Efficacy Of Cefacetrile In Treatment Of Subclinical And Clinical Mastitis In Dairy Cows

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ABSTRACT

Friesian dairy cows with subclinical and clinical mastitis were used to determine the efficiency of cefacetrile in treatment of mastitis.

The most common microorganisms inflecting mastitis includes E. coli and *Staph. aureus* which had been isolated and tested for their *in-vitro* sensitivity to various antibiotic drugs. The sensitivity tests revealed that E. coli and *Staph. aureus* were highly sensitive to cefacetrile in this study.

Milk of mastitic udders showed significant increase in pH value, chloride content and somatic cell count with a significant decrease in total solids (including fat and non fat solids), lactose content and casein level. One week post treatment, the previous changes were completely restored to normal control level.

Cefacetrile at a single intramammary dosage (353 mg), elicited a high curative percent in subclinical mastitis and moderate percent (55.5% with *E.coli* and 45.45% with *Staph. aureus*) in clinical mastitis.

Milk yield was significantly reduced in subclinical and clinical mastitis. One week post treatment, the milk yield was improved in both cases of mastitis displaying complete recovery in subclinical cases and nearly attaining its previous value in clinical cases.

It is concluded that cefacetrile is highly effective in treatment of subclinical mastitis and moderately effective in treatment of clinical mastitis. The observed effects on chemical constituents of mastitic milk were retrieved its normal control level, one week post treatment.

INTRODUCTION

Mastitis is a common problem among dairy cows in Egypt, causes economic losses to livestock. The financial losses from mastitis occur from reduced milk yield with poor quality and veterinary expenses for care and treatment *(1,2)*. The milk production losses attributed to mastitis ranged from 10 to 26% of total production according to the intensity of inflammatory process, prevalence of disease infectious agent, pathogenicity, and stage of lactation *(2)*. In addition to the significant economical losses, the infection can be considered as serious problem for public health *(3)*.

Treatment of mastitis has included both local and systemic application of antibiotic and other antimicrobial agents.

Extensive field trials proved that both subclinical and clinical mastitis developed resistance to most if not all, conventional antibiotics. The previous concept stressed the need for a novel antibiotic with a high efficacy.

Hence the aim of the present study was to evaluate the clinical and *in-vitro* efficacy of a single dose of cefacetrile in treatment of subclinical and clinical mastitis in lactating cows.

MATERIAL AND METHODS

Drug

Cefacetrile (Vitamast®) produced by Ciba-Geigy, Basel, Switzerland in the form of intramammary injectors each contain 353 mg.

Animals

Thirty, 4-8 years old Friezian dairy cows in a private farm at Sharkia province weighing from 400-450kg were used in this study. They were fed on balanced ration and water were supplied *ad-libitum*. All infections were found in one

quarter (infection in two quarters or more were excluded). Subclinical mastitis was present in 10 cases. Clinical mastitis were evident in 20 cases all were in acute stage of infection. They displayed clinical symptoms as swelling, heat, pain and abnormal mammary secretion as well as slight fever. Control value for milk yield were obtained from previous files for each infected animal before exposure to infection.

Milk sample

Milk samples were collected from subclinical and clinical mastitic udders one day prior to treatment, one day and one week post-treatment in sterile universal bottles for bacteriological examination *(4).*

Bacterial isolation

Cream-sediment inoculum of each milk samples were inoculated on Mannitol salt agar media for isolation of *Staph aureus* and MaConky agar for isolation of *E.coli* after inoculation of plates then incubated at 37°C for 48 hours. Positive growth was Gram-stained. *Staph aureus* was confirmed by tube coagulase test positive meanwhile *E.coli* were subcultured into eosin methylene blue medium. Chiny growth into eosin methylene blue (EMB) was confirmed as *E.coli* according to IMVC test (+/+/-/-) *(5).* Subclinical mastitis was diagnosed using Californea Mastitis Test (CMT) *(5).*

Sensitivity tests

The isolated micro-organisms (*E. coli* and *Staph aureus*) from mastitic milk were tested for their sensitivity *(6)* to cefacetrile and other antibiotics.

