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PREVALENCE OF SUBCLINICAL MASTITIS IN THRISSUR DISTRICT OF KERALA WITH SPECIAL REFERENCE TO ETIOLOGICAL AGENT AND ANTIBIOGRAM

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Abstract: A cross-sectional study was carried out to determine the prevalence of subclinical mastitis in lactating dairy cows in and around Thrissur district, Kerala. The study was carried out through field screening surveys by Draminski mastitis detector for each quarter milk sample, followed by bacteriological examination to identify the causative agents of intra-mammary infection. A total of 20 milking cows were examined, out of which 5 (25%) were found positive for subclinical mastitis on the basis of Draminski mastitis detector. Milk samples collected from 5 positive cows were subjected to microbiological culture for the isolation of pathogenic bacteria. Out of the 5 suspected samples examined for Gram's reaction 3 were Gram positive and 2 were Gram negative. Most of the isolates appeared as cocci in chain. The whole milk cultures from affected quarters showed 100 per cent sensitivity to enrofloxacin followed by Ceftriaxone/ Tazobactam (40%), gentamicin (20%) and Cotrimoxazole (20%). However, all other milk cultures were resistant to penicillin G,Cefalexin, Amphicillin, Amoxicillin Clavulanic acid and Cefoperazone.
Keywords: Dairy Cattle, sub-clinical mastitis,draminski mastitis detector, etiology, antibiogram.

Introduction

By selective breeding man developed dairy cattle having mammary glands with a production potential far in excess of the requirements of the newborn calf. Due to size, position and anatomic adjustment for rapid removal of large volumes of milk, the udders of present day dairy cows are prone to injuries and infections. Inflammation frequently follows such incidence producing the condition called mastitis. Recently mastitis is recognized as one of the devastating problem of milch animals which causes huge production losses to livestock industry. The etiological agent and the antibiotic sensitivity vary widely within the population depending on the management practices and geographical area. The existence of any sub-types of bacterial strains may attribute to the variations in antibiotic susceptibility. The emergence of drug resistant bacteria makes the treatment more complex. Experiences in attempt to control mastitis indicates that though the occurrence of inflammation in udder may not be completely preventable in all cows, the frequency of appearance among cows within a

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herd and the intensity of clinical attack may be lessened significantly by adopting practices like identification of causative pathogens, prompt treatment, appropriate udder and environment hygienic measures, culling, vaccination and dry cow therapy. Considering these points, a study was conducted with an objective to detect the prevalence of subclinical mastitis among 20 dairy animals housed in and around Thrissur district, Kerala. An effort was also made to identify the etiological agents responsible for the condition and study of their antibiogram.

Materials and Methods

A total of 20 animals from Thrissur district of Kerala were screened for the study. Milk samples were collected from all four quarters and screened for subclinical mastitis using electronic mastitis detector (Make and model -4×4Q; DRAMINSKI, Poland) as per the method described by Swarup *et al*. (1989). Positive samples were collected under sterile conditions and were transported to the laboratory for microbiological examinations. In order to isolate the bacteria brain heart infusion (BHI) culture media (HIMEDIA laboratories, Mumbai) were used. After obtaining bacterial growth in BHI medium, single colonies were picked up and subjected to Gram's staining in order to identify the morphology and Gram's reaction. In order to determine the bacterial susceptibility towards antibiotics modified Kirby-Bauer method (1966) was used. Muller Hinton agar medium was the medium of choice for detecting antibiotic susceptibility. Suspension of pure culture of isolated bacteria was prepared in BHI broth. The bacterial count in the suspension was standardized as that of standard tube (0.5 McFarland Standard). A sterile swab was dipped into the bacterial suspension and it was properly spread over the Mueller Hinton agar medium. Readymade antibiotic disc of ampicillin, cefoperazone, cotrimoxazole, amoxyclav, ceftriazone-tazobactum, entrofloxacin, gentamicin, pencillin G and cephalexin were placed on the surface of the medium using sterile forceps, and were incubated at 35°C for 18 hours as per the method suggested by the National Committee for Clinical Laboratory guidelines (2001). The diameter of zone of inhibition was measured (in mm) using antibiotic zone scale. The sensitivity of bacteria towards antibiotics were reported as sensitive (S) and resistant (R) according to method described by Sutra *et al*. (1994) [13].

Results and Discussion

Among 20 animals screened for the study only 5 dairy cows were identified as subclinicaly infected. None of the animals were detected for clinical mastitis. Data regarding clinical and subclinical mastitis are summarized in Table 1. Mastitis detector detects

electrical resistance in all the 4 quarters. Healthy quarters usually show readings between 330 and 360 units when analyzed using mastitis detector. Readings below 250 units indicated subclinical inflammation of the quarter. The infected milk sample led to increased salt content which resulted in lower resistance.

Table 1. Electrical resistance detected by Mastitis detector 4×4Q (DRAMINSKI, Poland)

Sl.no	Mastitis detector reading(Electrical resistance in units)				Remarks
	Fore Quarter		Hind Quarter		
	Right	Left	Right	Left	
1	300	320	290	340	-
2	300	390	400	410	-
3	310	280	270	320	-
4	250	240	290	190	Sub clinical
5	360	300	260	270	-
6	200	380	380	390	Sub clinical
7	280	300	320	320	-
8	290	290	260	290	-
9	310	270	250	260	-
10	410	360	280	300	-
11	260	290	310	200	Sub clinical
12	280	340	290	310	-
13	300	320	310	340	-
14	310	300	300	260	-
15	270	270	340	290	-
16	290	250	300	340	-
17	290	360	190	240	Sub clinical
18	310	290	270	280	-
19	420	340	280	300	-
20	350	240	420	410	Sub clinical

Morphology and Gram's reaction of the organisms present in suspected samples are given in Table 2. As per the table 2 out of the 5 suspected samples examined for Gram's reaction 3 were Gram positive and 2 were Gram negative. Most of the isolates appeared as cocci in chain. However bacilli and cocco-bacillary forms were also present.

