EFFICACY OF MOXIFLOXACIN AND MARBOFLOXACIN AGAINST MASTITIS IN BUFFALO

T H E S I S

Submitted

In partial fulfillment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCE

IN

VETERINARY CLINICAL MEDICINE, ETHICS AND JURISPRUDENCE

BY

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Enrollment No: - V/14/229

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(INDIA)

2022

DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled, "**EFFICACY OF MOXIFLOXACIN AND MARBOFLOXACIN AGAINST MASTITIS IN BUFFALO"** or part thereof has not been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis / publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

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Mr. **SHELKE AJAY ANANDRAO** has satisfactorily prosecuted his course of research for a period of not less than one semester and that the thesis entitled, **"EFFICACY OF MOXIFLOXACIN AND MARBOFLOXACIN AGAINST MASTITIS IN BUFFALO"** submitted by him is the result of original research work is sufficient to warrant its presentation to the examination in the subject of **VETERINARY CLINICAL MEDICINE, ETHICS AND JURISPRUDENCE** for the award of **MASTER OF VETERINARY SCIENCE** degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

We also certify that the thesis or part thereof has not been previously submitted by him for a degree of any other University.

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CERTIFICATE

This is to certify that the thesis entitled, **"EFFICACY OF MOXIFLOXACIN AND MARBOFLOXACIN AGAINST MASTITIS IN BUFFALO"** submitted by Mr. **SHELKE AJAY ANANDRAO** to the Maharashtra Animal and Fishery Sciences University in partial fulfillment of the requirement for the degree of **MASTER OF VETERINARY SCIENCE** has been approved by the Student's Advisory Committee after examination in collaboration with the external examiner..

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Place: Parbhani
Date: / /2022 (Shelke Ajay Anandrao)

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ABBREVATION

%	:	Percent
@	:	At the rate of
BW	:	Body weight
et al	:	Any other
Fig.	:	Figure
PCV	:	Packed cell volume
SCC	:	Somatic cell count
^{0}F	:	Degree Fahrenheit
i.e.	:	That is
D.F.	:	Degree of freedom
Inj.	:	Injection
Hb	:	Haemoglobin
g/dL	:	Gram per decilitre
RBC	:	Red blood cell
Ul	:	Microliter
TLC	:	Total leucocyte count
TEC	:	Total erythrocyte count
mg/kg	:	milligram per kilogram
cm^2	:	Centimeter square
cells/ml	:	Cells per millilitre
lakh/ml	:	Lakh per millilitre

Introduction

CHAPTER I

INTRODUCTION

The total livestock population of India is 535.78 million and total number of buffaloes in India is 109.85 million. About 20.5% of the total livestock population is contributed by buffaloes. In India 38.16 million buffaloes are in milking stage (20th Livestock census 2019). Asia has 167 million buffaloes which is 96% of the world buffalo population. India is the leading country in buffalo husbandry followed by Pakistan and China. The promising dairy buffalo breeds include Murrah, Jaffarabadi, Surti, Mehsana, Nagpuri, Pandharpuri and Marathwadi. Buffalo milk contains higher total solids and also it is rich source of fat than any other mammalian milk and it's energy value is 30.1gram. Buffalo milk is good source of Vitamin A and Vitamin D. Buffalo milk is suitable for various indigenous dairy products like Khoa, Ghee and Butter (Khedkar *et al.* 2016).

The family of buffalo is bovine and its scientific name is *Bubalus bubalis*. The domestic buffalo is one of the 40 livestock species used in world for food and agriculture production. The domestic buffalo again classified as riverine and swamp buffalo. Buffaloes are used for three purposes *i.e.* milk, meat and draught. Swamp type of buffalo is used for meat and draught purpose. Riverine buffaloes are primarily used for milk purpose and secondarily for meat purpose (Desta, 2012).

Mastitis is defined as inflammation of parenchyma of the mammary gland regardless of cause (Radostits *et al*. 2007). Mastitis is classified as clinical or subclinical mastitis depending upon clinical signs. Subclinical mastitis does not show visible effect on udder or milk quality but has effect on milk composition and mainly there is increase in somatic cell count. Clinical mastitis shows clinical symptoms like inflammation of udder and change in milk quality.

Bovine mastitis is a common disease of dairy cows and buffaloes characterized by physical, chemical, pathological and bacteriological changes in milk and glandular tissues (Samad, 2008). Mastitis is a serious problem of dairy

farmers all over the world due to decreased milk production of cattle and buffaloes causing severe economic losses (Kumar *et al*. 2007). Mastitis is considered as the number one disease amongst all livestock diseases according to surveys in the field (Khan and Khan, 2006).

Economic loss due to mastitis in India are estimated to be 6053.21 crores (Dua, 2001). Economic loss due to mastitis per animal per lactation are 2182.44 and 1272.36 INR for cattle and buffaloes respectively. Due to mastitis annual loss in the dairy industry is almost 2.37 thousand crore rupees in India (Lakshmi, 2016). The losses due to clinical mastitis are not only economical but also animal health and welfare, milk quality and quantity. Average decrease in milk yield due to clinical mastitis was 50% and due to subclinical mastitis was 17.5% (Shabaz *et al.* 2020).

Mastitis is one of the important production diseases of dairy animals all over the world, as it causes great economical loss due to lowered milk yield, decreased milk quality and production, increased cost of treatment, labour and discarded milk during infection. Predisposing factors for mastitis are poor management, bad hygiene, faulty milking practices, teat injuries and existing environmental population of pathogens.

Mastitis may be classified into two types *i.e.* clinical and subclinical (Duguma *et al.* 2014). *Staphylococcus spp., Streptococcus* spp*., Escherichia coli, Corynebacterium* spp*., Klebsiella* spp*. and Pseudomonas* spp. are some common bacteria which cause mastitis. Early detection of mastitis is important for lowering loss and improving recovery chances.

For the identification of clinical mastitis, there are numerous direct and indirect tests with variable efficacies such as, strip cup test, somatic cell count (SCC), culture, isolation and identification of causative agents, modified california mastitis test (MCMT), modified white side test (MWST), electrical conductivity of milk (EC) and ELISA.

The treatment of bovine mastitis is the most common reason that antibiotics are used for adult dairy cows. Before with holding antibiotic therapy, it

is important to assess the ability of affected cows to mount a successful immune response and when the immune system is compromised antimicrobial therapy may be recommended (Pamela, 2018).

Fluroquinolone group of antimicrobials inhibit the DNA gyrase enzyme and eventually DNA and RNA synthesis are still effective against majority of *Staphylococcus* and *Streptococcus* species isolates from buffalo mastitis cases (Chhabra *et al*. 2020).

Moxifloxacin is the 4th generation fluroquinolone. Moxifloxacin is an extended spectrum fluroquinolone which has improved coverage against Gram positive bacteria and good activity against Gram negative bacteria (Balfour and Lamb, 2000). Moxifloxacin has improved *in vitro* activity against Gram positive cocci, aerobic, anaerobic intracellular bacteria, as well as atypical organisms, such as *Mycoplasma* and *Chlamydia* as compared to older fluroquinolones. Moxifloaxacin is well absorbed after administration by different routes like intravenous, intramuscular and oral routes with bioavailability of 95 percent. Moxifloxacin found to be more effective than ciprofloxacin, enrofloxacin and levofloxacin (Meena *et al.* 2019).

The *Staphylococcus* species isolated from udder infections were found to be 100% sensitive to moxifloxacin, enrofloxacin, cefoperazone, amikacin, gentamicin, neomycin and chloramphenicol (Singh *et al.* 2014).

Marbofloxacin is third generatioin fluroquinolone. After systemic administration of marbofloxacin it has higher efficacy for the treatment of mastitis. After parental administration marbofloxacin have highest bioavailability *i.e*. about 100% (Thomas *et al*. 1994).

Considering the above facts, it has been decided to undertake the present study with the following objectives.

11. Objectives:

1. To study the incidence of clinical mastitis in buffaloes in and around Parbhani district.
2. To evaluate the efficacy of moxifloxacin and marbofloxacin against Clinical mastitis in buffaloes.

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

The present research work was undertaken to access the efficacy of moxifloxacin and marbofloxacin against clinical mastitis in buffaloes. The literature on incidence, clinical signs, haematological alterations, isolation of bacteria, antibiotic sensitivity test and treatment of clinical mastitis has been reviewed and presented under the different sub-headings as follows.

2.1. Incidence

Bilal *et al*. (2004) observed that prevalence of clinical mastitis in buffaloes was higher in peri urban (25.12%) than rural areas (19.74%). The incidence of clinical mastitis was highest during 4 to 6 month after calving both in peri-urban (45.76%) and rural areas (45.08%). The incidence of clinical mastitis was highest during the third lactation both in peri-urban (19.00%) and rural areas (22.98 %) respectively. The quarter wise incidence was highest in hind quarter (73.30%) than fore quarters (26.60%) in peri-urban area. In rural area hind quarters incidence was highest *i.e.* (63.10%) than fore quarters (36.80%).

Bachaya *et al.* (2005) found that quarter wise prevalence of mastitis in buffaloes was 58.75% and animal wise prevalence was 77.98% respectively.

Khan and Muhammad (2005) observed that overall incidence of subclinical mastitis in buffaloes was 27%, clinical mastitis was 4% and blind quarters were 10% respectively. In crossbred cows incidence of subclinical mastitis was 36%, clinical mastitis 5.5% and blind quarters 8 percent. Quarter wise prevalence was highest in hindquarters of crossbred cows than buffaloes (29%).

Joshi and Gokhale, (2006) observed incidence of clinical mastitis in buffalo as 1.99% and subclinical mastitis as 12.28%. Incidence of clinical mastitis as 11.51% and 3.94% in crossbred and local cows respectively.

Chisty *et al.* (2007) screened total 639 animals (cows were 370 and buffaloes were 269) and observed that prevalence of clinical mastitis in cattle was 16.72 % and in buffalo was 21.08% respectively.

Kumar *et al*. (2007) observed that prevalence of subclinical mastitis in buffaloes was 9.90%, latent mastitis was 4.24% and of non-specific mastitis was 15.80% respectively.

Sharma and Sindhu, (2007) examined 5707 samples of buffaloes and found that 2948 samples were positive for mastitis out of which 1070 cases were found to be positive for clinical mastitis and 1878 were positive for subclinical mastitis.

Kavitha *et al*. (2009) examined 1000 quarters of buffaloes and found that 31 quarters were positive for clinical mastitis and 17 quarters were positive for sub clinical mastitis.

Tufani *et al.* (2012) recorded prevalence of mastitis in cows during 2008-2009 as 8.08 percent. Lactation wise incidence of mastitis was 1st lactation 15.87%, 2nd lactation 15.87%, 3rd lactation 20.63%, 4th lactation 26.98%, 5th lactation 12.70 and 6th lactation 7.94 percent. The incidence of mastitis during early lactation was 52.38%, mid lactation was 26.98% and late lactation was 20.63 percent.

Abd-Elrahman, (2013) examined 500 housed buffaloes and informed that among 500 animals there were 9% cases of clinical mastitis and 18.5% cases of subclinical mastitis.

Akkewar (2013) examined 491 cows for clinical mastitis in and around Parbhani district and found that incidence of clinical mastitis was 6.92 percent.

Khanal and Pandit, (2013) studied prevalence of subclinical mastitis in cattle and buffaloes. Animal wise prevalence was 28.6% and quarter wise prevalence was 24.2 percent. Incidence of mastitis on the basis of quarter was highest in fore quarter (34.92%), followed by left hind quarter (31.76%), right hind quarter (28.57%) and right fore quarter (25.39%) respectively.

Ali *et al.* (2014) found that prevalence of subclinical mastitis of buffaloes as 41.8%, clinical mastitis was 13.6% and blind quarter was 9.7% respectively. The quarter wise prevalence was highest in the hind quarter and left side as compared to forequarter and right side.

Jingar *et al.* (2014) observed that the incidence of mastitis was lower in 1st parity than the rest of parity and it increased with parity number. Increase in parity results in increased incidence of mastitis in buffaloes and cows.

El-Naker *et al*. (2015) reported prevalence of mastitis in buffaloes was 19.90% and 5.90% for clinical and sub clinical mastitis respectively. A high incidence of clinical mastitis was observed during early lactation stage *i.e.* 51.60% and high incidence of subclinical mastitis was seen during late lactation stage *i.e.* 12.90 percent.

Prabhu *et al*. (2015) examined 68 buffaloes out of which 20 animals (29.41%) were positive for clinical mastitis and 48 animals (70.58%) for subclinical mastitis.

Abebe *et al.* (2016) observed that cow wise prevalence was 62.6%. For subclinical mastitis it was 59.2% and 3.4 percent for clinical mastitis.

Patbandha *et al.* (2016) examined 1356 milk samples from Jaffarabadi buffaloes and found that incidence of subclinical mastitis was 6.1% and clinical mastitis was 4.6 percent. The season wise incidence of clinical mastitis was highest during rainy season (6.3%) followed by winter season (4.2%) and summer season (3.4%).

Bhat *et al*. (2017) found animal wise incidence of clinical mastitis in bovines as 11.5% and quarter wise incidence as 3.94 percent.

Navaneethan *et al*. (2017) observed mastitis causing highest prevalent bacteria in bovines was *E.coli* found in right hind quarter (14.8%), left hind quarter (10.6%), left fore quarter (9.3%) and right fore quarter (6.6%).

Swami *et al*. (2017) concluded that prevalence of subclinical mastitis was highest in cattles (35.00%) than buffaloes (28.33%).

Salahuddin, (2018) examined 788 cattle in and around Parbhani district and found that prevalence of subclinical mastitis was 25.63% and clinical mastitis was 5.58 percent.

Sharma *et al*. (2018) examined 4452 quarter milk samples and found that 1503 (33.76%) samples were positive for subclinical mastitis and 809 (18.17%) samples were positive for clinical mastitis.

Kashyap *et al.* (2019) observed prevalence rate of subclinical mastitis in buffaloes as 68.33 percent. The prevalence of subclinical mastitis was highest during age group of 9 to 11 years *i.e.* 90.32 percent. Lactation wise prevalence of SCM was highest during mid lactation *i.e*. 76.47% followed by early lactation (67.27%) and late lactation (61.29%) respectively. A study of quarter wise incidence of SCM revealed that left hind quarters (30.83%) were more prone to SCM followed by right fore (19.16%), left fore (10%) and right hind quarter (8.33%).

Shelke *et al.* (2019) examined 512 cows in and around Parbhani district of Maharashtra and found that prevalence of subclinical mastitis was 26.17 percent.

Fukushima *et al.* (2020) observed that incidence of clinical mastitis in cows during 2014-2018 was 41.6 percent.

Mbindyo *et al*. (2020) found that overall prevalence of mastitis in cattle's on the basis of CMT and clinical examination was 80% out of which 6.8% cases were of clinical mastitis and 71.1% were subclinical mastitis.

Shabaz *et al*. (2020) observed prevalence of clinical and subclinical mastitis in dairy cattle from Chittoor District as 11.97% and 34.86% respectively and overall prevalence of mastitis was 46.84 percent.

Grima *et al*. (2021) examined 116 teat quarters and 57 lactating cows and found that 15.52% and 21.05% positive for *Staphylococcus aureus* from clinical mastitis samples.

Imran *et al*. (2021) examined 216 milk samples and found prevalence of mastitis in cattle and buffaloes as 23.14% and 18.50% respectively. Prevalence of mastitis at the age of 9-10 years was 35% and 32% in buffaloes and cattle respectively. Prevalence of subclinical mastitis was 56% and 55% in cattle and buffaloes respectively. Prevalence of acute mastitis in cattle and buffaloes was 4% and 5% respectively.

Khasanah *et al*. (2021) observed prevalence of mastitis in East Java as 68.18% and 66.72% at the cattle and quarter level respectively.

Krishnamoorthy *et al.* (2021) recorded worldwide prevalence of subclinical mastitis from bovines was 42% and clinical mastitis as 15 percent. In India prevalence of subclinical mastitis was 45% and clinical mastitis 18% respectively.

Patil *et al.* (2021) screened 81 lactating buffaloes and found 27 quarters of 18 buffaloes positive for subclinical mastitis *i.e.* animal wise prevalence of SCM was 22.22% and quarter wise prevalence was 11.33 percent.

Tezera and Ali (2021) recorded prevalence of mastitis in cows as 40.3% out of which clinical mastitis was 11.99% and subclinical mastitis 28.34 percent.

Thakur *et al.* (2021) examined 469 milking animals during July 2016 to June 2017 and found prevalence of mastitis as 42.86 percent. Prevalence of mastitis was higher in cows (47.40%) than buffaloes (40.20%). Quarter wise incidence was higher in hind quarters (32.31%) than fore quarters (15.46%).

Tumbare *et al.* (2021) examined 120 goats and found incidence of subclinical mastitis in goats was 65% in and around Parbhani district of Maharashtra.

2.2 Clinical examination

Bleul *et al*. (2006) observed that in 56 mastitis cases in cattle most frequent clinical findings were anorexia or reduced appetite in 50, tachycardia in 40, reduced ruminal motility and intestinal motility in 52 and no ruminal and intestinal motility in 39 cows.

Radostits *et al*. (2007) opined that mastitis includes abnormalities of secretion, consistency and temperature of mammary gland and frequently a systemic reaction. Abnormal gland is larger and firmer than other quarters. Animal shows signs of anorexia, pyrexia, depression or has decreased appetite or milk production. Abnormal milk flakes or clots are usually accompanied by discoloration and they are always significant, indicating a severe degree of inflammation, even when small and present only in the first few streams.

Sharma and Sindhu, (2007) observed inflammation of udder, hot, painful udder with the signs of pyrexia, tachycardia, tachypoenia in clinical mastitis in buffalo.

Khodery and Salama, (2008) observed the clinical signs in clinical mastitis like hotness, swelling, painful udder and serous excretion containing clots and flakes in milk in buffaloes.

Muhammad *et al*. (2011) observed in lactating cows and buffaloes mastitis positive milk showed abnormalities like abnormal colour, odour, consistency and presence of clots, blood, flakes and other visual abnormalities in milk.

Ghodasara *et al.* (2012) observed clinical sings in Jaffarabadi buffalo and cows in clinical mastitis cases as reduced milk yield, change in milk pH, visid mucoid gray white mammary secretion, secretion consisting of a few yellow clots in watery fluids and in some animals these secretion were with slightly blood tinged milk.

Khuswaha and Mohan, (2019) observed in bovine milk discoloration, presence of clots and large number of leukocytes, udder swelling, heat, pain and edema of mammary glands in clinical mastitis positive animals.

2.3. Haematological Examination

Zaki *et al*. (2008) reported that anemia in mastitis buffaloes was due to decrease in Hb, RBC and PCV levels in subclinical mastitis and no significant (P>0.05) changes were observed in Hb and TEC level of clinical mastitis infected animals as compared to healthy animals.

Modi *et al.* (2013) studied moxifloxacin @7.5 mg/kg body weight single time in sheep and studied haematological variation in sheep and found that therapeutic dose does not produce any non-significant difference values in haematological parameters in sheep as compared to control values.

Krishnappa *et al*. (2016) observed higher values of TLC in clinical mastitis positive buffaloes than healthy animals. There were significant changes in values of TEC, Hb and PCV in clinical mastitis as compared to healthy animals.

Garba *et al.* (2019) performed haematology in mastitis and non mastitis affected goats and found that there was significant difference in all haematological parameters except mean corpuscular haemoglobin where significance difference was observed ($p<0.05$).

Rathaur *et al.* (2020) studied haematological parameters of mastitis affected cows and found that significant decrease in ($p<0.01$) in TEC, Hb, PCV, neutrophilia and lymphopaenia.

Ramesh *et al.* (2021) eaxamined 110 bufffaloes for subclinical mastitis and on haematological examination it was found that Hb and TEC values were decreased significantly while PCV and lymphocyte values were non-significantly reduced. TLC and neutrophil values were increased significantly as compared to healthy animals.

2.4 Test on milk

2.4.1 Somatic cell count

Skrzypek *et al.* (2004) stated that SCC of healthy cows udder milk was in the range of 50,000 to 1,00,000 cells/ml. The SCC count of milk more than 2,00,000 cells/ml was assumed threshold distinguishing a healthy udder from infected or inflamed udder.

Juozaitiene *et al*. (2006) found in cows milk that when SCC increases from 1,00,000 to 8,00,000 and above then the milk yield of animals decreases to 14.4%, fat content by 14.7% and milk protein by 9.1 percent.

Moroni *et al*. (2006) studied 1912 samples from 42 buffaloes and observed that somatic cell count was distinctly greater in infected quarters with SCC>200,000 cell/mL had intramammary infection, whereas 98% of quarters with SCC below this threshold were not infected.

Das *et al.* (2018) examined 45 buffaloes from an organized farm and found that SCC value of subclinical mastitis was 7 lakh/ml of milk.

Supriya *et al*. (2010) studied prevalence of subclinical mastitis in cows with the help of somatic cell count and bacterial culture. On the basis of somatic cell count quarter wise incidence of subclinical mastitis was 22.5% and on the basis of bacterial isolation it was 32.4% respectively.

Sharma *et al.* (2011) studied different diagnostic tests for mastitis and found that most accurate test was somatic cell count i.e. 92.00% accuracy for bacteriological culture.

Patil *et al.* (2015) observed that somatic cell count in milk was highest in clinical mastitis or intra mammary infection than normal milk. SCC in buffaloes was higher in subclinical mastitis than normal milk. The SCC of clinical mastitis buffaloes was in the range of 10.21±0.220 (($\times 10^5$ cells/ml)

Aarsharaj *et al*. (2017) studied three different tests for mastitis *i.e.* somatic cell count (SCC), CMT and electrical conductivity. They found that most accurate

test for subclinical mastitis was SCC *i.e*. 58.5% than CMT (54.05%) and electrical conductivity (24.77%).

Sumon *et al*. (2020) concluded that threshold level of somatic cell count in cow was 100 x 10^3 cells/mL which was appropriate to identify intamammary infection of dairy cows.

2.4.2 Strip cup test

Jacobson and Olson (1935) used strip cup test in cows for clinical mastitis positive cases. It was used by stripping first four to five streams from each teat to find cows which show milks containing flakes or string masses and clots.

Pardo *et al.* (2007) used strip cup test to evaluate 734 CMT positive buffalo's samples and found that physical examination and strip cup test revealed absence of clinical alterations in milk and udder.

Blagitz *et al.* (2014) used strip cup test and CMT test for the diagnosis of clinical mastitis in ewes. The study was conducted in commercial flocks and 550 udder halves from ewes to diagnose clinical mastitis. Strip cup test evaluated presence of clots, flakes and any other abnormal secretions.

Hossain *et al.* (2016) examined 432 cases of dairy cows on the basis of clinical signs and strip cup test and found that 65 cases were positive for mastitis.

2.5. Isolation of bacteria

Gianneechini *et al*. (2002) observed on isolation of milk samples from 40 clinical mastitis cows cases and found most prevalent bacteria was *Staphylococcus aureus i.e*. 37.5 % and there was no bacterial growth in 32.5% of total milk samples. The most prevalent pathogen from subclinical mastitis was *Staphylococcus aureus* (62.8%) followed by *Streptococcus agalactiae* (11.3%), *Enterococcus* spp. (8%), coagulase-negative *Staphylococcus* (7.4%), *Streptococcus uberis* (6.4%), *Streptococcus dysgalactiae* (1.8%) and *E.coli* (1.5%).

