

THERAPEUTIC EFFICACY OF ENROFLOXACIN ALONE AND IN COMBINATION WITH LEVAMISOLE IN SUBCLINICAL MASTITIS IN CAMEL

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ABSTRACT

A total of 100-quarter milk samples were collected from 25 lactating she camels in sterilised test tubes and were subjected to Culture test, California Mastitis Test (CMT), Somatic Cell Count (SCC), pH, and Electrical Conductivity (EC) test before giving the treatment and then after fourth day following the treatment course to study the therapeutic efficacy of enrofloxacin as systemic therapy alone and in combination with levamisole in subclinical mastitis in camel. Treatment both with enrofloxacin alone and in combination with levamisole was effective in controlling subclinical mastitis in camel but the use of levamisole did not seem to increase the therapeutic efficacy of Enrofloxacin significantly.

Key words : Camel, enrofloxacin, levamisole, subclinical mastitis, therapeutic efficacy

Camel can prove to be an important source of milk in arid and semiarid areas like Rajasthan as its milk, like that of other dairy animals, contains all essential nutrients. In addition, it also possess therapeutic properties as it is considered good for people suffering with jaundice, tuberculosis, asthma and diabetes mellitus (Rao *et al*, 1970; Zagorski *et al*, 1998). Camel continues lactating even under stress condition like drought, when the production of other milch animals ceases.

Both quality and quantity of milk, like in other dairy animals, is adversely affected in mastitis, specially the subclinical one which is considered 15-40 times more prevalent than clinical form and accounts for greater losses in terms of milk production. Mastitis in camels was thought to occur rarely but various studies conducted in last 15-20 years at different places of world (Barbour *et al*, 1985, Abdurahman, 1996; Tuteja *et al*, 2003) suggested that mastitis is also a serious problem in camels.

The control of mastitis mainly depends upon three main principles i.e. elimination of existing infection, prevention of new infection and monitoring udder health status. Out of these, elimination of existing infection is the most important one and it can be best achieved by appropriate antibiotic therapy. Enrofloxacin is one of the most recent drugs being used in

veterinary practice in India. It is a bactericidal with broad spectrum of range and is effective at an extraordinary low concentration in comparison to other antimicrobial classes. Use of non-specific immuno-modulators such as levamisole has also been suggested in the control of mastitis and as an adjunct to the antibiotic therapy. By far no other worker had tried therapeutic trials on camel mastitis except Barbour *et al* (1985). However, many workers have tried treatment trials on mastitis in bovines.

So the present study was planned to study the therapeutic efficacy of enrofloxacin as systemic therapy alone and in combination with levamisole in subclinical mastitis in camel.

Materials and Methods

Milk samples were collected in sterilised test tubes and were subjected to Culture Test, California Mastitis Test (CMT), Somatic Cell Count (SCC), pH, and Electrical Conductivity (EC) test before providing the treatment.

Collection of milk samples : Milk samples were collected from apparently healthy lactating she camels from National Research Center on Camel, Jorbeer, Bikaner and camel herds from different areas in and around Bikaner. A total of 100-quarter milk samples were collected from 25 lactating camels aseptically after proper washing and disinfection of udders.

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Bacteriological examination - 0.01 ml of milk from each sample was streaked on five per cent ovine blood agar (BA) and MacConkey's lactose agar (MLA) plates with help of 4.0 - mm diameter sterile platinum loop and incubated aerobically at 37°C for 24-48 hours. The resulting growth from the respective plates of media was purified and identified on the basis of primary identification tests as per Quinn *et al* (1994).

The SCC of the milk samples was performed as described by Schalm *et al* (1971). For staining of milk smears, Giemsa stain was used. Modified California Mastitis Test (MCMT) was performed by standard method described by Schalm *et al* (1971). The Electrical Conductivity (EC) was measured with the help of digital conductivity meter (ELITE- DELUXE, (ATC) conductivity meter).

The pH of milk was determined using digital pH meter (μ pH system 362 by Systronics). The infected camels, as detected by the cultural examination, were divided randomly into 3 groups of 5 animals each and were subjected to systemic therapy. To these, the following drugs were given as follows:

Group I : Enrofloxacin* was administered intramuscularly at the dose rate of 5 mg per kg body weight once a day for 3 days.

