American Journal of Current & Applied Research in Microbiology

Research Article

Subclinical Mastitis of Cows in Swat Area: Prevalence and Associated Microorganisms

Shaukat Hayat[1], Haider Hayat[2], Farmanullah[3]*, Sardar Ali[1], Tariq Khan[4], Muhammad Altaf Hussain[3] and Javaid Iqbal[5]

[1]*Livestock & Dairy Development Department, (Extension) Khyber Pakhtunkhwa, Peshawar*
[2]*Department of Veterinary Pathology, University of Veterinary and Animal Sciences, Lahore*
[3]*Faculty of Veterinary and Animal Sciences, Lasbela University of Agriculture, Water and Marine Sciences, Uthal, Pakistan*
[4]*Department of Fisheries and Aquaculture, University of Veterinary & Animal Sciences, Lahore*
[5]*Department of Veterinary Anatomy & Histology, FAH&VS, Sind Agri, University, Tandojam*

***Address for Correspondence:** Farmanullah, Faculty of Veterinary and Animal Sciences, Lasbela University of Agriculture, Water and Marine Sciences, Uthal, Balochistan, Pakistan, E-mail: farman_aup@yahoo.com / farman.vas@luawms.edu.pk

Submitted: 19 November 2020; **Approved:** 21 December 2020; **Published:** 22 December 2020

Citation this article: Hayat S, Hayat H, Farmanullah, Ali S, Khan T, et al. Subclinical Mastitis of Cows in Swat Area: Prevalence and Associated Microorganisms. American J Current App Res Microbiol. 2020 Dec;2(1): 027-030.

ABSTRACT

This study was conducted to determine the prevalence rates of the subclinical mastitis in cows in Swat and also to investigate the associated organisms. In the study 200 lactating cows from four villages of Swat (Kula Deer, Bela, Takhta Band and Totano Bandai) were examined through surf field mastitis test for the presence of subclinical mastitis. The overall prevalence rates were found to be 49.0%, highest in Bela (56.0%), followed by Takhta Band (50.0%), Totano Bandai (48.0%) and Kula Deer (42.0%). Out of total 800 quarters 217 were found to be infected (27.12%). The SFMT positive samples when cultured at VR & DIC, Balogram, Swat, revealed eight bacterial species which are: *Staph aureus* (33.18%), *E coli* (21.50%), *Strep agalactea* (15.20%), *Strep pyogenes* (8.75%), *Strep dysgalactae* (5.99%), *Pseudomonas aeruginosa* (4.15%), *Bacillus cereus* (2.76%), *Proteus vulgaris* (1.84%), and (6.63%) with mixed growth.

Keywords: Cows; Prevalence; Subclinical mastitis; Swat; Microorganism

INTRODUCTION

The role of livestock in rural economy of Pakistan may be realized from the fact that 30-35 million rural population is engaged in livestock raising.

Livestock plays an important role in the economy of Pakistan by providing essential components of human diet in the form of milk, meat, eggs, etc. Among the diseases, mastitis is one of the most important infectious diseases of dairy animals, causing significant financial losses worldwide and is a major cause of antibiotics use in dairy cows [1]. Mastitis is the inflammation of the parenchymal cells of the mammary glands associated with microbial infections and physiological changes [2]. Mastitis is classified into two types-clinical and sub-clinical mastitis. In the clinical mastitis all the five cardinal signs of inflammation are present, while the sub-clinical form is bereft of any obvious manifestation of inflammation and thus can only be detected by the milk examination [3]. Sub-clinical mastitis is 3-40 times more common than the clinical mastitis and causes the greatest overall losses in most dairy herds [4]. Keeping in view, its importance, present study was designed to determine the prevalence rates of subclinical mastitis in cows in Swat, using Surf Field Mastitis Test (SFMT) and to identify the associated microorganisms in order to ensure specific antibiotic therapy.

MATERIALS AND METHODS

Collection of milk samples

This study on sub-clinical bovine mastitis was carried out to determine the prevalence rates in Swat area. The first part of the study was collection of milk samples for physical examination and apparently mastitis free milk samples were subjected to Surf Field Mastitis Test (SFMT) test, while the second part of the study was focused on morphological characteristics and confirmation through biochemical tests for identification of associated microorganism. Two hundred animals, cows were selected from four villages i.e., Bela, Takhta Band, Kula Deer and Totano Bandai of district Swat. The present study was carried out in the bacteriology and pathology laboratory section of Veterinary Research Institute (VRI) swat, Khyber Pakhtunkhwa. Samples were collected from the Sahiwal, Friesian and Jersey cross cows, respectively over a period of six months from October 2017 to February 20218. We were collected 800 milks samples from 200 healthy cows were collected from the four villages of Swat Bela, Totano Bandai, Kula Deer and Takhta Band, and aseptically in sterilized bijoux bottles. First of all the udders were washed with tap water and dried with separate tissue papers whereas the quarters (teats) were disinfected with a piece of cotton soaked in 70% ethyl alcohol. Then we had been send all samples for further investigation of diseases subclinical mastitis of cows from the Sahiwal, Friesian and Jersey cross cows, respectively.