Khan and Muhammad, (2005) isolated bacteria from mastitis affected buffaloes milk samples and found that most prevalent bacteria was *Staphylococcus aureus* (45%), *Streptococcus agalactiae* (23%), *E. coli* (18%) and *Bacillus* spp. (14%).

Khodery and Salama, (2008) examined 80 milk samples from 56 buffaloes and isolated *Staphylococcus aureus* from 7 samples, *Streptococcus uberis* from 3 samples and *Streptococcus agalactiae* from 1 sample of milk sample from buffalo.

Sumathi *et al.* (2008) performed bacterial isolation from clinical mastitis positive cows and found that most prevalent bacteria was *Staphylococcus aureus* (24%) followed by *E.coli* (20%), *Staphylcoccus epidermidis* and *Streptococcus* spp. (16%) and *Klebsiella* spp. (10%).

Botrel *et al.*(2010) examined 1770 clinical and subclinical samples from milking cows and isolate 1631 bacteria and most prevalent bacteria from mastitis was *Streptococcus uberis* (22.1%), followed by *E.coli* (16%) and coagulase positive *Staphylococcus* (15.8%). From subclinical mastitis samples most prevalent bacteria was coagulase positive *Staphylococci* (30.2%), coagulase negative *Staphylococci* (13.7%) and *Streptococcus dysgalactiae* (9.3%).

Pushpa *et al.* (2010) studied 65 clinical mastitis positive buffaloes and on isolation of bacteria found that most prevalent bacteria was *Staphylococcus aureus* 45 (70.7%), *E.coli* 10 (15.38%) and *Streptococcus agalactiae* 2 (3.07%).

Ali *et al*. (2011) cultured 234 samples for bacteria isolation from subclinical mastitis buffaloes and found that most prevalent bacteria was *Staphylococcus* (28.32%), followed by *E.coli*.(16.18%), *Pseudomonas* (13.29%), *Bacillus* (12.42%), *Streptococcus* (7.51%), *Salmonellae* (7.22%), *Corynebacterium* (6.64%), *Klebsiella* (5.20) and *Enterococci* (3.17%).

Baloch *et al*. (2011) examined 70 clinical mastitis positive milk samples from buffaloes and found most prevalent bacteria was *Staphylococcus aureus* (48.57%), *Bacillus cereus* (2.85%), *Streptococcus dysgalactiae* (2.85%),

Escherichia coli (10.0%), *Micrococcus luteus* (15.71%), *Proteus vulgaris* (4.28%), *Pseudomonas aeruginosa* (1.42%), *Streptococcus dysgalactiae* (11.42%), *Streptococcus uberis* (4.28%) and *Citrobacter* species (1.42).

Steeneveld *et al.* (*2011)* isolated milk samples from 20,000 clinical mastitis cows cases and found *Streptococcus uberis* and *Streptococcus dysgalactiae* in 40% cases, *Staphylococcus aureus* and *E.coli* in 30% cases.

Hegde *et al*. (2013) isolated 323 bacteria from milk samples from organized and unorganized farms and found *Sthaphylococcus aureus* and coagulase negative *Staphylococcus* 95 each, *E.coli* 48, *Streptococcus* in 85 isolates respectively.

Anwar *et al.* (2013) observed on isolation of milk samples that was *Staphylococcus aureus* bactera from Nili ravi breed of buffalo (40.42%) and Kundi breed (34.88%).

Jeykumar *et al.* (2013) examined 72 samples from cross breed cows and observed that most prevalent isolated bacteria was *Staphylococcus* spp. (44.44%) followed by *Streptococcus* spp. (5.5%), *E.coli* (41.66%) and *Klebsiella* spp. (8.33%).

Oliveira *et al.* (2013) studied 741 cases of cows and isolates found most prevalent bacteria was *Escherichia coli* (22.5%), environmental *Streptococcus* (12.8%), *Klebsiella* spp.(6.9%) and coagulase negative *Staphylococcus* (6.1%).

Pankaj *et al.* (2013) cultured 38 mastitis positive samples from murrah buffaloes and found 44 bacteria. Out of these coagulase positive *Staphylococcus* spp were 15.90%, coagulase negative *Staphylococcus* were 47.7%, *Streptococcus dysgalactiae* were 25%, *Streptococcus agalactiae* were 9.09%, *Streptococcus uberis* were 2.27% and there was also mixed infection of *Staphylococcus* spp. and *Streptococcus* spp. were 13.69 percent.

Chandrasekaran *et al.* (2014) isolated 401 clinical mastitis samples from dairy cows and found in *E.coli* 184(45.89%), *Staphylococcus aureus* in 162

(40.4%), *Bacillus* spp in 14 (3.49%), *Streptococcus* species in 13 (3.24%) and mixed infection in 16 (3.99%) samples respectively.

Charaya *et al*. (2014) examined 564 quarters milk samples of 144 clinical mastitis Murrah buffaloes. Out of which 320 (56.73%) quarters were found culturally positive showing isolation of *Staphylococcus aureus* in 140 (38.04%), *Streptococcus dysgalactiae* in 112 (30.43%), *Streptococcus agalactiae* in 13 (3.53%), *Escherichia coli* in 74 (20.10%) and *Corynebacterium pyogenes* in 29 (7.88%).

Jamali *et al.* (2014) examined 207 samples of bovine clinical mastitis and found that most prevalent bacteria were *Staphylococcus aureus i.e.* from 43 samples (20.1%).

Singh *et al*. (2016) isolated bacteria from 253 milk samples of Murrah buffaloes and found that predominant pathogen were *Staphylococcus* spp. *i.e.* 57.5%, *Streptococcus* 40% and *E.coli* 2.5 percent.

Verbeke *et al.* (2014) cultured 677 clinical mastitis positive cows samples and most frequently isolated organism was *Streptococccus uberis* (18.2%), *E.coli* (15.5%), *Staphylococcus aureus* (7.3%) and *Streptococcus dysgalactiae* (7.2%) respectively.

Castro *et al.* (2015) isolated clinical mastitis sample from cows and found that most prevalent pathogens were *Streptococcus dysgalactiae* (8.8%), *Streptococcus uberis* (8.3%) and *Staphylococcus aureus* (3.3%).

Abebe *et al.* (2016) found that most prevalent bacteria on isolation were *Sthapylococcus aureus i.e.*51.2% and 73.2% from subclinical and clinical mastitis samples of dairy herds respectively.

Bhat *et al*. (2017) isolates 23 milk samples from clinical mastitis bovines and observed that most frequent organism was *Staphylococcus aureus* (60.87%) followed by *Escherichia coli* (13.04%), coagulase negative *Staphylococci* (13.04%), *Streptococcus dysgalactiae* (8.69%) and *Streptococcus uberis* (4.35%).

Gao *et al*. (2017) examined 3288 samples from bovine herds and found that most common pathogens were *Escherichia coli* (14.4%), *Klebsiella* spp. (13.0%), coagulase negative *Staphylococcus aureus* (11.3%), *Streptococcus dysgalactiae* (10.5%) and *Staphylococcus aureus* (10.2%).

Vakkamaki *et al*. (2017) isolated mastitis positive samples from cows and found that most pathogenic organism was coagulase-negative *Staphylococci* (43%) followed by *Streptococcus uberis* (9%), *Streptococcus dysgalactiae* (8%), *Corynbacterium bovis* (7%), *Staphylococcus aureus* (21%) and *E.coli* (5%).

Bhutia *et al.* (2019) isolated bacteria from clinical mastitis positive buffaloes from period of 2007 to 2016. From the 3945 isolates the bacteria found were *Staphylococcus* spp. (28.7%), *Streptococcus* spp. (1%), *Corynbacterium* spp (1.1%) and Gram negative organisms (3.2%), other including mixed growth (3.3%) and no growth in (62.7%) respectively.

Cheng *et al.* (2019) isolated 541 bovine clinical mastitis samples and found the bacteria like the *Sthaphylococcus* spp. from103, *Klebsiella* spp. from 130, *Streptococcus* spp. from 101, *E.coli* from 100 and non aureus *Staphylococcus* from 107 samples respectively.

Manasa *et al*. (2019) examined 61 clinical mastitis positive samples of cows and on isolation of bacteria found that *Staphylococcus* spp. were 89% and *Staphylococcus aureus* were 54 percent. On isolation of 105 sub clinical mastitis positive samples found that *Staphylococcus* spp. were 71% and *Staphylococcus aureus* were 50 percent.

Morwal *et al*. (2019) on isolation of 24 clinical mastitis affected milk samples from cows and found that *Staphylococcus* spp. (44.44%), *Streptococcus* spp. (16.66%), *E. coli* (22.22%), *Bacillus* spp. (11.11%) and *Corynbacterium* spp.(5.50%).

Patel *et al*. (2019) isolated 28 Jaffarabadi buffaloes milk samples positive for clinical mastitis and found *Staphylococcus* spp. (25.95%), *Enterococcus* (10.80%), *E.coli* (8.88%) and *Streptococcus* (3.97%). Isolated bacteria from

subclinical mastitis was *Staphylococcus* spp. (10.09%), *Enterococcus* (8.72%), *E.coli* (0.38%) and *Streptococcus* (0.42%).

Chhabra *et al.* (2020) studied 71 culturally positive sub-clinical mastitis samples of buffaloes and isolated 94 organisms. Out of which most prevalent microorganism was *Staphylococcus* spp. *i.e*. (63.54%) followed by *Streptococcus* spp. (36.46%) and mixed infection of *Staphylococcus* and *Streptococcus* was (13.54%) respectively.

Mbindyo *et al.* (2020) cultured 1574 samples of dairy cattles for sub-clinical mastitis and found 1016 bacteria from the isolates. Most prevalent bacteria was coagulase-negative *Staphylococcus* (42.8%), *Streptococcus* (22.2%), *Staphylococcus aureus* (15.7%), *Psedomonas aeruginosa* (5.1%) and *Enterobacter* spp. (0.7%).

Waseem *et al.* (2020) examined 200 bovine clinical mastitis positive samples and on isolation found that *Staphylococcus* spp. have highest prevalence *i.e.* 46.4%, *Staphylococcus* and *Streptococcus* mixed infection was 20.8%, *Streptococcus* spp 18.4% and *E.coli* 14.4 percent.

Arya *et al*. (2021) examined 363 quarters for subclinical mastitis in buffaloes and found that 57 quarters milk samples positive for bacteriological isolation and major pathogenic bacteria were coagulase negative *Staphylococcus* 28 (49%) cases *Staphylococcus aureus* in 16 (28%) samples, *Streptococcus* spp. 9 (16%) samples and *Corynbacterium* spp. 4 (7%) samples.

Patil *et al.* (2021) isolated 27 positive milk samples of buffaloes positive for sub-clinical mastitis and found that most prevalent bacteria was *Staphylococcus* spp. (33.33%) and *E.coli* (14.81).

Rudenko *et al.* (2021) isolated 309 bacteria from milk samples of mastitis positive cows and found that *Staphylococcus aureus* in 18.80% samples, *E.coli* in 11.90% samples and *Staphylococcus uberis* in 11.70% samples.

Singha *et al.* (2021) isolated bacteria from clinical mastitis cases of cows and found *Streptococcus* (22.9%) and non aureus *Staphylococci* (20.3%).

2.7. Antibiotic sensitivity test

Joshi and Gokhale, (2006) found that most of the bacterial strains isolated from milk samples of cattle and buffaloes were sensitive to enrofloxacin (53.91%), oxytetracycline (17.39%), ampicillin (7.83%) and resistance to streptomycin.

Awandkar *et al.* (2009) studied antibiogram of clinical mastitis in bovine in and around Udgir district of Maharashtra and found that most sensitive antibiotic was ciprofloxacin followed by gentamicin, enrofloxacin, cloramphenicol and cefotaxim. Least effective antibiotic was cloxacillin followed by streptomycin, oxytetracycline, amoxicillin, doxycilin, ampicillin and ceftriaxone.

Hussain *et al*. (2012) found that *Staphylococcus aureus* isolated from mastitis positive buffaloes and cows, sensitive to oxytetracycline (95.65%), enrofloxacin (69.56%), amoxicillin (86.95%) and cloramphenicol(82.60%).

Chandrasekaran *et al*. (2014) found that *E. coli* from milk samples of acute mastitis positive cows was sensitive to enrofloxacin (79%) amoxicillin and sulbactam (74%) and gentamicin (73.1%). It was resistance to penicillin (63.0%), amoxicillin (52.1%) and oxytetracyclin (47.9%).

Singh *et al*. (2014) found that *Staphylococcus* spp. isolated from milk samples of cows was 100% sensitive to moxifloxacin, enrofloxacin, chloramphenicol, cefoperazone, amikacin, gentamycin and neomycin.

Das *et al.* (2018) isolate *Staphylococcus aureus* and performed antibiogram of isolates from bovine mastitis and found highest sensitivity to enrofloxacin (64%) followed by gentamcin (58%), cloxacilln (56%) and pefloxacin (54%).

Thomas *et al.* (2015) observed that *E.coli* from clinical mastitis positive cow milk was resistant to tetracycline (14.3%) and cefapirin (11.1%). *Staphylococcus aureus* resistance to penicillin G.

Singh *et al.* (2016) performed antibiotic sensitivity test of bovine mastitis milk and found that highest drug resistance against cefotaxime, ampicillin-sulbactam, cefixime and ceftriaxone. Majority of culture shows susceptibility to amikacin, chloramphenicol and gentamycin.

Yadav (2018) studied antibiogram on isolated *Staphylococcus aureus* from subclinical mastitis of cows samples from different farms of Maharashtra and found that highest resistance to the cephalexin (86.84%), followed by cotrimoxazole (82.89%), penicillin G (82.23%), ampicillin (81.57%), cefoperazone (76.94%), ceftriaxone (69.73%), cefotaxime (65.13%), amoxyclav (63.15%), tetracycline (54.60%), chloramphenicol (46.71%), ciprofloxacin (42.10%), gentamicin (38.81%), enrofloxacin (19.73%) and levofloxacin (19.73%) respectively.

Cheng *et al*. (2019) observed from clinical mastitis cows and found that *Staphylococcus aureus* most resistance to oxacillin (84%), penicillin (62%), tetracycline (34%) and clindamycin (33%). Streptococcus is highly resistance to tetracycline (59%). Prevalence of resistance to *Klebsiella* and *E.coli* was high to amoxicillin and clavulanate potassium (81%) and (38%) respectively. *Klebsiella* spp was resistance to tetracycline 32 percent.

Chhabra *et al.* (2020) observed on antibiogram study of subclinical mastitis positive buffaloes and found that the highest percent sensitivity of the *Staphylococcus* species isolates was 73.44% for moxifloxacin and 75% for enrofloxacin respectively.

Patil *et al.* (2021) compared the antibiogram of the bacterial isolates from subclinical mastitis of buffaloes to standard antibiotic discs determined by disc diffusion method and revealed highest sensitivity to gentamicin (77.77%) followed by enrofloxacin (70.37%) followed by ceftriaxone (55.55), moxifloxacin (51.85%), cefoperazone (40.74%) and tetracycline (33.33%).

Qolbaini *et al.* (2021) performed AST of 86 milk samples from subclinical mastitis of dairy cows and found that *Staphylococcus aureus* was susceptible to tetracycline, chlorphenicol, gentamycin, erythromycin and trimethoprim/sulfamethoxazole.

2.8 Treatment

2.8.1. Moxifloxacin

Malathum *et al.* (1999) studied *invitro* activity of moxifloxacin and found that moxifloxacin has greater potency than ciprofloxacin against *Staphylococcus aureus, Streptococcus* and *Enterococci.*

Entenza *et al*. (2001) found that moxifloxacin has excellent *in-vitro* and in vivo activity against both ciprofloxacin susceptible and first level resistant *Staphylococcus aureus* isolates.

Ince *et al.* (2003) studied activity and resistance of moxifloxacin in *Staphylococcus aureus* and found that moxifloxacin has enhanced potency against Gram positive *Staphylococcus aureus.*

Meunier *et al*. (2004) studied from 1994 to 2001 to determine the susceptibility of bovine pathogenic bacteria to marbofloxacin and found that *E.coli* isolated from gut was 82.7% in 2001 was susceptible to marbofloxacin. *E. coli* isolated from mammary diseases like mastitis was 98.96% suceptible to marbofloxacin in 2001.

Miravitlles, (2008) observed that moxifloxacin is effective against respiratory pathogens like Gram positive (*Streptococcus* spp.) and Gram negative (*Haemophilus influenza* and *Moraxella catarrhalis*) bacteria.

Carceles *et al.* (2007) studied moxifloxacin @ 5 mg/kg body weight in lactating goats and found that moxifloxacin have good penetration activity in milk after intramuscular administration.

Fernadenz *et al.* (2006) studied in goats effect of moxifloxacin and concluded that moxifloxacin have favourable pharmacokinetic effect in goats with good penetration to milk.

Sadariya *et al.* (2010) studied moxifloxacin @ 5 mg/kg body weight in Wister rat repeated intramuscular route after 24 hours interval for 14 days and found that all the haematological and biochemical values of rat were fluctuate within normal range during treatment period and mean values were not significantly differ from control values.

Kumar *et al.* (2013) studied effect of moxifloxacin in broilers and found that moxifloxacin have highest sensitivity against *Salmonella* isolates from broilers was 86.6% and against *E.coli* was 80 percent.

2.8.2. Marbofloxacin

Schneider *et al.* (2004) studied that after repeated intramuscular administration of marbofloxacin injection @ 2 mg/kg in cows have good efficacy against *E.coli* induced mastitis.

Kroemer *et al.* (2012) stated that marbofloxacin is used as treatment for acute *E.coli* mastitis in cows. Respiratory pathogens like *Pasteurella multocida* and *Mannheimia haemolytica* isolates were highly susceptible to marbofloxacin.

Grandemange, (2002) concluded in his study on use of marbofloxacin in bovine respiratory diseases that Inj. marbofloxacin is safe and effective against bovine respiratory disease.

Pillet *et al.* (2013) studied 100 acute clinical mastitis cows and given treatment of marbofloxacin @ 10 mg/kg single intravenous route once only and stated that it was effective and safe, effective against in acute mastitis cases of cows.

Garch *et al.* (2017) studied marbofloxacin suceptibilty from Europe causing respiratory disease in pigs and on isolation and study found that marbofloxacin is highly susceptible for *Actinobacillus pleuropneumoniae* or

Bordetella bronchiseptica. Marbofloxacin was found 0.1% resistance to *Pasturella multocida* and 1.4% resistance to *Haemophilus parasuis.*

Paulin *et al*. (2018) studied single dose of Inj. Marbofloxacin @ 10 mg/kg in respiratory condition of cattle *i.e*. *Pasteurella multocida, Mannheimia haemolytica* and *Histophilus somni.*

Mahapatra *et al.* (2018) stated that in acute bovine clinical mastitis use of marbofloxacin @ 8mg/kg intramuscular and supportive cefoperazone intramammary infusion along with Vitamin E and selenium is effective.

Fernandez *et al.* (2021) studied marbofloxacin @ 8.47 to 11.57 mg/kg body weight every 24 hours interval in contagious agalactia in goats and found that marbofloxacin is effective against contagious agalactia caused by *Mycoplasma agalactiae.*

Patil *et al.* (2021) observed that use of single dose of marbofloxacin in subclinical masitits cases of buffaloes and has efficacy of 58.33 percent. The subclinical mastitis positive buffaloes treated with marbofloxacin, Vitamin E, selenium and trisodium citrate have the highest efficacy *i.e.* 80% more than marbofloxacin alone.

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

3.1 Selection of animal

The lactating buffaloes for study will be screened with the help of clinical parameters and strip cup test. The work will be carried out at Department of Veterinary Clinical Medicine, Ethics & Jurisprudence, Veterinary Clinical Complex, COVAS Parbhani, veterinary polyclinics, veterinary dispensaries and nearby organized farms.

3.2 Incidence

A total of 129 buffaloes were screened with strip cup test for incidence study of clinical mastitis. The incidence of mastitis was correlated with age, lactation, quarter and breed. The mastitis positive samples of buffaloes were collected as per the treatment schedule and were further subjected to various milk tests for the diagnosis of clinical mastitis.

The incidence was expressed in percent positive by using the following formula.

$$\text{Incidence (\%)} = \frac{\text{No of animals positive}}{\text{No of animals tested}} \times 100$$

3.3 Clinical parameters

All individual clinical mastitis positive animals were studied for anamnesis and general mammary gland examination. The clinical parameters such as rectal temperature (^{0}F), heart rate (beats/min), respiration rate (/min) were taken on day 0 (before treatment) and 7th day (after treatment).

3.4 Udder parameters

All the clinical mastitis positive animals were examined for udder health. The parameters such as change in milk colour, consistency of milk, swelling of

udder, hotness of udder were studied on day 0 (before treatment) and on 7th day (after treatment).

3.5 Haematological parameters

Haematological evaluation was carried out by using automatic haemoanalyzer as per the method described by Jain, (1986). Following parameters were studied on day 0 (before treatment) and on 7th day (after treatment).

1. Haemoglobin (g/dl)
2. PCV (%)
3. Total erythrocyte count (TEC) ($x10^6$/ul)
4. Total leucocyte count (TLC) ($x10^3$/ul)
5. Differential leucocyte count (DLC) (%)

3.6 Test on milk

3.6.1 Somatic Cell Count (SCC) (per ml of milk)

Somatic cell count (SCC) is a useful predictor of intramammary infection that includes leucocytes such as neutrophils, macrophages, lymphocytes and erythrocytes totaling to approximately 75% and epithelial cells (25%). So, the measurement of somatic cells in milk is known as somatic cell count. It is widely considered as an indicator of udder health. Leucocyte increase in response to bacterial infection, tissue injury and stress. Somatic cells are indicators of both resistance and susceptibility of goats to mastitis and can be used to monitor the level of occurrence of subclinical mastitis in herds or individual goats. The SCC was determined as described by Schalm *et al.* (1971).

a. Preparation of milk smears

The test milk sample was thoroughly mixed by gentle shaking of the vials. The milk smears were prepared from the test samples on the pre-drawn one square cm ($1cm^2$) marked area over a clean, grease-free glass slide immediately after the collection of milk. Exactly 10 µl (0.01ml) of milk was taken with a micropipette and spread over the area of 1 cm^2 on a microscopic glass slide which was

uniformly smeared with a standard sterilized bacteriological platinum loop. The smear was allowed to air dry at room temperature while protecting from dust.

b. Staining of milk smear

These dry milk film slides were stained by Newman-Lampart single dip stain. Slides were placed in a covered Coplin jar containing the stain for 1-2 minutes. Excess of stain was drained off. The slides were gently washed in tap water and dried rapidly in the air. The dried stained smears were examined under the oil immersion lens of the light microscope.

c. Counting of cells

The counting of cells in 30 random fields was done under oil immersion objective lens (100x). The number of cells/ml of milk was estimated by multiplying the total number of cells in 30 fields with a working factor of the microscope (WF=21786.50) used.

