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Aetio-Prevalence Study on Bovine Sub Clinical Mastitis in Lactating Jersey Cross-Bred Cows

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A B S T R A C T

An investigation was conducted on subclinical mastitis at State University dairy farm, Mansbal in which 100 lactating Jersey cross bred cows were taken in to study. The animals showing clinical signs of mastitis were excluded from the study. Californian Mastitis test (CMT) and somatic cell count (SCC) were used for preliminary screening of animals for subclinical mastitis. Prevalence of subclinical mastitis on the basis of CMT was 52% while as the prevalence on the basis of SCC was 49%. On culture examination 45% was per cent prevalence of subclinical mastitis. The major mastitides isolated were *Staphylococci*, *Streptococci* and *E.coli* whereas the minor pathogens incriminated in mastitis were *Diplococcus*, *Corynebacterium*, *Pseudomonas*, *Micrococcus and Bacillus species.* Out of which *staphylococcus* spp. was 42.2%, *Streptococcus* spp. was 11.1%, *Escherichia coli* was 6.6%, *Diplococcus* spp. 2.2%, *Corynebacterium*spp.2.2%, *Pseudomonas*spp. 2.2%, mixed *Staphylococcus* and *Streptococcus* 22.2%, Gram negative but not *Escherichia coli*, 4.4% *Micrococcus* 2.22% and *Bacillus* species was 4.44%. Out of staphylococcal, 73.68% were coagulase negative while as coagulase positive were 26.31%.Out of streptococci, 60% were alpha haemolytic, 20% were beta haemolytic while as 20% were gamma haemolytic.

Introduction

Subclinical Mastitis is a common problem hindering the production growth in dairy farming. Dairy cattle are highly susceptible to mastitis because of increased metabolic stress to mammary gland (Radositits, *et al.*, 2006). It has been found that despite implementation of recommended control measures for control of mastitis, the menace of mastitis still continues to haunt the dairy enterprise. Annual losses in the dairy industry due to mastitis have been approximately 2 billion dollars in USA and 526 million dollars in India due to subclinical (Varshney and Naresh, 2004).) According to Sheare and Harris (2003), subclinical mastitis is important due to the fact that it is 15 to 40 times more prevalent than the clinical form (for every clinical case of mastitis there will be 15-40 sub clinical cases), it usually precedes the clinical form, is of longer duration, difficult to detect, adversely affects milk quality and production and constitutes a reservoir of microorganisms that lead to infection of other animals within the herd (Khan *et al.*, 2006).Unlike coagulase-positive *Staphylococcus aureus*, coagulase-negative staphylococci are of low virulence, and are rarely associated with clinical disease, although they frequently produce an

inflammatory response measurable as an elevated somaticcell count in the milk (Kudinha *et al.,* 2002). The study was conducted to determine the prevalence of subclinical mastitis along with the causative agent.

Materials and Methods

Collection of milk samples

The study was conducted at Mountain Livestock Research Institute (MLRI, Mansbal). A total of 100 lactating crossbred Jersy cows were selected for Californian Mastitis testing (CMT). The teats were cleaned with water, wiped dry with individual paper towels and disinfected with 70% ethyl alcohol on cotton. After discarding first few streaks of milk, milk was squirted from each teat in sterilised milk sampling tubes. The milk samples were transferred to laboratory at 5^0C and processed for Somatic cell count (SCC) and culturing on the same day.

Laboratory processing

SCC was estimated by Direct microscopic method. A cell count of > 500000/ml was considered positive for subclinical mastitis.

For cultural isolation of the causative agent, a loop full of milk sample was inoculated in nutrient broth and kept for 24 hours at 37^0C for development of turbidity. Next day a loop full of turbid nutrient broth was inoculated on Blood agar and kept for aerobic incubation at 37^0C for 24hrs. Isolate was not considered significant if less than 50 colonies grew from the sample of milk.

Identification of *Staphylococcus* spp., *Streptococci* spp., *Escherichia coli*, *Diplococcus spp.*, *Pseudomonas* spp., *Bacillus* spp., *Corynebacterium* spp. and *Micrococcus* spp. was done on the basis of colony morphology on blood agar, type of haemolysis, Grams stain, Catalase test, oxidase test and coagulase testing. Coagulase testing was done using both slide and tube methods.