Chemical analysis of milk

Milk samples were analyzed for total solids, non fat solids and chloride *(7),* pH value *(8),* lactose and casein level *(9)* and microscopic cell count *(10).*

Efficacy

The drug was used according to manufacture recommendation's i.e. one injector (contains 353 mg cefacetrile) administered intramammary per infected quarter.

Milk yield

Milk yield (kg/day) was calculated in subclinical and clinical mastitis in lactating cows just before treatment, one day and one week post-treatment.

Statistical analysis

Data were Statistically analysed using Student "t" tests *(11)*

RESULTS

Bacterial isolates

Sixteen isolates of *Staph aureus* and 10 isolates of E. coli strains were isolated from subclinical and clinical mastitic milk samples (Table 1).

Table1. Bacterial isolates from clinical and subclinical forms of mastitis in dairy cows.

Type of mastitis	Number of samples	Number of isolates	
		Staph. Aureus	*E.coli*
Clinical	20	11	5
Subclinical	10	5	5
Total	30	16	10

Sensitivity tests

E. coli and *Staph aureus* isolated from subclinical and clinical mastitic milk were highly sensitive to cefacetrile. Other antibiotics displayed varying degree of sensitivity i.e. microorganisms were more or less sensitive to these drugs (Table 2).

Table 2. Sensitivity tests of the microorganisms isolated from mastitic milk before treatment To various antibiotic (according to inhibitory zone)

Antibiotics	Organisms			
	Staph. Aureus		*E.coli*	
	Sensitivity %	Resistances %	Sensitivity %	Resistances %
Cefacetrile (30 µg)	100	-----	100	-----
Erythromycin (15µg)	76	24	66	34
Tetracycline (30µg)	25	75	21	79
Gentamycin (10 µg)	68	32	75	25
Ampicillin (10µg)	32	68	35	65
Pencillin (10 µg)	12	88	15	85
Kanamycin (5 µg)	74	26	71	29

Chemical constituent of milk

Milk of mastitic udders due to infection with *E.coli* or *Staph aureus* revealed significant increase in pH value P< 0.001, chloride content P< 0.01 and somatic cell count P< 0.05 with a significant decrease in total solids (including fat and non fat solids) P< 0.01, lactose content P< 0.01 and casein level P< 0.01 . The previous changes in total solids (fat and non fat solids) pH value, chloride content, lactose content and casein level as well as somatic cell count completely disappeared retaining its normal control level, one week post-treatment (Tables 3- 6).

Table 3.The effect of single dose of cefacetrile (353mg/quarter intramammary) on chemical constituent of milk from subclinical mastitic udders infected with *E. coli* (Mean ± S.E) (n = 5)

Type of milk	Total solids %	Fat %	Non fat solids %	Lactose (g/100ml)	Casein (g/100ml)	Chloride (mg/100ml)	pH value	Somatic cell count (10^3/ml)
Control	13.25±0.239	4.2±0.189	8.84±0.129	4.76±0.056	2.65±0.024	108.2±2.5	6.5±0.048	379±24.65
Mastitic milk pre-treatment (zero time)	** 10.14±0.125	* 3.21±0.106	*** 7.04±0.085	** 3.48±0.139	** 1.97±0.078	** 146.2±3.42	*** 7.38±0.061	* 798.5±68.71
Mastitic milk one day post-treatment	** 10.58±0.82	* 3.24±0.102	** 7.23±0.107	** 3.63±0.135	2.05±0.081	* 134.7±3.54	** 7.2±0.041	* 748.1±71.12
Mastitic milk one week post-treatment	12.15±0.165	3.48±0.087	8.64±0.072	4.26±0.105	2.45±0.039	123.5±3.89	6.74±0.007	435.2±18.75

* P< 0.05 ** P< 0.01 *** P< 0.001

Table 4. The effect of single dose of cefacetrile (353mg/quarter intramammary) on chemical constituent of milk from subclinical mastitic udders infected with *Staph. aureus* (Mean ± S.E) (n = 5)

