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RESEARCH ARTICLE

Studies on Subclinical Mastitis in Dairy Cows of Jammu and Kashmir

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ABSTRACT

A total of 800 individual quarter milk samples were subjected to the California mastitis test, 413 samples of 130 cows (74.61% crossbred and 25.38% native) were positive, 16 (81.25% crossbred and 18.75% native) of them were in the age group of 2-4 years. 71.53% cows within age group of 5-7 years had subclinical mastitis and only 16.15% cows within age of 7 years and above had subclinical mastitis. 18.11% California mastitis test positive sample upon cultures were cog+ *Staphylococci* positive followed by 14.60% which were *Streptococci* culture positive and only 14.60% milk samples were mixed culture positive. The most effective antibiotics were enrofloxacin (74.93%), gentamycin (67.74%), tetracycline (57.81%) and amoxicillin clavulanic acid (50.37%) respectively. These results show that subclinical mastitis accompanied with analysis of different factors and observations were made that crossbred cows were more struck by subclinical mastitis than native one, youngest cows had the most sensitivity, hot weather increases frequency, major bacterial pathogens was coagulase positive *Saphylococci* and most effective antibiotics was enrofloxacin. Despite large research efforts aimed to gain knowledge and to develop a new control tools for subclinical mastitis, the occurrence of this disease remains a substantial problem for dairy producers.

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INTRODUCTION

Mastitis, the inflammation of mammary gland remains a serious problem for dairy industry and has very serious ramifications for dairy industry worldwide (Abdel-Rady and Sayed, 2009). Mastitis in both clinical and subclinical forms is frustrating, costly and extremely complex disease that results in a marked reduction in the quality and quantity of milk (Harmon, 1994). Annual losses in the dairy industry due to mastitis were approximately 2 billion dollars in USA and 526 million dollars in India, in which subclinical mastitis is responsible for approximately 70% of these losses (Varshney and Naresh, 2004). Mastitis is a common disease entity of dairy cows, accompanied by physical, chemical, pathological and bacteriological changes in milk and glandular tissue (Samad, 2008). The disease is usually classified as acute, subclinical, and chronic based on aetio-pathological findings and observations (Tripathi and Chaltopadhyay, 1993). In India, among various pathogens responsible for mastitis, *Staphylococcus aureus* presents growing and formidable challenges for animal health concerns and remains predominant organism in crossbred cows (Singh and Kataria 2009; Peer *et al.,* 2009). Subclinical mastitis is a major problem affecting dairy cows all over the world and causes enormous losses for farmers and consequently influences the national income of the country (Ramachandrainh *et al.,* 1990). The occurrence of subclinical mastitis in dairy cows is often surprising to producers, because subclinically infected udder quarters can develop clinical mastitis and the rate of new infections can be high (Zdunczyk *et al.,* 2003). Cows with subclinical mastitis are those with no visible changes in the appearance of the milk and/or the udder, but milk production decreases by 10 to 20% with undesirable effect on its constituents and nutritional value rendering it of low quality and unfit for processing (Holdway, 1992). Although there are no visible or palpable external changes, the infection is present and inflammation has occurred in the udder (Blowey and Edmondson, 1995).

The invisible changes in subclinical mastitis can be recognized indirectly by several diagnostic methods including the California Mastitis Test (CMT), the Modified White Side Test (MWST), Somatic Cell Count (SCC), pH, chlorine and catalase tests. These tests are preferred to be screening tests for subclinical mastitis as they can be used easily, yielding rapid as well as satisfied results (Lesile *et al.,* 2002).

Somatic cell count can be measured quantitatively by CMT. It is a simple, easy and low cost screening test for subclinical mastitis at dairy farms (Dingwell *et al*., 2003). Validity of CMT in diagnosis of subclinical mastitis was established (Gharagozloo *et al*., 2003). The CMT gives an indirect estimate of SCC because it based upon a gelling reaction between the nucleic acid of the cells and a detergent reagent. The CMT is more perfect, efficient and reliable than other field and chemical tests for diagnosis of subclinical mastitis (Mahmoud, 1988; Viani *et al.,* 1990; Behera and Dwivedi, 1992; El-Balkemy *et al.,* 1997). El-Attar *et al.* (2002) reported that the CMT is more useful for sublicinical mastitis than MWST and moreover CMT was in a good agreement with bacteriological results (Park *et al.,* 1982). Identifying and eliminating subclinical mastitis may have significant economic benefits as preventing clinical mastitis and decreasing the amount of discarded milk (Dingwell *et al*., 2003). Subclinical mastitis is also of vital importance in its association with zoonotic diseases in which milk acts as a vehicle of pathogens causing tuberculosis and brucellosis (Shoshani *et al.,* 2000) and causes reduction in the quality and quantity of milk (Harmon, 1994), therefore its detection in its subclinical form is very important. Due to the aforementioned economic and public health importance, this investigation was conducted to study subclinical mastitis.