Studies in different countries have demonstrated different results. In a research performed in Canada by Dingwell *et al*. (2004) [6] Gram positive *Streptococcus sp.* were detected as the most common etiological agent of mastitis. In another research conducted by Green *et al*. (2002) [7] gram negative bacteria, *E. coli*, was the most common pathogen isolated from samples among 480 dairy cows. Hence it was clear that Gram positive and Gram negative organisms, both can act as causative agent for subclinical mastitis. In another research conducted in Tehran by Atyabi *et al*. (2006) [2] in order to determine the incidence of mastitis-causing bacteria, the frequency of coagulase-negative Staphylococcus, *S. agalactiae*, *S. dysgalactiae*, *E. coli*, and *S. Aureus* was reported as 30.27%, 22.11%, 11.43%, 10.16% and 2.89%, respectively. Bacteria constitute the most common etiological agent of mastitis. Among these the most common organism causing mastitis is *staphylococcus aureus* (Jones and Ward,1989). Bacteria that are known to cause mastitis include *Staphylococcus aureus, Streptococcus sp, Coryne bacterium bovis, Mycoplasma bovis, Escherichia coli, Klebsiella sp., Citrobacter sp., Enterobactersp, Pseudomonas sp*, *Coagulase negative staphylococcus sp., Actinomycespyogenes, Nocardia sp., Pasteurella sp.* etc (Nazer and Tavakoli, 1994). Etiology and pattern of the disease may vary greatly within the population due to geographical isolation and varied management practices. As per the observations of the present investigation animals were more prone to organisms of Gram positive nature.

Table 2. Morphology and Gram' reaction of the bacterial isolates from suspected samples

Sl.no	Morphology	Gram's reaction	Suspected organism
1	Cocci in chain	Gram positive	*Enterococci, Streptococci, Staphylococci. A. pyogenes*
2	Rods	Gram negative	*E. coli, Klebsiella sp., and Proteus sp.*
3	Cocco bacillary	Gram negative	*Pasteurella sp.*
4	Cocci	Gram positive	*Enterococci, Streptococci, Staphylococci, A. pyogenes*
5	Cocci	Gram positive	*Enterococci, Streptococci, Staphylococci, A. pyogenes*

The suspected samples were subjected to antibiogram study and zone of inhibition was measured using specific zone scale (Hi-Antibiotic zone scale–c). The result of antibiogram

study is presented in table 3. Outcome was compared with standard table (CLSI (formerly NCCLS) documents M2-A and M7-A) provided by the manufacturers and interpreted as sensitive or resistant. As per the table 3 enrofloxacin were detected as most efficient antibiotic against both Gram positive and Gram negative strains causing subclinical mastitis and penicillin as least one. From the antibiogram effect, it was observed that most of the gram negative organisms showed comparable sensitivity to ceftriazone-tazobactum, cotrimoxazole and gentamicin respectively. Cotrimoxazole, gentamicin and ceftriaxone-tazobactum were noted as effective antibiotics against Gram negative organisms.

A study conducted in Turkey by Kenar (2012) [8], it was observed that the most sensitive and resistant antibiotics against isolates of coagulase-negative staphylococci were cephalothin and trimethoprim, sulfamethoxazole, respectively. In a study conducted to investigate the antibiotic susceptibility of mastitis- pathogen by Brinda (2009) [4] in Romania, higher sensitivity of mastitis pathogens were reported to ampicillin and the most resistance was reported to tetracycline. These results are not consistent with our antibiotic susceptibility results. The variation in sensitivity among the results might be due to the climatic and hygiene conditions of the areas where these studies were done, so it requires further investigations on this field.

Table 3. Antibiogram of positive samples

SL.NO	CODES	ANTIBIOTICS	D-001	D-002	D-003	D-004	D-005
1	AMP	Ampicillin	*	*	*	*	*
2	CPZ	Cefoperazone	*	*	*	*	*
3	COT	Cotrimoxazole	*	S	*	*	*
4	AMC	Amoxicillin/clavulanate	*	*	*	*	*
5	CIT	Ceftriaxone/Tazobactam	S	S	*	*	*
6	EX	Enrofloxacin	S	S	S	S	S
7	GEN	Gentamicin	*	*	S	*	*
8	P	Penicillin G	*	*	*	*	*
9	CN	Cephalexin	*	*	*	*	*

S-sensitive *resistant

Conclusion

This study concludes that 25 % of the animals in Thrissur district showed subclinical mastitis and Gram positive organisms were the major etiological agent. Out of 5 positive samples, 3 samples with Gram positive cocci were sensitive to Enrofloxacin and Ceftriazone Tazobactum, 1 sample with Gram negative cocci were sensitive to Enrofloxacin and Gentamicin, and 1 sample with Gram negative bacilli was sensitive to Enrofloxacin, CeftriazoneTazobactum and Cotrimoxazole in the decreasing order of sensitivity. From the study it was clear that entrofloxacin was the drug of choice for the existing condition. Most of the Gram positive and Gram negative organisms were resistant to Cefalexin, Amphicillin, Pencillin G, Amoxicillin Clavulanic acid and Cefoperazone. These findings will help to avoid treatment failure due to be the indiscriminate use of antibacterials and emergence of micro flora resistant to different antibiotics as a major hindrance in the control of mastitis. Above all good knowledge of the environment and careful management of the identified risk factors will help in implementing preventative programmes to reduce the incidence of subclinical mastitis.

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