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EVALUATION OF NON-ANTIBIOTICS ALONE AND IN COMBINATION WITH CEPHRADINE IN THE CURE RATES IN CLINICAL BUBALINE MASTITIS

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ABSTRACT

Effect of 4 antibiotics *viz*; chlorpromazine Hcl, (CPZ), lidocaine Hcl (LID), Povidone-iodine (PI), and Dimethylsulphoxide (DMSO) either alone or their respective combinations with cephradine was evaluated. For this purpose 270 clinically mastitic quarters of 249 Nili-Ravi buffaloes were infused with either non-antibiotics alone (CPZ = 25mg + 39ml normal saline; Lid = 10ml (2%) + 30ml normal saline; PI = 10ml (10%) + 30ml normal saline; DMSO = 20ml + 20ml normal saline) or in combination with cephradine (Ceph) (500mg) for 5 days. The response to these 8 treatments was monitored in terms of Surf Field Mastitis Test (SFMT) and California Mastitis Test (CMT) based cure rates. Surf Field Mastitis Test (SFMT) based cure rate on day 14 post initiation of treatment was the highest (60%) in quarters treated with CPZ followed by those treated with PI (46.6%), Lid (43.3%), and DMSO (10%). Barring PI, all non-antibiotic antibacterials tested *in vivo* exhibited a synergy with cephradine (Ceph). Thus on day 14 post initiation of treatment, SFMT based cure rates in quarters treated with CPZ + Ceph, Lid + Ceph, PI + Ceph, and DMSO + Ceph were 66.6, 66.6, 50 and 66.6 percent, respectively. Surf Field Mastitis Test based quarter cure rates on day 28 post initiation of treatment with non-antibiotic antibacterials alone as well as their combinations with Ceph were similar to those recorded on day 14. California Mastitis Test (CMT) based percent quarter cure rates on day 14 and day 28 in non-antibiotic antibacterials treatment groups and in groups treated with non-antibiotic antibacterials + Ceph were almost similar to those recorded with SFMT on the corresponding time points.

Key words: Non antibiotics, cure rates, surf field mastitis test, California mastitis test, buffalo, clinical mastitis.

INTRODUCTION

With a population of over 28.4 million heads of dairy buffalo (*Bubalus bubalis*) and 25.5 million heads of cattle, Pakistan is one of the major dairying countries in the World (Economic Survey of Pakistan, 2007-08). Despite this huge number of cattle and buffalo, milk production is only 30 million tons per annum, which translates into only 2-3 liters of milk/animal/day. This undesirably low quantity of milk produced by a colossal dairy animal population can be ascribed to poor genetic potential, poor nutrition and management and suboptimal health of milk animals. The health of the milk-producing organ (udder) is of prime importance especially for the production of wholesome milk.

Mastitis (inflammation of the milk producing organ of mammals) is the most common and the most costly disease of the dairy industry all over the World (DeGraves and Fetrow, 1993; Allert, 1995). At the very least, every fifth dairy buffalo / cow is afflicted with this disease (Fazal-ur-Rehman, 1995). In Pakistan, statistics of current losses due to this disease are not available although it was estimated in 1978 that in Punjab Province alone, the total losses caused by clinical mastitis amount to Rs.240 million per annum (Chaudhry and Khan, 1978). It is pertinent to mention that this survey did not take into account the pecuniary losses associated with subclinical mastitis, the form of mastitis that is 15-40 times more prevalent than its clinical counterpart. These losses are mainly contributed by *Staphylococcus aureus*, followed by *Streptococcus agalactiae, Corynebacterium pyogenes* and *Escherichia coli*. It is pertinent to mention that these mastitogens can be controlled effectively by implementing the 5-point mastitis control plan as advocated by National Mastitis Council, Inc., USA (www.nmconlinbe.org). The components of this control program include (1) teat dipping; (2) dry cow therapy, (3) prompt treatment of clinical cases, (4) proper milking hygiene/use of adequately functioning milking equipment, and (5) culling chronically-infected cows. Two of these points involve the use of antibiotics therapy (Nickerson and Owens, 1990).

Several antibiotics are in use to treat and prevent intramammary infections. Extra-label and unsupervised uses have led to the development of resistance and in country like Pakistan, conventional antibiotics are prohibitively expensive for the resource poor dairy farmers. The present study was designed to ascertain whether chlorpromazine, lidocaine, DMSO and povidone-iodine could be used to treat clinical form of bubaline (= buffalo) mastitis.