Type of milk	Total solids %	Fat %	Non fat solids %	Lactose (g/100ml)	Casein (g/100ml)	Chloride (mg/100ml)	pH value	Somatic cell count (10^3/ml)
Control	13.25±0.239	4.2±0.189	8.84±0.129	4.76±0.056	2.65±0.024	108.2±2.5	6.5±0.048	379±24.65
Mastitic milk pre-treatment (zero time)	** 10.11±0.231	* 3.14±0.162	** 6.98±0.163	** 3.51±0.124	*** 1.99±0.028	** 146.3±2.29	* 6.95±0.027	** 831.5±56.47
Mastitic milk one day post- treatment	** 10.56±0.158	* 3.29±0.157	** 7.29±0.151	** 3.75±0.127	** 2.09±0.046	** 140.5±2.14	* 6.81±0. 21	* 757.3±61.29
Mastitic milk one week post- treatment	11.98±0.195	3.64±0.102	8.49±0.157	4.39±0.113	2.46±0.059	125.7±3.39	6.63±0.045	423.5±14.66

* P< 0.05 ** P< 0.01 *** P< 0.001

Table 5. The effect of single dose of cefacetrile (353mg/quarter intramammary) on chemical constituent of milk from clinical mastitic udders infected with *E. coli* (Mean ± S.E) (n = 5)

Type of milk	Total solids %	Fat %	Non fat solids %	Lactose (g/100ml)	Casein (g/100ml)	Chloride (mg/100ml)	pH value	Somatic cell count (10^3/ml)
Control	13.25±0.239	4.2±0.189	8.84±0.129	4.76±0.056	2.65±0.024	108.2±2.5	6.5±0.048	379±24.65
Mastitic milk pre treatment	* 9.11±0.851	* 2.94±0.047	*** 6.12±0.096	*** 2.86±0.085	*** 1.92±0.026	*** 153.2±3.51	** 7.24±0.052	*** 1118±69.57
Mastitic milk one day post- treatment	** 9.77±0.215	* 3.07±0.081	*** 6.71±0.215	** 3.57±0.131	*** 1.98±0.035	** 151.7±4.49	** 7.04±0.056	** 992.5±89.77
Mastitic milk one week post- treatment	11.46±0.439	3.51±0.088	7.84±0.346	3.99±0.267	2.34±0.089	129.5±7.66	6.4±0.105	501.2±62.46

* P< 0.05 ** P< 0.01 *** P< 0.001

Table 6. The effect of single dose of cefacetrile (353mg/quarter intramammary) on chemical constituent of milk from clinical mastitic udders infected with *Staph aureus* (Mean ± S.E) (n = 5)

Type of milk	Total solids %	Fat %	Non fat solids %	Lactose (g/100ml)	Casein (g/100ml)	Chloride (mg/100ml)	pH value	Somatic cell count (10^3/ml)
Control	13.25±0.239	4.2±0.189	8.84±0.129	4.76±0.056	2.65±0.024	108.2±2.5	6.5±0.048	379±24.65
Mastitic milk pre-treatment (zero time)	*** 9.32±0.165	* 2.97±0.062	*** 6.37±0.108	** 2.96±0.187	*** 1.89±0.029	*** 161.8±3.06	* 6.9±0.028	*** 1433±156.7
Mastitic milk one day post- treatment	** 9.57±0.287	* 3.06 ±0.098	*** 6.41±0.167	** 3.15±0.131	** 1.95±0.061	** 160.1±3.67	* 6.9±0.041	** 1247±148.5
Mastitic milk one week post- treatment	11.31±0.42	3.28±0.109	8.18±0.274	4.36±0.125	2.38±0.107	127.9±4.65	6.61±0.073	481.7±91.31

* P< 0.05 ** P< 0.01 *** P< 0.001

Efficacy of cefacetrile on mastitis

A single dose of cefacetrile, (353mg/quarter), was highly effective (100%) in treatment of subclinical mastitis caused by *E.coli* or *Staph aureus*. On the other hand, a single dose was moderately effective in clinical mastitis caused by *E.coli* or *Staph aureus* (Table 7).