Group II: Levamisole** was administered subcutaneously at the dose rate of 2.5 mg per kg body weight as a single dose along with enrofloxacin at the dose rate of 5 mg per kg body weight once a day for 3 days.

Group III : Kept as control group and no treatment was given in it.

After fourth day following the treatment course, the quarter milk samples from these animals were collected again in sterilised test tubes and subjected to culture and other diagnostic test (CMT, SCC, pH and EC).

The Mean \pm SE values of results for various tests in infected quarters before and after treatment were analysed statistically using 't-test' as per the standard method described by Snedecor and Cochran (1967).

Results and Discussion

The comparative efficacy of enrofloxacin and enrofloxacin + levamisole against the microorganisms

* Marketed by Intervet as Floxinⁱⁿ inj. (vet.) . Each ml. contains 100 mg of enrofloxacin.

** Marketed by Ranbaxy as Lemasol^a - 75 inj. (vet.) Each ml contains 75 mg of levamisole hydrochloride.

isolated from subclinical mastitis cases in camels is shown in the table 1. As is evident from the table, enrofloxacin when used alone, could clear 100 per cent of the infection due to streptococci and *Corynebacterium* spp. but the cure rate against staphylococci was observed to be only 42.85 per cent. Barbour *et al* (1985) performed preliminary study on therapeutics of camel mastitis with chloramphenicol, oxytetracycline and gentamicin and found that successful treatment depends upon correct choice of drug, proper dosage and milking of the animals during the course of treatment. But after that, no other worker tried therapeutic trials on camel mastitis. However, many workers have tried treatment trials on mastitis in bovines. Saluja (1999) and Sharma and Prasad (2003) recorded cure rates similar to present study with use of enrofloxacin (64.70 per cent and 61.53 per cent, respectively) in cattle. Overall higher efficacy of 80 per cent, 84.62 per cent and 100 per cent for treatment of mastitis with enrofloxacin have been reported in bovines by Maiti *et al* (1996), Akhtar *et al* (2003) and Kumar *et al* (1998), respectively.

When enrofloxacin + levamisole were used, 100 per cent of the streptococci and Bacillus spp. were cleared but the clearance of the staphylococci was only 40 per cent by this treatment. The overall efficacy was observed to be slightly better when enrofloxacin was used in combination with levamisole (62.5 per cent in comparison to 60 per cent with enrofloxacin alone). Kalorey *et al* (1993) and Singh *et al* (1996) also found the combination therapy with levamisole better than the use of antibiotic alone.

Variable results, as reported by different workers, could possibly be due to difference in antimicrobial sensitivity of the prevalent strain and the extent of damage to mammary tissue.

In both the treatments, cure rate for staphylococci was found lower than other microbes. However, during *in vitro* sensitivity testing by author, staphylococci showed 96.43 per cent sensitivity against enrofloxacin. So the *in vivo* results were not same as could be expected from *in vitro* testing results. This might occur due to differences in kinetics of the drug inside and outside the animal body. Wilson *et al* (1972) also made an analysis that in treatment of lactating quarters for staphylococci, a cure rate of 65 per cent was about the best that could be expected.

The comparison of the mean \pm SE values of SCC, EC and pH for enrofloxacin and enrofloxacin + levamisole before and after treatment is presented in table 2. The mean value of SCC in

Table 1. Comparative efficacy of enrofloxacin and enrofloxacin + levamisole against microorganisms isolated from subclinical mastitis in camels.

Organism	Enrofloxacin			Enrofloxacin + Levamisole		
	Quarters infected		Per cent cure	Quarters infected		Per cent cure
	Before treatment	After treatment		Before treatment	After treatment	
Staphylococci	7	4	42.85	5	3	40
Streptococci	2	-	100	2	-	100
Bacillus Spp.	-	-	-	1	-	100
Corynebacterium Spp.	1	-	100	-	-	-
Overall	10	4	60	8	3	62.5

Table 2. Mean ± SE values for SCC, EC and pH of infected quarters before and after treatment.