In this study for determining the efficiency of direct (culture and somatic cell counting) and indirect diagnostic tests (CMT, SFMT and WST) for subclinical mastitis. The study animals were stall fed with zero grazing and managed in small herds (2-3 heads per farm). All animals were hand milked with letdown of milk induced by suckling calves or hand massage. Standard mastitis control practices (e.g., pre and post milking antiseptic teat dipping and dry period antibiotic therapy) were not in place. Animals in the first week and last two months of lactation were excluded to avoid the possibility of false positives results [5]. After discarding first few streams of milk, 2 sets of QFS were collected at the time of morning milking. The first set of samples was collected aseptically (National Mastitis Council, Inc., USA 1990), placed in an ice box, transported to 458 Trop Anim Health Prod (2010), mastitis research laboratory swat kpk, Pakistan and used for cultural examination (National Mastitis Council, Inc. 1990) and direct microscopic Somatic Cell Counting (SCC) [6].

Surf Field Mastitis Test (SFMT)

The animals looked apparently free of mastitis. One thousand six hundred milk samples, four samples from each animal, one from each quarter, were collected aseptically in sterilized bijoux bottles. It needs to be mentioned at this stage, while collecting samples for sub-clinical mastitis, the samples found positive for clinical mastitis were not included in the samples count to be considered for sub-clinical mastitis, however the record of such samples was maintained properly and incorporated in the results. On the basis of reddish discolouration of milk due to presence of blood in milk, the samples were declared positive for clinical mastitis. The samples looking normal apparently were subjected to Surf Field Mastitis Test (SFMT) at the site of collection for diagnosis of sub-clinical mastitis. The samples found positive for sub-clinical mastitis were brought to veterinary research station (North). Balogram Swat in thermal boxes containing ice packs, for examination. Before samples were drawn in bijoux, the udders were washed with tap water and dried with separate tissue papers. The quarters (teats) were disinfected with a piece of cotton soaked in 70% ethyl alcohol. First few strips of milk were discarded to avoid contamination as much as possible.

The routinely used Surf Field Mastitis Test (SFMT), which is based on the principle that interaction between an anionic detergent (surf) and the cationic DNA of the somatic cells present in abnormal milk results in coagulation and gel formation [7]. SFMT solution was prepared by adding 3 grams of detergent Surf Excel (Lever Brothers Pakistan) to a measuring cylinder and adding distilled water up to 100 ml mark. An equal quantity of 3% Surf solution was added to a test sample on a plastic fabricated paddle with four rectangles for each quarter. The mixture was swirled for about one minute and then examined visually for the presence of small flocculates and/or gel which indicated presence of infection.

Bacteriological examination of the samples

Samples from SFMT positive animals were collected in a screw capped bottle and transferred in thermal boxes containing ice packs [8] to VR & DIC, Balogram, Swat, for further examination. Samples were incubated aerobically at 37°C for 24 hour and then centrifuged at 3000 rpm for 20 minutes. The supernatant was discarded and a loopful from the sediment and QFS was streaked on to 1 quadrant of blood agar (5% sheep RBCs) and MacConkey's agar (Difco Lab., Detroit, Michigan, USA) plate and incubated at 37°C for 48 hours. The growth of different micro-organisms was subjected to routine microbiological tests (e.g., Gram staining, catalase, oxidase, tube coagulase, CAMP test). Presumptively identified major mastitis pathogens (*Staphy aureus, Strept agalactiae* and *E coli)* were confirmed by using their respective commercially available identification kits (API Staph, API 20 *Strep* and API 20E; BioMerieux, France). Minor mastitis pathogens (coagulase negative *staphylococci*, *Nondiphtheriae Corynebacteria; diphtheroids like Corynebacterium bovis*) were identified by their growth characteristics and routine biochemical tests (catalase, oxidase, coagulase, etc) as per National Mastitis Council Inc. (1987).

RESULTS AND DISCUSSION

Animal wise prevalence

The prevalence rates in four villages of Swat recorded in present study were as: Bela 56%, Totano Bandai 48%, Kula Deer 42% and Takhta Band 50%, thus the highest prevalence was found in Bela (56%) and the lowest in Kula Deer (42%). The overall prevalence rate was found to be 49.00% shown in table 1.