3.6.2 Strip cup test

Strip cup test was performed as per the Constable *et al*. (2017).

Procedure

The milk was drawn on shiny, black plate that permitted the detection of discolouration of milk and clots, flakes and purulent material present in the milk. Comparison made between the milk of different quarters.

3.7 Isolation and identification of bacteria

The milk sample of treatment group animals was collected in sterile tubes and transported to the laboratory for further processing and microbiological analysis using standard protocols. The positive milk sample for clinical mastitis according to somatic cell count was cultured before treatment. The bacteria isolated from the milk sample were identified based on macro and microscopical morphology by Gram's stain and cultural characteristics and biochemical profiles (Krieg and holt 1984; Quinn *et al* 1994).

3.8 Antibiotic sensitivity test

The antibiotic sensitivity test was carried out on isolated bacteria from the milk sample of the buffalo as per (Bauer *et al.*1966).

Procedure

The plates were prepared using Muller Hinton Agar (M173) for use in the Baur- Kirby method for rapidly growing aerobic organisms.

Preparation of 30 inoculums- On pure culture was used and confirmed by the Gram staining before the susceptibility test.

Transferred 4-5 similar colonies with a wire loop to 5 ml Tryptone Soya Broth (Mo11) and incubate at 35^0C-37^0C for 2-8 hours until light to moderate turbidity develops. Compared the turbidity of the inoculum with that of standard 0.5 McFarland. Dip a sterile non-toxic cotton swab into standardized inoculum. Streaked the entire agar plate surface of the plate with the swab 3 times turning the plate at 600 angles between each streaking. Allowed the inoculum to dry for 5 to 15 minutes with lid in place. The disc was then applied using the aseptic technique. The discs deposited with centers at least 24 mm apart 350-370. Incubated immediately and examined after 16-18 hours or longer. Measured the zones showing complete inhibition.

Antibiotic discs

Commercially available antibiotic discs (Hi-Media laboratories Limited) were used to test sensitivity of the bacteria isolated against the antibiotics. Antibiotic discs used in present study were depicted in Table 3.1.

Table 3.1: Details of antibiotic disc used

Sr. no.	Antibiotic disc	Symbol	Dose
1	Moxifloxacin	MO	5 mcg
2	Marbofloxacin	Mar	5 mcg
3	Enrofloxacin	Ex	10 mcg
4	Ciprofloxacin	CIP	5 mcg

3.9 Treatment

Total 20 buffaloes suffering from clinical mastitis were divided randomly into 2 groups, comprising of ten animals each and treated as below. Table 3.2, Plate 3.1 and Plate 3.2.

Table 3.2: Schedule of treatment for different groups

Group	Treatment	Dose	Route	Duration
I	Inj Moxifloxacin	5 mg/kg	Intramuscular	5 days
II	Inj Marbofloxacin	2 mg/kg	Intramuscular	5 days

Supportive treatment- NSAID Tolfenamic acid given @ 2 mg/kg, Antihistaminics (Chlorpheniramine maleate @ 0.5 mg/kg), Multivitamins.

3.10 Statistical analysis

The statistical analysis was done with the help of two sample independent t test.

Plate 3.1 Therapeutic drug Inj. Moxifloxacin

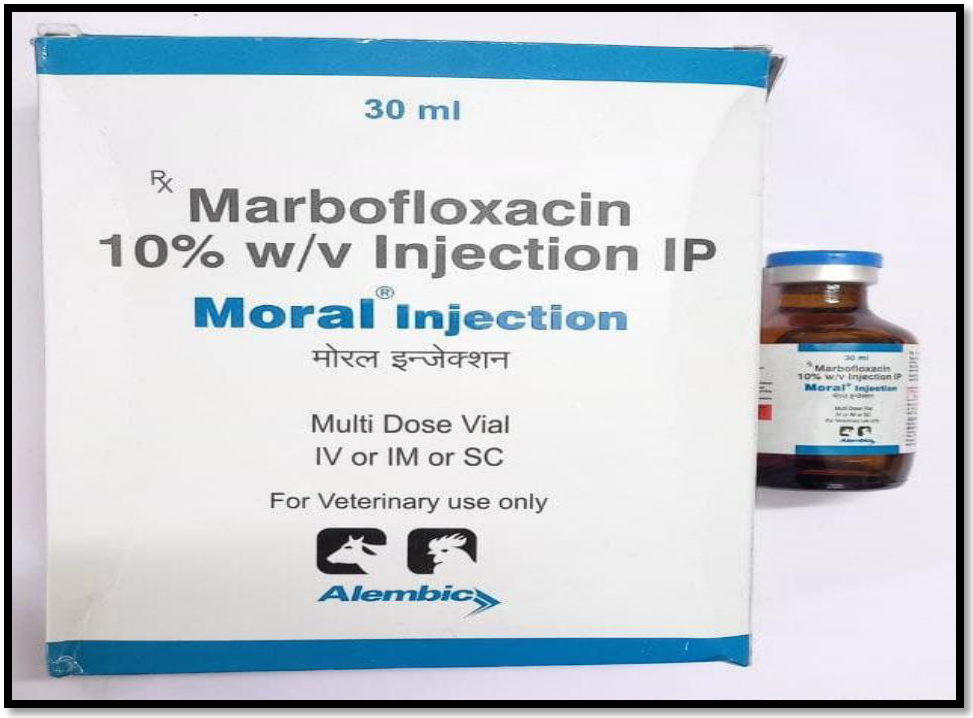

Plate 3.2 Therapeutic drug Inj. Marbofloxacin

Results and Discussion

CHAPTER IV

RESULTS AND DISCUSSION

The present study entitled “Efficacy of moxifloxacin and marbofloxacin against mastitis in buffalo” was carried out in and around Parbhani district to record the incidence of clinical mastitis in buffaloes and to evaluate efficacy of moxifloxacin and marbofloxacin against clinical mastitis in buffaloes. Total 129 buffaloes were evaluated for clinical mastitis using the strip cup test and somatic cell count throughout the current investigation, which carried out from May to December 2021.

Clinical mastitis milk and blood samples were taken from buffaloes from Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Parbhani and organized farms near Parbhani. A total of 20 buffaloes with clinical mastitis were thoroughly examined for incidence, clinical parameters, haematological changes, bacterial isolation and antibiotic sensitivity test and treatment given with moxifloxacin and marbofloxacin.

In present study, 20 clinical mastitis positive buffaloes were divided in two groups. Group I was treated with Inj. moxifloxacin @ 5 mg/kg body weight intramuscular for 5 days while group II was treated with Inj. marbofloxacin @ 2 mg/kg body weight intramuscular for 5 days. Both the groups were given same supportive treatment *i.e.* NSAIDs (Inj. Tolfenamic acid @ 2 mg/kg), Antihistaminics (Chlorpheniramine maleate @ 0.5 mg/kg) and Multivitamins. The efficacy of each group was evaluated on the basis of clinical signs, result of strip cup test and decrease in somatic cell count.

4.1 Incidence

For incidence lactating buffaloes were examined in and around Parbhani district with the help of strip cup test and somatic cell count.

The screening for clinical mastitis of buffaloes was carried out during May - December 2021 at different veterinary hospitals, veterinary polyclinics, veterinary clinical complex, college of veterinary and animal sciences, Parbhani

and different organized private farms. The lactating buffaloes with history of reduced milk production, swelling of teat, flakes or clots in milk were further subjected to strip cup test and somatic cell count study for confirmation of mastitis. In the present research work the overall incidence of clinical mastitis in buffaloes shown in Table 4.1 and Fig. 4.1.

Table 4.1 Incidence of clinical mastitis in buffaloes in and around Parbhani district

Sr. No	Place	No. of animals examined	No of animals positive for clinical mastitis	Percent Incidence
1	**VCC COVAS, Parbhani**	44	12	27.27%
2	**Private Buffalo Farm**	43	7	16.28%
3	**Veterinary dispensary**	42	5	11.90%
Total		**129**	**24**	**18.60**

In the current study, 24 buffaloes were found positive for clinical mastitis out of 129 examined buffaloes. In and around Parbhani district, the total incidence of clinical mastitis was 18.60 percent. The current findings were in similar with Chishty *et al*. (2007) and El-Naker *et al*. (2015) observed incidence 19.9 percent.

In contrary Tezera and Ali, (2021) and Mbindyo *et al.* (2020) reported overall incidence of clinical mastitis as 11.99% and 6.8% respectively, lower than present result. Fukushima *et al*. (2020) and Prabhu *et al.*(2015) reported overall incidence of clinical mastitis was 21.91% and 29.41% respectively higher than present result.

The difference observed in incidence might be because of variation in age, breed, lactation number, milk production, season, environment and managemental practices in the farms.

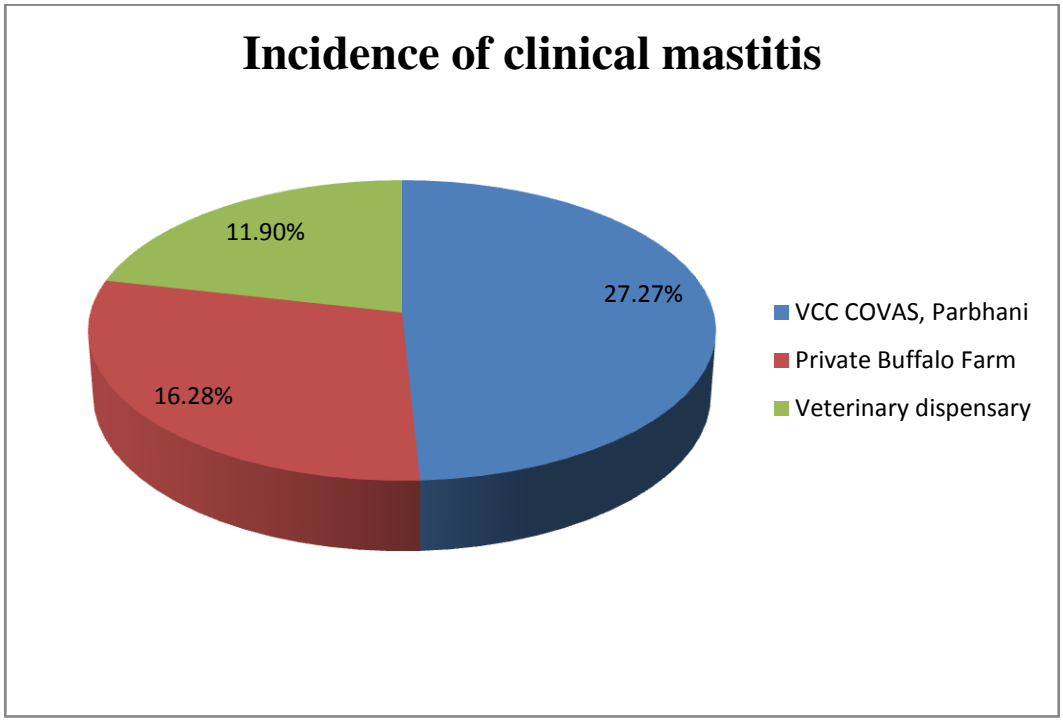

Fig. 4.1 Incidence of clinical mastitis in buffaloes in and around Parbhani district

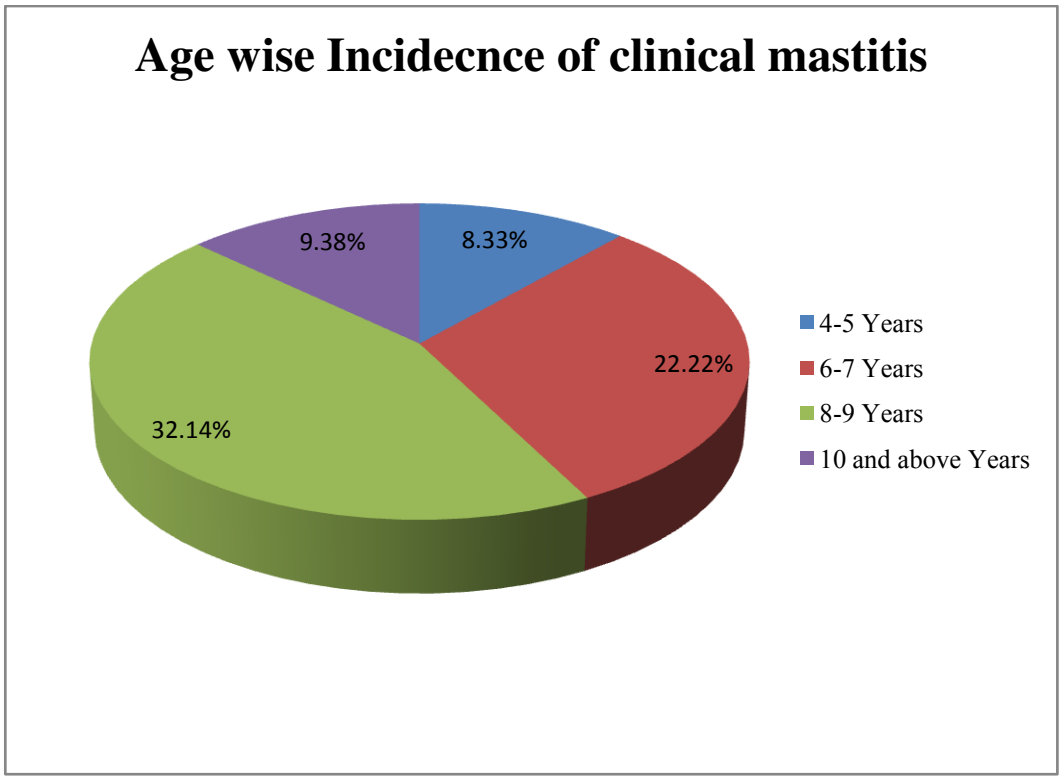

Fig. 4.2 Age wise incidence of clinical mastitis in buffaloes

4.1.1 Age wise

The age wise overall incidence of clinical mastitis is shown in Table 4.2 and Fig.4.2

Table 4.2 Age wise incidence of clinical mastitis in buffaloes

Sr. No	Age (Years)	No of animals screened	No. of animals positive for clinical mastitis	Percent Incidence
1	**4-5**	24	2	8.33%
2	**6-7**	45	10	22.22%
3	**8-9**	28	9	32.14%
4	**10 and above**	32	3	9.38%
Total		**129**	**24**	**18.60%**

The age wise incidence of clinical mastitis positive, 24 lactating buffaloes were in the age of 4-5 years, 2 animals were positive for clinical mastitis *i.e.* incidence was 8.33 percent. The age wise incidence in 6-7 years age group was 22.22% *i.e*. from 45 animals 10 were positive for clinical mastitis. The incidence in 8-9 years group was 32.14% *i.e*. from 28 animals 9 were positive for clinical mastitis. The animals having age of 10 years and above has shown incidence of 9.38% *i.e.* from 32 animals 3 been positive for clinical mastitis. The present values of incidence were agreement with Kumar *et al.* (2012).

4.1.2. Lactation wise

The lactation wise incidence of clinical mastitis in buffaloes is shown in Table 4.3 and Fig. 4.3

The lactation wise incidence of clinical mastitis in buffaloes was observed at different lactation number. The highest incidence was recorded at third lactation which was 40% followed by fourth lactation (20%), fifth lactation (13.33%), second lactation (12.5%) and first lactation (10%) respectively.

Table 4.3 Lactation wise incidence of clinical mastitis in buffaloes

Sr. No.	Lactation no.	Number of buffaloes screened	Number of buffaloes positive	Percent Incidence
1	**First**	20	2	10%
2	**Second**	24	3	12.5%
3	**Third**	20	8	40%
4	**Fourth**	35	7	20%
5	**Fifth**	30	4	13.33%
	Total	**129**	**24**	**18.60%**

Chances of clinical mastitis increase with increase in parity number. It might be due to the reason that from 1st parity onward milk production increases and it is highest in 4th parity. As the milk production increases udder immunity decreases as subsequently there is increase in diameter of teat canal and loosening of its sphincter, the environmental pathogens enter easily and proliferate due to decreased udder immunity (Tufani *et al*. 2012).

4.1.3 Quarter wise

The quarter wise incidence of clinical mastitis in buffaloes is depicted in Table 4.4 and Fig. 4.4.

The incidence of mastitis in right fore quarter was 4.6 % (Plate 4.2), in right hind quarter was 6.2 % (Plate 4.4), left hind quarter was 6.9 % (Plate 4.1) and left fore quarter was 3.1 % (Plate 4.3). This result was in agreement with the Navaneethan *et al*. (2017).

