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**Epidemiological, Diagnostic and Therapeutic  
Aspects of Subclinical Mastitis in Camel  
(*Camelus dromedarius*)**

उष्ट्र (कैमेलस ड्रॉमेडेरियस) में अनुशयनिक थनैला रोग के  
जनपादकीय, निदान एवं उपचार संबन्धी पहलू

**LENIN BHATT**

B.V.Sc. & A.H.

**THESIS**

**Master of Veterinary Science**

(Epidemiology and Preventive Veterinary Medicine)



**2004**

Department of Epidemiology and Preventive Veterinary Medicine  
College of Veterinary and Animal Science  
Rajasthan Agricultural University  
Bikaner – 334001 (Rajasthan)

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**THESIS**

Submitted to the  
Rajasthan Agricultural University, Bikaner  
in partial fulfillment of the requirements  
for the degree of

**Master of Veterinary Science**  
(Epidemiology and Preventive Veterinary Medicine)

**FACULTY OF VETERINARY & ANIMAL SCIENCE**

By

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Dated 09.01.2004

This is to certify that **LENIN BHATT** had successfully completed the **COMPREHENSIVE EXAMINATION** held on 17.06.2003 as required under the regulations for the degree of **MASTER OF VETERINARY SCIENCE**.



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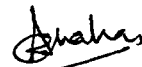
**CERTIFICATE II**

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This is to certify that this thesis entitled “**Epidemiological, Diagnostic and Therapeutic Aspects of Subclinical Mastitis in Camel (*Camelus dromedarius*)**” submitted for the degree of **Master of Veterinary Science** in the subject of **Epidemiology and Preventive Veterinary Medicine** embodies bonafide research work carried out by **Mr. Lenin Bhatt** under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged. The draft of the thesis was also approved by the advisory committee on 24.12.2003.



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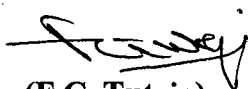
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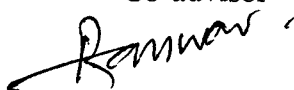
This is to certify that the thesis entitled “**Epidemiological, Diagnostic and Therapeutic Aspects of Subclinical Mastitis in Camel (*Camelus dromedarius*)**” submitted by **Mr. Lenin Bhatt** to Rajasthan Agricultural University, Bikaner, in partial fulfillment of the requirements for the degree of **Master of Veterinary Science** in the subject of **Epidemiology and Preventive Veterinary Medicine** after recommendation by the external examiner was defended by the candidate before the following members of the examination committee. The performance of the candidate in the oral examination on his thesis has been found satisfactory, we therefore, recommend that the thesis be approved.



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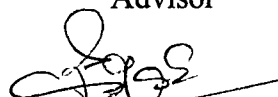
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This is to certify that **Mr. Lenin Bhatt**, of the **Department of Epidemiology and Preventive Veterinary Medicine**, College of Veterinary and Animal Science, Bikaner has made all corrections /modifications in the thesis entitled **“Epidemiological, Diagnostic and Therapeutic Aspects of Subclinical Mastitis in Camel (*Camelus dromedarius*)”** which were suggested by the external examiner and the advisory committee in the oral examination held on 26.02.2004. The final copies of the thesis duly bound and corrected were submitted on 27.02.2004, are enclosed herewith for approval.

  
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Enclosed one original and two copies of bound thesis. Forwarded to the Dean, Post Graduate Studies, RAU, Bikaner through the Dean, College of Veterinary and Animal Science, Bikaner.

  
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(Lenin Bhatt)

## LIST OF CONTENTS

<b>S.No.</b>	<b>Title</b>	<b>Page No.</b>
1.	INTRODUCTION	1-4
2.	REVIEW OF LITERATURE	5-28
3.	MATERIALS AND METHODS	29-36
4.	RESULTS AND DISCUSSION	37-60
5.	SUMMARY	61-63
6.	LITERATURE CITED	64-78
7.	ABSTRACT (English and Hindi)	
8.	APPENDIX	I - VI

## LIST OF TABLES

Table No.	Title	Page No.
1	<i>Prevalence of subclinical mastitis in camel</i>	37
2	<i>Relative frequency of various microorganisms in 41 isolates from subclinical mastitis cases in camels</i>	39
3	<i>Mean <math>\pm</math> SE values for SCC, EC and pH in 100 quarter milk samples from 25 camels</i>	41
4	<i>Comparison of mean values between infected and non infected quarters for various diagnostic tests</i>	44
5	<i>Results of CMT in 100 quarter milk samples from camels</i>	46
6	<i>Sensitivity and specificity and predictive values of CMT in subclinical mastitis in camels</i>	47
7	<i>Comparative efficacy of enrofloxacin and enrofloxacin + levamisole against microorganisms isolated from sub clinical mastitis in camels</i>	49
8	<i>Mean <math>\pm</math> SE values for SCC, EC and pH of infected quarters enrofloxacin and enrofloxacin + levamisole before and after treatment</i>	51
9	<i>Comparison of overall mean values of infected quarters for various diagnostic tests before and after treatment (n=18)</i>	51
10	<i>Comparison of mean <math>\pm</math> SE values for SCC, EC and pH in control group at 0 day and 4<sup>th</sup> day after treatment</i>	53
11	<i>In vitro chemotherapeutic sensitivity of mastitis isolates from camel</i>	54

## LIST OF FIGURES

Figure No.	Title	Page no.
1	<i>Prevalence of subclinical mastitis in camel</i>	37
2	<i>Relative frequency of various microorganisms in 41 isolates from subclinical mastitis cases in camels</i>	39
3	<i>Mean <math>\pm</math> SE values for SCC, EC and pH in 100 quarter milk samples from 25 camels</i>	42
4	<i>Results of CMT in 100 quarter milk samples from camels</i>	46
5	<i>Sensitivity and specificity and predictive values of CMT in subclinical mastitis in camels</i>	47
6	<i>Comparative efficacy of enrofloxacin and enrofloxacin + levamisole against microorganisms isolated from sub clinical mastitis in camels</i>	49
7	<i>Mean <math>\pm</math> SE values for SCC, EC and pH of infected quarters enrofloxacin and enrofloxacin + levamisole before and after treatment</i>	52
8	<i>In vitro sensitivity of Staphylococci against chemotherapeutic agents</i>	55
9	<i>In vitro sensitivity of Streptococci against chemotherapeutic agents</i>	55
10	<i>In vitro sensitivity of Corynebacterium spp. against chemotherapeutic agents</i>	56
11	<i>In vitro sensitivity of Bacillus spp. against chemotherapeutic agents</i>	56
12	<i>Overall Chemotherapeutic sensitivity of mastitis isolates from camels</i>	57

## LIST OF APPENDICES

<b>Appendices No.</b>	<b>Titles</b>	<b>Page No.</b>
I	<i>Results of Somatic Cell Count (SCC), Electrical Conductivity (EC), pH and California Mastitis Test (CMT) in non infected quarters from camels (n=59)</i>	I
II	<i>Results of culture examination, Somatic Cell Count (SCC), Electrical Conductivity (EC), pH and California Mastitis Test (CMT) in infected quarters from camels before treatment (n=41)</i>	III
III.	<i>Results of culture examination, Somatic Cell Count (SCC), Electrical Conductivity (EC), pH and California Mastitis Test (CMT) in infected quarters from camels after treatment (n=31)</i>	V

1.

## INTRODUCTION

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Camels are usually kept and exploited in areas that are usually inhospitable to other dairy animals. In arid and semi-arid areas like Rajasthan, camel also is an important source of milk. The average milk production capacity of a camel is 2.8 – 11 litres/day and it remains in lactation for 8 –18 months making a total yield of 800-3600 litres per lactation (Wilson, 1984). The role of camel as a milch animal is also important owing to its ability to continue lactating even under stress condition like drought, which is a usual feature in these areas.

The milk of camel, like that of other dairy animals, contains all the essential nutrients and provides a nutritious food supplying energy ( i.e. lactose and fat) body building units (i.e. proteins) and minerals. Camel milk has higher mineral contents including Ca, Na, Mg, Fe and Cu, low sugar and 40% lower cholesterol than that of cow milk.

Camel milk contains about 2-3 times more vitamin C than cow milk (Mehaia, 1994). This vitamin C is of paramount importance for human diet in arid and semi-arid areas where fruits and vegetables are scarce or not available. It also increases the shelf life of milk by increasing the acidity.

Camel milk also has therapeutic properties. In Somalia, it is used as a laxative food and in India, it is believed to treat different ailments like jaundice, T.B. and asthma (Rao *et al.* 1970). Camel milk is also very good for people suffering with diabetes mellitus as it is observed to contain about 40 – 60 IU of Insulin (Zagoroski *et al.*, 1998). When exposed to a scarcity of drinking water, total milk yield in cattle and buffaloes decreases and the milk produced has much higher dry matter content than normal, especially fat (Biance, 1965). Such milk

would not be a suitable diet for human beings exposed to same climatic and water stress. In contrast, the milk of dehydrated camel appears to be diluted (Yagil and Etzion, 1980) and there is also accompanying decline in fat content making it ideal for consumption under drought conditions.

Mastitis adversely affects both quality and quantity of milk. Mastitis not only reduces the productive life of animal but also causes changes in composition, consistency, aroma and taste of milk resulting in poor quality of milk. This poor quality results in lower returns to the producer and at the same time causes problems to the manufacturers during processing, as mastitis milk is not suitable for making fermented products.

Mastitis also has public health significance as some of the mastitis causing organisms, which are excreted in milk, are also pathogenic to human health e.g. *Mycobacterium tuberculosis*, *Brucella* spp. (Radwan *et al.*, 1995) etc. These pathogenic bacteria also retard the growth of useful starter microflora of body like *Lactobacillus acidophilus* and *Streptococcus lactis* (Yadav *et al.*, 1993).

There is extensive literature available on bovine mastitis and somewhat less reports on ovine and caprine but reports on prevalence and cause of mastitis in camels are rare, which led to the thought that camels are less susceptible to mastitis than other animals (Bolbol, 1982). But various studies conducted in last 15-20 years at different places of world e.g. Egypt (Mostafa *et al.*, 1987), India (Kapur *et al.*, 1982), Saudi Arabia (Barbour *et al.*, 1985, Hafez *et al.*, 1987), Somalia (Arush *et al.*, 1984, Abdurahman *et al.*, 1991), Sudan (Obeid, 1983) and U.A.E. (Quandil and Oudar, 1984) suggested that mastitis is also a serious problem in camels.

Mastitis is caused mainly by bacterial pathogens. Relative importance of different infections is likely to vary in different areas and countries. Therefore successful prevention and control of mastitis depends upon knowing the incidence and etiology of mastitis in a particular area. There are few data available regarding the etiology, occurrence and pathogenesis of mastitis in camels. Camel has also not been the subject of experimental mastitis studies, so the epidemiology and pathogenicity of mastitis causing organisms in camels remain unclear.

Mastitis occurs in both clinical and subclinical forms. Subclinical mastitis is 15-40 times more prevalent than clinical form and accounts for greater losses in terms of milk production. It is a herd problem that often goes unnoticed since no gross signs of inflammation and changes in the milk composition are evident and the milk and udder appear normal. These subclinical mastitis cases may later progress into clinical form causing more economic losses owing to cost of treatment, veterinarians fee, discarding of milk etc.

Detection of subclinical mastitis is difficult and depends upon various test procedures aimed at detecting the cause or the products of inflammation in milk. ✓Cultural examination is considered to be the golden test by International Dairy Federation (IDF) in order to establish any opinion about infection status of the udder.

Various indirect tests such as somatic cell count (SCC), California mastitis test (CMT), Electrical conductivity and pH estimation are based upon detection of products of inflammation or changes in milk and have a well established role as screening test for predicting disease status of mammary glands in cattle but their relevance for application to the camel is less known. These inflammatory markers can be reliable and easy source for detection of subclinical mastitis in camels also.

✓ There are three main principles of mastitis control 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. The control of mastitis can be achieved by appropriate antibiotic therapy. 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.

Due to wide and indiscriminate use of anti-microbials, there are chances of emergence of resistant bacterial strains and changes in their sensitivity pattern. Therefore, it is necessary to perform repeated drug trials based on sensitivity testing.

✓ Keeping all these facts in mind, the present study was planned with the following objectives :

1. To determine the prevalence of subclinical mastitis in camels.
2. To assess the role of inflammatory markers like California Mastitis Test (CMT), Somatic Cell Count (SCC), pH, and electrical conductivity in predicting the infection status of camel udder.
3. To isolate bacterial mastitis determinants in camel milk and to study their *in vitro* chemotherapeutic sensitivity pattern.
4. To study the therapeutic efficacy of enrofloxacin as systemic therapy alone and in combination with levamisole in subclinical mastitis in camel.

## 2.

### REVIEW OF LITERATURE

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The available literature has been compiled under the following headings:

1. Prevalence
2. Bacteriology
3. Somatic Cell Count
4. California Mastitis Test
5. pH estimation
6. Electrical Conductivity
7. Therapeutics
8. *In vitro* chemotherapeutic sensitivity

#### **Prevalence**

Obeid (1983) found an overall prevalence of 49.67 % of mastitis in traditionally managed camels in Sudan.

Barbour *et al.* (1985) studied 140 camel milk samples with different bacterial counts with CMT and found 55.71 % quarter-wise prevalence of subclinical and clinical mastitis in camels of Saudi Arabia.

Mostafa *et al.* (1987) found 54.5 % animal-wise prevalence of subclinical mastitis in camels as 30 out of 55 apparently healthy camels, from which milk samples were collected, yielded pathogenic bacteria.

Abdurahman *et al.* (1991) carried out a pilot mastitis survey based on interviews of owners of 40 camel herds in Sablaale district of S. Somalia and found that the prevalence of mastitis among these herds was 10.2 per cent.

Bakhiet *et al.* (1992) examined milk samples from 49 healthy dromedaries in Sudan and found bacteria in 45 % of samples (22/49).

Bakeer *et al.* (1994) did pathological and bacteriological studies on 300 udder tissues from 75 female camels slaughtered at an abattoir and found that 88 (29.3 %) of these samples were mastitic.

Abdurahaman *et al.* (1995) examined 391 milk samples from 101 camels from eastern Sudan and out of these, 43.5 % (170 samples) of them yielded pathogenic bacteria.

Abdurahman (1996a) studied 160 milk samples from lactating bacterian camels and found that intramammary infections were present in 22.5 % of samples.

Guliye (1996) showed that of milk samples from she camels reared within Negev desert in the Sinai Peninsula, 81.4 % were positive for presence of bacteria, of which 40.7 % had mixed isolates suggesting existence of subclinical mastitis in camel herds studied.

Obeid *et al.* (1996) found that 47.3 % of 336 milk samples from local Sudanese camels breeds were reactive in a rapid mastitis test.

Al-Ani and Al-Shareefi (1997) used 50 lactating camels from three different herds in Iraq for study on mastitis and found that 19 (38 %) of the examined udders had mastitis. Out of these 19 cases, 57.9 % were of subclinical mastitis while remaining 42 % were having clinical mastitis.

Mody *et al.* (1998) investigated the prevalence of mastitis in 146 adult female camels using California Mastitis Test in eleven village of Gujrat which revealed 30 cases (21 %) of subclinical mastitis of which 93 % samples showed presence of bacteria.

Almaw and Molla (2000) collected 753-quarter milk samples from traditionally managed lactating camels in eastern Ethiopia and noted 24.1 % animal wise prevalence mastitis on the basis of CMT.

Chaffer *et al.* (2000) found that out of 137 milk samples collected from 35 lactating camels in Israel, 35 % were positive for presence of pathogenic bacteria.

Al-jubori *et al.* (2001) screened 630 milk samples from 162 lactating camels in the United Arab Emirates and found incidence of subclinical mastitis to be 11.67 % on animal basis and 5.86 % on quarter basis.

Bekele and Molla (2001) examined 543-quarter milk samples in Afar region of northeastern Ethiopia to determine the prevalence of camel mastitis and found that 29.83 % samples yielded pathogenic bacteria.

Semereab and Molla (2001) assessed the bacteriological quality of raw camel milk in Ethiopia and found that 95 % of milk samples taken directly from the udder had aerobic plate count between  $4 \times 10^4$  and  $10^5$  cfu/ml.

Younan *et al.* (2001) conducted study to investigate the prevalence of *Streptococcus agalactiae* and *Staphylococcus aureus* in camel udder and found IMI with *Streptococcus agalactiae* in 12 % and IMI with *Staphylococcus aureus* in 11 % of all camel milk samples.

Tuteja *et al.* (2003) examined 282-quarter milk samples from 71 apparently healthy lactating camels by culture examination and Somatic Cell Count. He reported 39.72 % prevalence of subclinical mastitis among camels in Bikaner.

### **Bacteriology**

Zafer and Mustafa (1971) showed that members of the genus *Staphylococcus* followed by members of genera *Streptococcus* and *Corynebacterium* were prevalent microorganisms leading to mastitis in she camels.

Kospakov (1976b) isolated 87 strains of staphylococci from the udder tissue of camels.

Kapur *et al.* (1982) isolated *Klebsiella pneumoniae* and *E. coli* from a per acute case of mastitis in camel.

Obeid (1983) isolated *Staphylococcus aureus*, *Staphylococcus* spp., *Streptococcus* spp., *Micrococcus* spp. and Coliforms from milk samples of lactating camels which were 31.6, 11.2, 52.9, 3.0 and 11.3 per cent of total isolates, respectively.

Arush *et al.* (1984) isolated staphylococci, *Corynebacterium* and streptococci from 19 CMT positive milk samples of dromedary camels.

Hassanein *et al.* (1984) isolated *Corynebacterium pyogenes* from mastitis milk and udder tissues in camel.

Quandil and Oudar (1984) isolated *Bacillus cereus*, *Streptococcus uberis*, *Streptococcus agalactiae*, *Diplococcus pneumoniae*, *Staphylococcus aureus* and *E. coli* from 94 lactating camels in UAE. They also isolated one fungus – *Candida albicans*.

Barbour *et al.* (1985) found that most predominant bacterial isolates in mastitis milk in camels of Saudi Arabia were *Micrococcus* spp. (44.06 %), *Staphylococcus aureus* (29.66 %), *Streptococcus* spp. (8.47 %) and *Corynebacterium* spp. (6.77 %) followed by eight other floras.

Al-Mohizea (1986) investigated raw camel milk sold at market places and found large variations in microbial counts. *Staphylococcus aureus* was detected in all samples with some samples having *Salmonella* spp., *Bacillus cereus* and *Yersinia enterocolitica*.

Hafez *et al.* (1987) found *Micrococcus* spp., *Staphylococcus aureus*, *Streptococcus* spp., *Corynebacterium* spp. and others as the cause of mastitis in camels.

Mostafa *et al.* (1987) yielded 58 strains of *Clostridium perfringens*, coagulase positive *Staphylococcus aureus* and *E. coli* from apparently normal udders of she camels.

Ramadan *et al.* (1987) isolated *Pasturella hemolytica* and *Staphylococcus* spp. from cases of unilateral chronic mastitis in dromedaries.