MATERIALS AND METHODS

For this purpose, 270 clinically mastitic quarters of 249 Nili-Ravi lactating dairy buffaloes were selected. Animals previously treated for mastitis during the current lactation were not included in the panel of experimental subjects. Similarly only those quarters were selected which had lateral normal quarters. Animals selected were from those managed at Livestock Experimental Station, University of Agriculture, Faisalabad, and 6 private dairy farms (Bibi Jaan Dairy Farm, Aminpur Road, Faisalabad; Councillor Dairy Farm, Satiana Road, Faisalabad; Raja Dairy Farm, Sammundri Road, Faisalabad; Sheikh Dairy Farm, Rashidabad, Faisalabad; Chaudhry Dairy Farm, 102 RB, Faisalabad; Suleman Dairy Farm, Faisalabad). All experimental buffaloes were managed in 'tie-stall' *cum*. loose housing system during the experimental period. These buffaloes received a diet of concentrate mixture and green fodder. The treatment trial started in January and culminated in August, 2005. In a cut-and-carry feeding system, chopped green fodder plus chaffed wheat straw was fed *ad lib*. Berseem (*Trifolium alexandrinum*) and maize (*Zea mays*) were the main green fodders fed. Fresh drinking water was made available 3-5 times per day and animals hosed 1-3 times daily depending upon the weather. The buffaloes were hand-milked twice a day between 3-5 a.m. and 3-5 p.m. Standard mastitis control practices (e.g., post-milking antiseptic teat dipping, dry period antibiotic therapy, segregation or culling of mastitic animals) were not in place at any of the dairy farms.

Evaluation of non-antibiotic antibacterials in combination with antibiotic (cephradine) in the treatment of bubaline clinical mastitis: All 4 non-antibiotic antibacterials chlorpromazine (CPZ), lidocaine (Lid), povidon-iodine (PI), and Dimethylsulphoxide (DMSO) were evaluated in regimens similar to those given in Table 3.4 except that 500 mg of cephradine (Inj. Velosef™; Bristol-Myers Squibb, Pakistan) was additionally added to daily infusions of all 4 non-antibiotic antibacterials. Clinically mastitic quarters were infused with the respective non-antibiotic with the help of plastic part of sterile intravenous infusion catheter No. 20 (Vasocan Braunule™ Melsungen D-34209) attached to a sterile disposable syringe. Infusions were given for 5 days. Milk sample were collected again from the treated quarters on day 14 and day 28 of initiation of treatment (National Mastitis Council, Inc., 1990) for determination of bacteriological cure rates.

Evaluation parameters included Surf Field Mastitis Test (Muhammad *et al*., 1995) and California Mastitis Test (Schalm *et al*., 1971) based cure rates.

Statistical analysis (Steel et al., 1997): The data of somatic cell count was analysed using analysis of variance to compare mean somatic cell count before and on day 14 and day 28 post-initiation of treatment. Similarly, mean milk yield loss of the affected quarters was analysed by analysis of variance to compare the means before and after treatment.

RESULTS AND DISCUSSION

Mastitis screening tests (SFMT and CMT) based cure rates in quarters treated with non-antibiotic antibacterials alone and in combination with cephradine: Using Surf Field Mastitis Test (SFMT), testing of quarters treated with intramammary infusions of non-antibiotic antibacterials alone on day 14 post initiation of treatment, the highest cure rate 60% was achieved with chlorpromazine followed by povidone-iodine 46.6%, lidocaine 43.3% and dimethylsulphoxide 10 %, respectively (Table 2). Barring PI, all non-antibiotic antibacterials tested *in vivo* in the present study exhibited a synergy with cephradine (Ceph). Thus day-14 post initiation of treatment, SFMT based cure rates in quarters treated with chlorpromazine + cephradine, lidocaine + cephradine, povidone-iodine + cephradine, and dimethylsulphoxide + cephradine were 66.6, 66.6, 50 and 66.6 percent, respectively. Surf Field Mastitis Test based quarter cure rate on day 28 post initiation of treatment with non-antibiotic antibacterials alone as well as their combinations with Cephradine were similar to those recorded on day 14 Table 4.

As can be seen in Tables 2, 3 and 4 California Mastitis Test based percent quarter cure rates on day 14 and day 28 in non-antibiotic antibacterials treatment groups G_1 thru G_4 and in groups treated with non-antibiotic antibacterials + cephradine (G_6 thru G_9) were almost similar to those recorded with Surf Field Mastitis Test on the corresponding time points. This close proximity of percent quarter cure rates detected with these two animals-side screening tests attests their comparable efficiency of mastitis detection reported previously (Muhammad *et al*., 1995; Fazal-ur-Rehman, 1995).

