cells) in milk from the affected quarter. They can spread from one cow to another cow at or around the time of milking (Radostitis *et al.*, 1994) [4]. The major contagious pathogens are *Staphylococcus aureus*, *Streptococcus dysgalactiae* and *Streptococcus agalactiae*. In contrast the environmental pathogens are opportunistic invaders of the mammary gland and not adapt to survive within the host that is they invade, multiply, trigger host immune response and are rapidly eliminated by the host. The major environmental pathogens are Enterobacteriacae (particularly *E. coli*) and *Streptococcus uberis*. Many workers reported that *Staphylococcus* species is the chief etiological agent of mastitis in cattle and buffaloes (Singh *et al.*, 2005; Sharma *et al.*, 2007; Kumar *et al.*, 2010) [5, 6, 7]. This organism is ubiquitous in nature and can colonize in the skin as well as in the udder. About 90% of udder infections including both clinical and subclinical mastitis caused by *Staphylococcus aureus* (Ahmad *et al.*, 1988) [8]. The relative prevalence rate of various bacterial species isolated from the clinical and subclinical cases (Sori *et al.*, 2005) [9] showed that *Staphylococcus aureus* contributed 44.03% (clinical mastitis accounted for 34.04% and subclinical mastitis 49.43% of the above figure). Sentitula *et al.*, (2012) [10] tested 117 mastitic milk samples in NDRI yard among that 109 samples were found to be positive for Staphylococcus esp. *Staphylococcus* aureus accounted for 43.3%.
Season is the major factor which influences the animal health and makes them susceptible to the infection. In summer coliform mastitis is more prevalent because of summer temperature and humidity which increase the coliform counts in the bedding material (Smith *et al.*,1985; Erskine *et al.*,1988) [11, 12].

2. Materials and Methods

EMB agar for *E. coli* isolation and MSA (Mannitol salt agar) for isolation of *Staphylococcus aureus* were purchased from Hi-Media and TM-Media Company respectively. Potassium phosphate dibasic, Potassium dihydrogen orthophosphate were used for buffer preparation.

2.1 Selection of animals

The animals were selected from the herd maintained at Livestock Research

Centre (LRC) of NDRI, Karnal, Haryana. The animals were kept under normal routine management practice as followed at the institute's farm.

Initial screening of infected cows for clinical or subclinical mastitis was carried out on 92 KF and 73 Sahiwal cows. The milk of these cows was tested by modified California Mastitis Test (mCMT) for identification of sub-clinical mastitis. Cows suffering from clinical mastitis were diagnosed on the basis of clinical symptoms. Out of 165 animals, we randomly selected 24 Sahiwal and 24 KF which included 12 clinical and 12 subclinical cases distributed over three different seasons [4 cows of each breed with clinical and subclinical infection in each season i.e thermo neutral (October-November), winter (December to January) and summer (April 15th -May)]. Major mastitogens in milk were isolated from all these cows using selective agar media.

2.2 Microbiological analysis of milk

Mastitis infected cow's milk was processed on the same day of collection. Plate count agar, EMB agar and MSA agar were taken according to the number of samples to be processed.

2.2.1 Preparation of buffer

1.74 gram of Potassium phosphate dibasic (K2HPO4) and 1.36 gram of Potassium dihydrogen orthophosphate (KH2PO4) were added in 1 litre of distilled water and mixed gently. Of this, 9 ml of buffer taken in each test tube (5 test tubes per sample).

2.2.2 Preparation of agar

1.75% of Plate count agar, 11.1% of Mannitol salt agar and 3.6% of EMB agar were prepared. Then the agar and buffer were autoclaved.

2.2.3 Procedure (In laminar flow)

Autoclaved flask and test tubes were placed in laminar flow for further processing. Serial dilution of milk samples starting from 101 up to 105 were made with buffer solution. These dilutions were marked on test tubes. 0.1 ml of diluted sample (103 dilution for EMB and Mannitol salt agar and 104 dilution for plate count agar) was taken in the petridish. Then warm agar (about two third of volume of petridish) was poured in each dish. The contents were mixed properly by shaking. Contents covered with dish cover were allowed to cool slightly. After solidification of agar, all the plates were incubated in incubator at 37 ^{0}C (Inverted position) for about 24 to 36 hours. The colonies were counted with help of marker in each petridish.

3. Result

The table below shows the incidence of occurrence of clinical and subclinical mastitis in Sahiwal and KF cows (165) in NDRI farm. Highest number of subclinical mastitis cases (53) was found during summer followed by thermo neutral (41) and winter (32). Out of total 39 cases of clinical mastitis 16 were found during summer, 13 in thermo neutral and 10 in winter season.