Table 7. Efficacy of a single dose of cefacetrile (353mg/quarter intramammary) on subclinical and clinical mastitis in dairy cows (Mean ± S.E) (n = 5)

Type of mastitis	Causative organism	Number of infected quarters	Bacteriologically cured quarters	
			Number	%
Subclinical	*E. coli*	5	5	100
	Staph aureus	5	5	100
Clinical	*E. coli*	9	5	55.55
	Staph aureus	11	5	45.45

Milk yield

Milk yield (kg/day) was reduced in both subclinical and clinical mastitis in dairy cows. One week post treatment with cefacetrile (353mg/quarter), the milk yield completely retrieved to the previous levels in subclinical cases. Nevertheless, in clinical cases, milk yield was improved, yet it was still significantly less than its previous levels (Table 8).

Table 8. The effect of single dose of cefacetrile (353mg/quarter intramammary) on milk yield of mastitic udders (Mean ± S.E) (n = 5)

Type of milk	milk yield (kg/day)	
	Before treatment	one week post-treatment
Control	22.5 ± 0.23	22.5 ± 0.23
Subclinical E. coli	14.21±0.54*	21.5±0.46
Subclinical staph aureus	18.69±0.41**	21.5±0.65**
Clinical E. coli	15.85±0.04***	18.64±0.46
Clinical staph aureus	14.79±0.88***	18.51±0.42*

DISCUSSION

Mastitis in dairy cows is a serious problem as it causes high economic losses in dairy industries in Egypt *(12)*.

Mastitis in both subclinical and clinical forms is a serious disease but subclinical mastitis is the most serious as the infected animal shows no obvious symptoms and secretes apparently normal milk for long time during which causative organism spread infection in herd so bacteriological examination is still the most suitable diagnostic aid for accurate and reliable method to confirm the causative organisms *(13)*.

In the present investigation, it has been shown that *E.coli* and *Staph aureus* were the most causative organisms responsible for mastitis in dairy cows. These finding are similar to that previously reported by several authers *(14-18)*. The previous investigations mentioned that *E.coli* and *Staph aureus* were the most frequent isolates in the milk of both clinical and subclinical cases of mastitis.

Our results indicated that, the isolated microorganisms (*E.coli* and *Staph aureus*) were highly sensitive to cefacetrile. The drug is active against both gram-positive (*Staph aureus*) and gram-negative (*E.coli*), *(19)*. On similar ground, in the milk of mastitic udders gram-positive cocci (*Staph aureus*) were killed within 24 hours by a concentration of 2µg/ml of cefacetrile. At higher concentration (8µg/ml) only 6 hours were sufficient for destruction of E. coli. The minimum inhibitory concentration (MIC) of cefacetrile was between 0.1and 0.4µg/ml for gram-positive bacteria and between 0.5and 0.16µg/ml for gram-negative bacteria *(20)*.

The present study demonstrated that intramammary administration of a single dose of cefacetrile was highly effective (100%) in treatment of subclinical mastitis and moderately effective for clinical cases. These findings can be explained by assuming that, an effective therapeutic concentration of cefacetrile diffuse rapidly into the tissue and attain balance between tissue and milk. In support of the previous concept is the fact that, the drug concentration in milk was highest 12 hours after a single infusion *(21)*. The observed recovery in subclinical and clinical mastitis with a single dose is previously consistent with experimentally induced bovine mastitis *(20)*.

It has been recorded that, subclinical and clinical mastitis not only reduced milk quantity but also produced milk of inferior quality. The milk quantity and quality were improved one week post-treatment with cefacetrile. The obtained results are in coordination with that reported in previous studies *(18, 22)*.

In the present study, It has been observed that mastitis inflected some changes in the chemical constituent of milk. it displayed significant increase in pH value, chloride content and somatic cell count with a significant decrease in total solids (including fat and non fat solids), lactose content and casein level. These changes in milk composition may be due to decrease the synthetic capacity of the mammary gland which leads to decreased concentration of fat and casein in the milk *(23)*.