MATERIALS AND METHODS

Animals

A total of 250 dairy cows were examined at Teaching Veterinary Clinical Services Complex, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu and Kashmir, over two years from March 2012 till March 2014. The dairy cows were of crossbred cows and native breed. Also, they were of different ages. All dairy cows were apparently healthy with clinically sound udder secreting apparently normal milk. They lived nearly under the same conditions from point of habitat, hygiene and feeding systems. All animals were subjected to clinical and physical examinations with special interest towards the udder and teats. At the time of each examination, the breed of the cow, season, age of the cow, degree of quarter attack were recorded.

Milk samples

In a clean environment, thoroughly wiping the teats with 70% ethyl alcohol while paying extra attention to teat orifice was applied. After discarding the first few milk squirts, a total of 800 individual quarter milk samples were collected from 250 cows and subjected to the CMT.

California mastitis test (CMT)

In brief 3 ml of milk sample from each quarter was drawn in 4 shallow cups in the CMT paddle and then equal volume (3 ml) of the commercial available CMT reagents was squirted from a polyethylene wash bottle and added to each cup and mixed together by swirling the paddle in a circular motion for few seconds. The reaction developed almost immediately in the milk containing a high concentration of somatic cells. The peak of reaction was obtained within 10 seconds and scored. The CMT reaction/ results were read immediately as per manufacturer's recommendation and were scored for each quarter (teat) depending on the amount and thickness of gel formed as described by Ikram (1997) as shown in Table 1.

Bacteriological cultures

Each positive CMT milk sample was collected under aseptic conditions in sterile screw caped vials numbered to identify the particular quarter and were sent directly to the laboratory with a minimum of delay for on culture 10% sheep blood agar and MacConkey agar plates for 24 hours and then incubated under aerobic conditions at 37°C and analysed at 24 and 48 hours according to Carter and Cole (1990). Colonies were identified morphologically, microscopically and biochemically according to Quinn *et al.* (1994) and Waage *et al.* (1999).

Antibiotic susceptibility test

Antibiotic susceptibility testing was performed on CMT positive samples and evaluated according to the recommendations of Clinical and Laboratory Standards Institute (CLSI) with 8 different antibiotics namely cephalothin (KF) (30 µg), trimethoprim sulf amethoxazole (STX) (1.25-23.75 µg), amoxicillin clavulanic acid (AMC) (20-10 µg), enrofloxacin (ENR) (5 µg), gentamycin (GM) (10 µg), erythromycin (E) (5 µg), tetracycline (TE) (10 µg), and ampicillin (AM) (10 µg), all of which are widely used in veterinary practice in Jammu and Kashmir. In addition, oxacillin (OXA 1 µg) was used to determine

Table 1: Scoring of CMT results (Ikram, 1997)

Reading			Interpretation	
Aspect	Score		Infection	Related with the average cellular numeration (x 10^3/ml)
	Value	Cross		
Consistency normal or Gray color	0	_	Absent	100
Light gel disappearing after stirring or Purplish gray color	1	±	Infection risk by minor pathogenic	300
Light persistent gel-crumbly filaments or Purple gray	2	+	Subclinical mastitis	900
Immediate thickening viscous cluster at the bottom of the well	3	++	Subclinical mastitis	2700
Thick gel consistency of egg white color dark purple	4	+++	Subclinical mastitis near the clinical expression	8100

methicillin resistance of staphylococci. Antimicrobial susceptibility discs were obtained from Oxoid (Oxoid Limited England), except for ENR (Bayer Turk Kimya San, İstanbul, Turkey). By referring the measurement to a chart, the organisms classified into three categories: susceptible (S), intermediate sensitive (I) or resistant (R) to the antimicrobial drug.