Karamy (1990) did bacteriological studies on mastitis in she camel and found staphylococci to be the most prevalent organisms (34.4 % of total isolates) followed by *E. coli* (18.7 %), *Pasturella* spp. (18.7 %), *Streptococcus agalactiae* (15.6 %) and *Corynebacterium pyogenes* (12.6 %).

Bakhiet *et al.* (1992) isolated staphylococci (70.6 %) and streptococci (29.4 %) as udder organisms responsible for mastitis in camels.

Saad and Thabet (1993) examined 40 milk samples from cow camels for bacteriological quality and evidence of mastitis. The bacteria isolated included *Staphylococcus aureus* (5.9 %), coliforms (29.4 %), *Bacillus cereus* (23.5 %), *Micrococcus* spp. (23.5 %) and *Pseudomonas aeruginosa* (17.7 %).

Abdurahman *et al.* (1995) yielded *Streptococcus agalactiae* (40.58 %), other *Streptococcus* spp. (5.88 %), *Staphylococcus aureus* (12.35 %), coagulase negative staphylococci (40 %) and *E. coli* (1.17 %) from subclinical milk samples of dromedary camels.

Abdurahman (1996a) found *Staphylococcus aureus* (38.9 %) and coagulase negative staphylococci (61.1 %) as main organisms in subclinical mastitis milk samples from seven bacterian camels.

Hallah (1996) isolated *Clostridium perfringens* and other anaerobes along with *Corynebacterium* spp., staphylococci, streptococci and yeast from apparently normal udder tissues of she camel.

Obeid *et al.* (1996) considered streptococci, staphylococci, micrococci, *Aerobacter* spp. and *E. coli* (in descending order) as the main causative organisms of mastitis in camel.

Al-Ani and Al-Shareefi (1997) in their study on mastitis in camels reported that *Staphylococcus aureus* and *Corynebacterium pyogenes* were the main causes of chronic mastitis whereas *Staphylococcus epidermidis*, *Streptococcus* spp., *Pasturella hemolytica*, *E. coli* and *Micrococcus* spp. were the main causes of subclinical mastitis.

El-Jakee (1998) collected mammary tissues from 70 clinically normal quarters of she camels from Cairo abattoir for bacteriological examination. The bacterial isolates recovered were *Micrococcus* spp. (27.1 %), *Staphylococcus* spp. (21.4 %), *Streptococcus* spp. (17.1 %), *Corynebacterium* spp. (11.4 %), *Enterobacterium* spp. (4.3 %) and *Pasturella* spp. (4.3 %); 14.3% isolates were non-identified. In addition, 45 obligatory anaerobic bacteria were also isolated in mixed infection with aerobic bacteria. Same bacterial species were also isolated along with *Candida albicans* from mammary tissues of udders showing clinical symptoms of mastitis.

Mody *et al.* (1998) isolated staphylococci, streptococci, anthracoides, *Corynebacterium* and gram-negative bacteria (39 %, 25 %, 11 %, 4 % and 18 % of total isolates, respectively) from 146 camels in north Gujrat.

Alhendi (2000) isolated *Staphylococcus aureus*, *Streptococcus* spp., *Micrococcus* spp. *Corynebacterium* spp., *Pseudomonas aeruginosa*, *Pasturella* spp., *E. coli* and *Klebsiella* spp. from 295 cases of clinical mastitis in dromedaries.

Almaw and Molla (2000) regarded gram-positive cocci as the predominant pathogens associated with mastitis in camels which comprised 84 % and 85.7 % of the total isolates from 50 subclinical mastitis and 7 clinical mastitis cases, respectively.

Chaffer *et al.* (2000) found staphylococci to be the main bacteria present in milk samples of camels, comprising about 83.3 % of total isolates, along with *Bacillus* spp. (10.4 %), *Streptococcus disgalactiae* (4.2 %) and *Corynebacterium* spp. (2.1 %).

Sena *et al.* (2000) found that in samples of subclinical mastitis from camels, the per cent incidence was more due to *Streptococcus agalactiae* (67.44 %) and *Staphyococcus aureus* (13.95 %).

Al-jubori *et al.* (2001) on bacteriological examination of 630 milk samples from camels in UAE revealed that staphylococci were the chief etiological agents both in clinical and subclinical mastitis (41.09 % of total isolates), followed by *Streptococcus* spp. (21.92 %), *Enterobacterium* spp. (15.07 %) *Corynebacterium* spp. (9.59 %), *Micrococcus* spp. (5.48 %), *Pasturella* spp. (4.11 %) and *Pseudomonas aeruginosa* (2.74 %).

Bekele and Molla (2001) in their examination of lactating camels for mastitis found *Staphylococcus aureus*, coagulase negative staphylococci, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, other species of streptococci, *Pasturella haemolytica* and *E. coli* as the main mastitis pathogens.

Semereab and Molla (2001) assessed the bacteriological quality of raw camel milk in northeastern Ethiopia and found that milk samples from the udder mainly contained staphylococci (46.6 %), *E. coli* (8.3 %), streptococci (7.8 %) and *Bacillus* and *Pseudomonas* (7.1 %).

Guliye *et al.* (2002) isolated *Staphylococcus aureus*, *Micrococcus* spp., *Streptococcus* spp. and *E. coli* as most important organisms in camel milk.

Wernery and Kaaden (2002) believed that *Staphylococcus aureus*, streptococci and *Pasturella* were the primary causative organisms in pathogenesis of mastitis in camels. They considered micrococci, *E. Coli*, *Pseudomonas*, *Klebsiella*, *Bacteroides* and *Clostridium perfringens* as secondary agents.

Wernery *et al.* (2002) compared microbiological status of camel milk with bacteriological parameters laid down in milk hygiene regulations of EU and Germany and found that only 5.1 % samples did not meet the standard.

In study of Tuteja *et al.* (2003), *Staphylococcus epidermidis* was the most predominant organism (27.83 %) in etiology of infectious subclinical mastitis in

camels followed by unclassified *Streptococcus* spp. (20.87 %), *Staphylococcus aureus* (20 %), *Streptococcus agalactiae* (10.43 %), *Streptococcus dysgalactiae* (10.43 %), *Corynebacterium* spp. (9.57 %) and *Bacillus* spp. (0.87 %).

### **Somatic Cell Count (SCC)**

Milk from healthy glands contains leukocytes (lymphocytes, neutrophils, macrophages) that come from blood and epithelial cells that come from the glandular epithelium of the mammary gland. These cells (leukocytes and epithelial cells) are referred as "somatic cells". The SCC/ml of milk increases during inflammation and is thought to correspond to the degree of udder inflammation.

Bakshi (1963) reported that the leukocyte count test was the most reliable indirect test for detection of subclinical mastitis but it was next to cultural examination in efficiency.

Schalm and Lasmanis (1968) noticed that in subclinical and chronic mastitis, cell numbers were four to ten folds greater in stripping than in the foremilk. Some increase in cell numbers was recorded at the end of lactation because of fall in the milk volume.

Giesecke and Van Den Heever (1974) listed the factors affecting the SCC of milk. Somatic cell count increased with the lactation age and stage of lactation and was highest during the dry period. The SCC could vary with the milk yield and different fractions of milk. They also reported hourly and daily fluctuations in SCC. Other factors like corticosteroid therapy, intramammary infusion, transportation, change in diet and climate were also reported to influence SCC.

Kospakov (1976a) showed that average total cell counts in milk of camels with subclinical mastitis was between 7.4 to 12 million (leucocyte count between

7.2 – 11.7 million) against average total cell count of 1.3 million during normal lactation.

Fruganti and Valente (1980) reported cell count to be of particular value to detect aseptic secretory changes due to mechanical injuries, which might form an early stage of mastitis.

Roder and Gedek (1986) examined the association of different mastitis pathogens with milk cell count and reported that beta-hemolysis was associated with the highest cell count, followed by clumping factor than deoxyribonuclease.

In study of Mostafa *et al.* (1987), increase in cell count in milk from camels was correlated with the positive bacteriological findings.

Abdurahman *et al.* (1992) studied fine structure of leucocytes from udders of bacterial camels and the most important finding was the presence of large number of anucleated particles, round or ovoidal in shape with diameter of 1.5 – 27.5  $\mu\text{m}$ . He referred to them as cell fragments and found that these comprise 63-99 % of total particles in the milk pellets. Their structure was similar to cytoplasmic fragments found in goat milk (Wooding *et al.*, 1970; Dulin *et al.*, 1982). Cellular fragments were also reported to be present in cow milk but they differed structurally (Brooker, 1978).

Abdurahman *et al.* (1995) noted that in she camels, the mean values for log SCC in quarters infected with major pathogens, minor pathogens and non infected quarters were  $6.18 \pm 0.67$ ,  $5.85 \pm 0.62$  and  $5.96 \pm 0.76$  respectively with significant difference between non infected quarters and quarters with major pathogens.

Abdurahman (1996a) recorded mean SCC of  $12.16 \pm 0.16$  and  $13.37 \pm 0.26$  in non-infected and infected quarters respectively from camels. There was significant difference between values of non-infected and infected quarters.

Obeid *et al.* (1996) recorded leucocyte contents of milk samples from camel ranging from  $< 5 \times 10^5$  and  $> 7.75 \times 10^6$  cells/ml but they did not find any significant correlation between SCC and udder infection.

Al-Ani and Al-Shareefi (1997) reported SCC to range between  $6 \times 10^5$  and  $13 \times 10^5$  cells/ml. There was significant increase in milk cell counts associated with positive CMT.

Chaffer *et al.* (2000) found mean values of SCC in camel milk samples to be  $1.18 \times 10^5$  and  $3.08 \times 10^5$  cells/ml for non infected and infected quarters respectively having significant differences.

Sena *et al.* (2000) noted total leucocytes to be 280 to 376, 488 to 703 and above 856 thousand/ml in case of normal, subclinical and clinical mastitic milk samples respectively.

Guliye *et al.* (2002) recorded SCC ranging from  $1.01 \times 10^5$  and  $11.78 \times 10^6$  cells/ml in quarter milk samples from lactating dromedary camels and observed that quarter milk samples containing bacteria had a significantly higher mean value for SCC.

Tuteja *et al.* (2003) found mean SCC in camel milk samples ranging from  $4.8 \times 10^5$  to  $12.2 \times 10^6$  cells/ml in normal to clinically affected quarters. He found 65.60 % quarters to have SCC  $> 5,00,000$ /ml, the threshold level in cattle.

**California Mastitis Test (CMT)**

✓CMT is based upon the interaction of somatic cell DNA with detergent like Na-lauryl sulphate and the degree of gel formation is regarded as an estimate of the number of nucleated cells in milk.

✓ Schalm and Noorlander (1957) were the first to develop California Mastitis Test. They reported that CMT was the most commonly employed screening test for the diagnosis of mastitis and can be conveniently used in the field conditions.

Spencer and Simon (1960) evaluated the catalase test, cell count and CMT. The CMT was found to be the most useful over other tests for field application.

Obiger (1961) reported the advantages and disadvantages of rapid mastitis test including CMT and other laboratory methods for the diagnosis of mastitis and compared these tests on 1,500 milk samples. He considered none of these tests reliable in the detection of abnormal or infected milk and stressed that the results of rapid tests should be used together with bacteriological findings and cell picture.

Bhatnagar and Mehrotra (1969) modified CMT by employing 'Det' as a substitute for alkyl-aryl-sulphonate in CMT reagent. This test agreed with leucocyte count test to the extent of 95.20 per cent.

Pandit and Mehta (1969) substituted CMT reagent with sodium lauryl sulphate for the diagnosis of subclinical mastitis in buffaloes.

Sharma *et al.* (1969) compared sodium lauryl sulphate teepol test with the cultural examination and other indirect tests. They found this test to be inexpensive, rapid and reliable for the field diagnosis of subclinical mastitis.

Singh and Baxi (1980) used Mastied (Glaxo Laboratories), sodium lauryl sulphate and CMT for the diagnosis of subclinical mastitis in cows, buffaloes and

goats. They found all the three tests to be efficacious and easy to perform and interpret.

Barbour *et al.* (1985) found a positive correlation between camel milk samples collected from abnormal inflamed udders and samples positive in the CMT.

In study of Mostafa *et al.* (1987), positive CMT was correlated with bacteriological findings in 23 of 30 milk samples from lactating camels.

Abdurahman *et al.* (1995) noted mean CMT score of  $2.38 \pm 1.08$ ,  $1.64 \pm 0.78$  and  $1.85 \pm 0.98$  respectively in quarters infected with major pathogen, quarters infected with minor pathogens and non-infected quarters in camels.

Abdurahman (1996 a) recorded mean score of CMT in milk samples from bacterian camels as  $1.32 \pm 0.09$  and  $2.33 \pm 0.19$  in non-infected and infected quarters respectively. He suggested that CMT score is of value in predicting the infection status of the udder in camel.

In study of Al-Ani and Al-shareefi (1997), all the samples belonging to subclinical mastitis in camels gave CMT 2-3 scores.

Almaw and Molla (2000) Screened 753 quarter samples for subclinical mastitis by CMT, out of which 65 quarters (8.6 %) were positive by CMT. Out of these 65, 57 samples (87.7 %) yielded pathogenic bacteria on culture examination.

Sena *et al.* (2000) revealed CMT of grade 2 in case of subclinical mastitis in camel with positive correlation with TLC and stated that the test was useful for screening of subclinical mastitis.

Bekele and Molla (2001) found a positive correlation between positive CMT results and presence of major pathogens in camel milk samples and

suggested that CMT can be used as a screening test for detection of mastitis in camels.

Semereab and Molla (2001) found that among the CMT tested milk samples from camel udder, 47.3 % were positive.

Younan *et al.* (2001) evaluated CMT for detection of IMI by *Streptococcus agalactiae* and *Staphylococcus aureus* and found quarter level sensitivity of 77 % and 68 %, respectively.

Tuteja *et al.* (2003) reported that among all camel milk samples positive for CMT, 71.03 % were having CMT score 2 (trace) and the mean SCC was found to increase proportionately to CMT score.

### **pH estimation**

Yagil *et al.* (1984) studied the pH of camel milk and reported it to be  $6.4 \pm 0.03$ .

Yagil (1985) suggested that changes in DM concentration in dehydration in camels, especially under drought condition, might have some effect on pH of camel milk.

In study of Mostafa *et al.* (1987) increase in milk pH was correlated with positive bacteriological findings in milk samples from she camels.

Hamzawi and Hafez (1992) recorded mean ash content in milk of Friesian cows, Malti goats, Barki ewes and local camels and found that in camel milk, the

mean ash content was 0.810 % with alkalinity no. of 7.2 and noted that differences in ash content and alkalinity no. between species and between stages of lactation were statistically significant.

Guliye *et al.* (2000) conducted a study on compositional quality of camel milk and found that the milk pH was fairly constant having mean value of  $6.50 \pm 0.05$ .

Sena *et al.* (2000) noted that in the milk samples showing sub-clinical mastitis in camels, the pH was alkaline and ranged from 7.2 to 7.8 and in clinical cases, it was above 8.0. They found positive correlation between pH and CMT and pH and TLC.

Tuteja *et al.* (2003) measured pH of 282-quarter milk samples from camels and found that mean pH was within the normal range in all non-clinical quarters with SCM having mean pH of 6.39. However, in case of clinically infected quarters, there was a significant rise in mean pH.

### **Electrical Conductivity (EC)**

The major anions and cations present in milk, which have been studied in relation to secretory disorder in the mammary glands, are  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$ . The normal secretion of  $\text{Na}^+$  and  $\text{K}^+$  is controlled by active pumping systems on the basal and lateral membranes of the secretory cell (Linzell and Peaker 1971, Peaker 1975). Bacterial infection of the udder results in damage to the ductal and secretory epithelium, opening up of tight junction between secretory cells and increased permeability of the blood capillaries. Thus  $\text{Na}^+$  and  $\text{Cl}^-$  (which are high in extra cellular fluid) pour into the lumen of the alveolus and in order to maintain osmolarity,  $\text{K}^+$  level decreases proportionately (Wheelock *et al.*, 1966, Peaker

1975). The increase in concentrations of sodium and chloride and decrease in potassium therefore result into increase in the electrical conductivity (EC) of milk.

Malcolm *et al.* (1942) examined 42 specimens of milk out of which 74 per cent were correctly diagnosed by culture test, 90 per cent were diagnosed by electrical conductivity and 93 per cent by somatic cell count.

Linzell *et al.* (1974) reported that EC was an accurate and rapid method of diagnosing intramammary infection but there are certain conditions such as illness and oestrous which cause rise in EC in milk.

Linzell and Peaker (1975) reported that electrical conductivity of milk is a reflection of its  $\text{Cl}^-$ ,  $\text{Na}^+$  and  $\text{K}^+$  contents. Higher ionic strength leads to increase EC and, conductivity of fore milk at a single check was a highly accurate method for differentiation between infected and uninfected cows.

Massie *et al.* (1976) designed an apparatus for detection of sub clinical mastitis in a lactating cow, which comprises of a conductivity cell with two electrodes for passing electric current through the milk flowing between them. When the conductivity exceeds a pre-determined value, a visual or audible device (flashing light or alarm bell) is activated to warn the operator for a probable mastitic condition.

Oshima (1977) studied the detection of sub clinical mastitis by conductivity measurement and compared conductivity and other tests for detecting sub-clinical mastitis.

Blackshaw and McGrowan (1978) examined 73-quarter samples for sub clinical mastitis by simple electrical conductivity and bacteriological examination. They found 61 out of 73 samples positive by electrical conductivity and 14 by bacteriological examination. It was concluded that mastitis detector was over sensitive for the diagnosis of sub clinical mastitis.

Badran (1987) measured conductivity of fore milk and strippings of 20 healthy cows and 5 mastitic cows and concluded that in ordinary condition, conductivity of fore milk is a sensitive method for detection of sub clinical mastitis.

Nielen *et al.* (1993) studied the relationship between online electrical conductivity of milk and daily milk production on a low somatic cell count farm. They reported that rise of 1 milliseimon (ms) of mean electrical conductivity caused a decline of 0.86 kg of milk per day.

Chahar (2001) recorded EC in 300 milk samples from cattle. The mean  $\pm$  SE value of EC in subclinical mastitis milk was  $6.51 \pm 0.21$  mMho/cm and that of normal milk was  $5.31 \pm 0.10$  mMho/cm. She considered the threshold value for SCM to be 5.9 mMho/cm as all culturally positive samples had an EC value higher than that value.

Chavan *et al.* (2001) reported conductivity of milk having leucocyte count above 5 lacs as  $6.14 \pm 0.07 \times 10$  microMhos/cm, whereas in clinical mastitis, it was  $7.50 \pm 0.09 \times 10$  microMhos/cm.

Tanwar *et al.* (2001) studied the comparative efficacy of various diagnostic tests in diagnosis of subclinical mastitis in Rathi cows and found that EC appeared to be the best screening test under field condition with 100 % sensitivity. High correlation of electrical conductivity with SCC was also observed.