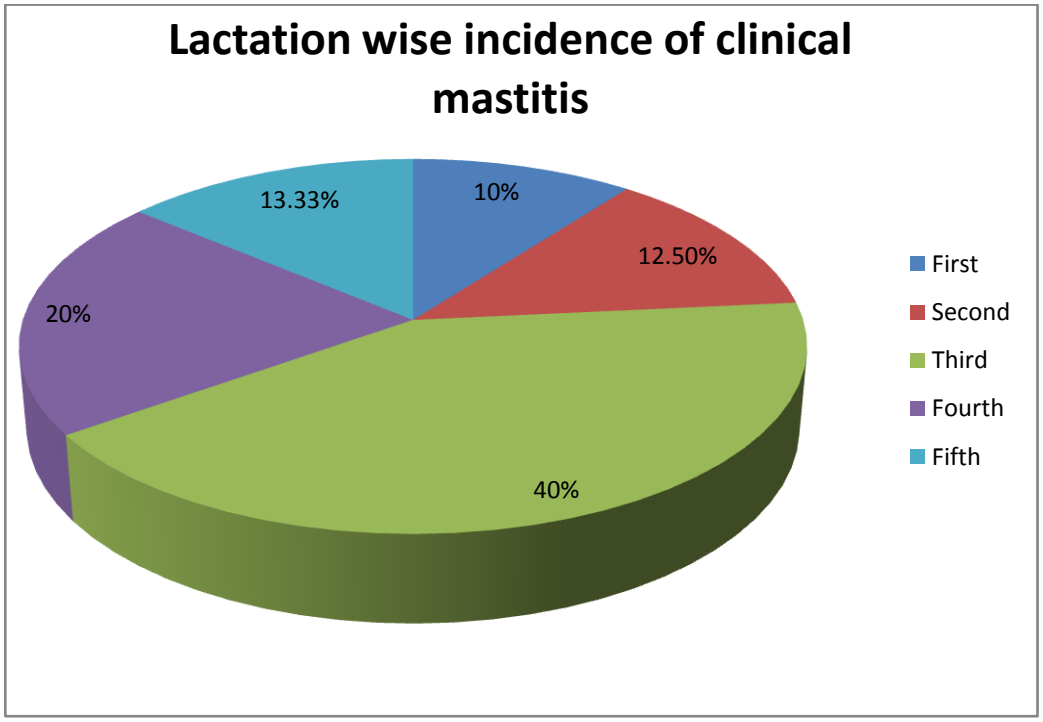

Fig. 4.3 Lactation wise incidence of clinical mastitis in buffaloes

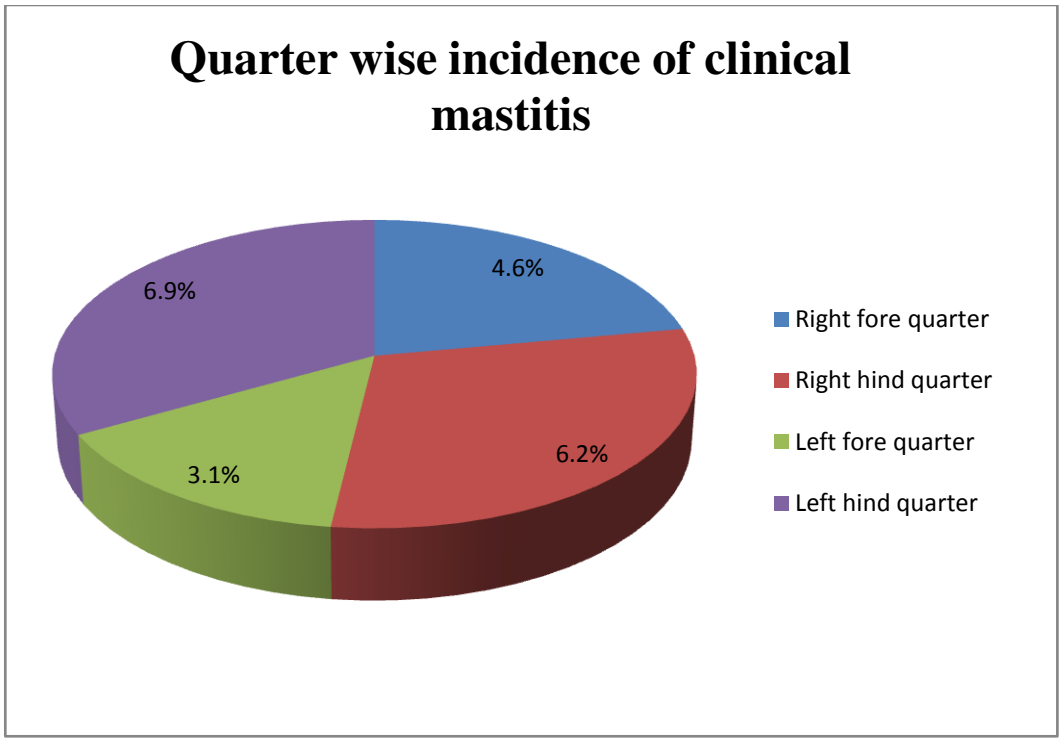

Fig. 4.4 Quarter wise incidence of clinical mastitis in buffaloes

Plate 4.1 Left Hind Quarter affected

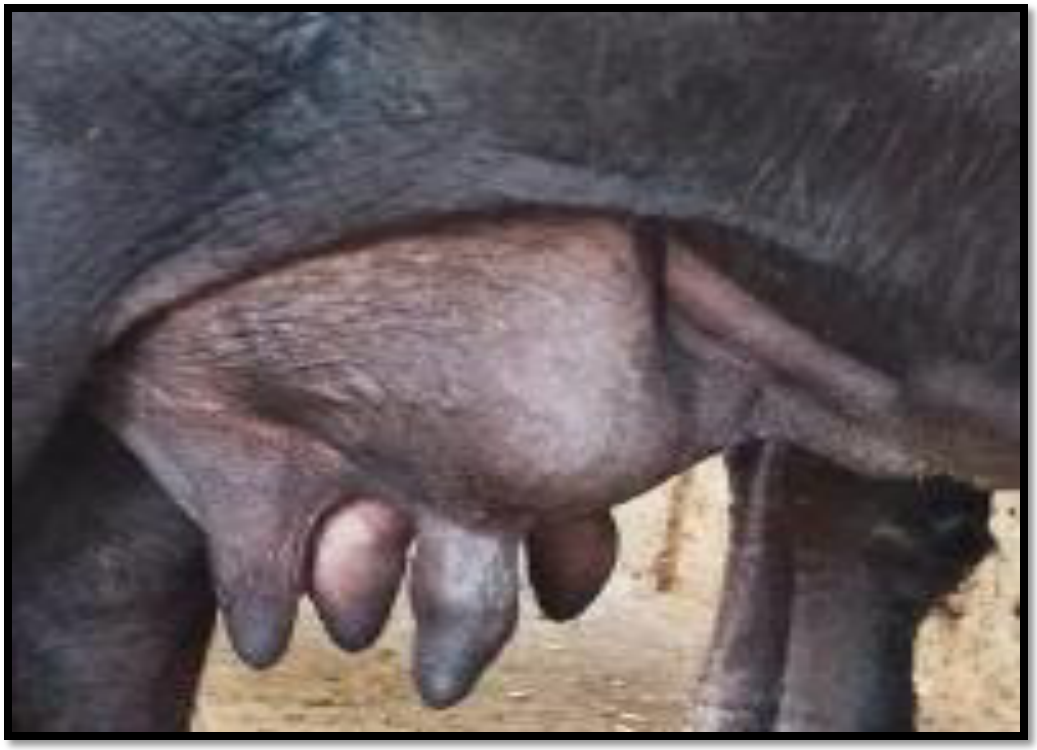

Plate 4.2 Right Fore quarter affected

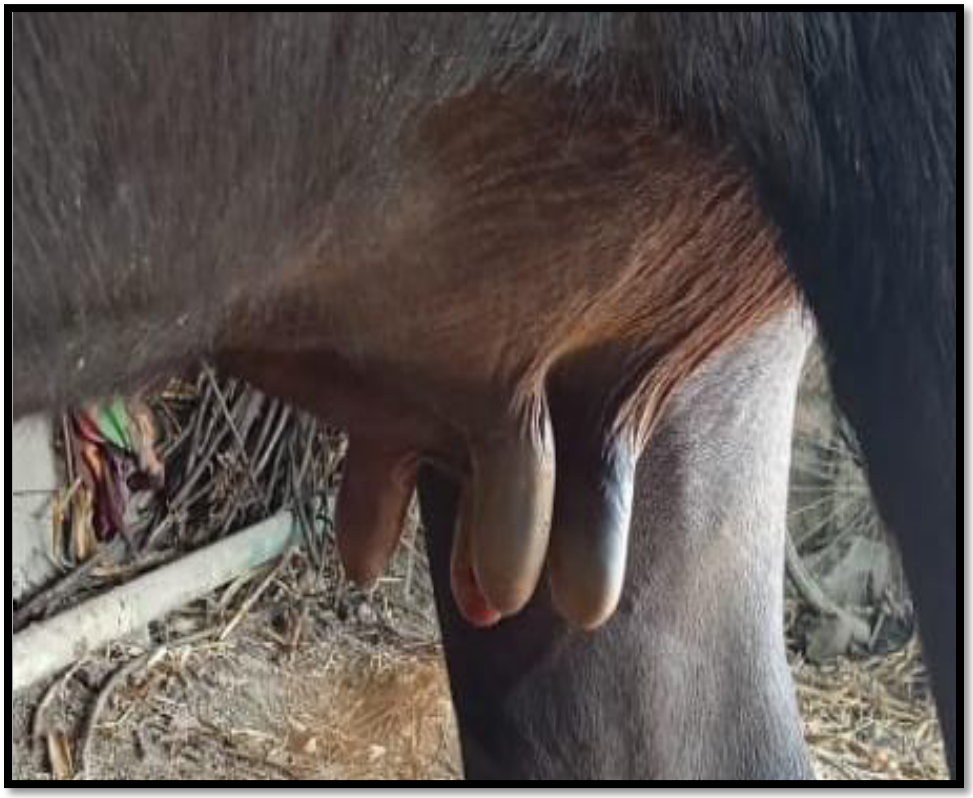

Plate 4.3 Left fore quarter and Hind quarter affected

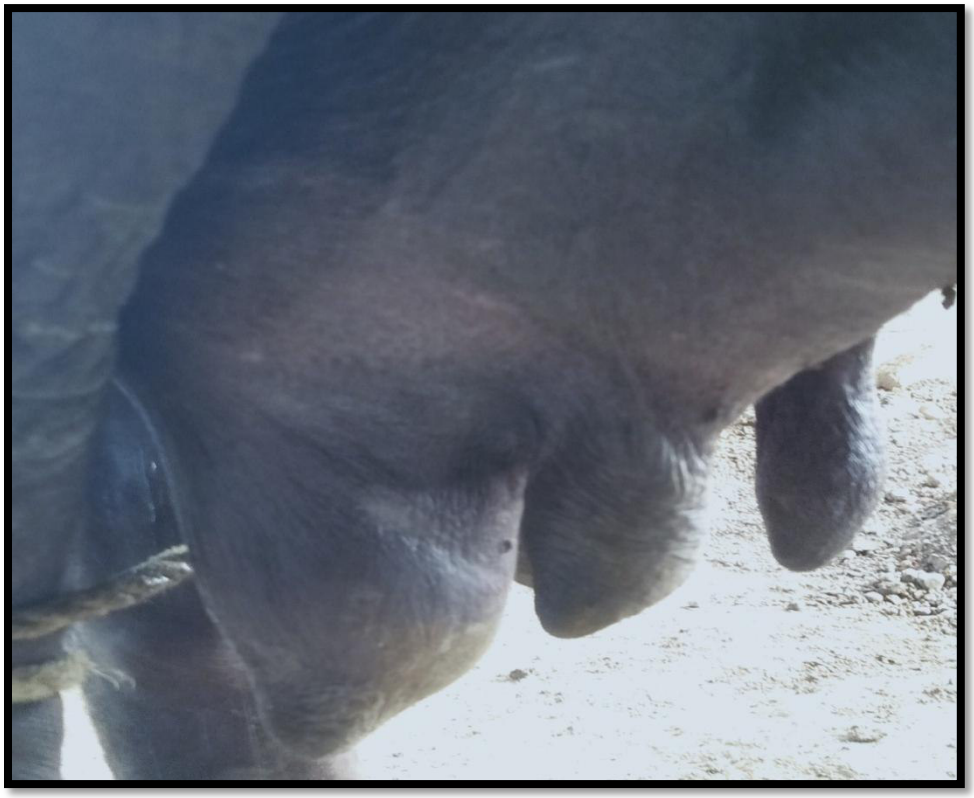

Plate 4.4 Right Hind quarter affected

Table 4.4 Quarter wise incidence of clinical mastitis in buffaloes

Sr. No.	Particular of quarter	Number of quarter examined	Number of quarter positive for mastitis	Quarter infection rate (QIR)
1	**Right fore quarter**	129	6	4.6 %
2	**Right hind quarter**	129	8	6.2 %
3	**Left fore quarter**	129	4	3.1 %
4	**Left hind quarter**	129	9	6.9 %
Total		**516**	**33**	**5.23 %**

The quarter wise incidence was recorded at fore quarter and hind quarter. The hind quarter showed higher incidence than the fore quarters. The results were in accordance with Sharma *et al*. (2011), Swami *et al.* (2017), Thakur *et al.* (2021), Shaikh *et al.* (2018) and Yadav *et al.* (2018). The high incidence of mastitis in hind quarter might be due to the high production capacity of hind quarters (Radostits *et al*. 2007) and also might be due to larger mass, greater vulnerability to direct trauma, relatively more closeness to the floor as compared to the fore quarters hence high chances of faecal, urine contamination in hind quarters (Hase *et al.* 2013).

4.1.4 Breed wise

The breed wise incidence was shown in Table 4.5 and Figure 4.5.

Table 4.5 Breed wise incidence of clinical mastitis in buffaloes

Sr. No.	Breed	No of animals examined	No of animals positive for mastitis	Incidence in percent
1	**Jaffarabadi**	40	8	20.00%
2	**Murrah**	33	6	18.18%
3	**Non- descript**	56	10	17.86%
	Total	**129**	**24**	**18.60%**

The incidence of clinical mastitis was higher in Jaffarabadi breed *i.e.* 20.00% and in Murrah buffaloes was 18.18 percent. This is in agreement with Jingar *et al*. (2014) who observed 22.78% to 32.89% of incidence of mastitis in murrah buffaloes during different parities. The incidence of clinical mastitis in non- descript animals was 17.86 percent. Variation in breed wise incidence might be due to variation in milk production, age of animals, housing system and individual variation of animals.

4.2 Udder Parameters

Changes in udder parameters before and after treatment were shown in Table 4.6.

On examination of udder in group I changes in colour of milk was observed in 9 (90%) cases on '0' day before initiation of treatment and on 7th day 3 (3.33%) cases after treatment. In group II change in colour of milk was observed on '0' day (before initiation of treatment) in 9 (90%) cases and it reduced to the 1 (11.11%) on 7th day of treatment.

Table 4.6 Udder parameters of group I and group II before and after treatment.

Sr. No	Udder Parameters	Group I		Group II	
		Day '0'	Day '7'	Day '0'	Day '7'
1	Change in colour of milk	9 (90%)	3 (33.33%)	9 (90%)	1 (11.11%)
2	Change in Consistency of Milk	10 (100%)	2 (20%)	10 (100%)	1 (10%)
3	Swelling of udder	9 (90%)	2 (22.22%)	9 (90%)	1 (11.11%)
4	Hotness present On Palpation of udder	8 (80%)	2 (25%)	8 (80%)	1 (12.5%)

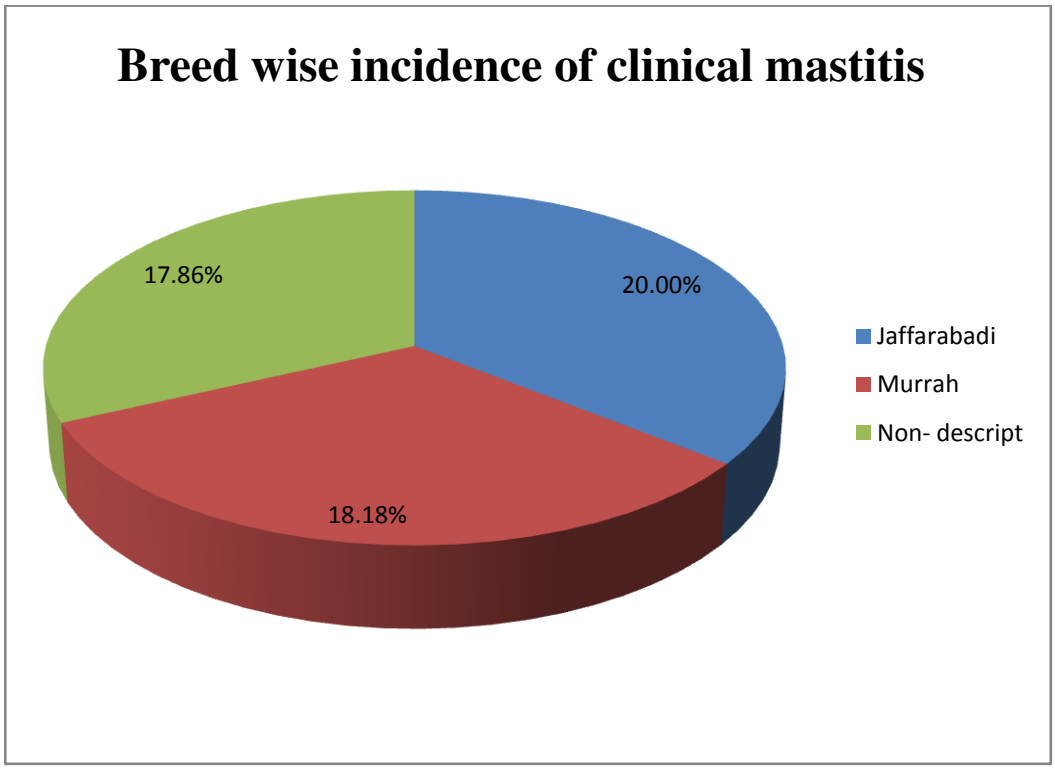

Fig. 4.5 Breed wise incidence of clinical mastitis in buffaloes

In group I on ‘0’ day 10 (100%) cases have shown change in consistency of milk and on 7th day after treatment the number was reduced to 2 (20%). In group II change in consistency of milk was in 10 (100%) cases on day ‘0’ (before initiation of treatment) and on 7th day (after treatment) it was 1 (10%).

In group I swelling of udder on ‘0’ day (before initiation of treatment) was observed in 9 (90%) cases and it reduced to 2 (22.22%) on 7th day of treatment. In group II udder swelling was present in 9 (90%) cases on ‘0’ day before initiation of treatment and it reduced to the 1 (11.11%) on 7th day after treatment.

In group I on ‘0’ day 8 (80%) cases have shown hotness on palpation of udder and it reduced to the 2 (25%) on 7th day after treatment. In group II on ‘0’ day 8 (80%) cases have shown hotness of udder and it reduced to the 1 (12.5%) on 7th day after treatment.

The abnormal colour of milk might be due to changes in vascularity during acute inflammation and flow of blood from body of animal to the udder. The shape and size of udder was changing grossly (Muhammad *et al.* 1997). The inflamed udder might be red, hot and hard on palpation and sometimes animal shows pain (Lafta, 2020).

In the mastitis affected buffaloes changes in colour of milk (abnormal colour), abnormal milk flakes, clots present in milk, swelling of udder, hotness of udder were observed in clinical mastitis animals and this is in agreement with Radostits *et al.* (2007), Khodery and Salama, (2008) and Muhammad *et al.* (2011).

4.3 Clinical parameters

The clinical parameters viz. rectal temperature, heart rate and respiration rate in the animals of group I and group II at day ‘0’ (before treatment) and on day 7 (after treatment) was studied and presented herewith as below.

4.3.1 Rectal temperature (^{0}F)

The mean rectal temperature (^{o}F) in group I and Group II on '0' day before treatment and 7th day after treatment are presented in Table 4.7 and Fig. 4.6

In buffalo normal rectal temperature (^{o}F) is in the range of 99.0 - 100.0 ^{o}F (Sinha, 2007).

The mean rectal temperature in clinical mastitis positive buffaloes was increased in both the treatment groups *i.e.* group I (102.71 ± 0.21) and group II (102.64 ± 0.22) before treatment on '0' day. Similar observations were reported by Sharma *et al.* (2007), Radostits *et al.* (2007), Sharma and Sindhu, (2007) and Bradley and Green, (2009). Elevated rectal temperature in clinical mastitis might be due to the local inflammation of mammary gland due to pathogens. Elevated rectal temperature in mastitis is due to systemic reaction caused due to the infection of mammary gland (Koop *et al.* 2010).

Table 4.7 Mean Rectal Temperature (^{o}F) in Inj. Moxifloxacin and Inj. Marbofloxacin treated groups before and after treatment.

Interval / **Treatment**		**Day '0'**	**Day '7'**	**Pooled**	**Two sample independent t test**		
					T statistics	**D.F.**	**Significance**
Group I Moxifloxacin		102.71^A ± 0.21	99.87^B ±0.17	101.29 ± 0.35	10.205	18	0.00
Group II Marbofloxacin		102.64^A ± 0.22	100.00^B ± 0.15	101.35 ± 0.32	9.545	18	0.00
Pooled		102.67 ± 0.15	99.97 ± 0.11				
Two sample independent t test	**T statistics**	0.227	0.908				
	D.F.	18	18				
	Significance	0.823	-0.855				

Means bearing different superscript (A, B) in each row differ significantly.

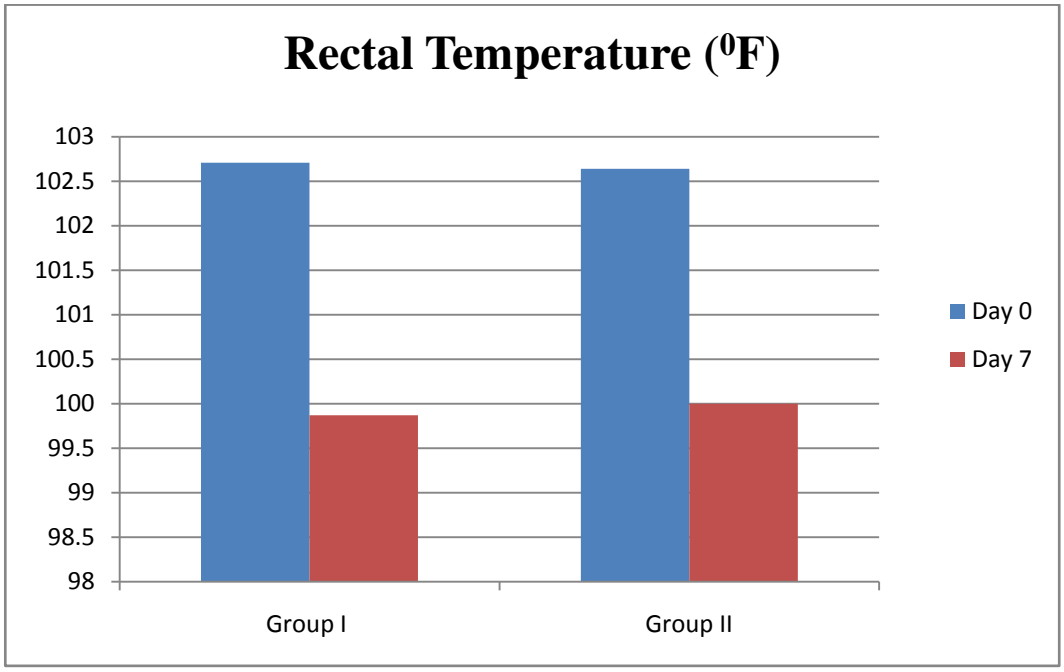

Fig. 4.6 Mean Rectal Temperature (^{0}F) in Inj. Moxifloxacin and Inj. Marbofloxacin treated groups before and after treatment.

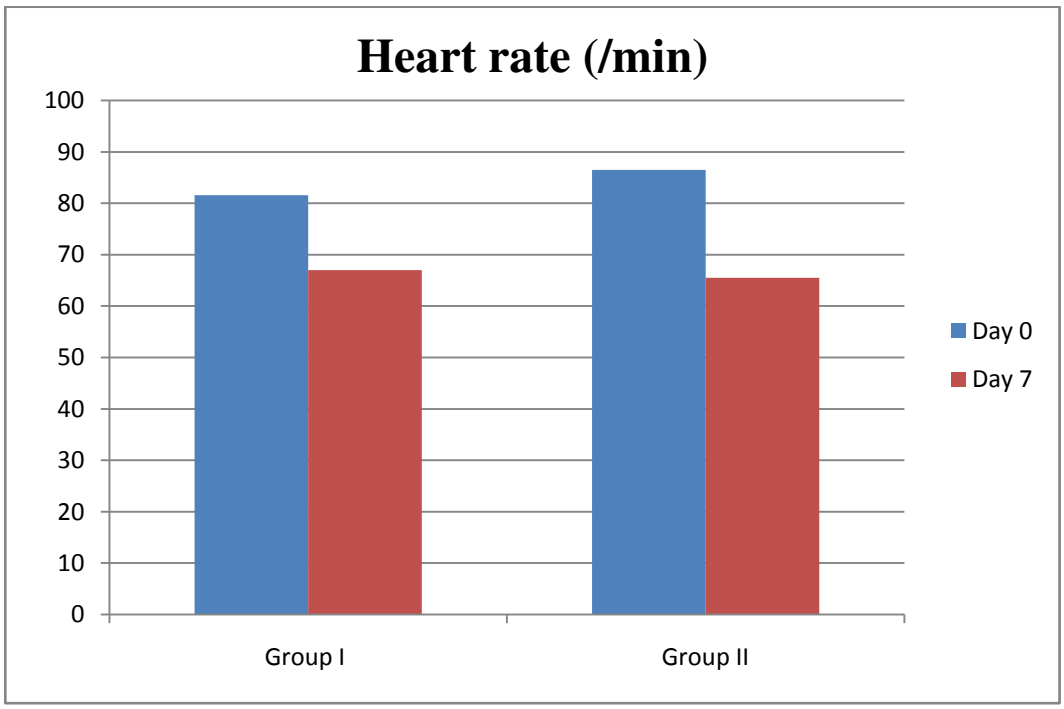

Fig. 4.7 Mean heart rate (/min) in Inj. Moxifloxacin and Inj Marbofloxacin treated groups before and after treatment.

The mean rectal temperature decreased significantly in the group I treated with Inj. Moxifloxacin from '0' day (102.71 ± 0.21) before initiation of treatment to 7th day (99.87 ± 0.17) after treatment. Rectal temperature was significantly decreased in group II treated with Inj. Marbofloxacin from '0' day before treatment (102.64 ± 0.22) to 7th day (100.00 ± 0.15) after treatment. Decreased rectal temperature after treatment might be because of reduced disease condition and reduced bacterial infection. Decrease in rectal temperature after treatment is in agreement with Sharma *et al.* (2007) and Qadri *et al*. (2016).

4.3.2 Heart rate (beats / min)

The mean heart rate (beats /min) in group I and group II on '0' day before treatment and on 7th day after treatment are presented in Table 4.8 and Fig. 4.7.

Table 4.8 Mean heart rate (/min) in Inj. Moxifloxacin and Inj Marbofloxacin treated groups before and after treatment.

Interval / Treatment		Day '0'	Day '7'	Pooled	Two sample independent t test		
					T statistics	D.F.	Significance
Group I Moxifloxacin		81.60^{A} ± 1.68	67.00^{B} ± 1.19	74.30 ± 1.95	7.083	18	0.00
Group II Marbofloxacin		86.50^{A} ± 1.57	65.50^{B} ± 1.39	76.75 ± 2.35	8.814	18	0.00
Pooled		83.80 ± 1.22	67.25 ± 0.89				
Two sample independent t test	**T statistics**	-1.912	-0.273				
	D.F.	18	18				
	Significance	0.072	0.788				

Means bearing different superscript (A, B) in each row differ significantly.

In buffaloes normal heart rate is in the range of 42-60 (beats/min) (Sinha, 2007).

The mean heart rate in clinical mastitis positive buffaloes was higher in both the treatment groups *i.e.* group I (81.60 ± 1.68) and group II (86.50 ± 1.57) before initiation of treatment on '0' day compared to normal heart rate. Similar observation was recorded by Radostits *et al*. (2007) and Sharma and Sindhu, (2007).

Any painful affection in superficial or deep tissue is responsible for increase in heart rate (Chakrabarti, 2000).

The mean heart rate in group I treated with Inj. Moxifloxacin decreased significantly from day '0' (81.60 ± 1.6) before initiation of treatment to day 7^{th} (67.00 ± 1.19) after treatment where as the mean heart rate in group II treated with Inj. marbofloxacin also significantly decreased from '0' day (86.50 ± 1.57) to 7^{th} day (67.50 ± 1.39) after treatment. The improvement in the animals heart rate after the treatment could be due to specific treatment of antibiotic and reduced disease condition. Decrease in heart rate after treatment is in agreement with the Sharma *et al.* (2007).

4.3.3 Respiration rate (Breath/min)

The mean respiration rate (breaths/min) in group I and group II on the '0' day before treatment and 7^{th} day after treatment are presented in Table 4.9 and Fig. 4.8.

In buffaloes normal respiratory rate is in the range of 22-28 (/min) (Sinha, 2007).