[95% Confidence Interval (CI), $p \pm 1.96\sqrt{(pq/n)} = 42.0\text{-}56.0\%$]

Comparison of prevalence rates among the villages through statistical analysis shows that the prevalence rates did not differ significantly ($\Sigma\chi2 = 1.999$, df = 3, $p > 0.5$ as at α level of 0.05 is 7.81 and at α level of 0.5 is 2.37).

Quarter wise prevalence

Out of total of 800 quarters (200 cows) examined, 217 were found positive for sub-clinical mastitis, and thus the overall quarter wise prevalence was recorded as 22.87% shown in table 1. Quarter wise prevalence by position was as: FL = 20.5%, FR = 30.5%, HL = 16.50% and HR = 41.00% and thus varied from 16.5% to 41.0%. Apparently the effect of position was quite substantial and warranted statistical analysis to evaluate the extent of variation due to position in udder. The data were, therefore, subjected to Chi Square analysis against the null hypothesis of uniform distribution of affected quarters in all the four positions in udder are shown in table 2.

The statistical analysis shows that at degree of freedom 3, the tabulated value of chi square at α level of 0.001 is = 16.27 ($\Sigma\chi2 = 26.57$, df = 3), hence the quarter-wise prevalence were not the same for all the four positions, but differ significantly from one position to another. Not all the four quarters ran the same risk of getting the sub-clinical mastitis ($\chi2 = 26.57$, df = 3, $p < 0.001$). Naturally it was be of interest to analyze whether quarters of front position differ from those of the hind position with respect to prevalence of sub-clinical mastitis. Similarly it would be of interest to know whether quarters on right side differ from those on left side with respect to risk of the disease. Data on these two aspects have been set out in table 3, and subjected to Chi Square analysis.

The statistical analysis shows that at degree of freedom 1, the tabulated value of chi square at α level of 0.05 is 3.84 and at α level of 0.001 is 10.83; hence the prevalence rates in left and right quarters differ significantly. Quarters on the right side of the animal were at higher risk of getting the disease ($\chi2 = 18.89$, df = 1, $p < 0.001$). The statistical analysis shows that at degree of freedom 1, the tabulated value of chi square at α level of 0.05 is 3.84, hence the prevalence rates in front and hind quarters are comparable and did not differ significantly ($\chi2 = 1.068$, df = 1, $p > 0.05$).

Bacterial spp associated with subclinical mastitis

The true positive 217 samples when cultured revealed eight bacterial species involved which are: *Staph aureus* in 72 (33.18%), *E coli* in 47 (21.50%), *Strept agalactiae* in 33 (15.20%), *Strept pyogenes* in 19 (8.75%), *Strept dysgalactae* in 13 (5.99%), *Pseudomonas aeruginosa* in 09 (4.15%), *Bacillus cereus* in 06 (2.76%) and *Proteus vulgaris* in 04 (1.84%) samples, 07 samples (3.22%) showed mixed infection of the *Staphy aureus* and *Strept agalactiae* while no bacterial species were detected in 06 samples (3.27%), although they were found positive for sub-clinical mastitis through SFMT shown in table 4.

The overall prevalence rate (49.0%) and quarter-wise prevalence (27.12%) is in line with the [9] results (36.67% CMT) and [10] results at Attock (quarter-wise 12.08% and animal-wise 44%) [11] results regarding bacterial species (*Staph aureus* 21.3%, *E coli* 15.9%, *Strept dysgalactiae* 15.6%, *Strept uberis* 11.1%, coagulase-negative *staphylococci* 6.2%, Arcanobacterium pyogenes 6.1% and Klebsiella species 4.2% and [12] results (*Staph aureus* 31.03%, *E coli* 24.31%, *Strept agalactiae* 10.34%, *Strept dysgalactiae* 10.34%, *Bacillus cereus* 10.32% and *Strept pyogenes* 6.89%) also support the results of the present study.

CONCLUSION

This study indicates that subclinical mastitis in cows (49.0%) is a major problem and threat to the livestock farmers of at Swat. The probable reasons might be the poor managemental practices adopted by the farmers and thus warrant immediate but sustainable attention. The disease control is possible through improving husbandry techniques, milking hygiene, adapting of dry cow therapy (sealing of the teats by acrylic latex at the time of drying off) and examination of milk fortnightly through surf field mastitis test.

Table 1: Prevalence rates and quarter-wise prevalence of sub-clinical mastitis in cows of swat.

S.No	Village	Animal Tested	Positive Animal	Prevalence (%)	Position	Left Side	Right Side	Total
1	Bela	50	28	56	Front Position	FL41/200	FR61/200	102/400
2	Takhta bandai	50	24	48				
3	Kula deer	50	21	42	Hind Position	HL 33/200	HR 82/200	115/400
4	Totano bandai	50	25	50				
5	Total	200	98	49	Total	74/400	143/400	217/800

Table 2: Test for uniformity of distribution with respect to position.