### **Therapeutics of subclinical mastitis**

Smith and Stables (1958) suggested that all cases of mastitis caused by *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus zooepidermicus* and *Streptococcus pneumoniae* should receive parenteral treatment with penicillin. Mastitis caused by *Streptococcus dysgalactiae* and *Streptococcus uberis* respond

well to penicillin, erythromycin and tetracycline but reinfection might occur quickly if the contributory causes are not corrected. Infections with *Streptococcus zooepidermicus* did not respond well to local treatment with penicillin, *Streptococcus pneumoniae* responded well to local treatment with penicillin with large doses but complete loss of function resulted in quarters allowed to go without treatment for any length of time.

Wilson *et al.* (1972) in a study, made an analysis that treatment of lactating quarters presented a problem. Clinical cases have to be treated and the recovery rate with streptococci was good. For staphylococci a cure rate of 65 % was about the best that could be expected.

Reyero *et al.* (1979) observed that levamisole could be used to stimulate the immune response *in vivo*.

Storper *et al.* (1981) infused (i)75 mg ampicillin and 200 mg cloxacillin sodium, (ii)250 mg kanamycin sulphate and 300000 units procaine penicillin G and (iii)100 mg ampicillin sodium and 200 mg cephalothine sodium at 24 hour intervals for eliminating staphylococci and streptococci from subclinically infected udders of lactating cows. The average cure rate of ampicillin and cloxacillin for *Staphylococcus aureus* infections was 47.5 per cent, ranging between 12.1 and 87.8 per cent in 3 herds. Kanamycin sulphate and 3,00,000 units procaine penicillin eliminated 79.2 and 88.6 per cent of *Staphylococcus aureus* infections in two cowherds. Ampicillin sodium and cephalothine sodium cured 66.0 per cent of *Staphylococcus aureus* infections in 5 herds, although in one herd only 15.6 per cent infections were eliminated.

Barbour *et al.* (1985) made preliminary observations on chemotherapy of mastitis cases in camels using chloramphenicol, oxytetracyclin and gentamicin and

concluded that successful treatment depended upon correct choice of drug, proper dosage and milking of animal during treatment.

Owens *et al.* (1988) demonstrated that combination therapy was more effective in curing *Staphylococcus aureus* infections than intramammary infusion alone during lactation.

Tripathy and Murty (1990) demonstrated that single intramammary infusion of cefacetrile @ 235 mg per quarter was effective in 40 % and 90 % of cows when treated without and with the knowledge of antibiogram pattern of the causative organisms, respectively.

Daley *et al.* (1992) in an experiment, observed that when IL-2 was given in conjugation with intramammary Na-cephapirin, therapeutic efficacy of experimental IMI caused by *Staphylococcus aureus* was improved by 20 to 30 % over that for quarters treated only with Na-cephapirin.

Bergmann (1993) in an experimental prototheca mastitis in the cows and its treatment with tetramisole hydrochloride found that clinical symptoms diminished within 3-24 hours after the first application in comparison with the control quarters. Milk cell counts were increased for 5 days after infection. He expected a more potent reduction of prototheca after application of levamisole hydrochloride.

Kalorey *et al.* (1993) detected subclinical mastitis by California Mastitis Test in 18 lactating cows, which were selected for study and noted that cultural examination of quarter milk samples before treatment showed staphylococci and streptococci in 60 % (untreated controls), 66.66 % (given trisodium citrate) and 83.33 % (given trisodium citrate and levamisole), while on day 6 after treatment the corresponding infection rates were 66.66 %, 46.66 % and 33.33 %, respectively.

Abdurahman (1996b) suggested that mastitis is usually not treated in traditionally managed camel herds and often took a natural course to chronicity resulting in induration of afflicted quarters which could lead to extensive fibrosis replacing secretory tissue and permanent loss of milk production.

Maiti *et al.* (1996) reported that daily injection of enrofloxacin @ 15 ml i/m for 3 days was effective in acute and subacute cases of mastitis in cattle with overall recovery rate of 80 per cent.

Singh *et al.* (1996) in a study, found that cefa-lak cured 78.88% cows and 79.41 % quarters infected with staphylococci, streptococci, *E. coli*, *Bacillus* spp. or unknown pathogens. The combined treatment with cefa-lak and levamisole cured 82.61 % cows and 80.64 % mastitic quarters.

Wilson *et al.* (1996) reported that florfenicol and cloxacillin did not differ significantly in efficacy versus clinical (n=85) or subclinical (n=71) bovine mastitis or for any etiological agent.

Kumar *et al.* (1996) administered enrofloxacin @ 4 mg/kg b.wt. by i/m route O.D. and 250 mg/quarter i/mm at 12 hours interval for 5 days in mastitic buffaloes and reported 100 % cure rate.

Morin *et al.* (1998) reported that in herds, in which mastitis is often caused by environmental bacteria, antibiotic and supportive treatment may result in a better outcome for cows with clinical mastitis than supportive treatment alone.

Pyorala and Pyorala (1998) found penicillin G successful in treating clinical mastitis caused by *Staphylococcus aureus* strains in young cows, but parenteral administration of spiramycin or enrofloxacin did not give satisfactory results in mastitis caused by penicillin-resistant *Staphylococcus aureus*.

Saluja (1999) on evaluation of five different treatment regimens in systemic lactating cow therapy found overall cure rate of 64.70 % and 60.00 % with enrofloxacin and enrofloxacin + levamisole, respectively.

Langoni *et al.* (2000) compared the efficacy of enrofloxacin in cattle (184 quarters) with SCM after systemic (5 mg/kg b.wt. i/m) and intramammary (250 mg in 10 ml distilled water) administration. The cure rates of quarters infected with *Staphylococcus aureus* were 72 and 85 per cent after intramammary and systemic treatments, respectively.

Srivastava *et al.* (2001) found that by i/m route, enrofloxacin was the most effective antibiotic in the treatment of mastitis in buffaloes.

Akhtar *et al.* (2003) tried treatment of affected mastitic quarters in crossbred cows with enrofloxacin along with diclofenac sodium. The treatment results showed that 84.62 % quarters were cured.

Jain and Kumar (2003) treated five cases of staphylococcal mastitis with lincomycin @ 300 mg in 6 ml distilled water via intramammary route twice daily for five days and found 80 per cent recovery (4 out of 5 cases). However these animals did not show adequate clinical response over the previous treatments- Ampicillin + cloxacillin (intramammary), enrofloxacin (intramuscularly) along with diclofenac sodium in recommended doses.

Sharma and Prasad (2003) performed therapeutic study with 4 different drugs to formulate suitable therapeutic regimen(s) for mastitis and found ampicillin + cloxacillin to be the most efficacious. The overall efficacy of enrofloxacin was on the tune of 66.67 % on animal basis and 61.53 % on quarter basis.

***In vitro* chemotherapeutic sensitivity of mastitis isolates**

✓Kapur *et al.* (1980) examined a large number of strains of *Staphylococcus epidermidis* originating from latent/subclinical cases of mastitis in bovines for *in vitro* drug sensitivity against 19 chemotherapeutic agents. Most of the strains (92 to 98.48 %) were found sensitive to streptomycin, oleandomycin, danamycin, rovamycin, gentamicin and erythromycin in the above order. Sensitivity to chlortetracycline, tetracycline, ampicillin, oxytetracycline, vibramycin, penicillin, cephaloridine and bacitracin varied from 68.56 to 86.42 per cent, while majority of the strains (95 %) were resistant to sulphadimidine.

Char *et al.* (1983) found that of the 45 strains of *Staphylococcus aureus* tested, 78 per cent were sensitive to chloramphenicol, 71 per cent to neomycin and 33 per cent to oxytetracycline and nitrofurantoin.

Barbour *et al.* (1985) performed *in vitro* antibiotic sensitivity on 118 mastitis bacterial isolates from camels against six antibiotics and found that chloramphenicol was the most effective.

Sudharma *et al.* (1985) tested antibiotic sensitivity of organisms associated with mastitis in cattle and goats. They reported that majority of staphylococcal isolates were sensitive to gentamicin (84 %), coliforms to neomycin (92.60 %) and streptococci to ampicillin (90.30 %).

Dholakia *et al.* (1987) investigated antibiogram of organisms isolated from mastitic milk samples and reported gentamicin and chloramphenicol to be the most effective drugs followed by neomycin and ampicillin.

Tripathy and Murty (1990) in antibiogram studies on 13 isolates indicated that 80 %, 100 % and 50 % of *Staphylococcus* spp., *Corynebacterium* spp. and *E. coli* and *Klebsiella* spp. respectively were sensitive to kanamycin.

Al-Ani and Al-Shareefi (1997) found that isolated bacteria from camel milk were sensitive to ampicillin, chloramphenicol, gentamicin, penicillin, streptomycin and tetracycline.

✓Kaya *et al.* (1998) performed antimicrobial sensitivity on 80 *Staphylococcus aureus* strains isolated from 141 cow milk samples and found the sensitivity pattern as : oxytetracycline (6.2 %), amoxycillin (10 %), enrofloxacin (32.5 %), erythromycin (35 %), danofloxacin (45%), gentamicin (55 %) and penicillin (76 %). The other isolated bacteria were resistant in varying degrees to other antibiotics.

Pushpa *et al.* (1998) tested 75 isolates by *in vitro* antimicrobial sensitivity test and observed that all the isolates were sensitive to more than one antibiotic, most to gentamicin and kanamycin, while others showed multiple drug resistance.

✓Swamy and Krishnamurthy (1998) found that most of the staphylococci were resistant to streptomycin, neomycin and penicillin while using the disk diffusion method.

Mody *et al.* (1998) found that gentamicin and chloramphenicol showed good sensitivity against bacterial isolates from camel milk but these isolates were resistance to nitrofurantoin, furazolidone and penicillin.

✓ Dhote *et al.* (1999) reported that majority of streptococcal and staphylococcal isolates were sensitive to ciprofloxacin and were least sensitive to amoxicillin and penicillin, respectively. The gram-negative bacteria were highly sensitive to pefloxacin and least to amoxicillin and cloxacillin. Overall, the isolates were highly sensitive to ciprofloxacin and least to amoxicillin.

✓ Saluja (1999) studied sensitivity of mastitis isolates against ten antimicrobials as streptomycin, spiramycin, chloramphenicol, oxytetracycline, ampicillin, cloxacillin, amoxicillin, amoxycylav, nitrofurantoin and enrofloxacin. He found enrofloxacin to be the most effective one which was 100 % effective against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae* and *Micrococcus* spp. and 93.1 % effective against *Streptococcus dysgalactiae*.

Tuteja (1999) performed *in vitro* chemotherapeutic sensitivity of 276 isolates recovered from IMI in buffaloes against 18 antimicrobials and found that more than 90 per cent of the isolates were sensitive to tetracycline, oxytetracycline, chloramphenicol, polymyxin-B, neomycin, kanamycin, oleandomycin, spiramycin, amoxicillin, amoxycylav and enrofloxacin except streptomycin, ampicillin, erythromycin, co-trimoxazole, nitrofurantoin and nalidixic acid.

Al-Jubori *et al.* (2001) studied antibiotic sensitivity pattern of mastitis isolates in camel and found that more than 85 % of predominant bacteria; *Staphylococcus* spp., *Streptococcus* spp. and *Corynebacterium* spp., were

sensitive to carbenicillin, gentamicin, kanamycin, erythromycin, ampicillin and amikacin, but resistant to colistin and trimethoprim + sulphamethoxazole.

✓ Dutta and Rangnekar (2001) studied *in vitro* anti-microbial sensitivity of different isolates from 46 samples from 29 cows affected with SCM. All the isolates were highly sensitive to enrofloxacin, majority of the isolates were moderately sensitive to gentamicin and oxytetracycline and resistant to ampicillin and streptomycin.

✓ Prasad (2001) studied the antibiogram pattern of 10 antibiotics against mastitis isolates from cows and buffaloes and found enrofloxacin to be the most effective with overall sensitivity of 93.07 % followed by gentamicin (80.78 %), cloxacillin (64.61 %) and chloramphenicol (59.23 %). Penicillin was least effective with only 17.69 % sensitivity.

Bulla *et al.* (2003) conducted *in-vitro* antimicrobial sensitivity testing of 52 bacterial isolates from SCM in buffaloes against 21 antimicrobial agents. All the 36 strains of staphylococci were sensitive to chloramphenicol, cloxacillin, ampicillin, enrofloxacin, oleandomycin, ampiclox and novobiocin. All the 14 strains of streptococci were found sensitive to penicillin-G, chloramphenicol, cloxacillin, ampicillin, enrofloxacin, oleandomycin, ampiclox and erythromycin.

✓ Ranjan *et al.* (2003) studied *in vitro* drug sensitivity pattern of bacterial isolates from bovine clinical mastitis and found enrofloxacin and gentamicin to be the most effective ones (85.91 %) followed by ampicillin, cloxacillin, streptomycin, amoxycillin and penicillin; tylosin was least effective (21.42 %).

Tuteja *et al.* (2003) found 100 % of the isolates from subclinical mastitis samples from camels sensitive to chloramphenicol, amoxycillin, cephalixin and amoxyclav . Sensitivity to cloxacillin, gentamicin, ciprofloxacin and penicillin was more than 80 per cent.

3.

## MATERIALS AND METHODS

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### 1. Collection of milk samples

Milk samples were collected from apparently healthy lactating 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.

The udders of camels were cleaned thoroughly with water and mopped to dry with clean cloth. The milkers' hands were thoroughly washed with soap and water and rinsed with 70 per cent alcohol. After discarding first few streams of milk, the teat apices were cleaned with cotton swab soaked in 70 per cent alcohol. Nearly 30 ml. of milk sample from each quarter was collected separately in sterile test tubes. Collection was first done from the near side and then from the offside to avoid contamination of the disinfected teat apices. The tubes were marked as right-fore (RF), right-hind (RH), left-fore (LF) and left-hind (LH). History and relevant information such as animal number, age, parity, stage of lactation, milk yield and physical condition of the udders were recorded.

### 2. Bacteriological examination

The milk samples collected aseptically were shaken thoroughly. With the help of a 4.0 mm diameter sterile platinum loop, 0.01 ml of the sample was streaked on five per cent ovine blood agar (BA) and MacConkey's lactose agar (MLA) plates. The plates were 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 primarily identification test as per Quinn *et al.* (1994) as follows-

### **Morphology**

Colonies of bacteria on nutrient agar plates were purified and bacteria were observed for their size, shape, arrangement, sporulation, capsulation and presence of any other distinctive feature.

### **Motility**

Motility was studied in hanging drop preparation of broth culture of bacteria.

### **Growth in air**

Growth in air was studied to confirm whether the bacterial isolates were able to grow under aerobic or anaerobic condition.

### **Gram's reaction**

Smears of young culture of bacterial isolates were stained by modified Gram's method of staining. The results of bacterioscopy were noted as Gram's positive, staining blue of primary stain and Gram's negative, those isolates taking stain of counter stains as pink.

### **Spore formation**

Spore formation was observed in smears prepared from bacterial colonies.

### **✓Catalase activity**

One drop of 3 per cent solution of hydrogen peroxide was placed on a clean glass slide. Pure culture was picked up from nutrient agar slant with an inoculating straight wire in front of flame and placed on the drop of hydrogen peroxide on glass slide. Culture was properly emulsified and coverslip was placed. The production of gas bubbles confirms a positive reaction.

### **Oxidase test**

Oxidase test confirms the production of cytochrome oxidase by certain bacteria. The culture from nutrient agar slant was picked up with an inoculating loop and rubbed on filter paper. Simultaneously a drop of oxidase reagent (N, N, N, N, - p - p phenylenediamine dihydrochloride) was added. Colonies producing oxidase gave coloured reaction. The colour on filter paper turns to purplish brown in a few seconds.

### **Oxidation and fermentation test**

Oxidation and fermentation test was used to differentiate oxidative bacteria from the fermenters. This test demonstrates the break down of sugars by oxidation or fermentation.

Hugh and Leifsons medium was used containing glucose and bromothymol blue as an indicator. Semisolid medium was inoculated in pairs by culture of bacteria to be tested. One tube of the pair was kept open while the other tube was covered with 1-2 mm layer of sterilized paraffin to provide anaerobic condition. The tubes were incubated for 24 hours.

Those bacteria that oxidized the sugar showed acid production and yellow discolouration of the medium in open tube while bacteria, which ferment sugar showed acid production and yellow discolouration of medium in both the paired tubes.

The cultures were stocked upon nutrient agar slants and kept at 4<sup>0</sup>C for further use.

### **Somatic Cell Count (SCC)**

The SCC of the milk samples were performed as described by Schalm *et al.* (1971). However, for staining of milk smears, Giemsa stain was used.

**3.1 Preparation of milk films :**

The milk sample was mixed thoroughly so as to obtain uniform distribution of the cells and allowed to stand for two to five minutes to permit air bubbles to rise and foam to disappear. Grease free slide was placed on a level area over a template to outline four one-cm<sup>2</sup> areas. With the help of micropipette 10 µl of milk was spread evenly over the first template on the left side. The procedure was repeated with samples from each quarter. Slides were air-dried and a few drops of xylene were poured over the milk smears and kept for 2 minutes to dissolve the fat globules of milk. The Smear was air dried and fixed with 99 % methanol for 2 minutes and washed with distilled water. Then it was stained with Giemsa stain for 30 min. After staining, the smear was kept in phosphate buffer solution (pH 7.0) in couplin jar for 5 min. and bloat dried. Somatic cells were stained clearly with deep blue nuclei against a light blue background.

**3.2 Calculation of working factor (WF) of the microscope:**

A binocular microscope was used with 10 X oculars and 1.9 mm oil immersion objective. The diameter of the field was measured with the help of a stage micrometer and the area of the field was calculated by using the formula  $\pi r^2$ . As the diameter of the field was 1.9 mm (0.019 cm), the area of the field equaled  $3.143 \times 0.0095^2$  or  $28.33 \times 10^{-5}$  sq. cm. and the number of fields that could be counted in 1 cm<sup>2</sup> was 3529.82. Since 0.01 ml (1/100) of the milk was spread over an area of 1 cm<sup>2</sup>, the volume of milk represented by each field would be  $1/3529.82 \times 1/100$  or  $1/352982$ . On this basis, each cell seen in a field taken at random would equal 352982 cells per ml. of milk. This is known as the microscopic factor (MF). The working factor (WF) was calculated by dividing the MF by the number of fields to be counted. Since 20 fields were counted, the working factor for the microscope used was 17649.1. The number of cells per ml of milk was calculated by multiplying the number of cells counted in 20 fields by a WF of 17649.1.

**3.3 Counting of cells:**

The stained slides were examined under oil immersion objective and the cells in 20 fields were counted. Total number of cells counted was multiplied by working factor of the microscope to obtain the number of cells per ml of milk.

**4. Modified California mastitis test (MCMIT)**

The test was performed by standard method as described by Schalm *et al.* (1971). In the present study Ezee (a Godrej detergent) was used in place of pure alkyl aryl sulphates or sulphonates of sodium or potassium as an anionic surface active agent. Cresol red was replaced by Bromocresol purple.