In a *S. aureus* mastitis treatment programme in Brazil, penicillin G (100000 IU), streptomycin (1000 mg) in an aqueous-oily or aqueous vehicles with or without the addition of 20% DMSO was used in three herds. An average of 70.6% bacteriologically negative glands were obtained using this combination of antibiotics in the aqueous-oily vehicle and 79.4% in the aqueous vehicle 120 hours after single application. The addition of DMSO in formulations had significant effect only in the herd with chronic mastitis (Figueiredo *et al*., 1993).

Ballarini (1972) studied the effect of DMSO as vehicle for antibiotic (penicillin) and corticosteroid (flumethasone) in the treatment of bovine mastitis. DMSO was used as a 90% solution. Cases of acute parenchymatous mastitis due to *E. coli* in 136 cows were

treated by repeated intramammary infusions of 0.25-0.50 mg flumethasone, associated with an appropriate antibiotic in DMSO. Favourable results were noticed in 95% of cases. Either during dry period or during lactation, 151 quarters were similarly treated with flumethasone or penicillin in DMSO; 90% of the acute cases responded to treatment during lactation. Of the chronic cases, only 24% responded to treatment during lactation, while 46% were cured by treatment during dry period. No advantages were detected from the use of DMSO as vehicle when treating infections localized in teat canal or milk system.

Table 1: Evaluation of non-antibiotic antibacterials in combination with antibiotic (cephradine) in the treatment of bubaline clinical mastitis

Non-antibiotic antibacterials + antibiotic	No. of quarters infused	Duration of treatment (days)
CPZ + Cephradine	30	5
Lid + Cephradine	30	5
DMSO + Cephradine	30	5
PI + Cephradine	30	5
Cephradine alone (control)	30	5

Table 2. Surf Field Mastitis Test (SFMT) based percent quarters cure rate in various non-antibiotics antibacterials/antibiotic groups on day 14 post initiation of treatment.

Non-antibiotic/antibiotic Groups	SFMT Reaction					Proportion of cured quarters to total treated quarters	Percent cure rate
	-	T	+	++	+++		
Chlorpromazine (G_1) (CPZ)	18	5	6	1	0	18/30	60
Lidocaine (G_2)(Lid)	13	7	8	2	0	13/30	43.3
Povidone-iodine (G_3) (PI)	14	7	7	2	0	14/30	46.6
Dimethylsulphoxide (G_4) (DMSO)	3	4	15	4	4	3/30	10
Cephradine (G_5) (Ceph)	19	8	3	0	0	19/30	63.3
CPZ + Ceph (G_6)	20	6	2	2	0	20/30	66.6
Lid + Ceph (G_7)	20	6	2	2	0	20/30	66.6
PI + Ceph (G_8)	15	7	6	2	0	15/30	50
DMSO + Ceph (G_9)	20	5	4	1	0	20/30	66.6

SFMT Surf Field Mastitis Test - Normal (Negative) T Trace + Weak Positive
++ Distinct positive +++ Strong positive
All treated quarters (n = 30) were clinical and reacted strongly (+++) on the day of first intramammary infusion.

Table 3. Surf Field Mastitis Test (SFMT) based percent quarter cure rate in various non-antibiotic antibacterials/antibiotic groups on day 28 post initiation of treatment.

Non-antibiotic/antibiotic Groups	SFMT Reaction					Proportion of cured quarters to total treated quarters	Percent cure rate
	-	T	+	++	+++		
Chlorpromazine (G_1) (CPZ)	18	5	6	1	0	18/30	60
Lidocaine (G_2) (Lid)	14	6	8	2	0	14/30	46.6
Povidone-iodine (G_3) (PI)	14	7	8	1	0	14/30	46.6
Dimethylsulphoxide (G_4) (DMSO)	3	5	14	4	4	2/30	10
Cephradine (G_5)(Ceph)	19	9	2	0	0	19/30	63.3
CPZ + Ceph (G_6)	20	7	2	1	0	20/30	66.6
Lid + Ceph (G_7)	20	6	2	2	0	20/30	66.6
PI + Ceph (G_8)	16	6	5	3	0	16/30	53.3
DMSO + Ceph (G_9)	20	5	4	1	0	20/30	66.6

SFMT Surf Field Mastitis Test - Normal (Negative) T Trace + Weak Positive
++ Distinct positive +++ Strong positive
All treated quarters (n = 30) were clinical and reacted strongly (+++) on the day of first intramammary infusion

Table 4 California Mastitis Test (CMT) based percent quarter cure rates in various non-antibiotic antibacterials / antibiotic groups on day 14 post initiation of treatment.