The milk samples of these mastitic cows were mostly found positive for *S. aureus*. Very few numbers of samples were found positive for *E. coli* as depicted in table below:

Table 1: Distribution of mastitogens in NDRI farm

Breed	Sahiwal		Karan Fries	
Mastitogen	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*
Percentage	83.33%	16.67%	91.66%	8.33%

4. Discussion

Different selective media were taken for differentiating the bacteria from mastitic milk. Mannitol salt agar showed positive results for *S. aureus*, growth of yellow colour colonies and the media colour turned to yellow. Metallic sheen on EMB indicated the growth of *E. coli*. Macconkey agar differentiated *E. coli from* other coliforms and there were pink colour colonies on Macconkey agar. Keviletsu & Yadav (2010) [13], reported that the occurrence of mastitis in Sahiwal and Murrah in NDRI farm was 26.43 and 18.91% respectively. Quarter wise prevalence of sub clinical mastitis was observed to be 31.16% in K.F cows (Ashutosh *et al.*, 2017) [14] similar to these observations we also observed lowest percentage of mastitis cases during winters. The data could be helpful in culling or segregation of cows for reducing spread of mastitis in the herd. Staphylococcal mastitis, usually caused by *Staphylococcus aureus*, a common form of bovine mastitis worldwide. About 90% of new udder infections including both clinical and subclinical mastitis were reported to be caused by *Staphylococcus aureus* (Ahmed *et al.*, 1988) [8]. Fox and Gay (1993) [15], found *Staphylococcus aureus* as a major agent of contagious bovine mastitis. Sentitula *et al.*, (2012) [10] by using biochemical and PCR based assays found that 43.3% mastitic milk samples positive for Staphylococcus in NDRI herd. The proportion of *S. aureus*, *Streptococcus epidermidis*, *Streptococcus agalactiae*, *Streptococcus uberis* and *Streptococcus dysgalactiae* among the mastitic cases was found as 64.9, 7.7, 5.1, 1.7, 48.7, 65.8 and 0.8%, respectively. In approximately half of the (52.1%) cases mastitis reoccurrence was observed. Further they observed that prevalence of *S. aureus*, *Streptococcus agalactiae*, *Streptococcus uberis, and Streptococcus epidermidis* in reoccurring mastitic cases was 73.7, 63.9, 45.9 and 6.6%, respectively. Few reports (Smith *et al.*, 1985; Erskine *et al.*, 1988) claimed prevalence of Coliform mastitis also in summers due to rising ambient temperature and humidity that increase the coliform counts in the bedding material. The results in this study suggested that the frequency of occurrence of *S. aureus* was greater than *E. coli* in mastitis infected KF and Sahiwal cows which could be used to further improve the treatment and management of the cows.

5. References

1. Watts JL. Etiological agents of bovine mastitis. Veterinary microbiology 1988;16(1):41-66.
2. Kuang Y, Jia H, Miyanaga K, Tanji Y. Effect of milk on antibacterial activity of tetracycline against *Escherichia coli* and *Staphylococcus aureus* isolated from bovine mastitis. Applied microbiology and biotechnology, 2009;84(1):135-142.
3. Blowey R, Edmondson P. Mastitis-causes, epidemiology

and control. Mastitis control in dairy herds (ed. R Blowey and P Edmondson) 1995, 27-45.
4. Radostits OM, Leslie KE, Fetrow J. Herd health: food animal production medicine (No. Ed. 2). WB Saunders Company 1994.
5. Singh R, Sharma Neelesh, Soodan JS, Sudhan, NA. Etiology and sensitivity of bacterial isolates from sub clinical mastitis in cattle from Jammu region. Journal of Research SKUAST-J 2005;4(2):223-224.
6. Sharma N, Maiti SK, Sharma KK. Prevalence, etiology and antibiogram of microorganisms associated with Sub-clinical mastitis in buffaloes in Durg, Chhattisgarh State (India). International Journal of Dairy Science 2007;2(2):145-151.
7. Kumar R, Yadav BR, Singh RS. Genetic determinants of antibiotic resistance in *Staphylococcus aureus* isolates from milk of mastitic crossbred cattle. Current microbiology 2010;60(5):379-386.
8. Ahmad J, Hussain I, Mahmood N, Munir R. Lactose determination as an aid to subclinical mastitis diagnosis. Pakistan Vet. J 1988;8:25-28.
9. Sori H, Zerihun A, Abdicho S. Dairy cattle mastitis in and around Sebeta, Ethiopia. Journal of Applied Research in Veterinary Medicine 2005;3(4):332.
10. Sentitula YR. Incidence of *Staphylococci* and *Streptococci* during winter in mastitic milk of sahiwal cow and murrah buffaloes. Ind. Journal of Microbiology 2012;52:153-159.
11. Smith KL, Todhunter DA, Schoenberger PS. Environmental mastitis: Cause, prevalence, prevention1, 2. Journal of Dairy Science 1985;68(6):1531-1553.
12. Erskine RJ, Eberhart RJ, Hutchinson LJ, Spencer SB, Campbell MA. Incidence and types of clinical mastitis in dairy herds with high and low somatic cell counts. Journal of the American Veterinary Medical Association 1988;192(6):761-765.
13. Keviletsu K, Yadav BR. Incidence of mastitis in Sahiwal cattle and Murrah buffaloes of a closed organized herd. Indian Journal of Animal Sciences 2010;80(5):467-469.
14. Ashutosh M, Pandita S, Yadav PS, Parkunan T. Quarter-wise prevalence of subclinical mastitis in crossbred cows. Indian Journal of Animal Research 2017;52(1):116-120.
15. Fox LK, Gay JM. Contagious mastitis. The Veterinary clinics of North America. Food animal practice 1993;9(3):475-487.