In both treatment groups, the mean respiration rate was higher in buffaloes with clinical mastitis *i.e.* group I (36.90 ± 2.84) and group II (36.40 ± 0.95) before initiation of treatment at day '0' as compared to the normal range of respiration rate in the buffaloes. Similar observations were also reported by Sharma and Sindhu, (2007) and Radostits *et al.* (2007).

Table 4.9 Mean Respiratory rate (breaths/Min) in Inj. Moxifloxacin and Inj. Marbofloxacin treated groups before and after treatment.

Interval / Treatment		Day '0'	Day '7'	Pooled	Two sample independent t test		
					T statistics	D.F.	Significance
Group I Moxifloxacin		$36.90^{A} \pm 2.84$	$23.90^{B} \pm 0.90$	30.40 ± 2.08	4.35	18	0.00
Group II Marbofloxacin		$36.40^{A} \pm 1.46$	$23.20^{B} \pm 1.06$	29.80 ± 1.66	9.23	18	0.00
Pooled		36.65 ± 1.46	23.55 ± 0.68				
Two sample independent t test	**T statistics**	0.167	0.50				
	D.F.	18	18				
	Significance	0.87	0.62				

Means bearing different superscript (A, B) in each row differ significantly.

A systemic change caused due to clinical mastitis in buffaloes *i.e.* intramamary infection due to bacteria with high temperature could result in increase in respiration rate. Increased respiratory rate might be because of fever in clinical mastitis (Chakrabarti, 2000).

In group I treated with Inj. Moxifloxacin the mean respiratory rate were significantly decreased from day '0' (36.90 ± 2.84) before initiation of treatment to day 7th (23.90 ± 0.90) after treatment. In group II treated with Inj. Marbofloxacin the mean respiratory rate were significantly decreased from day '0' (36.40 ± 1.46) before initiation of treatment to 7^{th} day (23.20 ± 1.06) after treatment. The improvement seen in respiration rate might be because animal recovered from disease condition with specific treatment given to the animal. This is in agreement with Sharma *et al.* (2007) and Qadri *et al*. (2016).

4.4 Haematological parameters

The haematological parameters were studied in all the 20 clinical mastitis positive buffaloes of group I and group II. The haematological parameters were examined in both treatment groups on day '0' before treatment and on day '7' after treatment *i.e.* haemoglobin (g/dl), total erythrocyte count (× 10^6 /µl), total leucocyte count (× 10^3 /µl), packed cell volume (%) and differential leucocyte count (%).

4.4.1 Haemoglobin (g/dL)

The mean haemoglobin concentration in group I and group II at '0' day before treatment and 7th day after treatment was presented in Table 4.10 and Figure 4.9.

In buffaloes normal haemoglobin concentration is ranges from 9 to 13.5 g/dL (Jain *et al*. 1986).

Table 4.10 Mean Haemoglobin concentration (g/dl) in Inj. Moxifloxacin and Inj. Marbofloxacin treated groups before and after treatment.

Interval / Treatment		Day '0'	Day '7'	Pooled	Two sample independent t test		
					T statistics	D.F.	Significacne
Group I Moxifloxacin		7.81 ± 0.18	8.24 ± 0.21	8.02 ± 0.14	-1.523	18	0.145
Group II Marbofloxacin		7.82 ± 0.24	8.27 ± 0.16	8.04 ± 0.15	-1.481	18	0.156
Pooled		7.81 ± 0.15	8.25 ± 0.12				
Two sample independent t test	**T statistics**	-0.032	-0.075				
	D.F.	18	18				
	Significance	0.975	0.941				

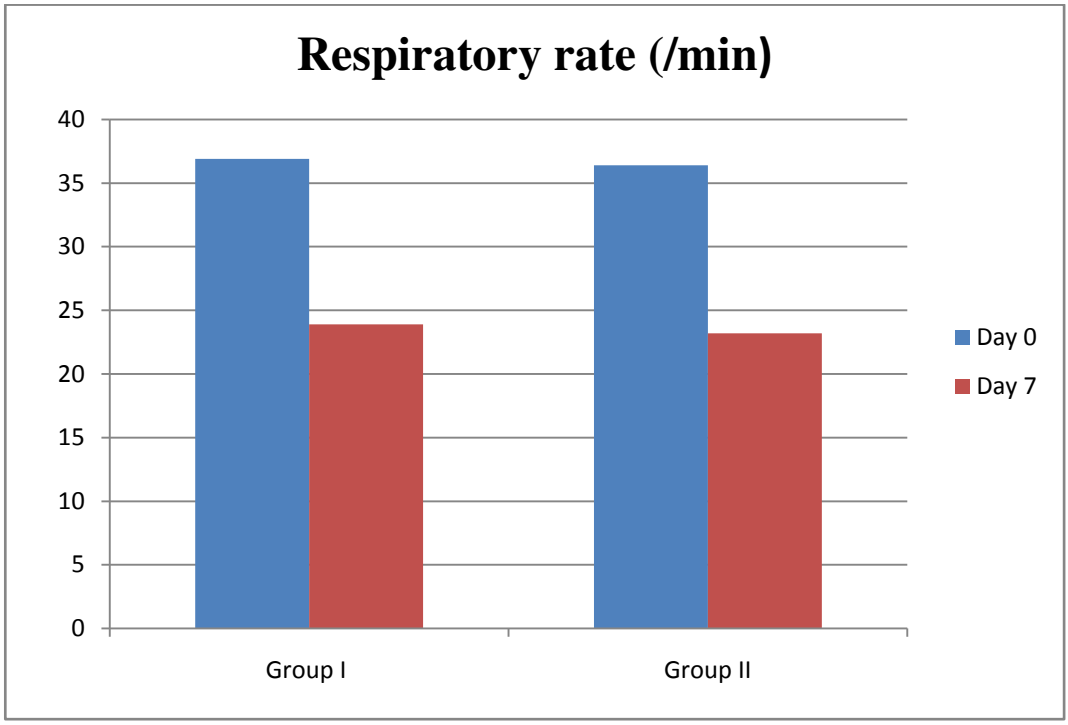

Fig. 4.8 Mean Respiratory rate (breaths/Min) in Inj. Moxifloxacin and Inj. Marbofloxacin treated groups before and after treatment.

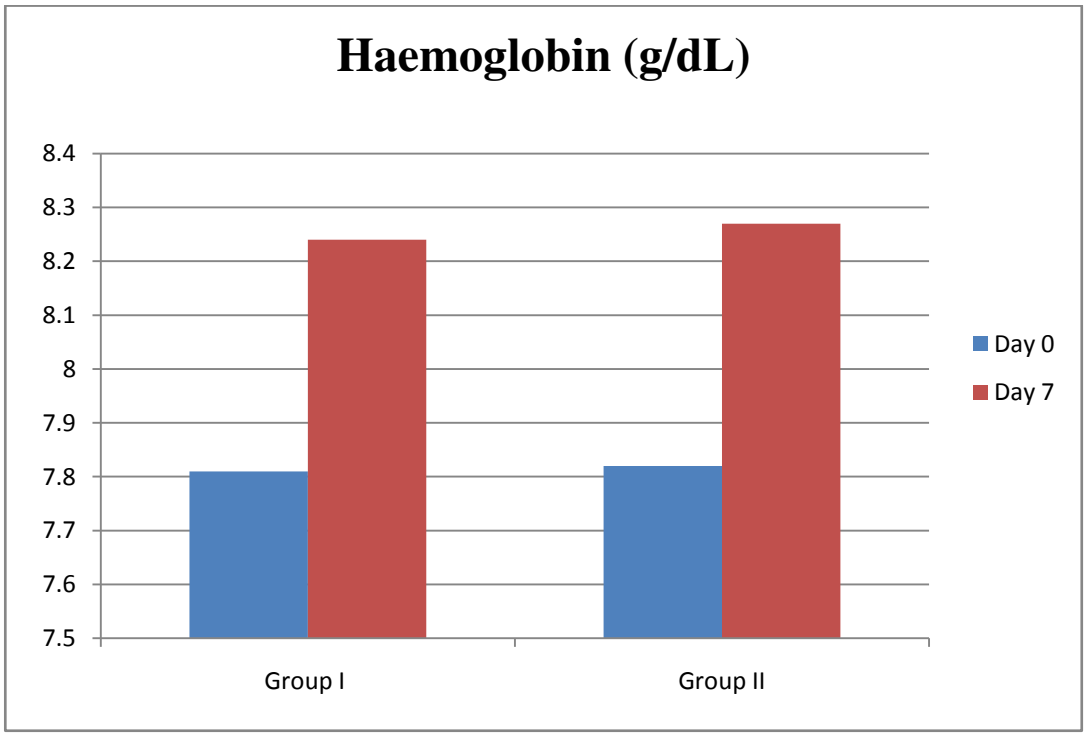

Fig 4.9 Mean Haemoglobin concentration (g/dl) in Inj. Moxifloxacin and Inj. Marbofloxacin treated groups before and after treatment.

The mean haemoglobin concentration values in clinical mastitis affected buffaloes were lower in group I (7.81 ± 0.18) and group II (7.82 ± 0.24) before initiation of treatment on day '0'. Similar observations were reported *i.e.* 7.80± 0.23 before treatment to 8.00± 0.18 after treatment by Qadri *et al*. (2018) and 7.2 ± 1.366 by Sayhood *et al.* 2018.

In clinical mastitis haemoglobin values were reduced which might be due to infection and inflammatory condition. Inflammation reduces dietary absorption of iron (Kelly, 1984).

In group I treated with Inj. Moxifloxacin, the mean haemoglobin was increased apparently but non significantly from day '0' (7.81 ± 0.18) before initiation of treatment to 7^{th} day (8.24 ± 0.21) after treatment. In group II treated with Inj. Marbofloxacin the mean haemoglobin values were increased apparently but non significantly from day '0' (7.82 ± 0.24) to the 7^{th} day (8.27 ± 0.16) after treatment. In present study increase in haemoglobin concentration after treatment might be due to reduced infection and inflammatory condition. Present findings are in agreement with Qadri *et al.* (2018).

4.4.2 Packed cell volume (%)

The mean packed cell volume concentration (%) in group I and group II on day '0' before treatment and 7^{th} day after treatment are presented in Table 4.11 and Fig. 4.10.

In buffaloes, packed cell volume (%) normal range is 22-34 percent (Jain *et al*. 1986).

The mean packed cell volume (%) in clinical mastitis positive buffaloes was in the normal range as compared to normal PCV level in buffaloes in both the treatment group *i.e*., group I (23.80 ± 80) and group II (23.95 ± 0.86) before initiation of treatment on day '0'. Similar observations were also reported by Zaki *et al*. (2008), Krishnappa *et al*. (2016) and Ramesh *et al.* (2021).

Table 4.11 The mean Packed cell volume (%) in Inj. Moxifloxacin and Inj. Marbofloxacin treated groups before and after treatment.

Interval / Treatment		Day '0'	Day '7'	Pooled	Two sample independent t test		
					T statistics	D.F.	Significance
Group I Moxifloxacin		23.80 ± 0.63	25.25 ± 0.62	24.52 ± 0.46	-1.623	18	0.12
Group II Marbofloxacin		23.95 ± 0.86	25.80 ± 0.65	24.87 ± 0.56	-1.703	18	0.106
Pooled		23.87 ± 0.52	25.52 ± 0.44				
Two sample independent t test	**T statistics**	-0.137	-0.609				
	D.F.	18	18				
	Significance	0.89	0.55				

4.4.3 Total erythrocyte count ($\times 10^6/\mu l$)

The mean total erythrocyte count concentration ($\times 10^6/\mu l$) in group I and group II on day '0' before treatment and on 7th day after treatment are presented in Table 4.12 and Fig. 4.11.

In buffaloes, total erythrocyte count is in the normal range of 5.07-8.27 $\times 10^6/\mu l$ (Jain *et al*. 1986).

The mean total erythrocyte count in clinical mastitis positive buffaloes was in the normal range as compared to normal TEC level in buffalo in all the treatment group *i.e*., group I (7.33 ± 0.08) and group II (7.39 ± 0.07) before initiation of treatment on day '0'. Similar observations were reported by Zaki *et al.* (2008) and Sarvesha *et al.* (2017).

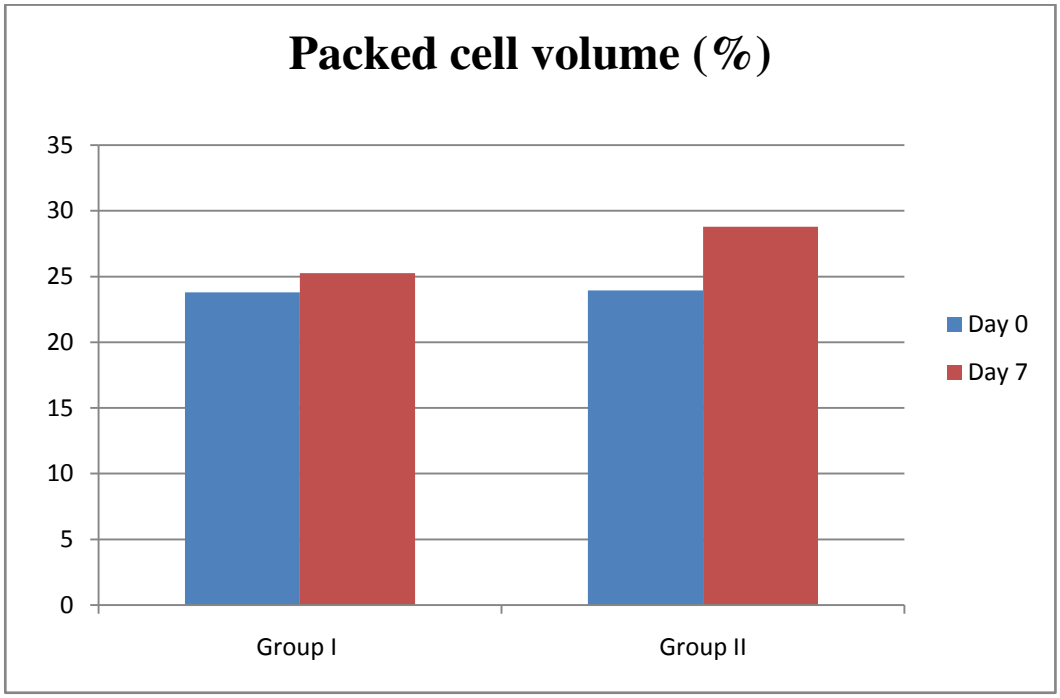

Fig. 4.10 The mean Packed cell volume (%) in Inj. Moxifloxacin and Inj. Marbofloxacin treated groups before and after treatment.

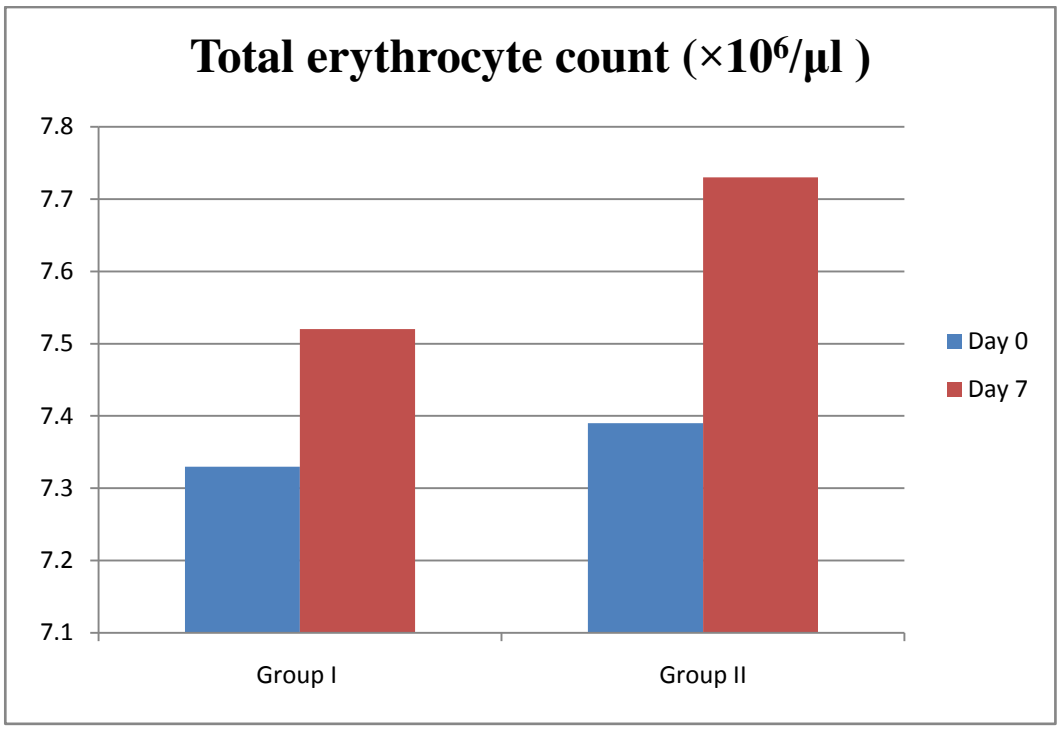

Fig. 4.11 The mean Total Erythrocyte Count Inj. Moxifloxacin and Inj. Marbofloxacin treated groups before and after treatments.

Table 4.12 The mean total erythrocyte count Inj. Moxifloxacin and Inj. Marbofloxacin treated animals before and after treatment.

Interval / Treatment		Day '0'	Day '7'	Pooled	Two sample independent t test		
					T statistics	D.F.	Significance
Group I Moxifloxacin		7.33 ± 0.08	7.52 ± 0.08	7.42 ± 0.06	-1.55	18	0.13
Group II Marbofloxacin		7.39 ± 0.07	7.73 ± 0.08	7.56 ± 0.06	-2.98	18	0.08
Pooled		7.36 ± 0.05	7.62 ± 0.06	.			
Two sample independent t test	**T statistics**	-0.509	-1.777				
	D.F.	18	18				
	Significance	0.617	0.092				

The mean total erythrocyte count of group I treated with Inj. Moxifloxacin showed apparent but non-significant increase from day '0' (7.33 ± 0.08) before initiation of treatment to the 7th day (7.52 ± 0.08) after treatment. The mean total erythrocyte count of group II treated with Inj. Marbofloxacin shown apparent but non-significant increase from 0th day (7.39 ± 0.07) before initiation of treatment to 7th day (7.73 ± 0.08) after treatment. This is in agreement with Zaki *et al.* (2010) and Qadri *et al.* (2018).

4.4.4. Total leucocyte count (×10^3/µl)

The mean total leucocyte count (×10^3/µl) in group I and group II on 'day 0' before treatment and 7th day after treatment is presented in Table 4.13 and Fig. 4.12.

In buffaloes, normal total leucocyte count ranges between 6.2-13.0 (x10^3 /ul) (Jain *et al*. 1986.).

Table 4.13 Mean Total Leucocyte Count (x10^3) in Inj. Moxifloxacin and Inj. Marbofloxacin treated groups before and after treatment.

Interval / **Treatment**		**Day '0'**	**Day '7'**	**Pooled**	**Two sample independent t test**		
					T statistics	**D.F.**	**Significance**
Group I Moxifloxacin		14.20^{A} ± 0.45	8.55^{B} ± 0.23	11.37 ± 0.69	11.00	18	0.00
Group II Marbofloxacin		14.96^{A} ± 0.23	8.11^{B} ± 0.25	11.53 ± 0.80	19.44	18	0.00
Pooled		14.58 ± 0.26	8.33 ± 0.17				
Two sample independent t test	**T statistics**	-1.47	1.25	Means bearing different superscript (A, B) in each row differ significantly.			
	D.F.	18	18				
	Significance	0.156	0.22				

The total leucocyte count in clinical mastitis positive buffaloes was higher in group I (14.20 ± 0.45) and group II (14.96 ± 0.23) on day '0' before initiation of treatment as compared to normal total leukocyte count. This is in agreement with Krishnappa *et al.* (2016).

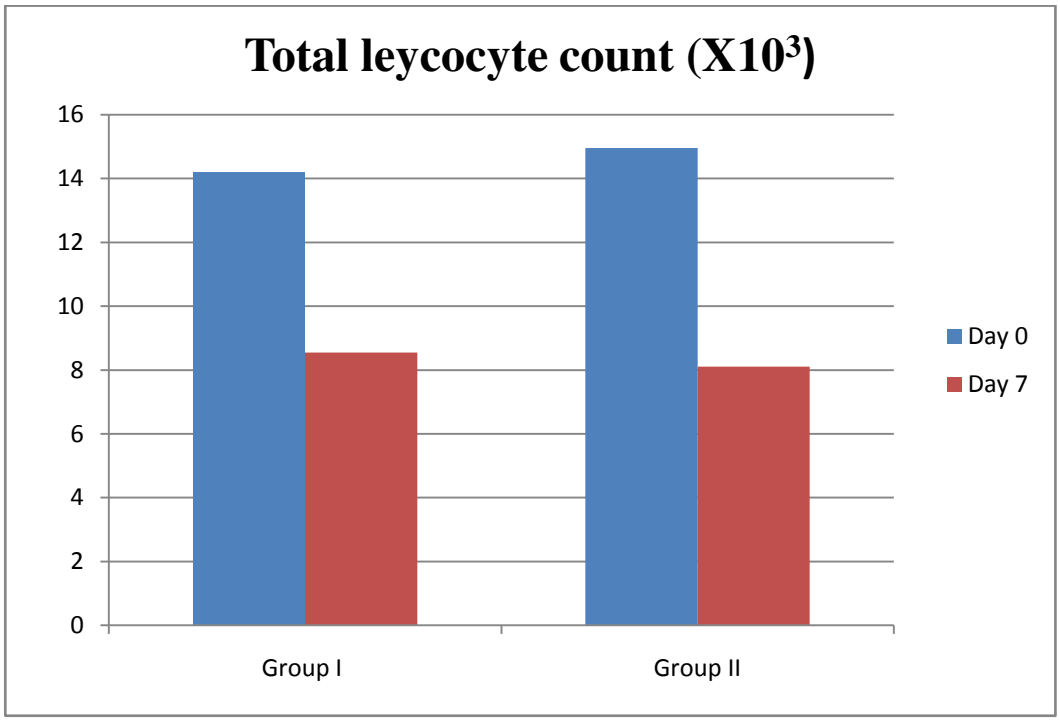

Fig. 4.12 Mean Total Leucocyte Count (x10^3) in Inj. Moxifloxacin and Inj. Marbofloxacin treated groups before and after treatment.

The total leucocyte count increases as a result of the invasion and spread of pathogenic micro-organisms in the mammary gland and systemic reaction of the body (Hristov *et al.* 2018).

In group I treated with Inj. Moxifloxacin the mean total leucocyte count significantly decreased from day '0' (14.20 ± 0.45) before treatment to day '7'after treatment (8.55 ± 0.23). In group II treated with Inj. Marbofloxacin the mean total leucocyte count significantly decreased from day '0' before treatment (14.96 ± 0.23) to 7th day after treatment (8.11 ± 0.25). Decrease in total leucocyte count after treatment might be due to decrease of microorganisms in the mammary gland. This result in agreement with Qadri *et al.* (2018).

4.4.5 Neutrophils (%)

The mean neutrophil count (%) in group I and group II on day '0' before initiation of treatment and 7th day after treatment are presented in Table 4.14 and Fig. 4.13.