S.No.	Test		Enrofloxacin (n=10)		Enrofloxacin + levamisole (n=8)	
			Before treatment	After treatment	Before treatment	After treatment
1.	SCC (x10 ⁵ cells/ml)	Mean±SE	2.612 ± 0.242	2.153 ± 0.223	2.470 ± 0.446	1.632 ± 0.258
2.	EC (mMho/cm)	Mean±SE	8.452 ± 0.133	8.171 ± 0.207	8.98 ± 0.261	8.56 ± 0.199
3.	pH	Mean±SE	6.415 ± 0.038	6.377 ± 0.029	6.506 ± 0.106	6.397 ± 0.034

group I (enrofloxacin) was 2.61×10^5 cells/ml before treatment, which decreased to 2.15×10^5 cells/ml after treatment. In group II (enrofloxacin+levamisole) the mean values for SCC were 2.47×10^5 cells/ml before treatment and 1.63×10^5 cells/ml after treatment the difference was non-significant in both the groups. The basal values for SCC in present study were lower than many studies done before (Kospakov, 1976; Abdurahman, 1996; Obeid *et al*, 1996; Al-Ani and Al-Shareefi, 1997; Sena *et al*, 2000 and Tuteja *et al*, 2003) but were similar to values found by Chaffer *et al*, 2000. It is possible that there may be an underestimation of cell counts in the present study in an attempt to avoid counting of cell fragments, which are said to be a predominant and constant feature in camel milk and are similar in size to somatic cells so may be counted as somatic cells in direct microscopic cell count (Abdurahman *et al*, 1992).

For EC in group I (Enrofloxacin), the mean value before treatment was 8.45 mMho/cm which decreased to 8.17 mMho/cm after treatment. In group II (Enrofloxacin + Levamisole) the mean values were 8.98 and 8.56 mMho/cm before and after treatment, respectively. In this also the decrease was non-significant in both groups. By far no attempt has been made to estimate EC of camel milk, however many workers have estimated EC of cow milk (Oshima, 1977 and Chahar, 2001). The value of EC of normal milk of camel was found to be higher than cow milk, in which EC value of 6.0 and above can be regarded as a clear indication of subclinical mastitis. The higher basal value of EC in camel milk

could be due to its higher chloride content (168 mg/dl) in comparison to cow milk (110 mg/dl) as the EC of milk mainly depends upon the concentration of chloride ions in milk.

For pH, the mean values before treatment were 6.42 and 6.51 in group I (Enrofloxacin) and group II (Enrofloxacin + levamisole) which decreased to 6.38 and 6.40 in group I and group II, respectively after treatment. For both the treatments, the values did not differ significantly before and after treatment.

Seven samples in group I (Enrofloxacin) and 6 samples in group II (Enrofloxacin + Levamisole) were positive for CMT before treatment. After treatment, these numbers decreased to 4 and 3 respectively for group I and group II.

In control group, spontaneous recovery of 11.11 per cent against staphylococci and 50 per cent for Corynebacterium spp. was observed but no spontaneous recovery was seen for streptococci and Bacillus spp. Overall 15.38 per cent of quarters in control group showed spontaneous recovery. Saluja (1998) also reported spontaneous cure rate of 13.04 per cent in quarters infected for subclinical mastitis in cattle from untreated groups. In this group, the mean values at 0 day and 4th day after treatment respectively were 2.34 and 1.90×10^5 cells/ml for SCC, 8.79 and 8.44 mMho/cm for EC and 6.55 and 6.45 for pH, the difference being non significant for all tests.

The overall (group I + group II) mean ± SE values of SCC in infected quarters before treatment

was $2.549 \pm 0.232 \times 10^5$ cells/ml which decreased significantly to $1.920 \pm 0.175 \times 10^5$ cells/ml of milk after treatment. The overall mean \pm SE values for EC and pH in infected quarters were 8.69 ± 0.15 and 6.46 ± 0.05 , respectively before treatment which decreased to 8.34 ± 0.15 and 6.39 ± 0.02 , respectively after the treatment. This decrease was non significant.

To conclude, treatment both with Enrofloxacin alone and in combination with levamisole were effective in controlling subclinical mastitis in camel but the use of levamisole did not seem to increase the therapeutic efficacy of enrofloxacin significantly.

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