The final composition of test reagent was as follows:

Ezee	: 2.0 ml
Sodium hydroxide (AR)	: 4.5 gm
Bromocresol purple	: 10 mg
Distilled water to make	: 1000 ml

**Procedure :**

A plastic paddle having four shallow cups was used. Equal amount (3 ml) of milk and the reagent were put into each cup of the paddle and the contents were mixed by a gentle circular motion of the paddle in a horizontal plane. The grading and interpretation was done as follows:

Score	Suggested meaning	Description of visible reaction
1	Negative	Mixture remains liquid
2	Trace	A slight slime with no tendency towards gel formation.
3	Weak	A distinct slime with no tendency towards gel formation.
4	Distinct positive	Mixture thickens immediately with gel formation. On continued swirling, mass moves around the periphery leaving the bottom of the cup exposed.
5	Strong positive	A distinct gel forms which tends to adhere to the bottom of the paddle and during swirling a distinct central peak is formed.

**5. Electrical conductivity**

Electrical conductivity of milk samples was measured with the help of digital conductivity meter (ELITE – DELUXE, (ATC) conductivity meter) by passing AC voltage through the milk with conductivity cell. Measurement of resistance of the milk was made and was converted suitably within instrument to display the conductivity directly.

First of all, mains head was connected to 230 V  $\pm$  10 per cent mains supply. Then the conductivity cell and ATC probe were connected to the input sockets provided on the rear side of the instrument. The conductivity cell and ATC probe were put in the solution whose specific conductivity was known (0.1 N potassium chloride solution) and 10 mmho range switch was selected. The instrument was calibrated to 12.88 with the help of CALIB knob. Then the conductivity cell and ATC probe were thoroughly washed with distilled water. About 30 ml of milk was taken in 50 ml beaker and conductivity cell and ATC probe were put into the milk. Direct reading was displayed on digital screen in mmho/cm. Conductivity cell was thoroughly washed with distilled water when it was changed from one milk sample to another.

**6. pH Reaction**

H<sup>+</sup> ion concentration of milk was determined immediately using digital pH meter ( $\mu$  pH system 362 by Systronics).

The instrument was first standardized using three point calibration with solutions of known pH 4.0, 7.0 and 9.2. About 30 ml milk was taken in a 50 ml beaker. The electrodes of digital pH meter were dipped in milk and pH button was pressed. After 30 seconds, the pH of milk was automatically displayed on digital screen.

## 7. Therapeutics

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 :

In group I, enrofloxacin\* was administered intramuscularly at the dose rate of 5 mg per kg body weight once a day for 3 days.

In 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 was kept as control group and no treatment was given.

After fourth day following the treatment course the quarter milk samples from these animals were collected again in sterilized test tubes and subjected to culture and other diagnostic test.

## 8. *In Vitro* chemotherapeutic sensitivity

Different strains of various organisms originating from udder infections from camels were subjected to in-vitro drug sensitivity testing using 8 antimicrobials by the disc diffusion method as described by Bauer et al. (1966). Nutrient broth in tubes were inoculated with the bacterial cultures. After 6-7 hours, when the bacteria were in the exponential phase of growth, sterile cotton swab was dipped in the broth and swabbed on the sensitivity testing agar plates. After 3-5 minutes, the antibiotic discs were placed with the aid of disc dispenser in front of flame. The plates were then incubated at 37°C for 24 hours and were observed for the zone of inhibition. The diameter of the zone of inhibition was determined with

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

\*\* Marketed by Ranbaxy as Lemasol® – 75 inj. (vet.) Each ml contains 75 mg of levamisole hydrochloride.

the help of a measuring scale and compared with the standard scale of inhibition for each antibiotic disc as per the instructions provided by the manufacturer (Hi – media). Following discs were used :

Amoxycillin	(Am)	30 mcg
Amikacin	(Ak)	30 mcg
Ceftriaxone	(Ci)	30 mcg
Ciprofloxacin	(Cf)	30 mcg
Cloxacillin	(Cx)	30 mcg
Chloramphenicol	(C)	30 mcg
Enrofloxacin	(Ex)	10 mcg
Gentamicin	(G)	30 mcg
Penicillin	(P)	10 IU
Streptomycin	(S)	25 mcg

4.

## RESULTS AND DISCUSSION

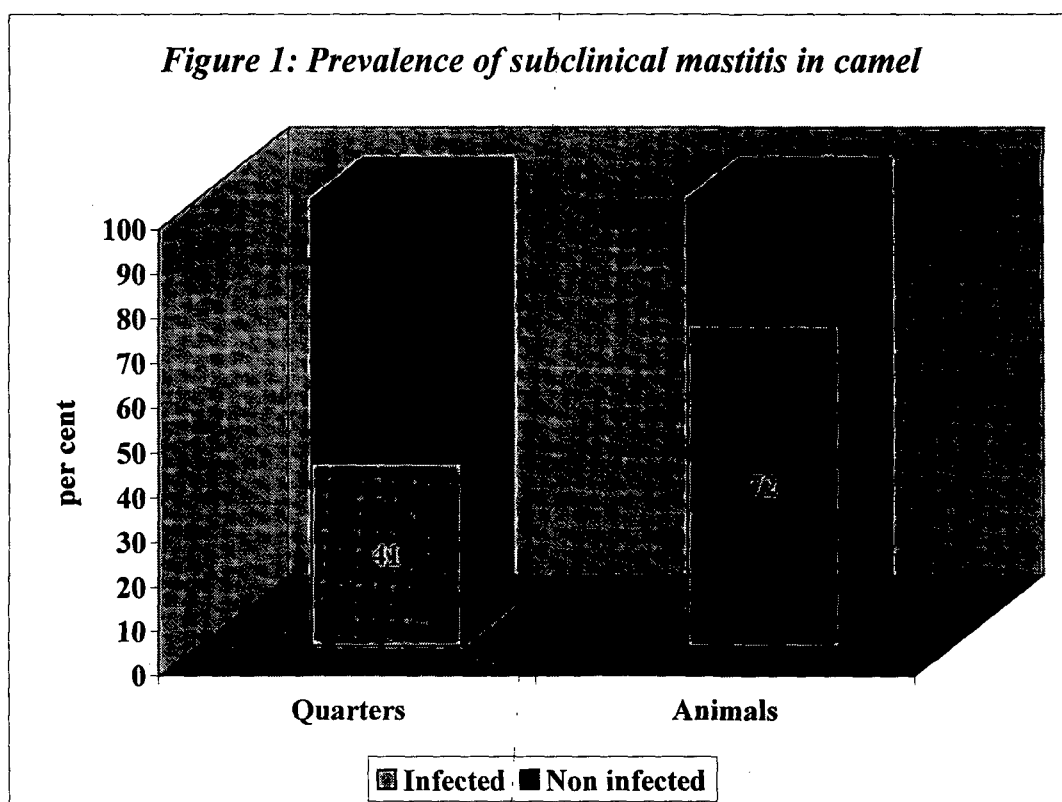
### 1. Prevalence

The result of culture examination of 100-quarter milk samples from 25 apparently healthy camels is presented in Table 1 and Figure 1.

*Table 1 : Prevalence of subclinical mastitis in camel*

Examined	Positive for culture examination	% Positive
Quarters (n=100)	41	41
Animals (n = 25)	18	72

*Figure 1: Prevalence of subclinical mastitis in camel*



On basis of culture examination, the quarterwise prevalence of subclinical mastitis in camels was recorded as 41 % (41/100). This finding supports the earlier findings by Abdurahman *et al.* (1995), Al-Ani and Al-

Shareefi (1997) and Tuteja *et al.* (2003). These authors reported the quarterwise prevalence of subclinical mastitis in camels as 43.5 %, 38 % and 39.72 % respectively. The prevalence rate of subclinical mastitis in present study is slightly higher in comparison to Chaffer *et al.* (2000), who recorded 35 % quarterwise prevalence of subclinical mastitis, and is higher than the observations of Abdurahman (1996a), Almaw and Molla (2000) and Bekele and Molla (2001). They found the prevalence of subclinical mastitis in camel to be 22.5 %, 24.1 % and 29.83 %, respectively. Al-jubori *et al.* (2001) reported a low quarterwise incidence of subclinical mastitis in camels (5.86 %). However, much higher prevalence of subclinical mastitis has been recorded in camels by Barbour *et al.* (1985), Obeid *et al.* (1996) and Guliye (1996). They found the prevalence of subclinical mastitis in camels as 57.28 %, 47.3 % and 81.4 %, respectively. Semereab and Molla (2001) found that 95 % of the milk samples taken directly from the udders of camels had high aerobic plate counts.

The animals-wise prevalence of subclinical mastitis in camels was recorded much higher (72 %) when compared with the quarter-wise prevalence. If any animal had only one quarter positive, then also it was considered as a positive case. This prevalence rate is higher than that has been observed so far by Obeid, 1983 (49.67 %), Mostafa *et al.*, 1987 (54.54 %), Bakhiet *et al.*, 1992 (45 %) and Mody *et al.*, 1998 (21 %). Younan *et al.* (2001) found 12 % and 11 % of all camels studied to have intra mammary infection with *Streptococcus agalactiae* and *Staphylococcus aureus*, respectively.

These differences in the infection rates, as reported by different workers, could be due to the differences in climatic, managerial and hygienic conditions.

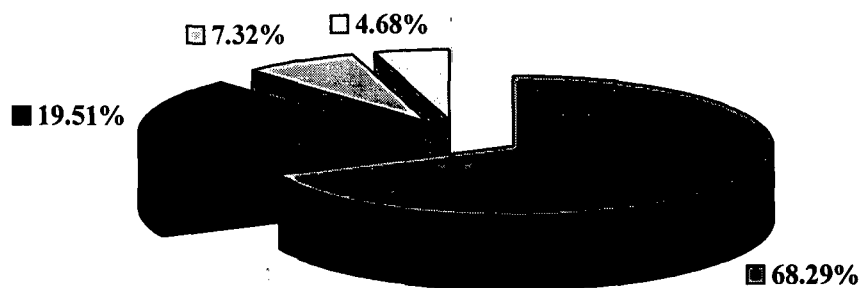
### **2. Bacterial determinants associated with subclinical mastitis in camels**

The bacterial isolates recovered from milk samples of 100 quarters from 25 apparently healthy camels and the relative frequency of appearance of these microorganisms in 41 isolates are given in Table 2 and Figure 2.

**Table 2.** *Relative frequency of various microorganisms in 41 isolates from subclinical mastitis cases in camels*

S.No.	Organism	No. of isolates	Per cent
1.	<i>Staphylococcus</i> spp.	28	68.29
2.	<i>Streptococcus</i> spp.	8	19.51
3.	<i>Corynebacterium</i> spp.	3	7.32
4.	<i>Bacillus</i> spp.	2	4.68

**Figure 2 :** *Relative frequency of various microorganism in 41 isolates from subclinical mastitis cases in camels*



■ *Staphylococcus* spp. ■ *Streptococcus* spp. □ *Corynebacterium* spp. □ *Bacillus* spp.

Amongst different mastitogenic agents, staphylococci were found to be the most predominant bacteria associated with subclinical mastitis in camels, accounting for 68.29 % of the total isolates. Streptococci were next with 19.51 % occurrence followed by *Corynebacterium* spp. and *Bacillus* spp. which comprised 7.32 and 4.68 % of total isolates, respectively.

Almost similar observations have been reported by Obeid (1983), Quandil and Oudar (1984), Arush *et al.* (1984), Barbour *et al.* (1985), Hafez *et*

*al.* (1987), Karamy (1990), Abdurahman *et al.* (1995), Alhendi (2000), Bekele and Molla (2001), Guliye (2002) and Tuteja *et al.* (2003). Organisms like *Micrococcus* spp., *Pasturella* spp., *E. coli*, *Pseudomonas* and *Klebsiella* were not encountered in the present study, though presence of these organisms has been reported from milk samples associated with subclinical mastitis in camels by some of the above workers. The presence of these organisms has also been found to be associated with cases of clinical mastitis in camels by Kapur *et al.* (1982), Ramadan *et al.* (1987), El-Jakee (1998), Alhendi (2000) and Almaw and Molla (2000). This is simply what one might expect as the subclinical mastitis cases in turn convert into clinical cases in long term if no proper attention is given for treatment of such cases. These micro organisms are also considered as major mastitis pathogens in dairy cows (Schalm *et al.*, 1971; Quinn *et al.*, 1994). Besides these, Mostafa *et al.* (1987), Hallah (1996) and El-jakee (1998) also reported association of anaerobic bacteria with subclinical mastitis in camels. Quandil and Oudar (1984) also isolated a fungus - *Candida albicans* from udders of lactating camels in Sudan.

Like other dairy animals, staphylococci and streptococci seem to be associated with most of the cases of subclinical mastitis in camels also. Findings of earlier workers *e.g.* Almaw and Molla (2000), Sena *et al.* (2000) and Tuteja *et al.* (2003) also support this fact. Wernery and Kaaden (2002) regarded staphylococci and streptococci along with *Pasturellae* as the primary causative organisms in pathogenesis of mastitis in camels. Barbour *et al.* (1985) regarded *Micrococcus* spp. as the main etiological agents of camel mastitis but Obeid (1983) considered this species to be non pathogenic. As camel has not been the subject of experimental mastitis studies, the pathogenicity of mastitis causing organisms remains unclear in this species.

In present study, staphylococci were the most predominant species among all bacterial isolates. Higher prevalence of staphylococci in comparison to other organisms has also been reported by Zafer and Moustafa (1971), Karamy (1990), Abdurahman *et al.* (1995), Abdurahman (1996a), Al-Ani and Al-Shareefi (1997), Mody *et al.* (1998), Al-jubori *et al.* (2001) and Semereab

and Molla (2001). They regarded staphylococci as the main etiological agent of camel mastitis. Kospokov (1976b) isolated 87 strains of staphylococci from the udder tissues of camels. In study of Bakhiet *et al.* (1992) and Chaffer *et al.* (2000), staphylococci comprise as high as 70.6 and 83 % of the total mastitis isolates, respectively. However, Obeid (1983), Obeid *et al.* (1996) and Sena *et al.* (2000) reported a higher incidence of streptococci in comparison to *Staphylococcus* spp.

### **3. Efficacy of various inflammatory markers**

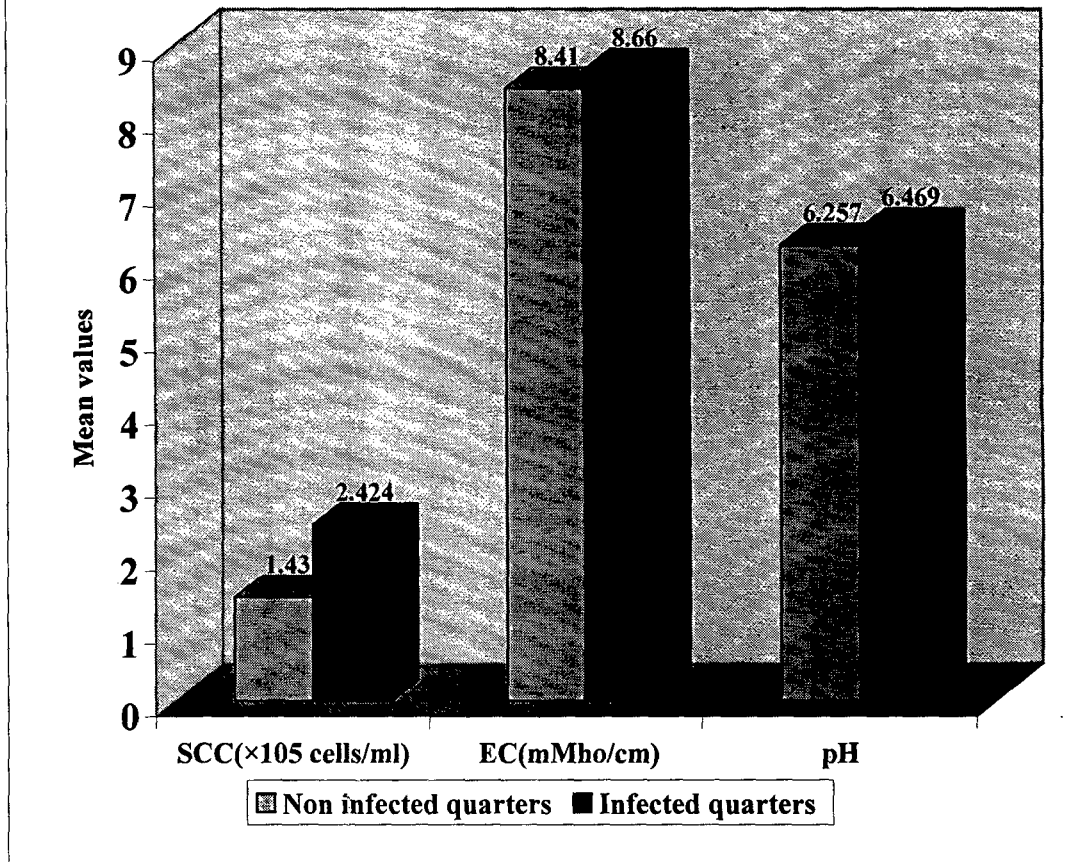
A total of 100 quarter milk samples from 25 apparently healthy camels were subjected to various diagnostic tests *i.e.* Somatic Cell Count (SCC), California Mastitis Test (CMT), Electrical Conductivity (EC) and estimation of pH, along with the culture examination to assess their role in detection of subclinical mastitis in camels. Results for SCC, EC and pH estimation are presented in Table 3 and are depicted in Figure 3.

**Table 3 : Mean ± SE values for SCC, EC and pH in 100 quarter milk samples from 25 camels**

S.No.	Test		Non infected quarters (n = 59)	Infected quarters (n = 41)
1.	SCC (×10 <sup>5</sup> cells/ml)	Mean ± SE	1.430 ± 0.047	2.424 ± 0.145 <sup>***</sup>
		Range	0.882-1.941	1.059-3.883
2.	EC (mMho/cm)	Mean ± SE	8.41 ± 0.067	8.66 ± 0.081 <sup>*</sup>
		Range	7.12-9.75	7.45-10.71
3.	pH	Mean ± SE	6.257 ± 0.059	6.469 ± 0.034 <sup>**</sup>
		Range	5.405-6.768	6.188-7.239

Note : Values with superscript \* have P<0.05, \*\* have P≤0.01 and \*\*\* have P≤0.001

**Figure 3 : Mean values for SCC,EC and pH in 100-  
quarter milk samples from camels**



The mean somatic cell count was recorded to be  $1.430 \times 10^5$  cells/ml in non infected quarters ranging from  $0.882 \times 10^5$  to  $1.941 \times 10^5$  cells/ml (with SE of  $\pm 0.047 \times 10^5$  cells/ml). In infected quarters the mean somatic cell count was  $2.424 \times 10^5$  cells/ml with SE as  $\pm 0.145 \times 10^5$  cells/ml. It ranged between  $1.059 \times 10^5$  to  $3.883 \times 10^5$  cells/ml.