The normal range of neutrophil in buffaloes is 13-54 (%) (Jain *et al*. 1986).

The mean neutrophil percentage in clinical mastitis positive buffaloes was higher in both the treatment groups *i.e*., group I and II was (65.90 ± 2.01 and 68.30 ± 1.30) before initiation of treatment on 'day 0' as compared to normal neutrophil count. Increased neutrophil concentration in mastitis might be due to bacterial infection and neutrophil act as first line of defense by animal immune system against bacteria. Similar observations were also observed by Krishnappa *et al*. (2016) and Das *et al.* (2018).

The systemic effects might be because of mediators of inflammation. In the initial stage of inflammation, the acute phase protein are released that stimulate the first line of defense mechanism *i.e.* neutrophils at the site of inflammation (Qadri *et al*. 2018)

4.14 Mean Neutrophil percentage in Inj. Moxifloxacin and Inj. Marbofloxacin treated animals before and after treatment at different intervals.

Interval / Treatment		Day '0'	Day '7'	Pooled	Two sample independent t test		
					T statistics	D.F.	Sign
Group I Moxifloxacin		65.90^{A} ± 2.01	31.10^{B} ± 0.86	48.50 ± 4.13	15.86	18	0.00
Group II Marbofloxacin		68.30^{A} ± 1.31	32.60^{B} ± 1.43	50.45 ± 4.20	18.30	18	0.00
Pooled		67.10 ± 1.20	31.85 ± 0.83				
Two sample independent t test	**T statistics**	-0.99	-0.89				
	D.F.	18	18				
	Sign	0.33	0.38				

Means bearing different superscript (A, B) in each row differ significantly.

The group I treated with Inj. Moxifloxacin and group II treated with Inj. Marbofloxacin the mean neutrophil percent significantly decreased *i.e.* in group I from (65.90 ± 2.01) on day '0' to (31.10 ± 0.86) on 7th day and in group II from day '0' (68.30 ± 1.31) to 7th day (32.60 ± 1.43). Decrease in neutrophil concentration after treatment might be because of reduced bacterial infection. The result was in agreement with Singh *et al.* (2014) and Qadri *et al.* (2018).

4.4.6 Lymphocyte (%)

The mean lymphocyte count of group I and group II on day '0' before treatment and 7th day after treatment was presented in Table 4.15 and Fig. 4.14.

The normal range of lymphocyte percentage in buffaloes is 26-75 percent (Jain *et al*. 1986).

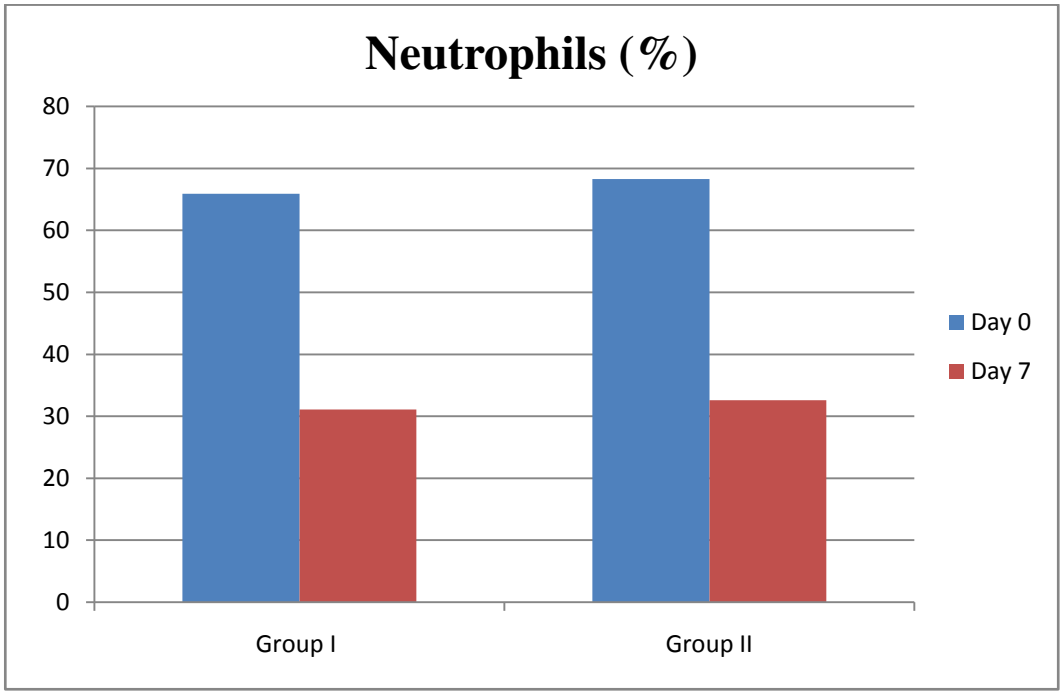

Fig. 4.13 Mean Neutrophil (%) in Inj. Moxifloxacin and Inj. Marbofloxacin treated animals before and after treatment

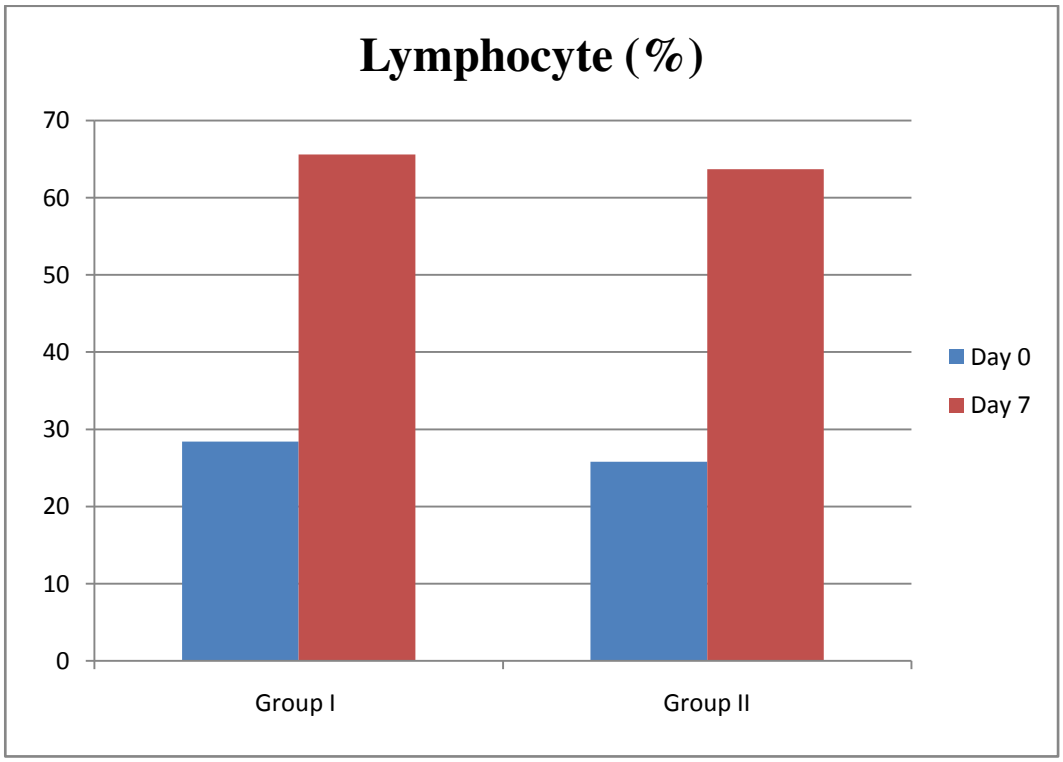

Fig. 4.14 Mean Lymphocyte (%) in Inj. Moxifloxacin and Inj. Marbofloxacin treated groups before and after treatment

Table 4.15 Mean Lymphocyte (%) in Inj. Moxifloxacin and Inj. Marbofloxacin treated groups before and after treatment.

Interval / Treatment		Day '0'	Day '7'	Pooled	Two sample independent t test		
					T statistics	D.F.	Significance
Group I Moxifloxacin		28.40^{A} ± 1.56	65.60^{B} ± 0.93	47.00 ± 4.35	-20.416	18	0.00
Group II Marbofloxacin		25.80^{A} ± 1.24	63.70^{B} ± 1.37	44.75 ± 4.44	-20.43	18	0.00
Pooled		27.10 ± 1.01	64.65 ± 0.83				
Two sample independent t test	**T statistics**	1.30	1.14				
	D.F.	18	18				
	Significacne	0.21	0.26				

Means bearing different superscript (A, B) in each row differ significantly.

The mean lymphocyte percentage in clinical mastitis positive buffaloes were lower in both the treatment groups *i.e.* group I (28.40 ± 1.56) and group II (25.80 ± 1.24) as compared with normal range of lymphocyte in buffaloes and this is agreement with Krishnappa *et al.* (2016).

The decrease in lymphocyte might be due to variation in comparative numbers of various white blood cells (Singh *et al.,* 2013).

The mean lymphocyte count of group I treated with Inj. Moxifloxacin showed significant increase in lymphocyte count from day '0' (28.40 ± 1.56) before initiation of treatment to the 7^{th} day (65.60 ± 0.93) after treatment. The mean lymphocyte count in group II also significantly increased from 0^{th} day (25.80 ± 1.24) before initiation of treatment to the 7^{th} day (63.70 ± 1.37) after treatment. The increase in lymphocyte count after treatment might be because of

relative variation of white blood cells (Singh *et al.,*2013). This is in agreement with the Babji *et al.* (2020).

4.4.7 Monocyte (%)

The mean monocyte count (%) in group I and group II on day '0' before initiation of treatment and 7th day after treatment is presented in Table 4.16 and Fig. 4.15.

The normal monocyte (%) range in buffaloes is 1-11.5 % (Jain *et al*. 1986).

Table 4.16 Mean Monocyte (%) in Inj Moxifloxacin and Inj Marbofloxacin treated groups before and after treatment.

Interval / Treatment		Day '0'	Day '7'	Pooled	Two sample independent t test		
					T statistics	D.F.	Significance
Group I Moxifloxacin		3.30^{A} ± 0.42	2.00^{B} ± 0.2	2.65 ± 0.29	2.51	18	0.02
Group II Marbofloxacin		3.70^{A} ± 0.44	2.40^{B} ± 0.37	3.05 ± 0.32	2.23	18	0.03
Pooled		3.50 ± 0.30	2.20 ± 2.36				
Two sample independent t test	**T statistics**	-0.64	-0.84				
	D.F.	18	18				
	Significacne	0.52	0.41				

Means bearing different superscript (A, B) in each row differ significantly.

The mean monocyte percentage in clinical mastitis positive buffaloes was in the normal range in both treatment groups *i.e*., group I (3.30 ± 0.42) and group II (3.70 ± 0.44) before initiation of treatment on day '0' as compared to normal monocyte percentage.

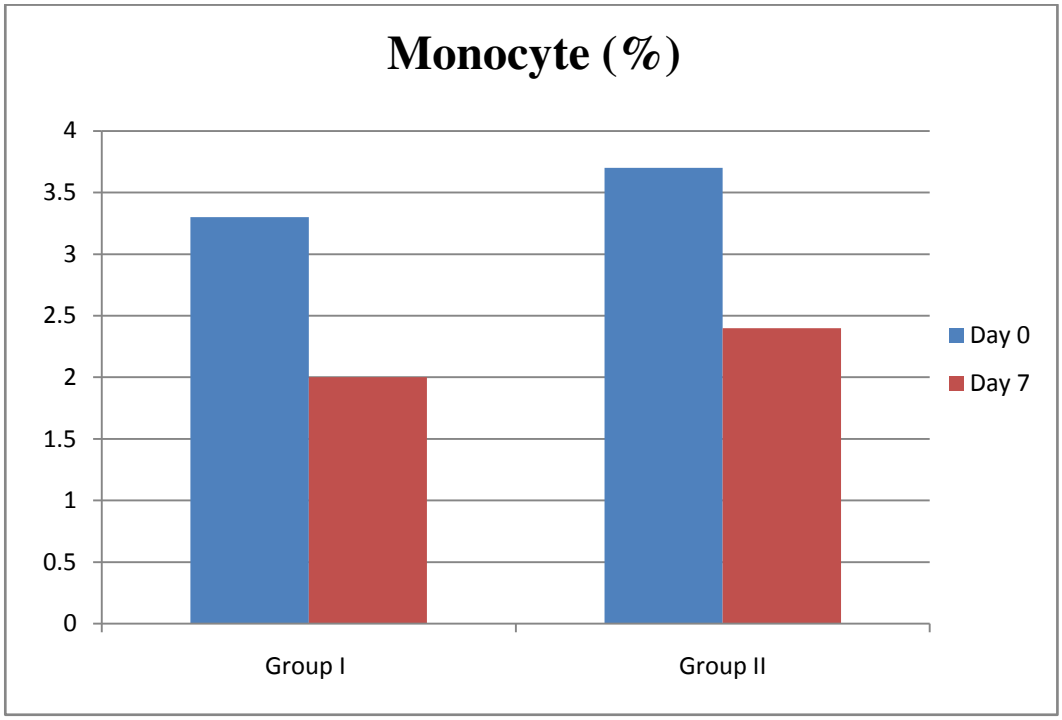

Fig. 4.15 Mean Monocyte (%) in Inj Moxifloxacin and Inj Marbofloxacin treated groups before and after treatment

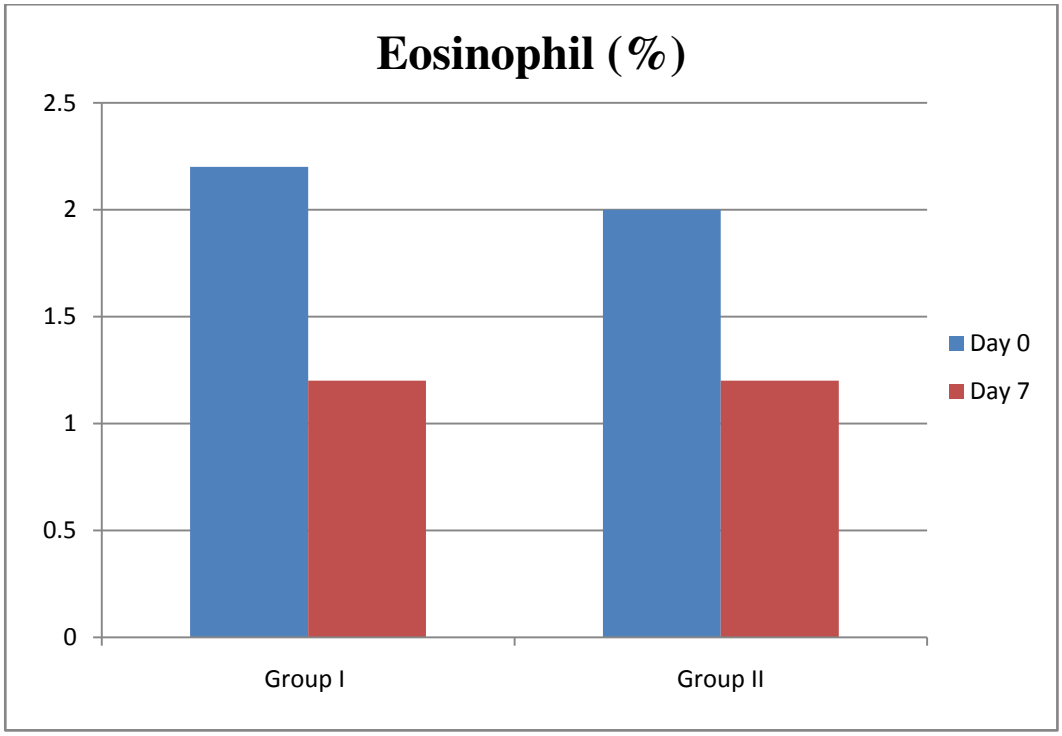

Fig. 4.16 Mean Eosinophil (%) in Inj. Moxifloxacin and Inj. Marbofloxacin treated groups before and after treatment

4.4.8 Eosinophil (%)

The mean eosinophil (%) in group I and group II on day '0' before initiation of treatment and 7th day after treatment is presented in Table 4.17 and Fig. 4.16.

The normal range of eosinophil (%) in buffaloes is 2-14 percent (Jain *et al*. 1986).

Table 4.17 Mean Eosinophil percentage in Inj. Moxifloxacin and Inj. Marbofloxacin treated groups before and after treatment.

Interval / **Treatment**		**Day '0'**	**Day '7'**	**Pooled**	**Two sample independent t test**		
					T statistics	**D.F.**	**Significance**
Group I Moxifloxacin		2.20^{A} ± 0.20	1.20^{B} ± 0.20	1.70 ± 0.17	3.53	18	0.00
Group II Marbofloxacin		2.00^{A} ± 0.21	1.20^{B} ± 0.33	1.60 ± 0.15	3.20	18	0.00
Pooled		2.10 ± 0.14	1.20 ± 0.11				
Two sample independent t test	**T statistics**	0.68	0.00				
	D.F.	18	18				
	Significacne	0.50	1.00				

Means bearing different superscript (A, B) in each row differ significantly.

The mean eosinophil percentage in clinical mastitis positive buffaloes was in normal range in both treatment groups *i.e.* group I (2.20 ± 0.20) and group II (2.00 ± 0.21) on day '0' before initiation of treatment as compared to normal eosinophil percent. This is in agreement with Krishnappa *et al*. (2016).

4.4.9 Basophils (%)

The mean basophil percentage in group I and group II on day '0' before treatment and 7th day after treatment are presented in Table 4.18 and Figure 4.17.

The normal range of basophil (%) in buffalo is 0-3.5 percent, (Jain *et al*. 1986).

Table 4.18 Mean Basophil (%) in Inj. Moxifloxacin and Inj. Marbofloxacin treated animals before and after treatment.

Interval / Treatment		Day '0'	Day '7'	Pooled	Two sample independent t test		
					T statistics	D.F.	Significance
Group I Moxifloxacin		0.20 ± 0.13	0.10 ± 0.10	0.15 ± 0.08	0.60	18	0.55
Group II Marbofloxacin		0.20 ± 0.13	0.10 ± 0.10	0.15 ± 0.08	0.60	18	0.55
Pooled		0.20 ± 0.92	0.10 ± 0.06				
Two sample independent test	**T statistics**	0.00	0.00				
	D.F.	18	18				
	Significance	1.00	1.00				

The range of mean basophil percentage in treatment group I was 0.10 ± 0.10 to 0.20 ± 0.13. The range of mean basophil percent in treatment group II was 0.20 ± 0.10 to 0.20 ± 0.13.

The mean basophil percent in group I ((0.12 ± 0.13) and group II (0.20 ± 0.13) before initiation of treatment was in the normal range. This is agreement with Khan *et al.* (1997) and Krishnappa *et al*. (2016).

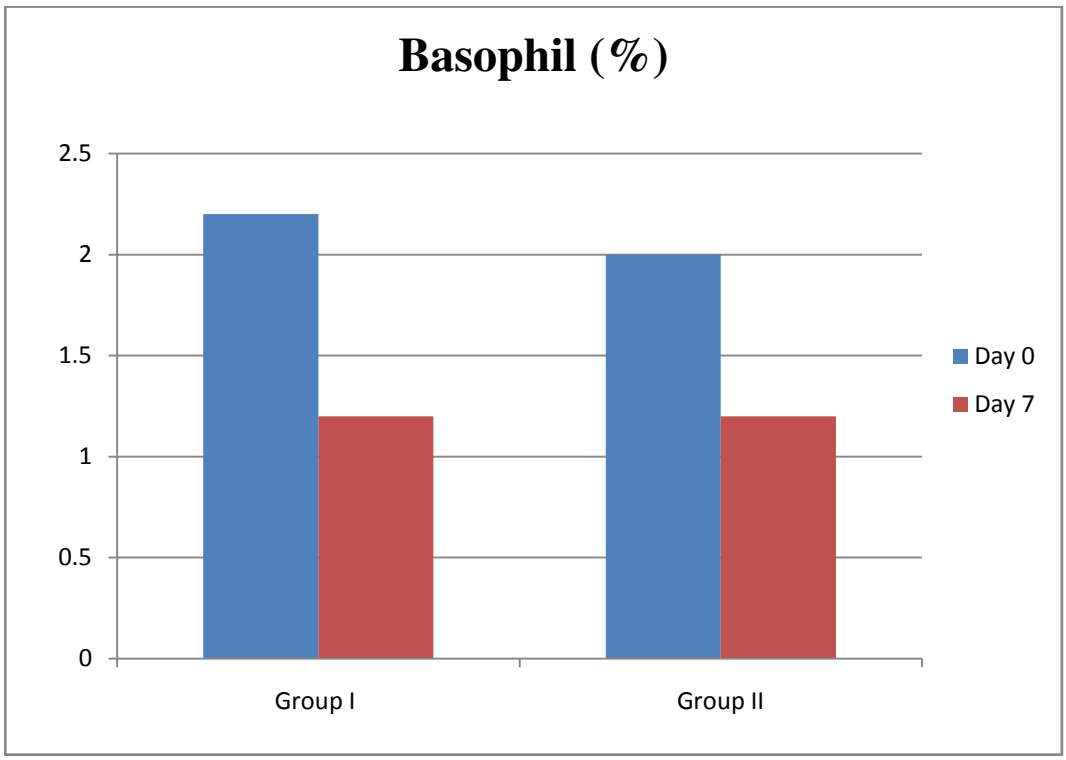

Fig. 4.17 Mean Basophil (%) in Inj. Moxifloxacin and Inj. Marbofloxacin treated animals before and after treatment.