Non-antibiotic/antibiotic Groups	CMT Reaction					Proportion of cured quarters to total treated quarters	Percent cure rate
	-	T	+	++	+++		
Chlorpromazine (G_1) (CPZ)	18	4	7	1	0	18/30	60
Lidocaine (G_2) (Lid)	13	7	8	2	0	13/30	43.3
Povidone-iodine (G_3) (PI)	14	8	7	1	0	14/30	46.6
Dimethyl Sulphoxide (G_4) (DMSO)	2	4	16	4	4	2/30	6.6
Cephradine (G_5) (Ceph)	19	8	3	0	0	19/30	63.3
CPZ + Ceph (G_6)	20	4	4	2	0	20/30	66.6
Lid + Ceph (G_7)	20	5	5	0	0	20/30	66.6
PI + Ceph (G_8)	15	7	6	2	0	15/30	50
DMSO + Ceph (G_9)	20	4	5	1	0	20/30	66.6

CMT California Mastitis Test - Normal (Negative) T Trace + Weak Positive
++ Distinct positive +++ Strong positive
All treated quarters (n = 30) were clinical and reacted strongly (+++) on the day of first intramammary infusion

Table 5 California Mastitis Test (CMT) based percent quarter cure rates in various non-antibiotic antibacterials alone and in combination with cephradine on day 14 and day 28 post initiation of treatment.

Non-antibiotic/antibiotic Groups	CMT Reaction					Proportion of cured quarters to total treated quarters	Percent cure rate
	-	T	+	++	+++		
Chlorpromazine (G_1) (CPZ)	19	3	7	1	0	19/30	63.3
Lidocaine (G_2) (Lid)	14	8	6	2	0	14/30	46.6
Povidone-iodine (G_3) (PI)	15	7	7	1	0	15/30	10
Dimethyl Sulphoxide (G_4) (DMSO)	3	5	14	4	4	3/30	10
Cephradine (G_5)(Ceph)	19	8	3	0	0	19/30	63.3
CPZ + Ceph (G_6)	20	5	3	2	0	20/30	66.6
Lid + Ceph (G_7)	20	6	2	2	0	20/30	66.6
PI + Ceph (G_8)	16	8	6	2	0	16/30	53.3
DMSO + Ceph (G_9)	20	4	5	1	0	20/30	66.6

CMT California Mastitis Test - Normal (Negative) T Trace + Weak Positive
++ Distinct positive +++ Strong positive
All treated quarters (n = 30) were clinical and reacted strongly (+++) on the day of first intramammary infusion

Table 6. Surf Field Mastitis Test and California Mastitis Test based percent cure rates of non-antibiotic antibacterials alone and in combination with cephradine on day 14 and day 28 post initiation of treatment.

Non-antibiotic/antibacterials Groups	SFMT on day 14 %	SFMT on day 28 %	CMT on day 14 %	CMT on day 28 %
Chlorpromazine (G_1) (CPZ)	60	60	60	63.3
Lidocaine (G_2) (Lid)	43.3	46.66	43.3	46.6
Povidone-iodine (G_3) (PI)	46.6	46.6	46.6	50
Dimethyl Sulphoxide (G_4) (DMSO)	10	10	6.6	10
Cephradine (G_5) (Ceph)	63.3	63.3	63.3	63.3
CPZ + Ceph (G_6)	66.6	66.6	66.6	66.6
Lid + Ceph (G_7)	66.6	66.6	66.6	66.6
PI + Ceph (G_8)	50	53.3	50	53.3
DMSO + Ceph (G_9)	66.6	66.6	66.6	66.6

SFMT = Surf Field Mastitis Test
CMT = California Mastitis Test
All quarters (n = 30) were clinical and reacted strongly (+++) on the day of first intramammary infusion.

The sensitivity of 132 samples (isolates) cultured from infected mammary glands was studied in 3 herds of cattle from the milk basin of Belo Horizonte, Brazil. DMSO added to the culture medium in concentrations of 10 and 15% did not modify the behavior of the isolates which were initially resistant to penicillin (75%) and streptomycin (38%). However, it increased the sensitivity of isolates that were initially only slightly sensitive and at 6% and 10% concentrations, DMSO did not inhibit the growth of any of the isolate, while at 15% it inhibited 27.6% and at 20% it inhibited 100% of the isolates (Andrade *et al*., 1993).

A special type of synergy is produced by the combination of conventional antibiotics and non-antibiotic antibacterials (e.g. β-lactam antibiotics and phenothiazines). Strains of *S. aureus* that are resistant to methacillin (MIC 100 mg/L) became sensitive to concentrations of methacillin as low as 6.2 mg/L when they were cultured in medium containing chlorpromazine to which the organism was resistant. Other chlorpromazine related compounds can reduce the MIC even further to 1.6 mg/L. The same effect was seen when penicillin resistant corynebacterium isolates were cultured in the presence of low concentration of chlorpromazine and penicillin (Kristiansen *et al*., 1993).

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