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Original Article

Antimicrobial efficacy of combination of lincomycin and spiramycin (Lispiracin™) as systemic dry cow therapy for controlling bovine mastitis

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Abstract
To determine the efficacy of combination of lincomycin and spiramycin (Lispiracin™) for controlling bovine mastitis through systemic dry period therapy, present study was designed. A total of 20 dry pregnant cows were selected randomly at the end of lactation and divided into two equal G1 and G2 groups. Group G1 was treated with lincomycin@ 5mg/kg (IM) and spiramycin@ 10 mg/kg (IM) (Inj. lispiracin®) at end of lactation and at 14th day pre calving while group G2 was kept as control. Samples of milk were collected aseptically at dry off and at day 14th post-calving. The efficacy of treatment was determined through prevalence of mastitis (sub-clinical and clinical) before and after parturition and bacteriological cure rate. Quarter and animal wise prevalence of both clinical and sub-clinical mastitis after systemic dry cow therapy with lispiracin™ group (G1) was lower than control group (G2). This was evaluated through Surf Field Mastitis Test, Somatic cell count, isolation, identification and purification of microbiological cultures. The mean score of surf field mastitis test of G1 group before treatment and at day 14thpost calving was significantly different ($p<0.05$) as compared to G2 group Somatic Cell Count was significantly reduced from 8.0 at dry off to 3.50 ($P<0.05$) after day14th post calving in lispiracin™ group. Quarter wise prevalence (%) of clinical mastitis in G1 group was lower than G2 group. The cure rate of infected quarters at day 14th post calving was 86.6% ($p< 0.01$) with lispiracin™ treated group than that of control group which was 11.11%.It was concluded that systemic dry period therapy especially with combination of lincomycin and spiramycin (lispiracin™) helped in controlling bovine Mastitis.

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INTRODUCTION

Mastitis is a managemental disease of dairy sector which leads to the significant economic losses throughout the world. It deteriorates both quantity and quality of milk production (Aqib *et al.,* 2000). In Pakistan, mastitis ranked as top most production limiting disease of dairy animals (Pinzon-sanchez *et al.,* 2011). It spreads from one animal to other hence affecting the whole herd (Aqib *et al.,* 2000). An intra-mammary infection influences the changes in the mammary gland that leads to the mastitis. S*taphylococcus aureus* is the most prevalent microorganism of mammary gland which is the main source if intra-mammary infections mostly in subclinical mastitic infections (Cengiz *et al.,* 2015). Lack of an effective mastitis control plan leads to intramammary infections during dry period (Dingwell, 2002).

Prevention of mastitis is only possible with programmed and well managed measures. Various strategies has been applied to control bovine mastitis but systemic dry cow therapy is effective one. The antibiotics given immediately

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after the last days of milking are referred as dry cow therapy. Different strategies have been adopted to control mastitis but, systemic dry period therapy is the most effective one. About 70 to 80% existing infection is wiped out by the use of systemic dry period therapy and it also provides safety from new infection by 50 to 75% (Dodd, 1983; Grohn *et al.;* 2004).

The use of antibiotics in dry period therapy is one of basic tools for the control of bovine mastitis. The use of antibiotics in combination has been proved effective during dry period for controlling mastitis problem. To get the full functional effect of dry cow therapy, it should be used in combination (Costa *et al.;* 1996). According to research, 70 to 80% infection is wiped out by the use of dry cow therapy and it also provides safety from infection by 50 to 75% (Radostit *et al.;* 2000; Grohn *et al.;* 2004).

Therefore the current study was designed to evaluate the efficacy of combination of lincomycin and spiramycin as systemic dry period therapy in the control of bovine mastitis with an objective to find out a better antibiotic for dry period therapy to control the mastitis, thus preventing new intramammary infections and eliminating the existing intramammary infections.

MATERIALS AND METHODS

Experimental Animals

A total of 20 dry pregnant cows (n=20) were selected from institutional dairy farm and randomly divided into two equal groups viz G1 and G2. Animals in group G1 (n=10) was treated with lincomycin (5mg/kg IM) and spiramycin (10mg/kg IM) (Inj. Lispiracin®; Leads pharma, Pakistan) in combination at end of lactation and at 14th days pre calving. Group 2 (n=10) was kept without any treatment and served as control group.