While counting somatic cells, the important finding observed was the anucleated particles the 'cell fragments', the presence of which has been reported to be a predominant and constant feature in camel milk (Abdurahman *et al.*, 1992). They studied the fine structure of these round or ovoidal particles and found them comparable to the fragments found in goat milk (Wooding *et al.*, 1970; Dulin *et al.*, 1982), containing many vacuoles with endoplasmic

reticulam and mitochondria. These cell fragments may constitute upto 95 % of total particles in milk. The origin of these fragments has not been studied, however their similarities with goat milk suggest an apocrine secretion, as in goats. The finding of these cell fragments in camel milk has an important practical implication as being similar in size, they may be counted as somatic cells in direct microscopic cell count, making both enumeration and differentiation of somatic cells difficult. In present study and attempted was made to count only nucleated cells.

The result for mean SCC as recorded in present study is almost similar as was observed by Chaffer *et al.* (2000), who found mean values of SCC in camel milk samples to be  $1.18 \times 10^5$  and  $3.08 \times 10^5$  cells/ml for non infected and infected quarters, respectively. Much higher SCC have been recorded by Kospakov (1976a), Abdurahman (1996a), Obeid *et al.* (1996), Al-Ani and Al-Shareefi (1997), Sena *et al.* (2000) and Tuteja *et al.* (2003). It is possible that there can be an underestimation of cell counts in the present study in an attempt to avoid counting of cell fragments, which may constitute upto 95 % of total particles in camel milk. In present study however, no attempt was made to count these cell fragments.

The mean  $\pm$  SE values for EC, as observed in present investigation, were  $8.41 \pm 0.067$  and  $8.66 \pm 0.081$  mMho/cm in case of non infected and infected quarters, respectively. It ranged between 7.12 to 9.75 mMho/cm in case of non infected quarters and from 7.45 to 10.71 mMho/cm in case of infected quarters. By far no attempt has been made to estimate EC of camel milk, however many workers have estimated EC of cow milk and found it to be of value in predicting the cases of subclinical mastitis in bovine. 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.

In non infected quarters, the mean  $\pm$  SE value of milk pH was  $6.257 \pm 0.059$  ranging between 5.405 to 6.768 whereas, the infected quarters have mean  $\pm$  SE values as  $6.469 \pm 0.034$  with range of 6.188 to 7.239. Yagil *et al.* (1984) and Guliye *et al.* (2000) found the pH of normal camel milk to be  $6.4 \pm 0.03$  and  $6.50 \pm 0.05$ , respectively. Tuteja *et al.* (2003) found the pH of milk from quarters of camels with subclinical mastitis to be 6.39 which is almost similar to the value recorded in present investigation. However, Sena *et al.* (2000) found the pH of milk from subclinical mastitis cases in camels to be higher (7.2 to 7.8).

Many samples that were negative on culture examination showed high value for these inflammatory markers. This variability could be due to presence of inflammation for any reason other than infection as a positive bacteriology although shows the presence of infection but negative culture does not exclude an inflammatory process in quarter.

The differences in the overall mean values of infected and non infected quarters for SCC, EC and pH are shown in Table 4.

**Table 4 :** *Comparison of mean values between infected and non infected quarters for various diagnostic tests*

S.No.	Test	Infected quarter	Non Infected quarter
1.	SCC ( $\times 10^5$ Cells/ml)	$2.424 \pm 0.145^{***}$	$1.430 \pm 0.047$
2.	EC (mMho/cm)	$8.66 \pm 0.081^*$	$8.41 \pm 0.067$
3.	pH	$6.469 \pm 0.034^{**}$	$6.257 \pm 0.059$

Note : Values with superscript \* have  $P < 0.05$ , \*\* have  $P \leq 0.01$  and \*\*\* have  $P \leq 0.001$

The mean value for SCC in infected quarters was higher than the mean value of non infected quarters with a difference of  $0.994 \times 10^5$  cells/ml. This difference was highly significant ( $P \leq 0.001$ ) which indicates the potentiality of SCC in prediction of inflammatory status of camel udder. Similar results were obtained by Kospakov (1976a), Sena *et al.* (2000) and Tuteja *et al.* (2003). They reported an increase in SCC from normal level in case of subclinical mastitis. Abdurahman (1996a), Chaffer *et al.* (2000) and Guliye (2002) also observed significant differences between SCC values of non infected and

infected quarters. However, Obeid *et al.* (1996) did not find any significant correlation between SCC and udder infection in camels. Abdurahman *et al.* (1995) noted significant difference in log SCC value between non infected quarters and quarters infected with major pathogens but the difference between log SCC value of non infected quarters and quarters infected with minor pathogens was non significant.

The difference between EC of infected quarters (mean = 8.66) and non infected quarters (mean = 8.41) was found to be 0.25 which was significantly higher ( $P < 0.05$ ).

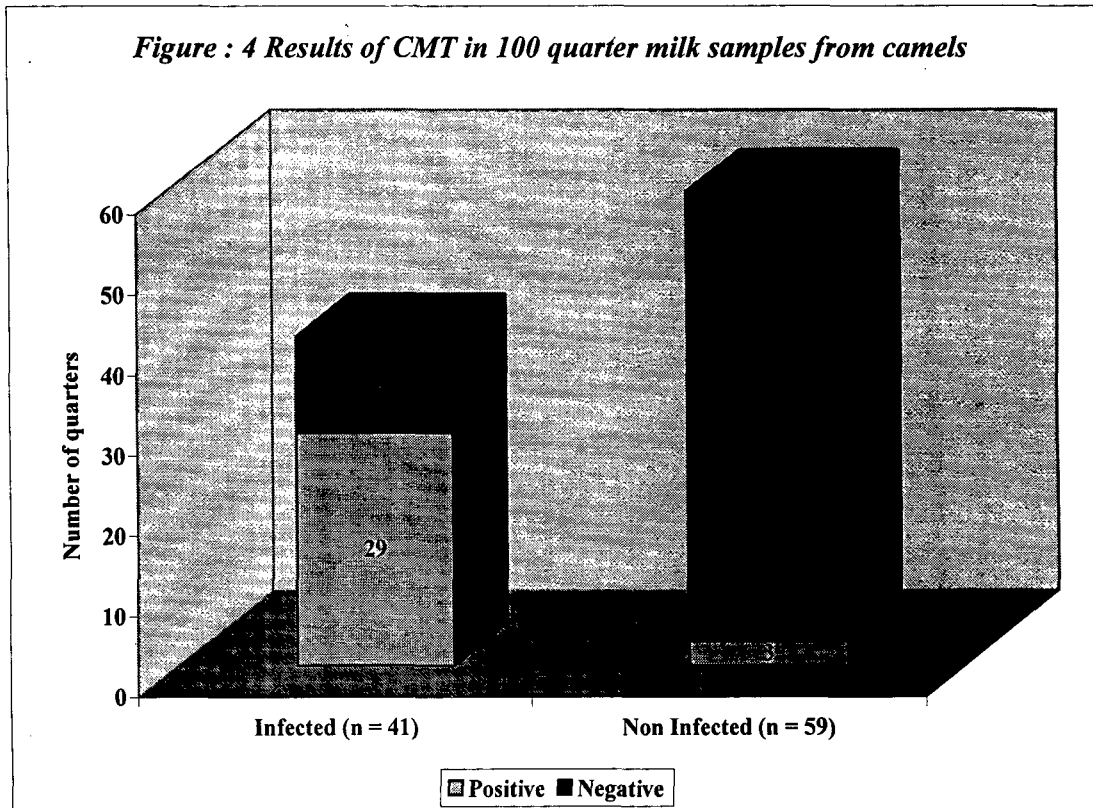
The infected quarters also had higher mean value for pH of milk (6.469) than the non infected quarters (6.257) having a significantly higher difference of 0.212 ( $P \leq 0.01$ ). Sena *et al.* (2000) and Tuteja *et al.* (2003) also reported a significant rise in milk pH of camel milk in case of clinically infected quarters. In study of Mostafa *et al.* (1987) increase in milk pH was correlated with positive bacteriological findings. Yagil (1985) suggested that changes in DM concentration in dehydration in camels, especially under drought condition, might have some effect on pH of camel milk.

In cattle, a threshold value of 5 lack cells/ml has been established for SCC and according to combination of this SCC value and culture examination, mastitis has been classified into three types in cattle – subclinical (culture positive,  $SCC > 5$  lack), latent (culture positive,  $SCC < 5$  lack) and non specific (culture negative,  $SCC > 5$  lack). But no such classification can be done in camels as there is no such threshold value fixed for SCC in camels and in absence of background information and experimental data it is difficult to establish threshold values of inflammatory markers in camels. In present study also, although the mean value for SCC in culturally positive quarters along with mean values of pH and EC differed significantly from quarters that were culturally negative, but the individual values overlapped between infected and non infected quarters. So it is necessary to establish the basal values for these inflammatory markers in camels along with their physiological variation.

The result of California Mastitis Test (CMT) of 100 quarter milk samples from 25 camels are shown in the Table 5 and Figure 5.

**Table 5 : Results of CMT in 100 quarter milk samples from camels**

Quarters	Positive	Negative
Infected (n = 41)	29	12
Non Infected (n = 59)	3	56



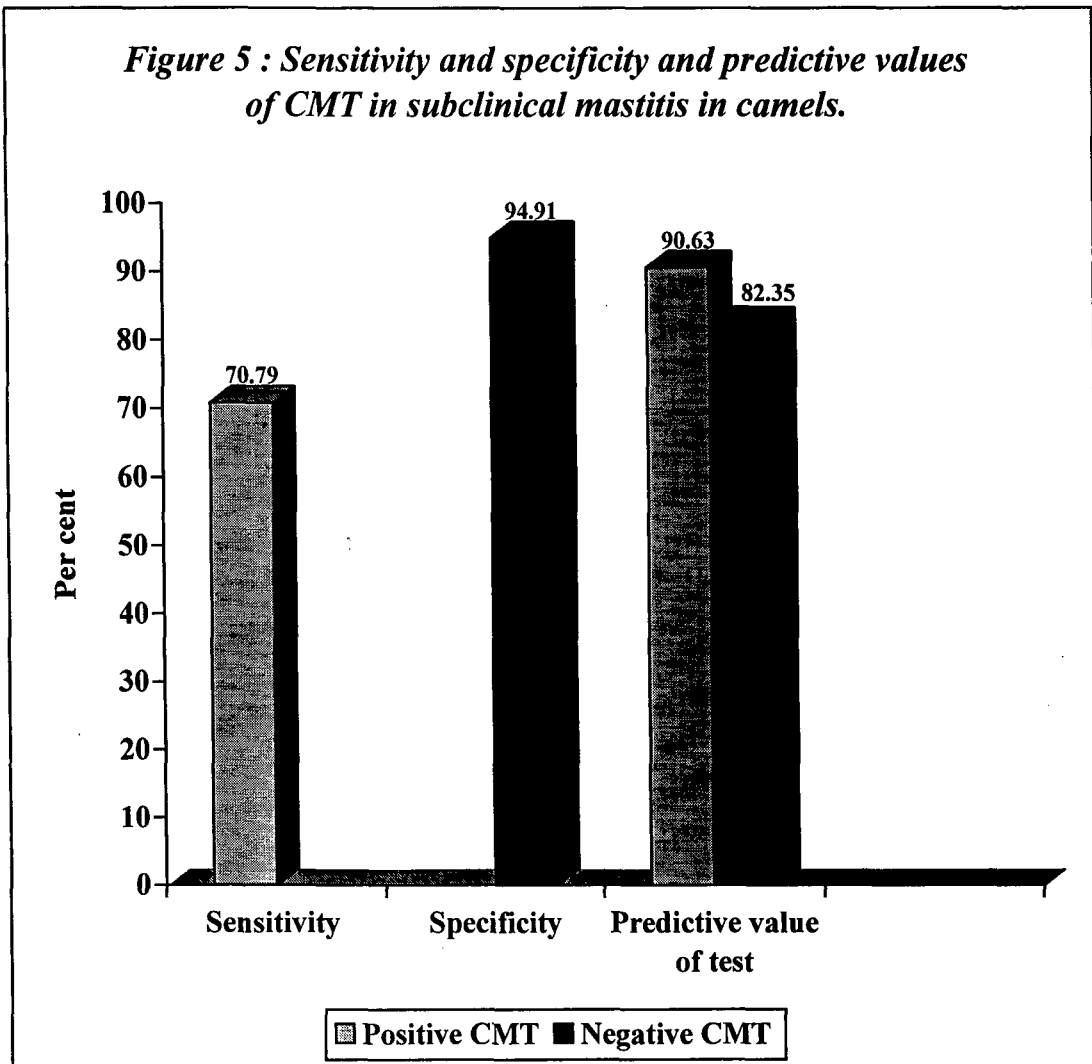
Out of total 41 infected quarters, only 29 showed positive CMT results and rest 12 were negative for CMT. Among total 59 non infected quarters, 56 were also negative for CMT and only 3 gave positive reaction in CMT. Almaw and Molla (2000) also found 7 samples out of 65 CMT positive reactors which gave negative results on microbiological culture. This could be possible because the samples might have been taken during the convalescent phase of the infection but with high leucocyte count giving a CMT positive result.

The predictive values of CMT along with sensitivity and specificity as observed in the present investigation are given in Table 6 and Figure 5.

**Table 6 :** *Sensitivity and specificity and predictive values of CMT in subclinical mastitis in camels*

	Positive reaction for CMT	Negative reaction for CMT
Infected quarters (n=41)	29	12
Non infected quarters (n = 59)	3	56
Sensitivity	$29/41 = 70.79 \%$	-
Specificity	-	$56/59 = 94.91 \%$
Predictive value of test	$29/32 = 90.63 \%$	$56/68 = 82.35 \%$

**Figure 5 :** *Sensitivity and specificity and predictive values of CMT in subclinical mastitis in camels.*



The sensitivity and specificity recorded for CMT were 70.79 % and 94.91 %, respectively. The predictive value of positive CMT was observed to be higher (90.63 %) than the predictive value of negative test (82.35 %). It indicates that CMT was able to detect only 70.79 % of the total positive cases of subclinical mastitis in camels but when a sample gives positive CMT, there are 90.63 % chances that it will be positive culturally also. Barbour *et al.* (1985), Mostafa *et al.* (1987), Abdurahman *et al.* (1995), Bekele and Molla (2001) and Tuteja *et al.* (2003) found positive correlation between positive CMT result and presence of udder infection in camels. Abdurahman (1996a) and Sena *et al.* (2000) also suggested that CMT is of value as a screening test for detection of mastitis in camels. Younan *et al.* (2001) recorded of 77 and 68 % quarter level sensitivity of CMT for detection of IMI by *Streptococcus agalactiae* and *Staphylococcus aureus*, respectively.

In the present study SCC, EC and pH differed significantly between infected and non infected quarters and CMT also showed predictive value of 90.63 % for positive reaction. It showed ability of these tests in indicating the infection status of udder in camels, however the number of animals studied was small. So further investigation using large number of animals are necessary to understand the dynamics of mastitis in camels before such indicators could be routinely used in predicting udder infections in camels.

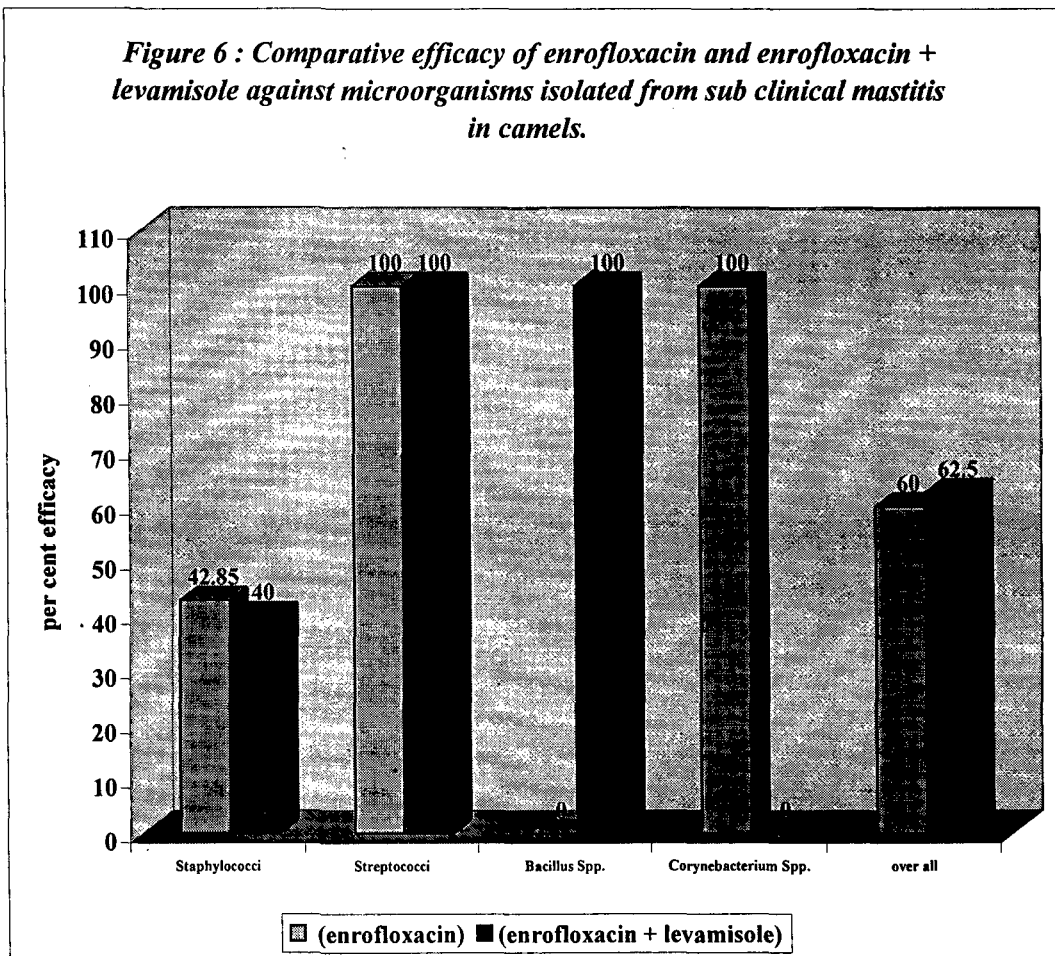
#### **4. Therapeutics of the SCM**

The comparative efficacy of enrofloxacin and enrofloxacin + levamisole against microorganisms isolated from subclinical mastitis cases in camels is shown in the Table 7 and Figure 6.

**Table 7:** Comparative efficacy of enrofloxacin and enrofloxacin + levamisole against microorganisms isolated from sub clinical mastitis in camels

Organism	T <sub>1</sub> (enrofloxacin)			T <sub>2</sub> (enrofloxacin + levamisole)		
	Quarters infected		% cure	Quarters infected		% 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

**Figure 6:** Comparative efficacy of enrofloxacin and enrofloxacin + levamisole against microorganisms isolated from sub clinical mastitis in camels.



As is evident from the table, enrofloxacin when used alone, could clear 100 % of the infection due to streptococci and *Corynebacterium* spp. but the cure rate against staphylococci was observed to be only 42.85 %.

When enrofloxacin + levamisole were used, 100 % of the streptococci and *Bacillus* spp. were cleared but the clearance of the staphylococci was only 40 % by this treatment.