Statistical analysis

The data recorded, wherever applicable, was statistically analyzed using chai square test.

RESULTS

Relation between the subclinical mastitis and CMT

The results of relation between the subclinical mastitis and CMT with scores are shown in Table 2. Out of the 800 quarter milk samples examined by the CMT, 51.61 % samples were positive with 19.12%, 23.37% and 9.12% having the score of 1, 2 and 3 respectively, whereas 48.37% samples were negative with (0) score.

Relation between subclinical mastitis and breed

Relation between the subclinical mastitis and breed is presented in table 3. Results indicate that of the total 250 cows tested in this investigation only 130 cows had subclinical mastitis which included 97 crossbred cows and 33 native cows with percentage of 38.80% and 13.20% and overall percentage of 52.00%.

Relation between the subclinical mastitis and age

Relation between the subclinical mastitis and age are presented in table 4. The present results indicate that among the 130 CMT positive cows 12.30% belonged to the age group of 2-4 years, of which 81.25% cows were crossbred and 18.75% native respectively, whereas 71.53% cows within age group of 5-7 years had subclinical mastitis which included 76.34% crossbred and 23.65% native cows and only 16.15% cows within age of 7 years and above had subclinical mastitis which included 61.90% crossbred and 38.09% native respectively. Thus incidence of subclinical mastitis increases with age and then declines with decline in the milk production of cows.

Relation between the subclinical mastitis and bacterio-logical culture

Relation between the subclinical mastitis and bacteriological culture is shown in table 5 and the from results it was clear that 18.11 % CMT positive sample upon cultures were cog+ Staphylococci positive followed by 14.60% which were *Streptococci* culture positive and only 14.60% milk samples were mixed culture positive respectively. Also the CMT negative quarter milk samples showed on growth upon culture. Thus most prevalent isolated bacteria were coagulase positive *Staphylococci* followed by *Streptococci* and coagulase negative *Escherichia coli* respectively.

Relation between the subclinical mastitis and antibiotic sensitivity test (AST)

Relation between the subclinical mastitis and antibiotic sensitivity test (AST) are presented in the table 6. All the 403 CMT positive milk samples were subjected to Antimicrobial susceptibility using 8 antibiotics or antibiotic combinations (KF, TS, AMC, ENR, GM, E, TE, and AM,) commonly used in Jammu and Kashmir. The most effective antibiotics were ENR (74.93%), GM (67.74%), TE (57.81%), and AMC (50.37%) respectively, while other antibiotics included in the study were less than 50% susceptibility to causative organisms respectively. 10 % of CMT positive samples were methicillin resistance (*Staphylococci*) which was determined by using oxacillin (OXA 1 µg).

Table 2: Relation between the positive CMT and the infection status of the quarters

CMT score	Number of quarters	Percentage (%)
3	153	19.12
2	187	23.37
1	73	9.12
0	387	48.37
Total	800	100

Where 0, 1, 2 and 3 indicate, absence of infection(–), infection by minor pathogen (±), subclinical mastitis(+) and subclinical mastitis(++).

Table 3: Relation between the subclinical mastitis and breed

Breed	Total no of animals	No of animals with subclinical mastitis	Percentage
Crossbred	163	97	38.80
Native	87	33	13.20
Total	250	130	52.00

Table 4: Relation between the subclinical mastitis and age

Age in Year	Crossbred (%)	Native (%)	Total (%)
2-4	81.25	18.75	12.30
5-7	76.34	23.65	71.53
7 and above	61.90	38.09	16.15
Total no.	97	33	130

Table 5: Relation between the subclinical mastitis and bacterio-logical culture

Bacteria	Subclinical mastitis	
	Number	Percentage
cog+ Staphylococci	73	17.67
Streptococci	52	12.59
Escherichia coli Cog-	47	11.38
Staphylococci	45	10.89
Bacillus spp.	13	3.14
Corynebacterium spp.	35	8.47
Enterococcous spp.	11	2.62
Pseudomonas spp.	33	7.99
Pasteurella spp.	19	4.71
Mixed growth	55	13.64
Negative	19	4.60
Total	413	100