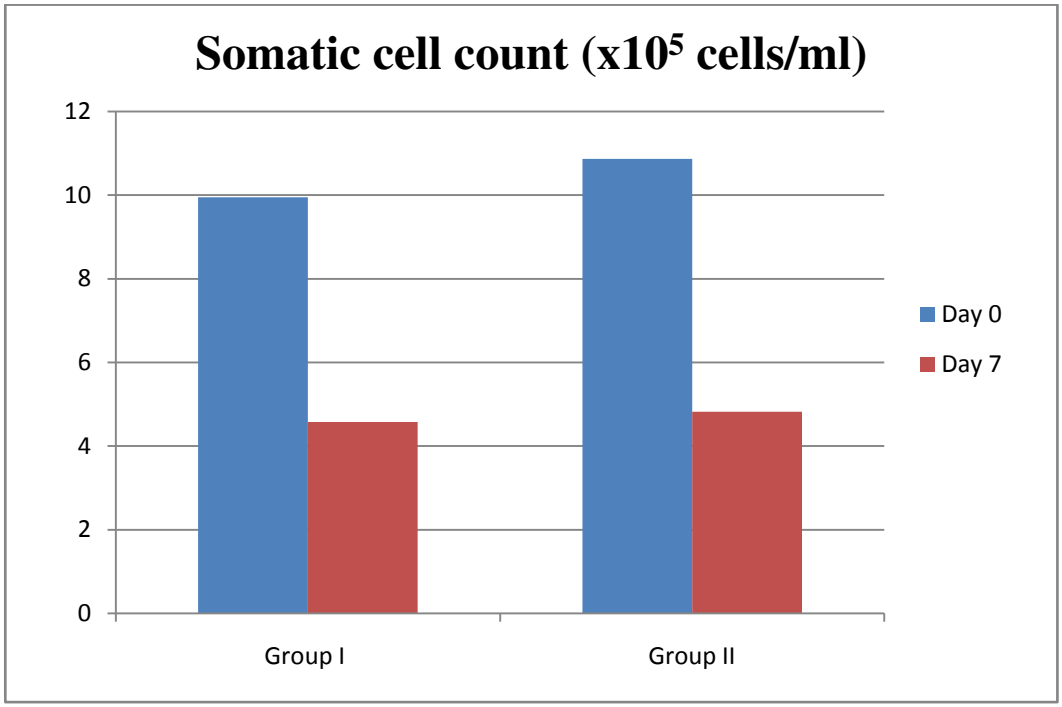

Fig. 4.18. Mean somatic cell count in Inj. Moxifloxacin and Inj. Marbofloxacin treated groups before and after treatment

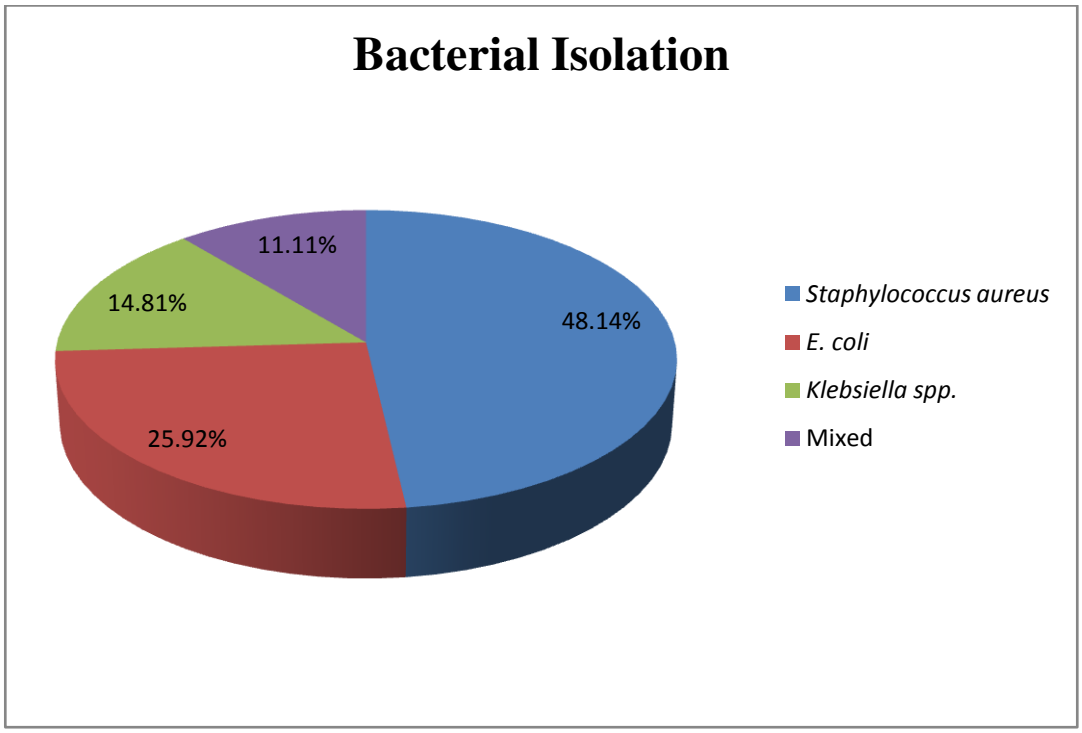

Fig. 4.19 Different genus of bacteria isolated from clinical mastitis positive buffaloes

4.5 Test on Milk

4.5.1 Somatic cell count (x10^5 cells/ml)

The mean somatic cell count (x10^5 cells/ml) in group I and group II on day '0' before treatment and 7th after treatment are presented in Table 4.19 and Fig. 4.18.

The range of normal SCC in milk is 0-200000 cells/ml (Radiostits *et al.* 2007).

The mean SCC of group I (9.95 ± 0.31) and group II (10.87 ± 0.41) before initiation of treatment at day '0' was higher than normal range. Increase in somatic cell count on day '0' as compared to the normal range is might be because of reflection of the duration and severity of infection, conversely this could be because of inadequate immune cell function and there by increased number of somatic cell count. This is in agreement with Das *et al.* (2018).

Table 4.19 Mean somatic cell count in Inj. Moxifloxacin and Inj. Marbofloxacin treated groups before and after treatment.

Interval / Treatment		Day '0'	Day '7'	Pooled	Two sample independent t test		
					T statistics	D.F.	Significance
Group I Moxifloxacin		9.95^{A} ± 0.31	4.58^{B} ± 0.31	7.26 ± 0.65	11.98	18	0.00
Group II Marbofloxacin		10.87^{A} ± 0.41	4.82^{B} ± 0.19	7.84 ± 0.72	13.30	18	0.00
Pooled		10.42 ± 0.27	4.70 ± 0.18				
Two sample independent t test	**T statistics**	-1.77	-0.64				
	D.F.	18	18				
	Sign	0.09	0.52				

Means bearing different superscript (A, B) in each row column differ significantly.

The mean SCC in group I treated with Inj. Moxifloxacin was significantly decreased from 9.95 ± 0.31 $x10^5$ cells/ml on day 0 before initiation of treatment to the 4.58 ± 0.31 $x10^5$ cells/ml on 7th day after treatment. The mean SCC count of group II treated with Inj. Marbofloxacin significantly decreased from 10.87 ± 0.41 $x10^5$ cells/ml day '0' before initiation of treatment to the 4.82 ± 0.19 $x10^5$ cells/ml day 7th after treatment.

Decrease in SCC after treatment might be because of reduced infection in the mammary gland due to specific antibiotic therapy. The mean SCC decreased significantly after treatment is in agreement with Bradley and Green, (2009).

4.5.2 Strip cup test

Strip cup test was performed as per (Constable *et al.* 2017).The results of strip cup test was shown in Table 4.20.

Total 129 animals were examined with the help of strip cup test and 24 animals were found positive for strip cup test. Out of 24 positive animals for mastitis in buffaloes, clots in milk was present in 15 animals (65.5%), discolouration of milk was present in 22 animals (91.67%) and blood in milk was present in 2 animals (8.33%). Out of 129 animals examined for clinical mastitis 24 animals found positive with the help of strip cup test and incidence was 18.60% this is in agreement with Hossain *et al.* (2016).

Table 4.20 Results of strip cup test.

Sr. No.	Examination	Animals Positive	No of animals examined	Percentage
1	Clots present	15	24	65.5%
2	Discolouration of milk	22	24	91.67%
3	Blood in milk	2	24	8.33%

4.6 Isolation and Identification

In present study, different bacterial species isolated from 20 clinical mastitis positive buffaloes are depicted in Table 4.21 and Fig. 4.19.

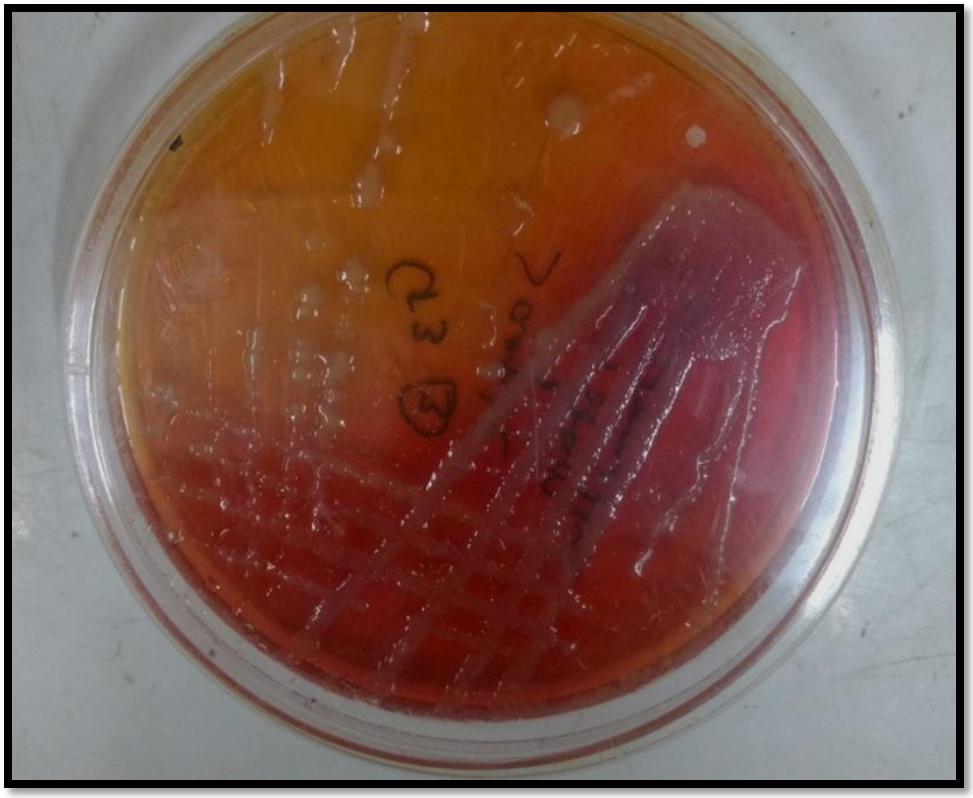

Plate 4.5 *Staphylococcus aures* on Mannitol Salt Agar plate

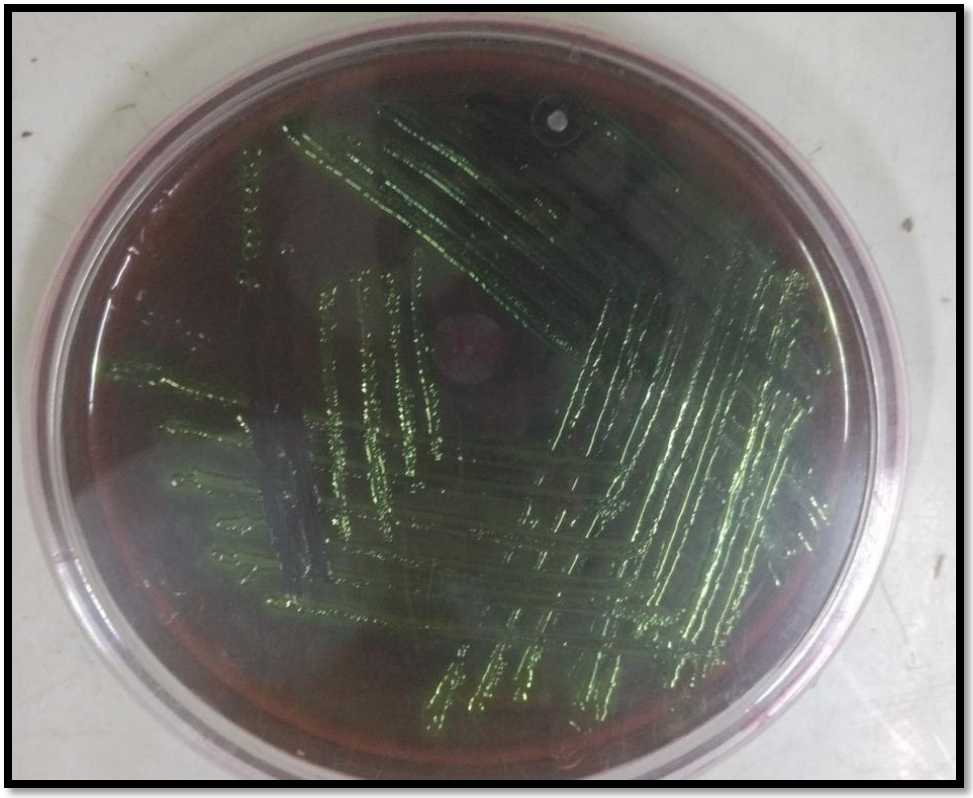

Plate 4.6 *E. coli* on EMB agar

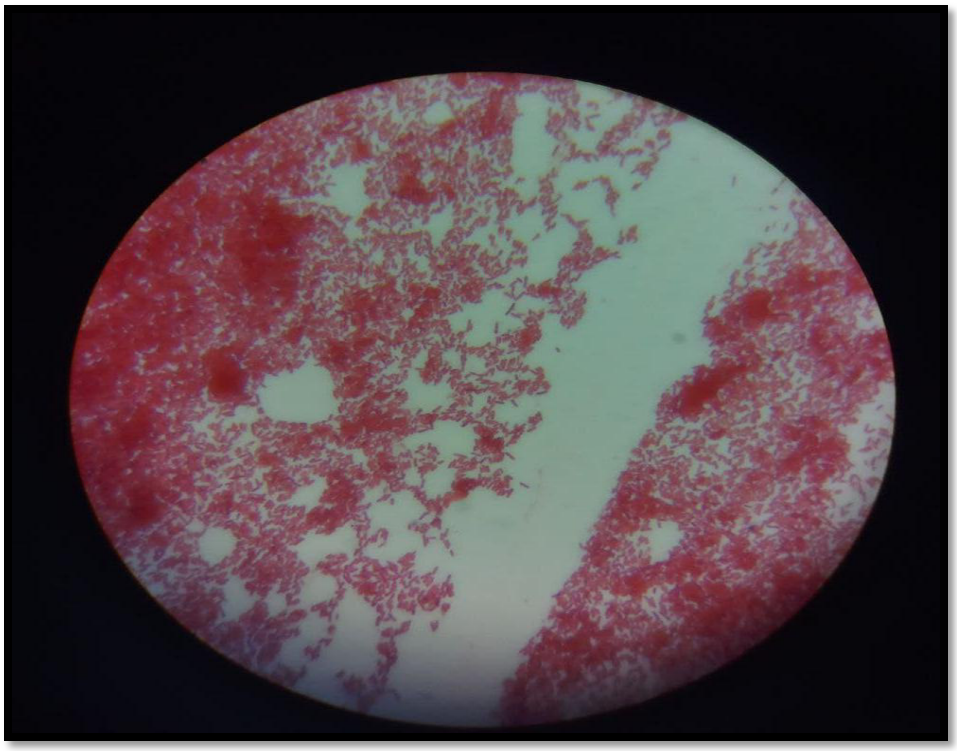

Plate 4.7 Gram negative staining of *E. coli*

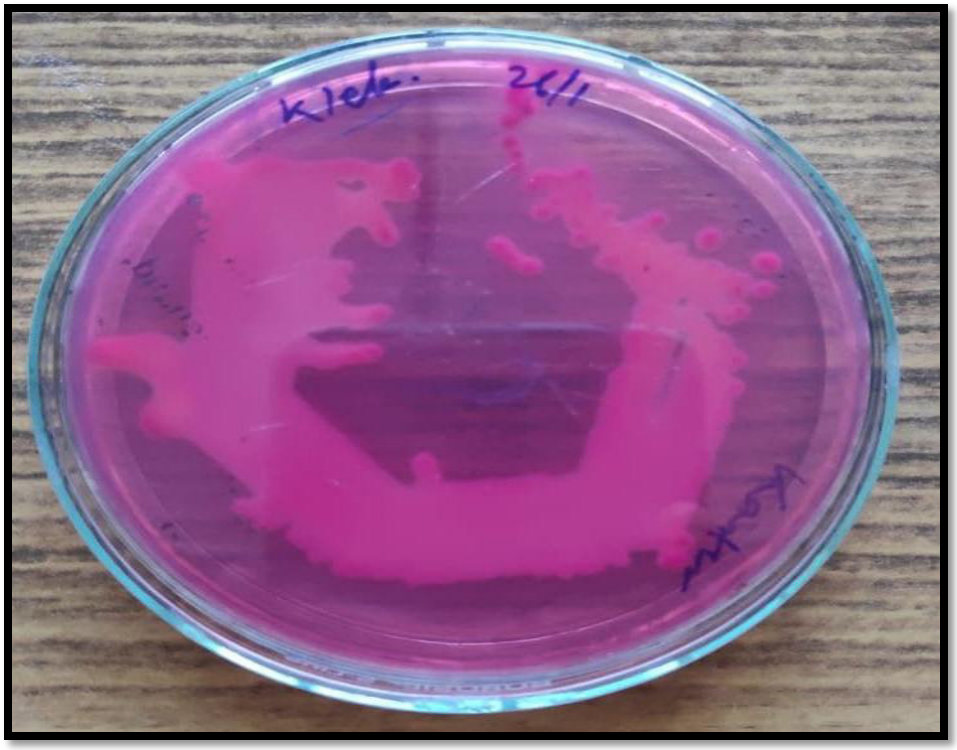

Plate 4.8 Growth of *Klebsiella* on EMB agar

Table 4.21 Different genus of bacteria isolated from clinical mastitis positive buffaloes.

Sr. No.	Bacteria isolates	No. of Isolates	Percentage Incidence
1	***Staphylococcus aureus***	13	48.14%
2	***Escherichia coli***	**7**	25.92%
3	***Klebsiella spp.***	4	14.81%
4	**Mixed**	3	11.11%
Total		**27**	**100**

Total 20 clinical mastitis positive buffaloes (27 quarters) samples were studied for cultural examination. Most predominant mastitis causing bacteria found were *Staphylococcus aureus i.e.* (48.14). Similar observation were reported by Anwar *et al.* (2013), Jeykumar *el al.* (2013), Morwal *et al.*(2019) and Mbindyo *et al.* (2020) (Plate 4.5).

Second most bacteria found on isolation was *E.coli i.e.* 25.92 percent. Similar observation was reported by Oliveira *et al.* (2013), Charaya *et al*. (2014) and Morwal *et al*. (2019) (Plate 4.6 and 4.7)

Klebsiella spp. were observed 11.11% and this is in agrrement with Sumathi *et al.* (2008). The growth of *Klebsiella* on EMB agar shown in Plate 4.8.

The higher incidence of *Staphylococcus aureus* in this study might be due to its ubiquitous nature and its ability to colonize the skin as well as udder. These organisms are contagious and spread from animal to animal during milking and better suceptibilty in the environment (Shukla *et al.* 1998).

4.7 Antibiotic Sensitivity Test

The details of antibiotic sensitivity test on milk of animals from both the treatment groups are shown in Table 4.22 and Fig. 4.20.

The antibiotic sensitivity test for susceptibility of bacteria was performed as per the method described by Bauer *et al.* (1966).

There were 4 discs used for antibiotic sensitivity test *i.e.* moxifloxacin, marbofloxacin, enrofloxacin and ciprofloxacin.

Table 4.22 Details antibiotic sensitivity of clinical mastitis samples

Sr. No.	Antibiotic disc	Percent sensitivity of isolates and resistance	
		Sensitive	Resistance
1	Marbofloxacin	70%	30%
2	Moxifloxacin	50%	50%
3	Enrofloxacin	60%	40%
4	Ciprofloxacin	50%	50%

The present study showed that samples were highly susceptible to Marbofloxacin (70%), followed by Moxifloxacin (50%), Enrofloxacin (60%) and Ciprofloxacin (50%).

The similar observations were reported by Chandrasekaran *et al.* 2014 and Chhabra *et al.* 2020.

4.8 Treatment

Twenty buffaloes suffering from clinical mastitis were divided randomly into two groups *i.e.* Group I and Group II and given treatment as detailed in Table 4.23, Fig.4.19, Plate 4.9 and Plate 4.10.

Table 4.23 Comparative efficacy of Group I and Group II

Sr. No.	Group	No of animals affected	No of animals recovered	Percentage efficacy
1	I (Moxifloxacin)	10	5	50%
2	II (Marbofloxacin)	10	7	70%

The comparative efficacies in treatment groups were calculated on the basis of improvement in clinical signs, strip cup test and somatic cell count after treatment. The efficacy of marbofloxacin was found to be more (70%) as

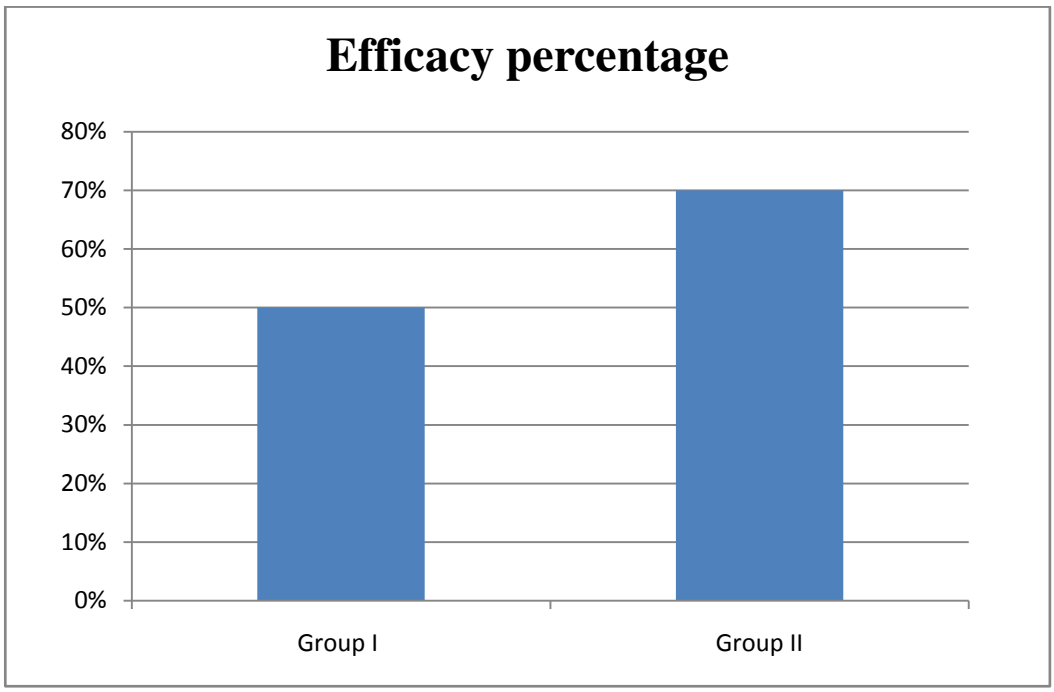

Fig. 4.20 Comparative efficacy of Group I and Group II

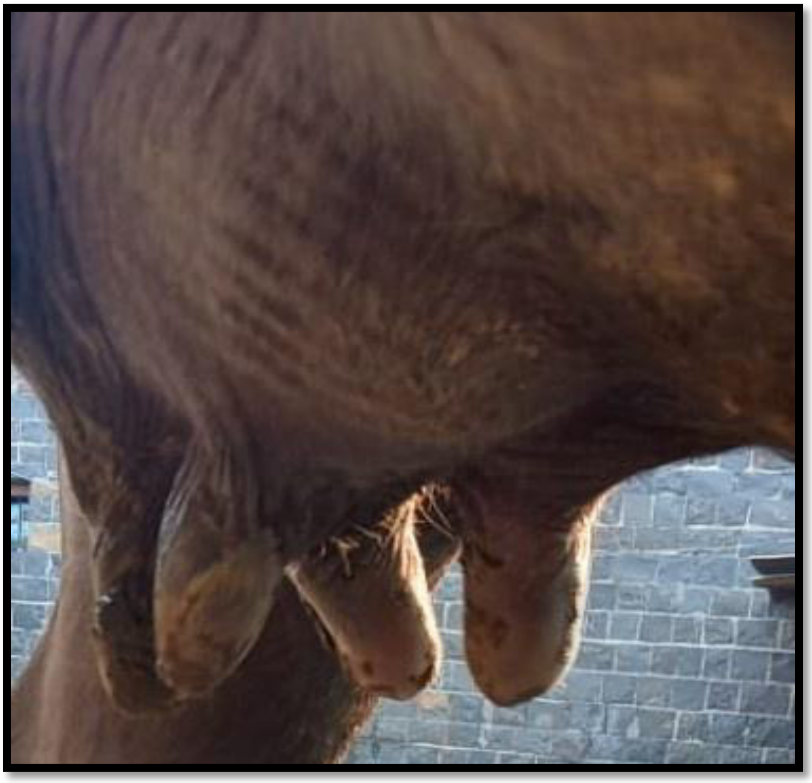

Plate 4.9 Group I animal treated with Inj. Moxifloxacin showing clinical recovery.