Results and Discussion

Californian mastitis test

At Mountain Livestock research institute, Mansbal, 100 cross bred Jersey cows were screened with Californian Mastitis test for presence of Subclinical mastitis**.** Out of 100 cows, 52 showed precipitation with or without gel formation. Thus prevalence of subclinical mastitis at Mansbal was 52% as shown below:

Out of 400 quarters of 100 cows, 157 quarters were positive for subclinical mastitis on the basis of Californian Mastitis test. Hence the quarter prevalence was 39.25 %.

The selected samples were stained with modified Newman Lampert stain for somatic cell count (SCC). Out of all samples 49 samples showed somatic cell count of more than 500000/ml of milk and prevalence of subclinical mastitis on the basis of SCC was 49% as shown below:

Cultural identification

The samples were cultured for bacteriological isolation of the causative agent. Out of all samples 45 samples showed positive growth of bacteria. Thus percent prevalence on the basis of cultural examination was 45%. The main causative agent were *staphylococcus* as 42.2%, *Streptococcus* was 11.1%, *Escherichia coli* was 6.6%, *Diplococcus* 2.2%, *Corynebacterium* 2.2%, *Pseudomonas* 2.2%, *mixed Staphylococcus* and *Streptococcus* 22.2%, Gram negative but not E.coli 4.4%, *Micrococcus* 2.22% and *Bacillus* species was 4.44%.

Californian mastitis test

CMT	No. of cows		Infection
N	48	No gel formation	Absent
T	32	Slight precipitate	Infection risk by minor pathogen
1+	14	Precipitation but without gel	subclinical
2+	5	Gel formation	Serious subclinical
3+	1	Quick gel formation	Subclinical mastitis near the expression of clinical mastitis

Somatic cell count

Somatic cell count/ml	No. of samples	Disease
< 5 00000	51	No
>500000	49	Yes

Cultural identification

Major causative agent	Nos	Average somatic cell count	Per cent prevalence
Staphylococcal	19	2033333	42.2
Streptococcal	5	1400000	11.1
E. coli	3	1500000	6.6
Diplococcus	1	1100000	2.2
Corynebacterium	1	900000	2.2
Pseudomonas	1	950000	2.2
Mixed *Staphylococcus* and *Streptococcus*	10	1800000	22.2
Gram negative but not E.coli	2	1100000	4.4
Micrococci	1	800000	2.22
Bacillus	2	900000	4.44

Out of Staphylococcal, 73.68% were coagulase negative while as coagulase positive were 26.31%. Out of streptococci, 60% were alpha haemolytic, 20% were beta haemolytic while as 20% were gamma haemolytic as shown below:

From the above results it is obvious that the dairy farm of Mountain Livestock Research Institute (MLRI, Mansbal) is having a presence of subclinical mastitis in cross bred jersey lactating cows with a prevalence of 52% and with a quarter prevalence of 39.25%. These findings are in accordance with Bhat *et al.*, 2016 who found an overall prevalence of subclinical mastitis in organised farms of Jammu to be 59.43 per cent (%) and quarter level prevalence to be at 34.78%.Eriksson, 2013 has also reported that the prevalence of subclinical mastitis in Surveys from different developing countries have shown a SCM prevalence of 52.4 – 88.6 % at cow level and 26.7 – 63.2 % at quarter-level. But the findings of Akhoon *et al.*, 2015are contrary who found prevalence of subclinical mastitis as 29.41% at Mansbal dairy farm. The reports of prevalence of sub clinical mastitis ranging from 29 per cent to 97 per cent have also been published different workers (Nickerson *et al.*, 1995; Nath and Dutta, 2007; Junaidu *et al.*, 2011 and Sharma *et al.*, 2012). This variation could be due to difference in breeds, farm management, level of production and differences in study methods for studying mastitis employed by the investigators. (Bhat *et al.*, 2016).

The major mastitides isolated were staphylococcus (42.2%) of which coagulase negative staphylococcus were 73.68% and

coagulase positive were 26.31%. Our findings are in agreement with Bhat *et al.,* 2016 who also found higher prevalence of staphylococcal subclinical mastitis to be 66.67%. Coagulase-negative staphylococci are normal flora of healthy teat skin and constitute a constant source of bacteria to colonise the teat end. The results of the current study show that coagulase-negative staphylococci play a major role in causing bovine sub clinical mastitis and it agrees with Kudinha *et al.,* 2002 who reported higher prevalence of Coagulase negative staphylococcal mastitis (22.9%) than coagulase positive (17.1%).Eriksson, 2013 also found that the most common udder pathogens were Coagulase Negative Staphylococci (CNS) in different areas of south Zambia.

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