Collection of Milk Samples

Milk sample (10 ml) was collected from each quarter aseptically according to guidelines of National Mastitis Council (NMC) (Muhammad *et al.,* 1995). Each teat end was scrubbed dynamically with cotton gauze saturated with alcohol (70%). Antibiotic treatments were given to the animals immediately following mammary secretions collections.

Collection of milk samples post calving

Milk samples (10 ml) from each quarter was collected aseptically at day 14th post calving (Hogan *et al.,* 1999).The collected samples were placed in crushed ice and shifted to Mastitis Research Laboratory, Department of Clinical Medicine and Surgery, University of Agriculture, faisalabad for isolation and biocharacterisation of prevalent mastitis pathogens.

Diagnosis of subclinical mastitis

All the collected samples of milk were subjected to the surf field mastitis test (Ahmad *et al.,* 1995).

Somatic cell count

Somatic cell count of all milk samples was determined before treatment and on day 14th post calving. It was carried out by using kit Porta SCC® (Porta, 2010).

Isolation and Identification bacteria

Samples of milk were processed for the bacteriology within 24 hrs of collection following storage at 4°C. Microbiological procedures described by the NMC Inc., USA (1990) were followed for culturing the samples of milk and detection of mastitis pathogens. The samples of milk were shaken eight times to get a standardized distribution of the pathogens. About 0.01ml of milk samples were dispersed onto the blood ager plates using a platinum-rhodium loop. Samples were cultured on a 100mm plate and were incubated at 37°C for forty eight hours. A quarter is infected if there are 5 or more similar colonies present on the plate (Roberson *et al;* 1988). The morphological and cultural characteristics of primary growth were studied by examination and observation of colony characteristics and preparation of smears from various colonies. These smears were stained with Gram's staining method and examination was under the microscope.

The primary growth was purified by subculturing on selective and differential media. The selective and differential media used was MacConkey's agar for streptococcus species and blood agar for hemolytic species. Each isolate was recognized on the basis of morphological and cultural characteristics, hemolytic, motility and biochemical characteristics. Coccal isolates (gram positive) presumptively were recognized as micrococci or staphylococcus. The genus of the bacteria was determined through observation of colony

morphology hemolysis pattern, gram stain and catalase test.

Statistical analysis

Percentage prevalence of mastitis was calculated in the both groups by chi square test. The cure rate of infected quarters among groups was calculated by using chi square test comparing treated group and the control. Both groups were compared with each other using two proportional Z-tests. All the values were considered significant at P<0.05.

RESULTS AND DISCUSSION

The mean score of surf field mastitis test of G1 group (1.667±0.222) before treatment and at day 14th (1.223±0.221) post calving was significantly different (p<0.05) as compared to G2 control group as shown in the Table I.

The mean score of somatic Cell Count of G1 group before treatment and at day 14th (1.223±0.221) post calving was also significantly different (p<0.05) as compared to G2 group as shown in the Table II.

Table I: Comparison of mean±SE of SFMT of mastitic cows before treatment and at day 14th post calving

Groups	Pre-Treat	14th day Post calving	Overall Mean
G 1	1.667±0.222	1.223±0.221	1.445±0.166
G 2	1.638±0.139	1.916±0.138	1.777±0.165
Overall Mean	1.652±0.041	1.569±0.042	1.611±0.0415

Table II: Comparison of mean score of SCC of mastitic cows before treatment and at day 14th post calving

Groups	Pre Treatment	At day 14th post calving	Overall Mean
G 1	8.00	3.50	5.75
G 2	7.75	9.25	8.5
Overall Mean	7.87	6.62	7.125

The prevalence of clinical mastitis was 15% in G1 group before treatment and was 10% after treatment which was less as compared to G2 group. Animal wise prevalence (%) of clinical mastitis at day 14th postcalving was higher in G2 than G1 group. Out of total 20 animals, 85% were subclinically mastitic before treatment. In G1 group, % animals were subclinically mastitic and in G2 group, 80% animals were subclinically mastitic. Animal wise prevalence (%) of sub-clinical mastitis before treatment was higher in G1 group than G2 group. Quarter wise percent prevalence of clinical mastitis in G1 group was lower than G2 group while Quarter wise percent prevalence of subclinical mastitis before treatment was 75%. Quarter wise prevalence (%) of subclinical mastitis before treatment was 37.5%. Quarter wise percent prevalence (%) of subclinical mastitis before treatment was higher in G2 group than in G1 group. Quarter based cure rate of infected quarters of G1 group at day 14th post calving was 86.6% which was higher as compared to control group. So, the quarter based cure rate of infected quarters treated with Lispiracin™ at day 14th post calving was highest as compared to control group. In G1 group, prevalence of *Streptococcus agalactiae, Staphylococcus aureus* and *E. coli* was significantly different (p<0.05) as compared to G2 control group as shown in the Table III.