S.No	Position	Observed Affected Quarters	Expected Affected Quarters	χ^2
1	Front Left (FL)	41	54.25	3.23
2	Front Right (FR)	61	54.25	0.83
3	Hind Left (HL)	33	54.25	8.32
4	Hind Right (HR)	82	54.25	14.19
5	Total	217	217	26.57

Table 3: Comparison in left and right side quarters, and comparison of prevalence between front and hind positions of cows (Figures in parenthesis are frequencies expected on the basis of Null hypothesis).

S. No	Samples Status	Left Side	Right Side	Total	Samples Status	Front Position	Hind Position	Total
1	Positive	74 (101.5)	143 (101.5)	217	Positive	102 (108.5)	115 (108.5)	217
2	Negative	326 (298.5)	257 (298.5)	583	Negative	298 (291.5)	285 (291.5)	583
3		400	400	800		400	400	800

Table 4: Bacterial species isolated from SFMT positive samples (*n* = 217).

Name of Bacterial Specie	Samples	% age
Staph Aureus	72	33.18
E cloi	47	21.65
Strept Agalactiae	33	15.20
Strept Pyogenes	19	8.75
Strept Dysgalactiae	13	5.99
Pseudomonas aeruginosa	09	4.15
Staph aureus + Strep Agalactiae (mixed infection)	07	3.22
Bacillus cereus	06	2.76
Proteus vulgaris	04	1.84
Samples with no growth	07	3.22

ACKNOWLEDGEMENT

I express sincere thanks to our Research Team/Group for their kind collaboration and assistance. Special thanks to Professor Dr. Javaid Iqbal for their supervision and guidance. I am also very appreciative to the coauthors for their critical and technical improvement of our manuscript.

Author Contributions

Shaukat Hayat and Haider Hayat wrote the original draft of the manuscript. Shaukat Hayat, Muhammad Altaf Hussain, Sardar Ali and Javaid Iqbal conceived and designed the experiment. Farmanullah, Shaukat Hayat and Tariq Khan helped in data collection and management. Sardar Ali, Haider Hayat, and Farmanullah. helped in data analysis.

REFERENCES

1. Mitchell JM, Griffiths MW, McEwen SA, McNab WB, Yee AJ. Antimicrobial drug residues in milk and meat: causes, concerns, prevalence, regulations, tests, and test performance. J Food Prot. 1998 Jun;61(6):742-56. doi: 10.4315/0362-028x-61.6.742. PMID: 9709262.
2. Radostitis OM, Blood DC, Gray CC. Veterinary medicine. 8th edition. Billiere Tindall, London, 1996; 563-614.
3. Hillerton JE. Balancing mastitis and quality. Proc. British Mastitis Conference, Stonelergh, UK. 1999; 1-7.
4. Schultz LH, Brown RW, Jasper DE, Natzke P. Current concepts of bovine mastitis. The National Mastitis Council, Inc. Washington Dc, USA. 2nd edition. 1978; 6-9.
5. Radostits OM, Gay CC, Blood DC, Hinchcliff KW. Veterinary medicine. WB. Sanunders Co, Philadelphia, USA. 2007.
6. Schalm OW, Carroll G, Jain JC. Bovine mastitis. Lea and Febiger, Philadelphia, USA. 1971.
7. Thiers FO, Benites NR, Costa EO. Correlation between direct somatic cell count and the california mastitis test (cmt) in cows' milk. Napgama. 1999; 2: 9-12.
8. Gabbar MAK. Manual for field veterinarians on dispatch of specimens for laboratory diagnosis. Field Document No. 3, 2nd edition. Central Veterinary Diagnostic Laboratory, Tandojam, Sindh, Pakistan. 1992.
9. Hunderra S, Ademe, Z, Sintayehu S. Dairy cows mastitis in and around Sebeta. Ethiopia. J Dairy Sci. 2005; 56: 109-115.
10. HA Bachaya, Z Iqbal, G Muhammad, A Yousaf, HM Ali. Subclinical mastitis in buffaloes in attock district of Punjab (Pakistan). Pakistan Vet J. 2005; 25: 134-136.
11. Ericsson Unnerstad H, Lindberg A, Persson Waller K, Ekman T, Artursson K, Nilsson-Ost M, Bengtsson B. Microbial aetiology of acute clinical mastitis and agent-specific risk factors. Vet Microbiol. 2009 May 28;137(1-2):90-7. doi: 10.1016/j.vetmic.2008.12.005. Epub 2008 Dec 11. PMID: 19155148.
12. Holt JG, Krieg NR, Sneath PHA, Stanley JT, William ST. Bergey's manual of determinative bacteriology. 9th Edition. William and Wilkins, Baltinmore, Maryland. 1994