In both the treatments cure rate for staphylococci was lower than other microbes. However, in *in vitro* sensitivity testing, staphylococci showed 96.43 % sensitivity against enrofloxacin. So the *in vivo* results were not same as could be expected for *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 % was about the best that could be expected.

The overall efficacy of treatment was 60 % for T<sub>1</sub> (enrofloxacin) and 62.5 % for T<sub>2</sub> (enrofloxacin + levamisole). Barbour *et al.* (1985) performed preliminary study on therapeutics of camel mastitis with chloramphenicol, oxytetracylin and gentamicin and found that successful treatment depends upon correct choice of drug, proper dosage and the milking of animals during the course of treatment. But after that no other worker tried therapeutic trials on camel mastitis. However, many workers 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 % and 61.53 % respectively) in cattle. Overall higher efficacy of 80 %, 84.62 % and 100 % for treatment with enrofloxacin have been reported in bovine by Maiti *et al.* (1996), Akhtar *et al.* (2003) and Kumar *et al.* (1998), respectively.

The overall efficacy was observed to be slightly better when enrofloxacin was used in combination with levamisole. 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 case of control group, spontaneous recovery of 11.11 % against staphylococci and 50 % for *Corynebacterium* spp. was observed but no spontaneous recovery was seen for streptococci and *Bacillus* spp. Overall 15.38 % of quarters in control group showed spontaneous recovery. Saluja (1998) also reported spontaneous cure rate of 13.04 % in quarters infected for subclinical mastitis in cattle from untreated groups.

The comparison of the mean  $\pm$  SE values of SCC, EC and pH for enrofloxacin and enrofloxacin + levamisole before and after treatment are presented in Table 8 and 9 and Figure 7.

**Table 8:** Mean  $\pm$  SE values for SCC, EC and pH of infected quarters before and after treatment

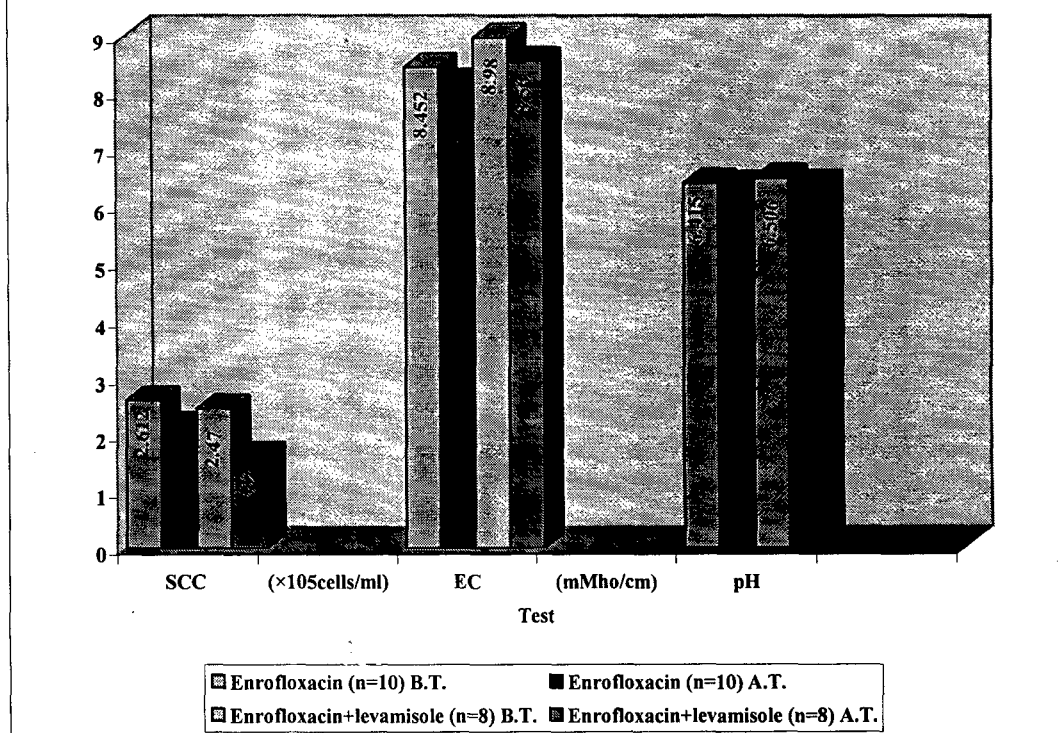
S.No.	Test		Enrofloxacin (n=10)		Enrofloxacin+levamisole (n=8)	
			Before treatment	After treatment	Before treatment	After treatment
1.	SCC ( $\times 10^5$ cells/ml)	Mean $\pm$ SE	2.612 $\pm$ 0.242	2.153 $\pm$ 0.223	2.470 $\pm$ 0.446	1.632 $\pm$ 0.258
		Range	1.412-3.883	0.882-3.000	1.059-3.883	0.882-2.294
2.	EC (mMho/cm)	Mean $\pm$ SE	8.452 $\pm$ 0.133	8.171 $\pm$ 0.207	8.98 $\pm$ 0.261	8.56 $\pm$ 0.199
		Range	7.45-8.92	7.03-8.97	8.28-10.71	7.99-9.69
3.	pH	Mean $\pm$ SE	6.415 $\pm$ 0.038	6.377 $\pm$ 0.029	6.506 $\pm$ 0.106	6.397 $\pm$ 0.034
		Range	6.188-6.560	6.192-6.484	6.334-7.239	6.282-6.573

**Table 9:** Comparison of overall mean values of infected quarters for various diagnostic tests before and after treatment (n=18)

S.No.	Test	Before treatment	After treatment
1.	SCC ( $\times 10^5$ Cells/ml)	2.549	1.920*
2.	EC (mMho/cm)	8.69	8.34 <sup>NS</sup>
3.	pH	6.455	6.386 <sup>NS</sup>

Note : Values with superscripts \* have  $P < 0.05$  and <sup>NS</sup> have  $P \geq 0.05$

*Figure 7 : Mean  $\pm$  SE values for SCC, EC and pH of infected quarters with enrofloxacin and enrofloxacin+levamisole before and after treatment*



As shown in table the mean  $\pm$  SE value of SCC in T<sub>1</sub> (enrofloxacin) was  $2.61 \pm 0.24 \times 10^5$  cells/ml before treatment, which decreased to  $2.15 \pm 0.22 \times 10^5$  cells/ml after treatment. In T<sub>2</sub> (Enrofloxacin+levamisole) the values for SCC were  $2.47 \pm 0.45 \times 10^5$  cells/ml before treatment and  $1.63 \pm 0.26 \times 10^5$  cells/ml after treatment. Overall the before treatment value of  $2.55 \pm 0.23 \times 10^5$  cells/ml decreased to  $1.92 \pm 0.18 \times 10^5$  cells/ml after treatment.

For EC in T<sub>1</sub> (Enrofloxacin) the mean  $\pm$  SE value before treatment was  $8.45 \pm 0.13$  mMho/cm which decreased to  $8.17 \pm 0.21$  mMho/cm after treatment. In T<sub>2</sub> (Enrofloxacin+levamisole) the mean  $\pm$  SE values were  $8.98 \pm 0.26$  and  $8.56 \pm 0.20$  mMho/cm before and after treatment, respectively. The overall mean value decreased from  $8.69 \pm 0.15$  mMho/cm before treatment to  $8.34 \pm 0.15$  mMho/cm after treatment.

For pH, the mean  $\pm$  SE values before treatment were  $6.42 \pm 0.04$  and  $6.51 \pm 0.11$  in T<sub>1</sub> (Enrofloxacin) and T<sub>2</sub> (Enrofloxacin+levamisole) groups.

The overall mean  $\pm$  SE value was  $6.46 \pm 0.05$  before treatment which decreased to  $6.39 \pm 0.02$  after the treatment.

A decreased was observed in individual and overall values of SCC, EC and pH before and after treatment but the lower values of SCC, EC and pH as observed after treatment were only numerically lower except for overall SCC value which was statistically lower ( $P < 0.05$ ) than value observed before treatment.

The mean  $\pm$  SE values of SCC, EC and pH in control group are shown in Table 10.

**Table 10 :** Comparison of mean  $\pm$  SE values for SCC, EC and pH in control group at 0 day and 4<sup>th</sup> day after treatment

Test		0 day	4 <sup>th</sup> day after treatment
SCC ( $\times 10^5$ cells/ml)	Mean $\pm$ SE	2.335 $\pm$ 0.237	1.901 $\pm$ 0.178
	Range	1.059-3.706	0.882-2.824
EC (mMho/cm)	Mean $\pm$ SE	8.79 $\pm$ 0.143	8.44 $\pm$ 0.193
	Range	7.91-9.74	7.11-9.77
pH	Mean $\pm$ SE	6.550 $\pm$ 0.064	6.445 $\pm$ 0.051
	Range	6.243-6.993	6.204-6.797

In control group the mean  $\pm$  SE value at 0 day and 4<sup>th</sup> day after treatment respectively were  $2.34 \pm 0.23$  and  $1.90 \pm 0.18 \times 10^5$  cells/ml for SCC,  $8.79 \pm 0.14$  and  $8.44 \pm 0.19$  mMho/cm for EC and  $6.55 \pm 0.06$  and  $6.45 \pm 0.05$  for pH.

Before treatment 13 out of 18 quarters in treatment groups T<sub>1</sub> and T<sub>2</sub> reacted positive for CMT while after treatment only 7 quarters gave positive reaction in CMT.

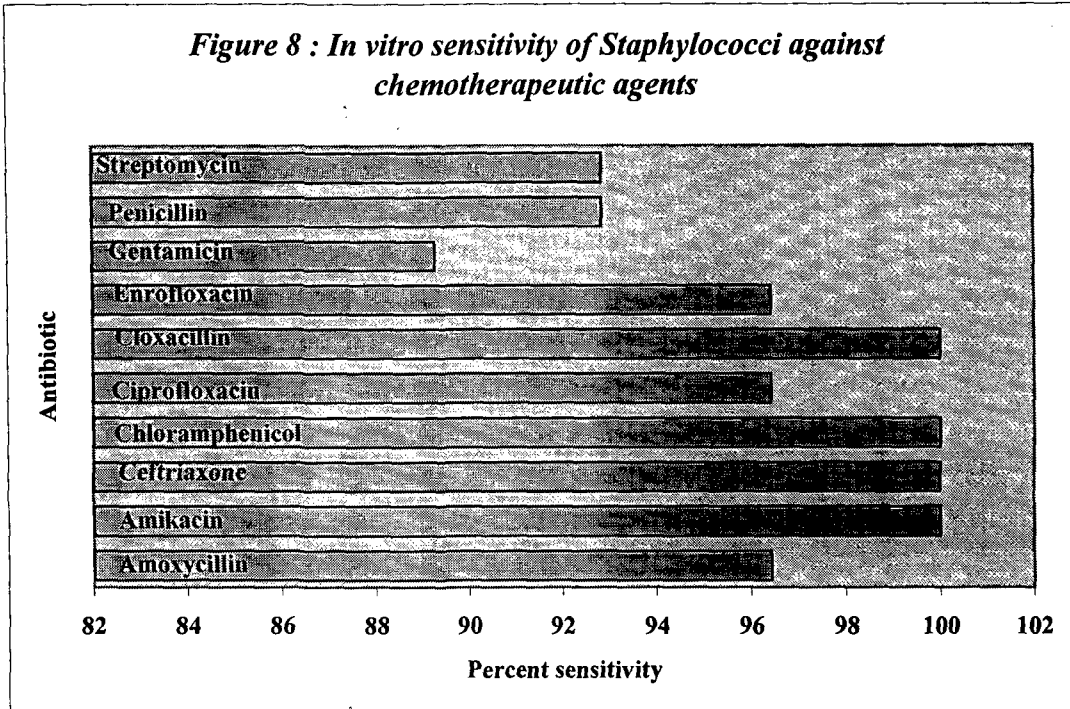
**5. *In vitro* chemotherapeutic sensitivity**

The *in vitro* chemotherapeutic sensitivity of 10 antibiotics against the bacterial isolates from SCM cases from camels as recorded in present investigation are presented in Table 11 and Figure 8, 9, 10, 11 and 12.

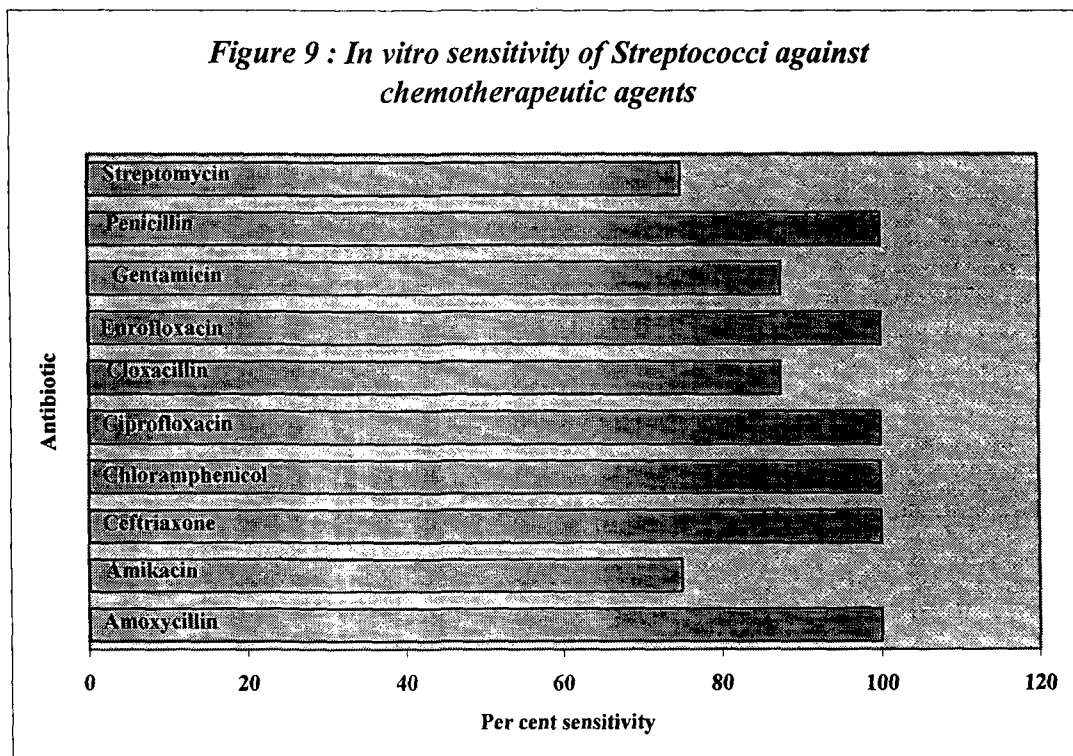
**Table 11: In vitro chemotherapeutic sensitivity of 41 mastitis isolates from camel**

Antibiotic		<i>Staphylococcus</i> spp.	<i>Streptococcus</i> spp.	<i>Corynebacterium</i> spp.	<i>Bacillus</i> spp.	Overall per cent sensitivity
Amoxicillin	S	27	8	3	2	97.56
	I	1	-	-	-	2.44
	R	-	-	-	-	0.00
Amikacin	S	28	6	2	2	92.68
	I	-	1	-	-	2.44
	R	-	1	1	-	4.88
Ceftriaxone	S	28	8	3	2	100
	I	-	-	-	-	0.00
	R	-	-	-	-	0.00
Chloramphenicol	S	28	8	3	2	100
	I	-	-	-	-	0.00
	R	-	-	-	-	0.00
Ciprofloxacin	S	27	8	3	1	95.12
	I	-	-	-	1	2.44
	R	1	-	-	-	2.44
Cloxacillin	S	28	7	3	2	97.56
	I	-	1	-	-	2.44
	R	-	-	-	-	0.00
Enrofloxacin	S	27	8	3	2	97.56
	I	1	-	-	-	2.44
	R	-	-	-	-	0.00
Gentamicin	S	25	7	3	2	90.24
	I	-	1	-	-	2.44
	R	3	-	-	-	7.32
Penicillin	S	26	8	3	1	92.68
	I	2	-	-	-	4.88
	R	-	-	-	1	2.44
Streptomycin	S	26	6	2	2	87.80
	I	1	-	1	-	4.88
	R	1	2	-	-	7.32

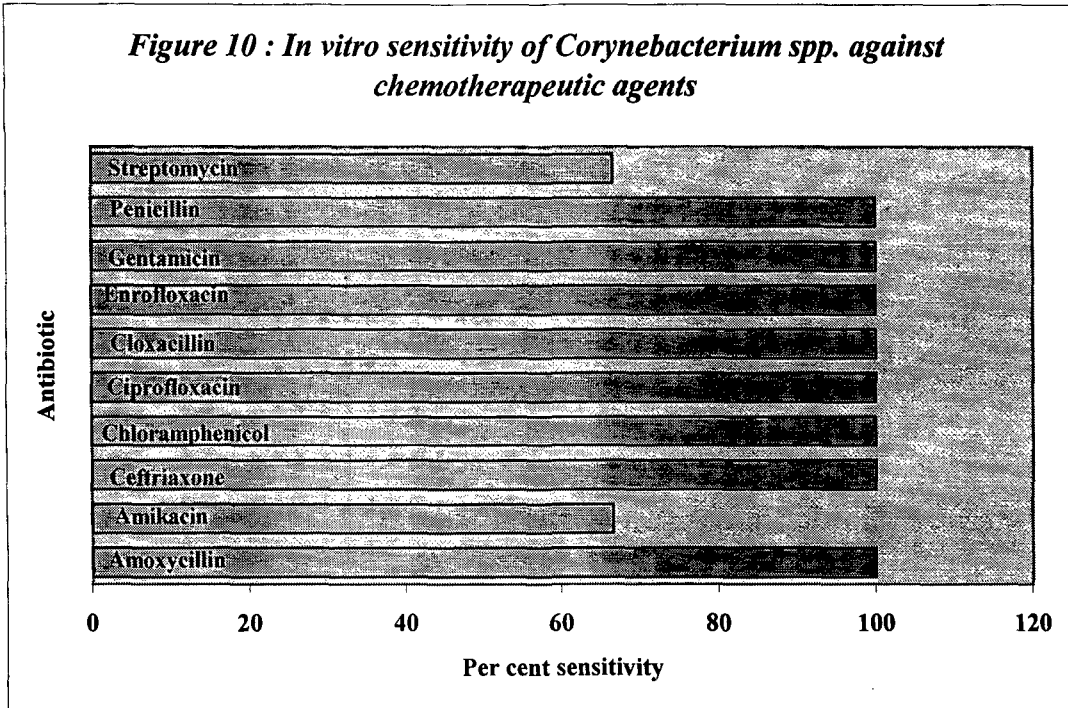
*Figure 8 : In vitro sensitivity of Staphylococci against chemotherapeutic agents*



*Figure 9 : In vitro sensitivity of Streptococci against chemotherapeutic agents*



*Figure 10 : In vitro sensitivity of Corynebacterium spp. against chemotherapeutic agents*



*Figure 11 : In vitro sensitivity of Bacillus spp. against chemotherapeutic agents*