Plate 4.10 Group II animal treated with Inj. Marbofloxacin showing clinical recovery.

compared to moxifloxacin (50%). However, further study is required to confirm the findings of research study.

Summary and Conclusions

CHAPTER V

SUMMARY AND CONCLUSIONS

Present study was investigated from May - December 2021, in which 129 lactating buffaloes were examined for clinical mastitis with the help of strip cup test, somatic cell count and clinical signs. Blood and milk sample of buffaloes positive with clinical mastitis collected to isolate mastitis causing bacteria from the milk, to study haematological changes in mastitis and to perform different direct and indirect test on milk sample of clinical mastitis positive buffaloes. Total 20 clinical mastitis positive buffaloes were studied for clinical parameters, haematological changes, incidence of mastitis, bacterial culture and therapeutic management with Inj. Moxifloxacin and Inj. Marbofloxacin on day '0' before initiation of treatment and day '7' after treatment.

In present research work 20 clinical mastitis positive buffaloes were divided into 2 groups, 10 animals in each. Group I was treated with Inj. Moxifloxacin @ 5 mg/kg body weight for 5 days and group II was treated with Inj. Marbofloxacin @ 2 mg/kg body weight for 5 days. Supportive treatment was given same *i.e.* antihistaminics, multivitamins and NSAIDs. The efficacy of treatment in both groups was studied on the basis of decrease in somatic cell count, changes in clinical parameters and strip cup test.

Out of 129 buffaloes examined on the basis of strip cup test and clinical signs 24 buffaloes were found positive for clinical mastitis resulting in overall incidence of 18.60 percent. Age wise incidence was highest in 8-9 years (32.14%) followed by 6-7 years (22.22%), 10 years and above (9.38%) and 4-5 years (8.33%). Lactation wise incidence was found to be highest in third lactation (40%), followed by fourth (20%), fifth (13.33%), second (12.5%) and first (10%). Quarter wise incidence was highest in left hind quarter (6.9%), followed by right hind quarter (6.2%), right fore quarter (4.6%) and left fore quarter (3.1%). Breed wise incidence was highest in Jaffarabadi buffaloes (20%) followed by Murrah buffaloes (18.18) and non- descript buffaloes (17.86%).

Mean rectal temperature, heart rate and respiratory rate showed significant improvement in both the groups after the treatment as compared to day '0' before initiation of treatment.

In present research study, haematological parameters were studied to understand severity of clinical mastitis positive animals. Haematological parameters showed significant improvement included total leucocyte count, neutrophils count and lymphocyte. However, non-significantly improvement was observed in haemoglobin, total erythrocyte count, packed cell volume, eosinophil count, basophil count and monocyte count.

The strip cup test was quick and effective to diagnose milk flakes, clots and colour of milk in clinical mastitis cases. Out of 516 quarters 27 (5.23%) quarters were positive for clinical mastitis. The other indirect test performed for diagnosis of clinical mastitis was somatic cell count.

In present research work, total 20 clinical mastitis positive buffaloes were studied for bacterial culture examination. Out of 20 quarters positive for clinical mastitis 27 isolates found highest bacteria were *Staphylococcus aureus* (48.14%), followed by *E. coli* (25.92%), *Klebsiella* spp. (14.81%) and mixed infection (11.11%). On antibiotic sensitivity test samples were found sensitive to Marbofloxacin (70%), Moxifloxacin (50%), Enrofloxacin (60%) and Ciprofloxacin (50%).

Comparison of efficacies of marbofloxacin and moxifloxacin has shown that marbofloxacin @ 2 mg/kg body weight intramuscularly for 5 days was more (70%) effective as compared to the efficacy of moxifloxacin @ 5 mg/kg body weight intramuscularly for 5 days (50%).

Hence, it is concluded that Inj. Marbofloxacin @ 2 mg/kg body weight for 5 days is more effective in clinical mastitis than Inj. Moxifloxacin @ 5 mg/kg body weight.

CONCLUSIONS

The following conclusions are taken from observations made in current clinical mastitis studies:

1. Out of 129 lactating buffaloes examined with the help of strip cup test and somatic cell count, 24 buffaloes were found positive for clinical mastitis. In and around Parbhani district, the overall incidence of clinical mastitis in buffaloes was 18.60 percent.
2. Higher incidence of clinical mastitis was found in third lactation (40%) followed by fourth (20%), fifth (13.33%), second (12.5%) and first 10%.
3. Age wise incidence of clinical mastitis was highest in 8-9 years (32.14%), followed by 6-7 years (22.22%), 10 years and above (9.38%) and 4-5 years (8.33%).
4. Quarter wise incidence was highest in left hind quarter (6.9%), followed by right hind quarter (6.2%), right fore quarter (4.6%) and left fore quarter (3.1%).
5. Breed wise incidence was highest in Jaffarabadi buffaloes (20%), followed by Murrah (18.18%) and Non- descript buffaloes (17.68%).
6. The clinical parameters like rectal temperature, heart rate and respiratory rate showed significantly improvement in both group I and II after treatment with Inj. Moxifloxacin and Inj. Marbofloxacin.
7. Haematological parameters shows significant improvement in total leucocyte count, neutrophil and lymphocyte count and there was non significant improvement in total erythrocyte count, haemoglobin, packed cell count, basophil, monocyte and eosinophil in both treatment groups.
8. *Staphylococucus aureus* was reported as most frequently isolated bacteria from mastitis (48.14%), followed by *E. coli* (25.92), *Klebsiella* spp. (14.81) and mixed infection (11.11%).
9. The antibiotic sensitivity test for all the bacteria showed highest sensitivity to marbofloxacin (70%) followed by enrofloxacin (60%), moxifloxacin (50%) and ciprofloxacin (50%).

10. Comparison of efficacies of marbofloxacin and moxifloxacin has shown that Inj. marbofloxacin @ 2 mg/kg body weight intramuscularly for 5 days was more (70%) effective than Inj. moxifloxacin @ 5 mg/kg body weight intramuscularly for 5 days (50%).

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Appendix

Appendix

Rectal temperature

Total number of animals	Group I		Group II	
	Day 0	Day 7	Day 0	Day 7
1	102.5	100.2	103	100.1
2	103.0	100.5	102.3	99.6
3	103.5	100	103.4	99.8
4	101.9	99.5	102.2	99.9
5	102.5	100.1	103.2	100.4
6	102.1	99.9	101.9	99.9
7	103.1	98.5	101.5	99.8
8	104.0	99.7	102.8	99.6
9	102.0	100.2	103.7	101.2
10	102.5	100.1	102.4	100.4
Pooled mean	**102.71**	**99.87**	**102.64**	**100.07**

Heart rate

Total number of animals	Group I		Group II	
	Day 0	Day 7	Day 0	Day 7
1	80	70	89	76
2	78	68	86	67
3	76	66	90	62
4	85	60	83	68
5	79	62	85	65
6	81	67	75	69
7	84	72	92	71
8	74	71	85	70
9	90	68	84	61
10	89	66	91	66
Pooled mean	**81.6**	**67**	**86**	**67.5**

Respiratory rate

Total number of animals	Group I		Group II	
	Day 0	Day 7	Day 0	Day 7
1	44	26	35	26
2	42	25	32	25
3	13	24	39	24
4	35	22	38	23
5	39	20	42	27
6	38	21	37	28
7	34	23	33	21
8	40	27	36	20
9	41	29	34	18
10	43	22	38	20
Pooled mean	**36.9**	**23.9**	**36.4**	**23.2**

Haemoglobin

Total number of animals	Group I		Group II	
	Day 0	Day 7	Day 0	Day 7
1	7.5	8.2	7.4	7.9
2	8.2	8	8.4	8.6
3	7.9	9.2	9.2	8.8
4	7.2	7.5	7.4	7.9
5	7.9	8.4	7.9	8.4
6	8.4	8.6	7.3	8.7
7	9	9.4	6.5	7.3
8	7.5	7.8	7.4	7.9
9	7.3	7.4	8	8.2
10	7.2	7.9	8.7	8.9
Pooled mean	**7.81**	**8.24**	**7.82**	**8.26**

Packed cell volume

Total number of animals	Group I		Group II	
	Day 0	Day 7	Day 0	Day 7
1	22.5	24.2	22.1	24.4
2	25.5	26.6	25.1	26.1
3	24.23	27.2	28.6	27.8
4	21.8	22.9	22.3	24.5
5	24.4	25.2	25.6	26.3
6	26.1	27.2	21.9	28.1
7	27.2	28.2	20.1	22.3
8	23.1	24.4	22.6	24.3
9	21.2	22.3	23.4	25.2
10	22	24.3	27.8	29
Pooled mean	**23.80**	**25.25**	**23.95**	**25.8**

Total erythrocyte count

Total number of animals	Group I		Group II	
	Day 0	Day 7	Day 0	Day 7
1	7.2	7.5	7.3	7.8
2	7.3	7.4	7.4	7.6
3	7.1	8.2	7.1	7.8
4	7.5	7.4	7.6	7.3
5	8	7.3	7.1	7.9
6	7.1	7.4	7.5	7.5
7	7	7.3	7.4	8
8	7.3	7.6	7.6	7.4
9	7.4	7.6	7.1	7.9
10	7.4	7.5	7.8	8.1
Pooled mean	**7.33**	**7.52**	**7.39**	**7.73**

Total leucocyte count

Total number of animals	Group I		Group II	
	Day 0	Day 7	Day 0	Day 7
1	14.9	7.8	14.4	9.2
2	15.8	8.9	15.1	7.6
3	13.2	9.3	15.7	7.9
4	15.4	7.6	14.3	9.1
5	13.8	7.9	13.9	8.4
6	15.6	7.5	14.8	9.2
7	14.3	9.2	15.9	7.6
8	14.6	8.9	14.3	7.8
9	11	9.1	16.1	6.9
10	13.4	9.3	15.1	7.4
Pooled mean	**14.2**	**8.55**	**14.96**	**8.11**

Neutrophils

Total number of animals	Group I		Group II	
	Day 0	Day 7	Day 0	Day 7
1	66	35	65	34
2	74	33	68	26
3	70	26	74	42
4	55	31	72	32
5	64	31	67	34
6	69	29	69	35
7	70	34	68	32
8	55	29	59	26
9	70	30	70	33
10	66	33	71	32
Pooled mean	**65.9**	**31.1**	**68.3**	**32.6**

Lymphocyte

Total number of animals	Group I		Group II	
	Day 0	Day 7	Day 0	Day 7
1	30	63	28	62
2	20	64	25	69
3	26	72	20	55
4	36	63	24	66
5	29	65	27	62
6	26	68	24	60
7	25	63	24	64
8	36	66	35	70
9	26	68	27	65
10	30	64	24	64
Pooled mean	**28.4**	**65.6**	**25.8**	**63.7**

Monocyte

Total number of animals	Group I		Group II	
	Day 0	Day 7	Day 0	Day 7
1	2	1	4	2
2	3	2	5	4
3	2	1	2	1
4	6	4	2	1
5	4	2	4	3
6	3	2	6	4
7	3	2	5	3
8	5	3	4	3
9	2	1	2	1
10	3	2	3	2
Pooled mean	**3.3**	**2**	**3.7**	**2.4**

Eosinophil

Total number of animals	Group I		Group II	
	Day 0	Day 7	Day 0	Day 7
1	2	1	3	2
2	2	1	2	1
3	2	1	3	2
4	3	2	2	1
5	3	2	2	1
6	2	1	1	1
7	2	1	2	1
8	3	2	2	1
9	2	1	1	1
10	1	0	2	1
Pooled mean	**2.2**	**1.2**	**2**	**1.2**

Basophil

Total number of animals	Group I		Group II	
	Day 0	Day 7	Day 0	Day 7
1	0	0	0	0
2	1	0	0	0
3	0	0	1	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	1	0
8	1	0	0	0
9	0	0	0	0
10	0	1	0	1
Pooled mean	**0.2**	**0.1**	**0.2**	**0.1**

Somatic cell count

Total number of animals	Group I		Group II	
	Day 0	Day 7	Day 0	Day 7
1	10	4.3	11	4.5
2	11	4	10	4.9
3	11.3	6	12	5.3
4	9.6	5.5	11	5.2
5	8.5	5	13	4.5
6	9.4	3.5	9.7	6.1
7	9.7	4.2	9.6	5.1
8	8.5	6	11.5	4.1
9	11.1	4.1	8.9	4.3
10	10.4	3.2	12	4.2
Pooled mean	**9.95**	**4.58**	**10.87**	**4.82**

Vitae

VITAE

The author Mr. Shelke Ajay Anandrao was born on 12th November 1995 at Kawalgaon Ta. Purna, Dist. Parbhani of Maharashtra state.

He has completed his primary education at Kawalgaon wadi, Tq. Purna, Dist. Parbhani. He has completed his Higher Secondary Education from Bal Vidya Mandir, Parbhani and Higher Education from Bal Vidya Mandir Junior college, Parbhani during 2011 and 2013 respectively.

He has special interest in animal welfare and entrepreneurship so he was admitted to Nagpur Veterinary College, Nagpur and completed his graduation of B. V. Sc and AH in 2019.

As he has special interest in animal suffering and affection towards animals he admitted to the Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary and Animal Sciences, MAFSU, Parbhani.

He has special interest in sports. He is chess team representative of university at West Zonal inter-university competition at Bhopal in 2016. He is representative of Mallakhamb at inter-university competition held at Kurukshetra during 2018.

He was actively participated in the National Service Scheme (NSS) unit of Nagpur Veterinary College, Nagpur and also successfully completed B-certificate of National Cadet Corp (NCC) at 1 Mah R&V Sqn. at Nagpur during his under-graduation. He is life member of the Veterinary Internal and Preventive Medicine Society. He has published two research articles and one abstract in national journals.

Thesis Abstract

THESIS ABSTRACT

a)	Title of the thesis (In Capital letters)	:	**EFFICACY OF MOXIFLOXACIN AND MARBOFLOXACIN AGAINST MASTITIS IN BUFFALO**
b)	Full name of student	:	**SHELKE AJAY ANANDRAO**
c)	Name and address of Major Advisor	:	**Dr. Siddiqui Md Ferozoddin Md Fasihuddin** Assistant Professor Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary and Animal Sciences, MAFSU, Parbhani- 431402 (M.S.).
d)	Degree to be awarded	:	M. V. Sc.
e)	Year of award of degree	:	2022
f)	Major subject	:	Veterinary Clinical Medicine, Ethics and Jurisprudence
g)	Total number of pages in the thesis	:	59
h)	Number of words in the abstract	:	252
i)	Signature of Student	:	
j)	Signature, Name and address of forwarding authority (HOD/SH)	:	**Dr. S. T. Borikar**

ABSTRACT

The present research work entitled "Efficacy of moxifloxacin and marbofloxacin against mastitis in buffalo" was carried out to record incidence, haematological parameters, clinical parameters and efficacy of moxifloxacin and marbofloxacin in clinical mastitis in buffaloes in and around Parbhani district.

For study of incidence of mastitis 129 animals were examined from which 24 animals were found to be positive for clinical mastitis. The overall incidence of clinical mastitis was 18.60 percent. Quarter wise incidence observed was 5.23 percent. Four to five years of age group showed incidence of 8.33% followed by six to seven years (22.22%), eight to nine years (32.14%) and ten years and above was (9.38%). Lactation wise incidence was highest in third lactation (40%), followed by fourth (20%), fifth (13.33%), second (12.5%) and first (10%). Breed wise incidence was highest in Jaffarabadi buffaloes (20%) followed by Murrah buffaloes (18.18%) and non-descript buffaloes (17.86%).

The clinical parameters like rectal temperature, heart rate and respiratory rate showed significant improvement in treatment group I and II. Haematolgical studies showed significant improvement in total leucocyte count, neutrophil and lymphocyte count while apparent non-significant improvement in haemoglobin, PCV, basophil, eosinophil, monocyte count.

On bacterial isolation most frequent bacteria observed were *Staphylococcus aureus* (48.14%), followed by *E.coli* (25.92%), *Klebsiella* spp. (14.81%) and mixed infection (11.11%). On antibiotic sensitivity test samples were found sensitive to Marbofloxacin (70%), Moxifloxacin (50%), Enrofloxacin (60%) and Ciprofloxacin (50%).

Comparison of efficacies of marbofloxacin and moxifloxacin has shown that marbofloxacin @ 2 mg/kg body weight intramuscularly for 5 days was more (70%) effective than moxifloxacin @ 5 mg/kg body weight intramuscularly for 5 days (50%).

प्रबंध सारांश

प्रबंधाचे नाव	:	म्हशींमधील स्तनदाहाच्या उपचाराकरीत मॉक्सिफ्लोक्सासिन आणी मारबोफ्लोक्सासिन ची कार्यक्षमता
विद्यार्थ्याचे नाव	:	शेळके अजय आनंदराव
प्रमुख मार्गदर्शकाचे नाव व हुदा	:	डॉ. सिद्दीकी मो. फेरोझोद्दीन मो. फसिहोद्दीन सहाय्यक प्राध्यापक, पशुवैद्यकीय औषधशास्त्र, नितीशास्त्र व न्यायवैद्यकशास्त्र, पशुवैद्यक व पशुविज्ञान महाविद्यालय , म. प. म. वि. विद्यापीठ, परभणी
प्रदान करण्यात येणारी पदवी	:	एम. व्ही. एस. सी.
पदवी प्रदान वर्ष	:	२०२२
मुख्य विभाग	:	पशुवैद्यकीय औषधशास्त्र, नितीशास्त्र व न्यायवैद्यकशास्त्र विभाग
प्रबंधातील एकूण पाने	:	५९
सारांशातील एकूण शब्द संख्या	:	३००
विद्यार्थ्याची स्वाक्षरी	:	
पुढे पाठवणाऱ्या अधिकाऱ्याचे नाव व हुध्दा	:	डॉ. एस. टी. बोरीकर

सारांश

सदर संशोधन प्रकल्प “म्हशींमधील स्तनदाहाच्या उपचाराकरीत मॉक्सिफ्लोक्सासिन आणी मारबोफ्लोक्सासिन ची कार्यक्षमता" म्हशींमधील स्तनदाह रोगाविरुद्ध मारबोफ्लोक्सासिन व मॉँक्सिफ्लोक्सासिन या प्रतिजैविकांची कार्यक्षमता तपासणे तसेच परभणी व आजूबाजूच्या परिसरात म्हशींमधील स्तनदाह रोगाची नोंद करणे, रक्तचाचण्या करणे तसेच क्लिनिकल पॅरामीटर्सचा अभ्यास करण्यासाठी करण्यात मे ते डिसेंबर २०२१ दरम्यान, करण्यात आला.

सदर संशोधन प्रकल्पात दूध देणाऱ्या १२९ म्हशी स्तनदाहासाठी तपासण्यात आल्या यापैकी २४ म्हशी बाधित आढळल्या. परभणी व आजूबाजूच्या भागात स्तनदाह रोगाची नोंद १८.६० % आढळून आली. चार ते पाच वर्षे वयोगटातील ८.३३ % म्हशी, तसेच सहा ते सात वर्ष वयोगटातील २२.२२ %, आठ ते नऊ वर्षे वयोगटातील ३२.१४ % व दहा वर्षे व वरील वयोगटातील ९.३७ % म्हशी स्तनदाह रोगाने बाधीत आढळल्या. तिसऱ्या वितात सर्वात जास्त म्हशी स्तनदाह रोगासाठी सकारात्मक आढळल्या (४०%), तसेच चौथ्या वितात २०%, पाचव्या वितात १३.३३%, दुसऱ्या वितात १२.५०% तर पहिल्या वितात सर्वात कमी १०% म्हशी स्तनदाहाने बाधित आढळल्या. जाफरबादी जातीच्या म्हशी मध्ये सर्वात जास्त स्तनदाह २०% आदळून आला तसेच मुराह जातीच्या १८.१८% आणी इतर १७.८६% म्हशी स्तनदाह बाधित आढळल्या.

शारीरिक तापमान, हृदयाचे ठोके आणि श्वसनदर यासारख्या शारीरिक चाचण्यांमध्ये उपचारानंतर दोन्ही गटांत लक्षणीय सुधारणा आढळली. रक्तचाचणीत हिमोग्लोबीन, पॅक सेलची मात्रा, एकूण लाल पेशींची संख्या, न्युट्रोफिल, लिंफोसाइट, मोनोसाईट आणी इओसिनोफील च्या गणनेत लक्षणीय सुधारणा दिसून आली. दुधाच्या प्रत्यक्ष निदान चाचणीत स्ट्रीप कप टेस्ट उपयोगी पडली तसेच स्तनदाहाच्या स्थितीचे मूल्यांकन करण्यासाठी दुधाच्या नमुन्यावर सोमॅटीक सेल काउंन्ट चाचणी करण्यात आली.

जीवाणू तपासणीत असे आढळले की म्हशींमध्ये स्तनदाहासाठी स्टाफायलोकोकॉकस ऑरियास हा जीवाणू सर्वात जास्त कारणीभूत आढळून आला. तसेच इश्चरशिया कोलाय, क्लेबसिएल्ला स्पेसिज आणी इतर जीवाणू स्तनदाह साठी कारणीभूत आढळले. प्रतिजैविके संवेदनशीलता चाचणी दुधावर करण्यात आली व त्यातून मारबोफ्लोक्सासिन (७०%), एनरोफ्लोक्सासिन (६०%), मॉँक्सिफ्लोक्सासिन (५०%) आणी सिप्रोफ्लोक्सासिन (५०%) संवेदनशील आदळून आले.

म्हशींमधल स्तनदाहाच्या उपचारा करीता मारबोफ्लोक्सासिन (प्रतिकिलो वजनास २ मिलिग्रॅम स्नायुतुन एकदा पाच दिवसांसाठी) व मॉँक्सिफ्लोक्सासिन (प्रतिकिलो वजनास ५ मिलिग्रॅम स्नायुतुन एकदा पाच दिवसांसाठी) यांचा तुलनात्मक अभ्यास केला असता मारबोफ्लोक्सासिन (७०%) हे मॉँक्सिफ्लोक्सासिन (५०%) पेक्षा जास्त परिणामकारक आढळले.