In the current study, the prevalence of clinical mastitis post systemic antibiotic therapy with lispiracin™ was 0% and quarter wise prevalence of clinical mastitis post-systemic antibiotic therapy with lispiracin™ was also 0%. Prevalence of sub-clinical mastitis post-systemic antibiotic therapy with lispiracin™ was 20% and quarter wise prevalence of sub-clinical mastitis post systemic antibiotic therapy with lispiracin™ was 5% which were lower than control group. In control group, Prevalence of clinical mastitis post calving was 20% and quarter wise

prevalence of clinical mastitis post calving was 10%. Prevalence of sub-clinical mastitis post calving was 80% and quarter wise prevalence of sub-clinical mastitis post calving 30% which were very high than lispiracin™ group. The mean score of SCC (Somatic Cell Count) of Lispiracin™ group was decreased from 8.00 to 3.50 ($P<0.05$) as compared to control group. This study was near to Serieys *et al*. (2004) who depicted that somatic cell count was reduced using systemic dry cow therapy. Ataee *et al*. (2009) studied the efficacy of systemic antibiotic administration during dry period. They showed the somatic cell count score 4.2 and 5.1 for tylosin and cefquinome.

Table III: Prevalence of cultured bacteria in each group before treatment and at day 14 post calving.

Bacteria isolated	G1 (Lispiracin™ group)				G2 (Control group)			
	At Dry off		At Day 14 post calving		At Dry off		At Day 14 post calving	
	No.	%	No.	%	No.	%	No.	%
Staphylococcus aureus	8	20	1	2.5	10	25	12	30
Streptococcus aglactiae	5	12.5	1	2.5	6	15	7	17.5
E. Coli	2	5	0	0	2	5	3	7.5
Total	15	37.5	3	5	18	45	22	55

The somatic cell count scores of present study were lower than those. Quarter based cure rate of infected quarters treated with lispiracin™ was 86.6% while in control group was 11.11% that was near to the previous study (Hovareshti *et al.,* 2007). They got 83% cure rate with tylosin intramuscularly comparing with other intramammary antibiotic preparations. The present study is similar with Soback *et al.* (1990) findings using systemic dry cow therapy with norfloxacinnicotinate due to large distribution volume, long half life and active against mastitic pathogens. The prevalence of *Staphylococcus aureus, Streptococcus aglactiae* and *E. coli* after treatment was 2.5, 2.5 and 0% in lispiracin™ group while in control group; prevalence was 30, 17.5 and 7.5%. This study was near to Calvinho *et al.* (2007) which showed the prevalence of *staphylococcus aureus* 21.59%. Similar types of findings are reported in previous studies (Batra *et al.* (1998), Costa *et al.* (1996). Use of the high concentration of the drug can lower the infection in the dry period, damaged tissues may gain their original shape before calving and mastitis is reduced in the calving period (Nickerson and Owens, 1994). Experiments have clarified that the dry period therapy is beneficial against the *Streptococcus agalactiae* and *Staphylococcus aureus* competing the intramammary infections (Dodd, 1983).In the herd having low somatic cell count, the chances of mastitis has become lowered after using the antibiotics in dry period. The advantages of systemic dry period therapy may have better distribution of antibiotic in the mammary tissue which can lead to cure the intramammary infection (IMI) and prevention of the new intramammary infection (Boddie and Nickerson, 1986). Systemic antibiotic therapy in dry period has been attempted in a better way to improve the cure rates of intramammary infections (Tarabla and Canavesio, 2003). In conclusion, systemic dry period therapy at dry off and before parturition is an effective tool to control mastitis in bovines. It should be included in other mangemental practices to control mastitis.

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