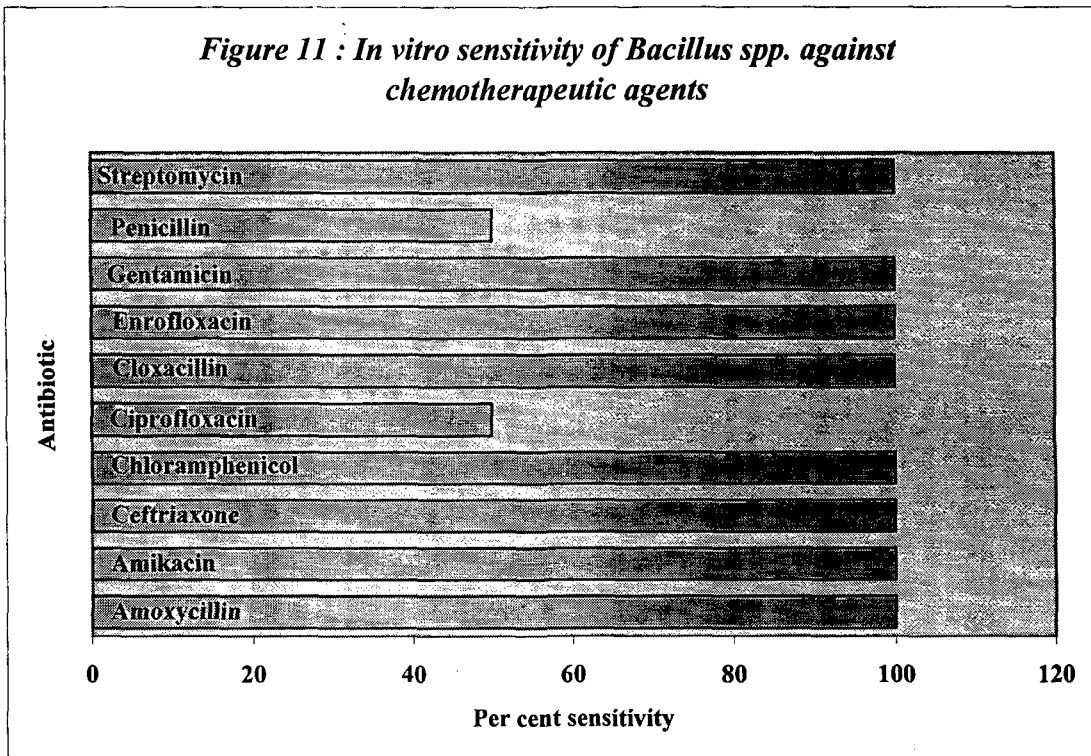
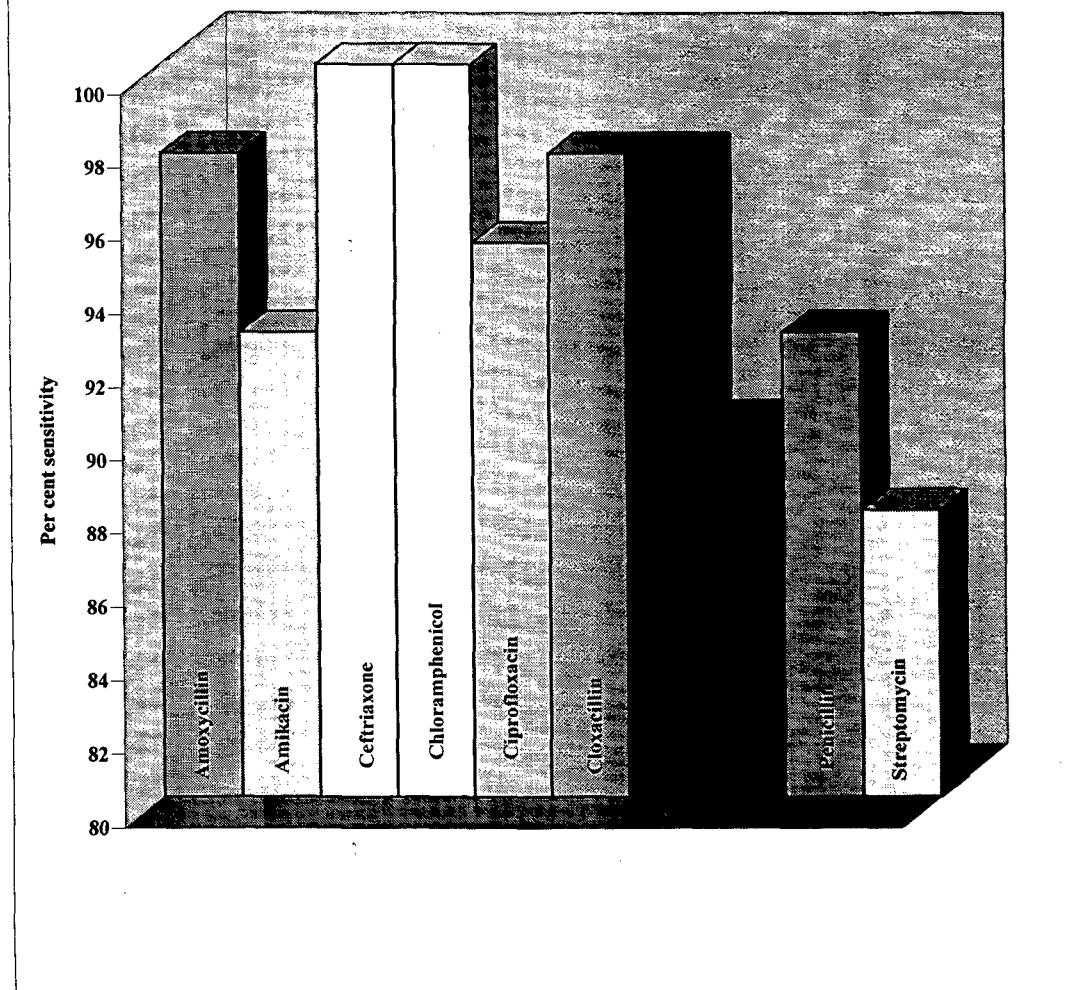


Figure 12 : Overall Chemotherapeutic sensitivity of mastitis isolates from camels



Studies on *in vitro* chemotherapeutic sensitivity of the isolates from subclinical mastitis cases in camels revealed that staphylococci showed 100 % sensitivity against chloramphenicol, ceftriaxone, amikacin and cloxacillin whereas 96.43 % sensitivity was recorded for enrofloxacin, ciprofloxacin and amoxycillin followed by penicillin (92.86 %) streptomycin (92.86 %) and gentamicin (89.29 %). Among streptococci 100 % sensitivity was recorded against amoxycillin, ceftriaxone, chloramphenicol, ciprofloxacin, enrofloxacin and penicillin followed by cloxacillin and gentamicin (87.5 %) and amikacin and streptomycin (75 %). *Corynebacterium* spp. were 100 % sensitive against

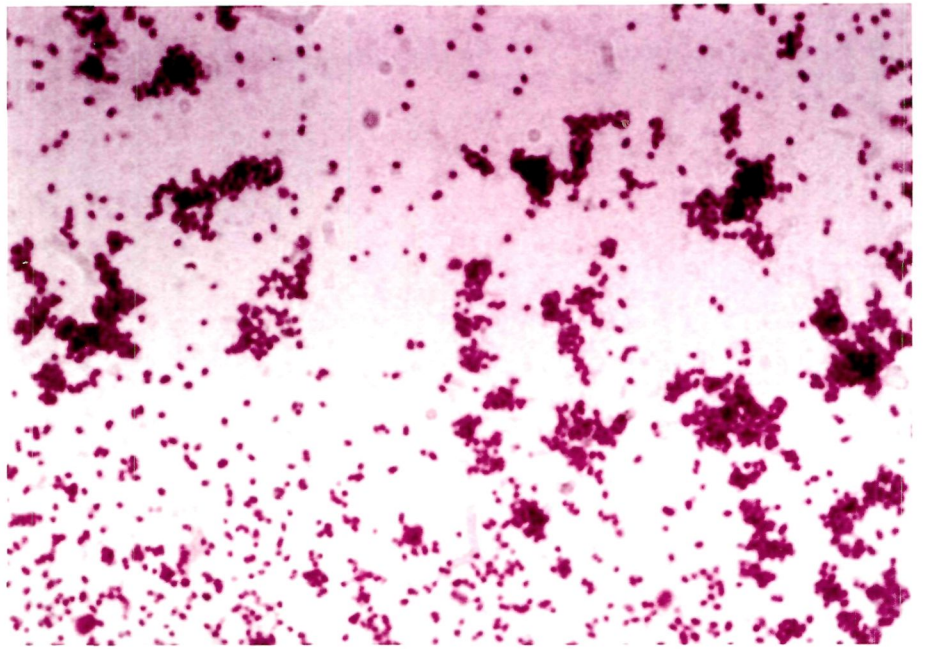
most of the antibiotics except amikacin and streptomycin (66.67 %) while *Bacillus* spp. showed 100 % sensitivity to all but ciprofloxacin and penicillin, for which 50 % of strains were resistant.

High sensitivity of staphylococci from camel milk to chloramphenicol, gentamicin, streptomycin and amikacin were also observed by Barbour *et al.* (1985) and Al-Jubori *et al.* (2001) but Al-Ani and Al-Shareefi (1997) and Mody *et al.* (1998) found sensitivity of Staphylococci from camels to be poor against chloramphenicol, gentamicin, streptomycin and penicillin. Tuteja *et al.* (2003) reported very high sensitivity of staphylococci from camel udders to ceftriaxone, chloramphenicol, amoxycillin, cloxacillin and gentamicin (all 100 %) and also to ciprofloxacin (95.65 %) and penicillin (86.96 %). Almost similar results were obtained for *Streptococcus* spp. and *Corynebacterium* spp.

In present study, the overall sensitivity against camel mastitis isolates was highest for ceftriaxone and chloramphenicol (100 %) followed by other antibiotics *i.e.* enrofloxacin, amoxycillin and cloxacillin (97.56 %), ciprofloxacin (95.12 %), amikacin and penicillin (92.68 %), gentamicin (90.24 %) and streptomycin (87.80 %). Barbour *et al.* (1985), Mody *et al.* (1998), Al-jubori *et al.* (2001) and Tuteja *et al.* (2003) have also recorded overall high sensitivity of mastitis isolates from camels against chloramphenicol, ceftriaxone, amoxycillin, cloxacillin, gentamicin, ciprofloxacin and amikacin though Al-Ani and Al-Shareefi (1997) found chloramphenicol and streptomycin to have overall poor sensitivity against camel subclinical mastitis isolates.

These results indicate the variability in the antimicrobial sensitivity pattern for microbes isolated from SCM cases in camels and suggest the importance of epidemiological knowledge of herd at any particular area to guide initial therapy.

**Plate -1 staphylococci  
from subclinical  
mastitic milk**



**Plate -2 streptococci  
from subclinical  
mastitic milk**



**Plate -3 somatic cell (a)  
and cell fragments (b) in milk  
smear from subclinical  
mastitic case**

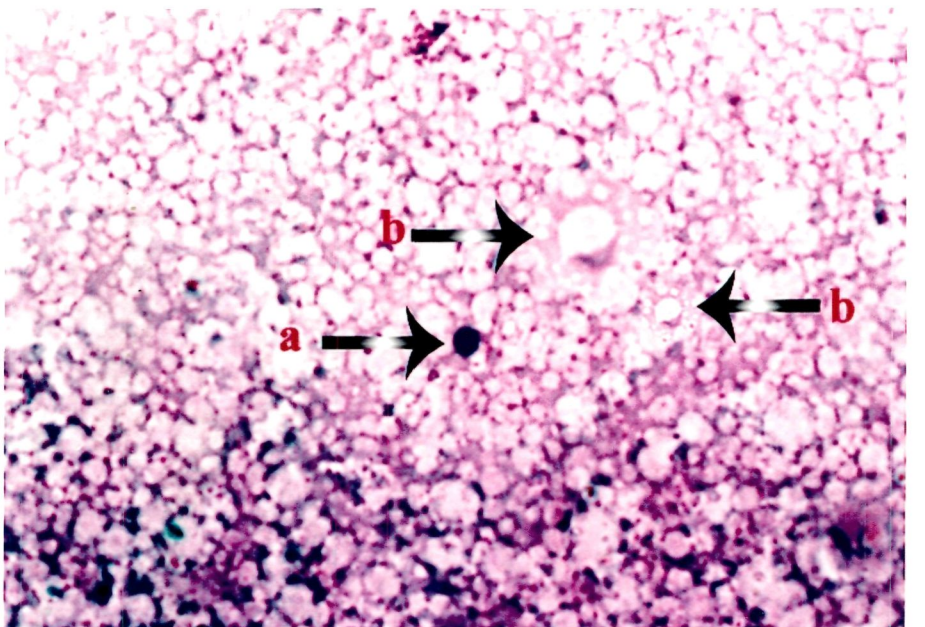


Plate -4 *Corynebacterium*  
spp. from subclinical  
mastitic milk

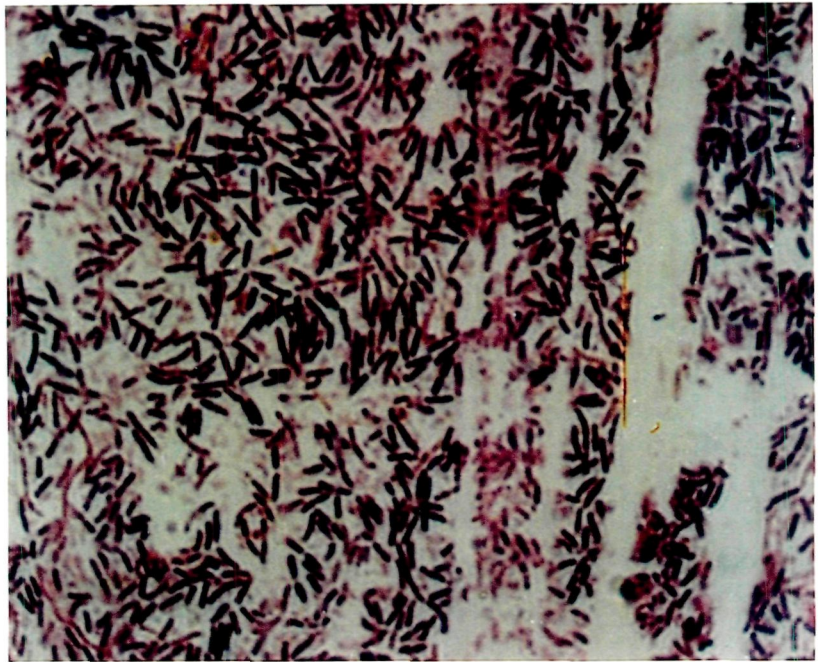


Plate -5 *Bacillus* spp. from  
subclinical mastitic milk

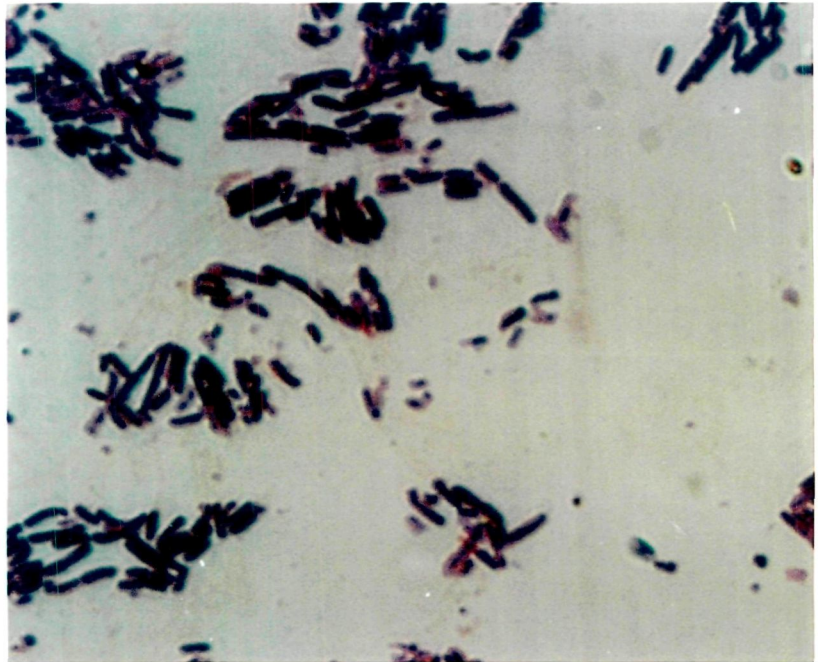
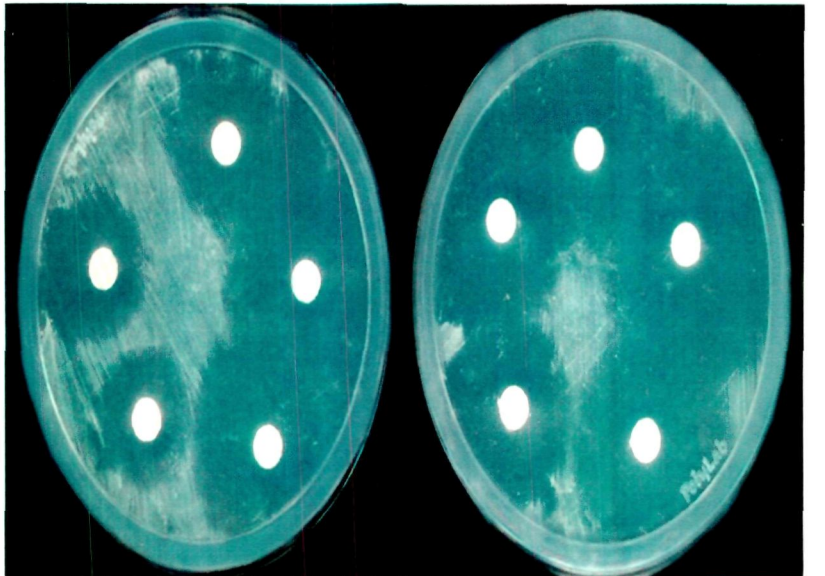


Plate -6 antibiotic sensitivity  
pattern of mastitogens



## SUMMARY

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A total of 100-quarter milk samples from 25 apparently healthy camels were collected and subjected to culture examination, Somatic Cell Count (SCC), California Mastitis Test (CMT), Electrical Conductivity (EC) and pH to find out the prevalence of subclinical mastitis, to determine the bacterial isolates and to assess the role of these tests in predicting the infection status of camel udder. In addition, efficacy of enrofloxacin alone and in combination with levamisole and *in vitro* antibiotic sensitivity pattern against these bacterial isolates were also studied.

The prevalence of subclinical mastitis on the basis of culture examination was 41 % (41/100) quarters wise and 72 % (18/25) animals wise.

Amongst different mastitogenic agents, staphylococci were found to be the most predominant bacteria associated with subclinical mastitis in camels, accounting for 68.29 % of the total isolates. Streptococci were next with 19.51 % occurrence followed by *Corynebacterium* spp. and *Bacillus* spp. which accounted for 7.32 % and 4.68 % of the total isolates, respectively.