The increase in pH value in subclinical and clinical mastitic milk is probably a consequence of transmission of sodium chloride and alkalinity to milk through blood as a result of alteration in the permeability of infected mammary gland *(24,25)*.

On the other hand, the observed increase in somatic cell count in mastitic milk reflects a different stage of udder inflammation imputable to the invasion and multiplication of invading microorganisms to the mammary tissue *(26)*.

The decrease in total solids percent of milk in subclinical and clinical mastitis is most probably imputable to reduction of capillary blood flow, a common finding in mastitis, that would consequently limit the level of glucose, fatty acids, amino acids and other nutrients precursors, available to the mammary gland for milk synthesis *(1)*.

The recorded decrease in lactose content may be due to deleterious effects on the manufacture of lactose by udder tissue as a sequel of intramammary infection *(25)*. Nevertheless the reduction of lactose in mastitic milk is not related to the capacity of the gland to produce lactose since the production of casein and fat is not reduced at nearly the same rate. It seems that impaired lactose production is probably related to an altered osmotic equilibrium induced by mastitis. Sodium chloride enters milk from blood as a result of altered permeability and rises the osmotic pressure of milk. Osmotic pressure of milk is brought into equilibrium by reduction in the secretion of lactose *(26)*.

The previous alteration in increase of pH value, chloride content , somatic cell count , total solids (including fat and non fat solids), lactose content and casein level completely disappeared one week post treatment.

Hence from the findings of this study it could be concluded that cefacetrile is highly effective in treatment of subclinical mastitis and moderately effective in clinical cases. One week post treatment the milk quantity was nearly similr to its previous value. Moreover, the recorded adverse effect on chemical constituent

of milk were reversible to control value after seven days post treatment.

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الملخص العربي

كفاءة السيفاسيترل في علاج التهاب الضرع تحت الإكلينيكي و الإكلينيكي في ابقار اللبن

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لقد أجريت هذه الدراسة لإستبيان دور احد مشتقات السيفالوسبورين (السيفاسيترل) في علاج التهاب الضرع في ابقار اللبن.

لقد تم عزل الميكروب القولونى والعنقودي من الحالات المصابة بالتهاب الضرع ولقد تبين من اختبار الحساسية ان الميكروبات المعزولة من الحالات المرضية لها حساسية كاملة لدواء السيفاسيترل.

لقد تبين ان الإصابة بالتهاب الضرع سبب زيادة معنوية في تركيز ايون الهيدروجين والعدد الكلى للخلايا الجسمية وكذلك نسبه الكلورين في اللبن بينما حدث نقص معنوي في كميه المواد الصلبة (الدهون و المواد الصلبة الغير دهنيه) واللاكتوز والكازين وبعد أسبوع من العلاج بالسيفاسيترل عادت المستويات السابقة للمستوى الطبيعي لها.

ولقد أتضح من هذه الدراسة أن السيفاسيترل له كفاءة علاجيه عالية في علاج التهاب الضرع دون الإكلينيكي في أبقار اللبن بينما كانت كفاءة الدواء في الحالات الإكلينيكية متوسطة التأثير.

ولقد لوحظ ان التهاب الضرع الإكلينيكي دون الإكلينيكي يسبب نقصا معنويا في كميه ادرار اللبن في الأبقار وبعد أسبوع من العلاج حدث تحسن ملحوظ في معدل إنتاج اللبن ولكن لم يصل لمستواه قبل الإصابة .

يستخلص من هذه الدراسة ان السيفاسيترل له كفاءة علاجيه عالية في علاج التهاب الضرع دون الإكلينيكي و كفاءة متوسطة في التهاب الضرع الإكلينيكي ولقد اتضح أيضا ان التهاب الضرع في الأبقار له أثار سلبية على محتويات اللبن ولكنها تعود للمستوى الطبيعي بعد أسبوع من العلاج.