DISCUSSION

The present study on subclinical mastitis and its treatment was done because the subclinical mastitis is considered 15-40 times more prevalent than clinical mastitis and accounts for greater losses in terms of milk production (Harmon, 1994), serves as a reservoir of infectious organisms, and accounts to approximately 70% of economic losses due to all farms of mastitis (Varshney and Naresh, 2004). From the aforementioned results overall incidence of subclinical mastitis in 250 daiy cows by the CMT was 52% .Our results are in agreement with

Table 6: Relation between the subclinical mastitis and antibiotic sensitivity test (AST)

	KF	AMC	TS	ENR	GM	E	TE	AM
Susceptible	84	154	102	209	199	80	167	73
Intermediate	100	49	90	93	74	89	66	74
Total susceptible	184	203	192	302	273	169	233	147
Resistance	219	200	211	101	130	234	170	256
Percentage	45.65	50.37	47.64	74.93	67.74	41.93	57.81	36.47

Where KF, TS, AMC, ENR, GM, E, TE and AM indicate cephalothin, trimethoprim sulf amethoxazole, amoxicillin clavulanic acid, enrofloxacin, gentamycin, erythromycin, tetracycline and ampicillin respectively

results of Singh and Baxi (1988) who reported 54.0% incidence of SCM in cows in whole India, however lesser incidences were obtained by Coni *et al.* (1983), Alexandrova (1986), Mahmoud (1988), and Ismail and Hatem (1998), in Italy, Bulgaria, Egypt and Saudi Arabia, respectively. Major bacterial pathogens were coagulase positive *Staphylococci* followed by *Streptococci* and coagulase negative *Escherichia coli* respectively. A same correlation between CMT and bacterial isolation has been previously demonstrated by Egan (1982). The positive CMT without isolation of the causative agent may attributed to failure to isolate organisms by the employed cultural techniques (selective media for *Mycoplasmas, Haemophilus and fungi* were not employed) samples with unspecified mixed cultures and 4.71% of sampled cows for bacterial culture based on CMT score had no growth due to short lived infections that have been cleared by the cow or infections that are characterized by intermittent shedding of bacteria (*S. aglactiae, S. aureus, Mycoplasma*). From the results it was clear that main three bacteria causing subclinical mastitis were coagulase positive *Staphylococci, Streptococci,* and coagulase negative *Escherichia coli* respectively while *Pseudomonas, Corynebacterium pyogenes* and some *Pastreulla* were less commonly involved. Therefore result of our study are in agreement with El-Balkemy *et al.,* (1997); El-Attar *et al.,* (2002); Dego and Tareke, (2003). Subclinical mastitis has a multi factorial nature that involves interaction between host, agent and environment (Thrusfield, 2005). For this reason, the studied factors included breed and bacterial culture (Bendixen *et al.,* 1988), age (Hultgren 2002), management, environment (McDougall, 2003) and hygiene (Ward *et al.,* 2002). With studying the breed factor it was found the native breed cows (37%) were resistant as compared to cross breed cows (59%). Ghazi and Niar (2006) also found native breed more resistant compared to the imported ones. This is primarily due to the genetic resistance (Payne and Wilson, 1999), and also due to the adaptation of these cows to native environment and climate. With regard to the influence of season it was noticed that subclinical mastitis frequency in the examined dairy cows was more significant in hot weather than in cold weather. These observations can be attributed to poor feeding, unbalanced rations and bad hygiene during the hot weather as the green fodders become deficient, in addition to, increasing the insect population. That led to decrease in immunity and consequently the mastitis frequency increases, while during the cold weather, clover becomes somewhat enough and insect population become limited to spread infection. Our results are in agreement with results of Bendixen *et al.* (1988) who also made same observation. The results showed, that 48.37%, 9.12%, 23.37%,and 19.12% of quarters gave 0, ±, +, and ++ score, of CMT respectively which is less as compared to Attia *et al.* (2003) who found 30 and 75% gave +++ and ++ score respectively. Additionally, transmission of subclinical occurs not only among cows but also among quarters within a cow (Lam *et al.,* 1997). Also the results from AST indicated that most effective antibiotics were ENR (74.93%), GM (67.74%), TE (57.81%), and AMC (50.37%) respectively. In conclusion crossbred cows were more struck by subclinical mastitis than native one, youngest cows had the most sensitivity, hot weather increased frequency, major bacterial pathogens was coagulase positive *staphylococci* and most effective antibiotics was enrofloxacin.

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