The mean somatic cell count was recorded to be  $1.430 \times 10^5$  cells/ml of milk in non infected quarters (SE of  $\pm 0.047 \times 10^5$  cells/ml) ranging from  $0.882 \times 10^5$  to  $1.941 \times 10^5$  cells/ml. In infected quarters the mean somatic cell count was  $2.424 \times 10^5$  cells/ml (SE as  $\pm 0.145 \times 10^5$  cells/ml). It ranged between  $1.059 \times 10^5$  to  $3.883 \times 10^5$  cells/ml.

The mean  $\pm$  SE values for EC as observed in present investigation were  $8.41 \pm 0.067$  and  $8.66 \pm 0.081$  mMho/cm in case of non infected and infected quarters, respectively. It ranged between 7.12 to 9.75 mMho/cm in case of non infected quarters and from 7.45 to 10.71 mMho/cm in case of infected quarters.

In non infected quarters, the mean  $\pm$  SE value of milk pH was  $6.257 \pm 0.06$  ranging between 5.40 to 6.77 whereas, the infected quarters have mean  $\pm$  SE values as  $6.47 \pm 0.03$  with range of 6.19 to 7.24.

In present study all the three parameters (SCC, EC and pH) differed significantly between infected and non infected quarters.

Out of total 41 infected quarters, only 29 showed positive CMT results and rest 12 were negative for CMT. Among total 59 non infected quarters, 56 were also negative for CMT and only 3 gave positive reaction in CMT. The sensitivity and specificity recorded for CMT were 70.79 % and 94.91 %, respectively. The predictive value of positive CMT was observed to be higher (90.63 %) than the predictive value of negative test (82.35 %).

In the treatment of subclinical mastitis, when enrofloxacin alone was used it cleared 100 % of the infection due to streptococci and *Corynebacterium* spp. but the cure rate against staphylococci was observed to be only 42.85 per cent. When enrofloxacin + levamisole were used for treatment, 100 % of the streptococci and *Bacillus* spp. were cleared but the clearance of the staphylococci was only 40 % by this treatment. The overall efficacy of treatment was 60 % in T<sub>1</sub> (enrofloxacin) and 62.5 % for T<sub>2</sub> (enrofloxacin + levamisole).

The mean  $\pm$  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 mean  $\pm$  SE values for EC and pH in infected quarters were  $8.69 \pm 0.15$  and  $6.46 \pm 0.05$ , respectively before treatment which decreased to  $8.34 \pm 0.15$  and  $6.39 \pm 0.02$  respectively after the treatment. This decrease was non significant.

The CMT showed positive reaction in 13 quarters before treatment while only 7 quarters were positive for CMT after treatment.

*In vitro* chemotherapeutic sensitivity of the isolates from subclinical mastitis cases in camels revealed 100 % sensitivity of staphylococci against chloramphenicol, ceftriaxone, amikacin and cloxacillin whereas 96.43 % sensitivity was recorded for enrofloxacin, ciprofloxacin and amoxycillin

followed by penicillin (92.86 %) streptomycin (92.86 %) and gentamicin (89.29 %). Among streptococci 100 % sensitivity was recorded against amoxycillin, ceftriaxone, chloramphenicol, ciprofloxacin, enrofloxacin and penicillin followed by cloxacillin and gentamicin (87.5 %) and amikacin and streptomycin (75 %). *Corynebacterium* spp. showed 100 % sensitivity against most of the antibiotics except amikacin and streptomycin (66.67 %) while *Bacillus* spp. were 100 % sensitive to all but ciprofloxacin and penicillin, for which 50 % of strains were resistant.

The overall sensitivity against camel mastitis isolates was highest for ceftriaxone and chloramphenicol (100 %) followed by other antibiotics *i.e.* enrofloxacin (97.56 %), amoxycillin (97.56 %), cloxacillin (97.56 %), ciprofloxacin (95.12 %), amikacin (92.68 %), penicillin (92.68 %), gentamicin (90.24 %) and streptomycin (87.80 %).

### **CONCLUSION**

The following conclusions were drawn from the present investigation –

1. Quarter wise and animal wise prevalence of subclinical mastitis in camels was found to be 41 per cent and 72 per cent respectively.
2. Staphylococci were the most predominant bacteria associated with subclinical mastitis in camels.
3. The values for indirect tests like SCC, CMT, pH estimation and EC differed significantly between non infected and infected quarters but further study is required before using these parameters as indicators of subclinical mastitis in camel.
4. The treatment with enrofloxacin and enrofloxacin + levamisole resulted in 60 per cent and 62.5 per cent cure respectively.
5. Chloramphenicol, ceftriaxone, amoxycillin, cloxacillin and enrofloxacin showed more than 95 per cent *in vitro* sensitivity against bacterial isolates from camel milk.

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# **Epidemiological, Diagnostic and Therapeutic Aspects of Subclinical Mastitis in Camel (*Camelus dromedarius*)**

**M.V.Sc. Thesis**

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## **ABSTRACT**

**A total of 100-quarter milk samples from 25 apparently healthy camels were collected and subjected to culture examination, Somatic Cell Count (SCC), California Mastitis Test (CMT), Electrical Conductivity (EC) and pH to find out the prevalence of subclinical mastitis, to determine the bacterial isolates associated with subclinical mastitis in camels and to assess the role of these tests in predicting the infection status of camel udder. Further, efficacy of enrofloxacin alone and in combination with levamisole and *in vitro* antibiotic sensitivity pattern against these bacterial isolates were also studied.**

**The prevalence of subclinical mastitis was found to be 41 per cent on quarter basis and 72 per cent on animals basis. Staphylococci were the most prevalent organisms (68.29 %) among bacterial isolates followed by streptococci (19.51 %), *Corynebacterium* spp. (7.32 %) and *Bacillus* spp. (4.68 %). Mean values of somatic cell count, electrical conductivity and pH of culturally positive quarters were significantly higher than the mean values of culturally negative quarters. The California Mastitis Test showed 70.79 % sensitivity.**

**In present study, treatment with enrofloxacin alone was found to be 60 % effective while treatment with enrofloxacin + levamisole resulted in a cure rate of 62.5 per cent. Spontaneous recovery was seen in 15.38 per cent quarters in control group. There was significant decrease in the mean value of somatic cell count after treatment but the decrease in pH and EC was not significant.**

***In vitro* antibiotic sensitivity of 41 isolates against 10 antimicrobials revealed more than 95 % sensitivity for chloramphenicol, ceftriaxone, amoxycillin, cloxacillin, enrofloxacin and ciprofloxacin.**

# उष्ट्र (कैमेलस डॉमेस्टेरिस) में अनुशयनिक थनैला रोग के जनपादकीय, निदान एवं उपचार संबन्धी पहलू

जानपदिक रोग विज्ञान एवं निवारक पशु औषध विज्ञान विभाग  
पशु चिकित्सा एवं पशु विज्ञान महाविद्यालय  
राजस्थान कृषि विश्वविद्यालय, बीकानेर

शोधकर्ता :

लेनिन भट्ट

मुख्य समादेष्टा :

डॉ. (श्रीमती) अन्जु चाहर

## अनुक्षेपण

उष्ट्रों में अनुशयनिक थनैला रोग की व्यापकता नापने, विभिन्न परीक्षणों की क्षमता जांचने एवं दूध में मौजूद जीवाणुओं के प्रकारों का पता लगाने के लिये प्रत्यक्ष रूप से स्वस्थ दिखने वाले २५ मादा उष्ट्रों के १०० थनों के दूध के नमूने एकत्र किये गये तथा उनमें जैविक परीक्षण, दैहिक कोशिका गणना, कैलिफोर्निया थनैला परीक्षण, विद्युत चालकता एवं अम्लीय सांद्रता की जांच की गई। इसी के साथ ऐनरोफ्लोक्सेसिन की, अकेले तथा लेवामिसोल के साथ मिलकर उपचार की क्षमता तथा इन जीवाणुओं की प्रतिजैविक सूक्ष्मग्राहिता का भी अध्ययन किया गया।

अनुशयनिक थनैला रोग का प्रसार थनों के आधार पर ४१ प्रतिशत तथा उष्ट्रों के आधार पर ७२ प्रतिशत पाया गया। इन नमूनों से प्राप्त जीवाणुओं में *स्टेफाइलोकोकस* जीवाणु का प्रसार सर्वाधिक (६८.२९ प्रतिशत) पाया गया। इसके बाद *स्ट्रेप्टोकोकस* (१९.५१ प्रतिशत), *कोराइनीबैक्टीरियम* (७.३२ प्रतिशत) तथा *बेसीलस* (४.६८ प्रतिशत) जीवाणु थे। जीवाणु संक्रमित थनों में दैहिक कोशिका गणना, विद्युत चालकता एवं अम्लीय सांद्रता के औसत मान जीवाणु असंक्रमित थनों के औसत मानों की अपेक्षा प्रभावी रूप से अधिक पाये गये। कैलिफोर्निया थनैला परीक्षण की संवेदनशीलता ७०.७९ प्रतिशत पाई गई।

इस अध्ययन में अकेले ऐनरोफ्लोक्सेसिन द्वारा उपचार दर ६० प्रतिशत थी जबकि ऐनरोफ्लोक्सेसिन एवं लेवामिसोल की सम्मिलित उपचार क्षमता ६२.५ प्रतिशत दर्ज की गई। नियन्त्रण समूह में से १५.३८ प्रतिशत थनों में स्वतः उपचार देखा गया। उपचार के पश्चात दैहिक कोशिका गणना के औसत मान में उपचार पूर्व से प्रभावी कमी देखी गई जबकि विद्युत चालकता एवं अम्लीय सांद्रता में दर्ज की गई कमी सांख्यिकी रूप से अप्रभावी थी।

उष्ट्रों के ४१ थनों से निकाले गये जीवाणुओं के १० प्रतिजैविकों के खिलाफ प्रतिजैविक सूक्ष्मग्राहिता परीक्षण में पाया गया कि ९५ प्रतिशत से अधिक जीवाणु क्लोरेमफेनिकॉल, सेफ्ट्राइएक्सोन, एमोक्सीसिलिन, क्लोक्सासिलिन, ऐनरोफ्लोक्सेसिन एवं सिपरोफ्लोक्सेसिन के प्रति संवेदनशील थे।

*Appendix – I: Results of Somatic Cell Count (SCC), Electrical Conductivity (EC), pH and California Mastitis Test (CMT) in non infected quarters from camels (n=59)*

S.No.	SCC ( $\times 10^5$ cells/ml)	EC (mMho/cm)	pH	CMT
1	1.235	8.69	6.332	-
2	1.941	8.74	6.260	-
3	1.941	8.56	6.355	-
4	1.412	8.72	6.316	-
5	1.588	8.37	6.368	-
6	1.765	8.45	6.356	-
7	1.059	8.45	6.312	-
8	1.588	7.12	6.541	-
9	1.765	7.92	6.335	-
10	1.412	7.67	6.374	-
11	1.941	8.67	6.278	-
12	1.059	8.44	6.344	-
13	1.588	8.48	6.139	-
14	1.235	9.66	6.439	+
15	1.588	9.75	6.768	-
16	1.235	8.62	5.405	+
17	0.882	8.71	6.274	-
18	0.882	8.20	6.297	-
19	1.235	8.26	6.340	-
20	1.235	8.50	6.154	-
21	1.765	8.64	6.396	-
22	0.882	8.64	6.408	-
23	1.509	9.37	6.233	-
24	1.235	9.53	6.280	-
25	1.588	8.01	6.065	-
26	1.765	8.57	6.269	-
27	1.941	9.17	6.395	-
28	1.941	8.75	6.258	-
29	1.059	8.34	6.646	-

S.No.	SCC ( $\times 10^5$ cells/ml)	EC (mMho/cm)	pH	CMT
30	1.588	7.98	6.090	-
31	1.588	7.54	6.049	-
32	1.412	8.40	6.386	-
33	1.412	7.84	6.258	-
34	1.235	7.45	6.518	-
35	1.059	8.74	6.260	+
36	1.588	9.02	6.402	-
37	1.765	8.97	6.484	-
38	1.059	8.76	6.471	-
39	0.882	8.73	6.470	-
40	1.941	8.42	6.323	-
41	1.765	8.63	6.136	-
42	1.765	8.48	6.332	-
43	1.941	8.34	6.450	-
44	0.882	8.53	6.308	-
45	1.059	8.78	6.425	-
46	1.235	7.92	6.126	-
47	1.765	7.92	6.187	-
48	1.765	8.25	6.388	-
49	1.588	7.57	6.391	-
50	0.882	8.05	6.325	-
51	1.765	7.98	6.255	-
52	1.588	8.21	5.916	-
53	0.882	8.15	6.423	-
54	1.941	7.92	6.276	-
55	1.412	8.04	6.198	-
56	0.882	8.12	6.450	-
57	1.059	8.12	6.172	-
58	1.059	7.88	6.512	-
59	1.765	8.30	6.246	-

**Appendix – II : Results of culture examination, Somatic Cell Count (SCC), Electrical Conductivity (EC), pH and California Mastitis Test (CMT) in infected quarters from camels before treatment (n=41)**

S.No.	Isolates	SCC ( $\times 10^5$ cells/ml)	EC (mMho/cm)	pH	CMT
1	<i>Staphylococcus</i> spp.	2.471	8.61	6.457	+
2	<i>Staphylococcus</i> spp.	3.000	8.59	6.454	+
3	<i>Staphylococcus</i> spp.	2.294	8.89	6.430	+
4	<i>Staphylococcus</i> spp.	3.530	8.17	6.303	+
5	<i>Staphylococcus</i> spp.	3.883	7.45	6.518	+
6	<i>Staphylococcus</i> spp.	2.647	8.92	6.560	-
7	<i>Staphylococcus</i> spp.	3.000	8.63	6.552	-
8	<i>Streptococcus</i> spp.	1.765	8.30	6.347	-
9	<i>Streptococcus</i> spp.	1.412	8.43	6.336	+
10	<i>Corynebacterium</i> spp.	2.118	8.53	6.188	+
11	<i>Staphylococcus</i> spp.	3.883	8.28	6.373	+
12	<i>Staphylococcus</i> spp.	3.883	8.80	6.400	+
13	<i>Staphylococcus</i> spp.	3.177	9.02	6.432	+
14	<i>Staphylococcus</i> spp.	1.235	8.52	6.334	-
15	<i>Staphylococcus</i> spp.	3.530	8.70	6.403	+
16	<i>Streptococcus</i> spp.	1.765	10.71	7.239	-
17	<i>Streptococcus</i> spp.	1.059	8.91	6.465	+
18	<i>Bacillus</i> spp.	1.235	8.92	6.402	+
19	<i>Staphylococcus</i> spp.	3.353	8.60	6.889	+
20	<i>Staphylococcus</i> spp.	2.471	9.59	6.993	+
21	<i>Staphylococcus</i> spp.	2.471	7.91	6.617	+
22	<i>Staphylococcus</i> spp.	1.588	8.78	6.454	-
23	<i>Staphylococcus</i> spp.	3.177	8.54	6.520	-

S.No.	Isolates	SCC ( $\times 10^5$ cells/ml)	EC (mMho/cm)	pH	CMT
24	<i>Staphylococcus</i> spp.	2.294	8.88	6.410	-
25	<i>Staphylococcus</i> spp.	3.706	8.81	6.397	+
26	<i>Staphylococcus</i> spp.	1.412	8.34	6.627	-
27	<i>Staphylococcus</i> spp.	3.000	8.54	6.828	+
28	<i>Streptococcus</i> spp.	2.824	8.43	6.454	-
29	<i>Corynebacterium</i> spp.	1.059	9.40	6.243	+
30	<i>Corynebacterium</i> spp.	1.412	9.74	6.266	+
31	<i>Bacillus</i> spp.	1.588	8.75	6.455	+
32	<i>Staphylococcus</i> spp.	1.765	8.74	6.258	-
33	<i>Staphylococcus</i> spp.	1.412	8.60	6.889	-
34	<i>Staphylococcus</i> spp.	3.530	8.75	6.454	+
35	<i>Staphylococcus</i> spp.	2.294	8.43	6.455	+
36	<i>Staphylococcus</i> spp.	3.706	8.30	6.336	+
37	<i>Staphylococcus</i> spp.	1.588	8.17	6.188	+
38	<i>Staphylococcus</i> spp.	2.118	8.45	6.312	+
39	<i>Streptococcus</i> spp.	1.941	8.52	6.334	+
40	<i>Streptococcus</i> spp.	3.706	8.43	6.347	+
41	<i>Streptococcus</i> spp.	1.059	8.35	6.303	+

*Appendix – III : Results of culture examination, Somatic Cell Count (SCC), Electrical Conductivity (EC), pH and California Mastitis Test (CMT) in infected quarters from camels after treatment (n=31)*

S.No.	Isolates	SCC ( $\times 10^5$ cells/ml)	EC (mMho/cm)	pH	CMT
1	<i>Staphylococcus</i> spp.	2.471	8.97	6.484	+
2	<i>Staphylococcus</i> spp.	2.647	8.73	6.399	+
3	<i>Staphylococcus</i> spp.	2.471	8.63	6.349	+
4	-	3.000	7.92	6.284	-
5	-	2.647	7.03	6.470	-
6	-	2.118	8.37	6.453	-
7	<i>Staphylococcus</i> spp.	2.471	8.78	6.420	+
8	-	0.882	7.61	6.405	-
9	-	1.059	7.39	6.314	-
10	-	1.765	8.28	6.192	-
11	<i>Staphylococcus</i> spp.	2.294	7.99	6.282	+
12	-	2.824	8.66	6.392	-
13	-	1.765	9.02	6.379	-
14	<i>Staphylococcus</i> spp.	1.235	8.48	6.470	-
15	<i>Staphylococcus</i> spp.	2.118	8.34	6.389	+
16	-	1.059	9.69	6.573	-
17	-	0.882	8.10	6.282	+
18	-	0.882	8.19	6.412	-
19	<i>Staphylococcus</i> spp.	2.647	8.10	6.797	+
20	<i>Staphylococcus</i> spp.	1.588	8.71	6.713	+
21	<i>Staphylococcus</i> spp.	1.765	7.11	6.486	-
22	<i>Staphylococcus</i> spp.	1.588	8.14	6.374	-
23	<i>Staphylococcus</i> spp.	2.824	8.07	6.498	-

S.No.	Isolates	SCC ( $\times 10^5$ cells/ml)	EC (mMho/cm)	pH	CMT
24	-	2.118	8.94	6.366	-
25	<i>Staphylococcus</i> spp.	1.941	8.03	6.301	+
26	<i>Staphylococcus</i> spp.	1.588	7.75	6.447	-
27	<i>Staphylococcus</i> spp.	2.824	8.62	6.657	-
28	<i>Streptococcus</i> spp.	2.471	8.55	6.414	-
29	<i>Corynebacterium</i> spp.	0.882	9.40	6.204	+
30	-	1.059	9.77	6.250	-
31	<i>Bacillus</i> spp.	0.882	8.56	6.282	+