ANTIBIOGRAM AND TREATMENT OF BOVINE ENDOMETRITIS WITH REFERENCE TO DISTRIBUTION OF CERTAIN ANTIMICROBIALS IN UTERINE TISSUES

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

PUSA (SAMASTIPUR), BIHAR

In partial fulfilment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

IN

ANIMAL REPRODUCTION

GYNAECOLOGY AND OBSTETRICS

By

Ankesh Kumar

Registration No.- M/VOG/23/1999-2000

Department of Animal Reproduction

Gynaecology and Obstetrics

BIHAR VETERINARY COLLEGE

PATNA - 800014, BIHAR (INDIA)

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2002

In Reverent

Dedication

to

My Mother

Dr. M. H. Akhtar
Ph. D.
Associate Professor and Head
Department of Animal Reproduction,
Gynaecology & Obstetrics
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Rajendra Agricultural University,
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CERTIFICATE – I

This is to certify that the thesis entitled **"ANTIBIOGRAM AND TREATMENT OF BOVINE ENDOMETRITIS WITH REFERENCE TO DISTRIBUTION OF CERTAIN ANTIMICROBIALS IN UTERINE TISSUES"** submitted in partial fulfilment of the requirements for the degree of Master of Veterinary Science (Animal Reproduction, Gynaecology and Obstetrics) of the faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar, Pusa is the record of bonafide research carried out by **Dr. Ankesh Kumar** under my supervision and guidance. No part of the thesis has been submitted for any other Degree or Diploma.

It is further certified that such help or information received during the course of this investigation and preparation of the thesis have been duly acknowledged.

12.07.02

(M. H. Akhtar)
Major Advisor

Endorsed :

12.07.02

(Chairman / Head of the Department)

CERTIFICATE – II

We, the undersigned members of the Advisory Committee of **Dr. Ankesh Kumar**, a candidate for the degree of Master of Veterinary Science with Major in Animal Reproduction, Gynaecology and Obstetrics, have gone through the manuscript of the thesis and agree that the thesis entitled "**ANTIBIOGRAM AND TREATMENT OF BOVINE ENDOMETRITIS WITH REFERENCE TO DISTRIBUTION OF CERTAIN ANTIMICROBIALS IN UTERINE TISSUES**" may be submitted by **Dr. Ankesh Kumar** in partial fulfilment of the requirements for the degree.

12.07.02

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CERTIFICATE – III

This is to certify that the thesis entitled "**ANTIBIOGRAM AND TREATMENT OF BOVINE ENDOMETRITIS WITH REFERENCE TO DISTRIBUTION OF CERTAIN ANTIMICROBIALS IN UTERINE TISSUES**" submitted by **Dr. Ankesh Kumar** in partial fulfilment of the requirements for the degree of Master of Veterinary Science (Animal Reproduction, Gynaecology and Obstetrics) of the faculty of Post-Graduate studies, Rajendra Agricultural University, Bihar, Pusa was examined and approved on 8-10-..... 2002.

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12.07.2002

(Ankesh Kumar)

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Chapter-I

INTRODUCTION

INTRODUCTION

Dairy industry is expanding vastly now-a-days due to high production of milk. Full genetic potential in term of milk production from the cows and buffaloes can be exploited only when the animals are of excellent reproductive order. A normal fertile cow should calve first at two years of age and then in every 12 months with a maximum service period of 85 days post partum.

Infectious infertility in dairy animals is one of the most important causes of reproductive failure on account of bad housing and poor hygienic condition with low feeding standards. Kodagali *et al.* (1980) reported that total of about 30 % buffaloes, which came for A.I., were suffering form genital infections.

Husbandry and sanitation practices, commonly employed in dairy cows of parturition, exposes to a broad range of bacterial contamination and provide an increased possibility for post partum infections which may result in mucopurulent vaginal discharge. In a large number of cows, which developed mucopurulent vaginal discharge, the uterine infections often resolved spontaneously (Olson *et al.* 1986). However, in some cows the discharge persists or becomes foul smelling which requires treatment.

Among several factors responsible for reproductive failure in farm animals, endometritis is one of the major gynaecological

problems and it ranks first both in heifers and cows (Sreeramulu, 1995). In fact, endometritis stand to be the toughest challenge to the veterinarian engaged in augmenting reproductive potential in dairy animals in field conditions. The increasing number of cases of endometritis is affecting the productivity of lactating animals leading to an enormous loss to the owners in particular and nation as a whole.

Various therapeutic agents have been advocated and tried for the treatment of endometritis in dairy animals. The objective of all the therapy is to stimulate the uterine muscle through higher vascularity either by increasing blood supply or to create a healthy environment in the uterus by checking and controlling the uterine infections. Normally uterus has the tremendous power of involution and evacuation in cycling animals and it needs only to provide a sterile environment by checking the infections with suitable antimicrobials for curing various degrees of endometritis. It may complicate and need alternative therapy in case of ovarian dysfunctions and persistence of corpus luteum (CL). Chauhan and Takkar (1983) suggest that intrauterine antimicrobial therapy is superior to prostaglandins.

The indiscriminate and prolonged use of antimicrobials in the absence of *in-vitro* sensitivity has contributed to the emergence of resistant strains of bacteria. Thus isolation, preliminary identification and determination of drug sensitivity of the causative organisms have

become very important for effective therapy of gynaecological infections and to limit the development of drug resistant. Numbers of drugs have been used from time to time by various workers against bacterial isolates from cases of endometritis. (Sharma *et al.*, 1993; Gupte and Deopurkar 1993 and Anjaneyulu *et al.*, 1999).

Before the advent of antibiotics, preferred treatment for bovine genital infections was intravenous infusion of solutions with disinfectant effect, which aimed at combating the microorganisms at the site where they caused clinical signs. When antibiotics became available, the route of their administration to over come uterine infection remained the same because of the inherent advantages. It is however, imperative that a minimum therapeutic concentration in the uterine tissues be maintained which is a prerequisite for successful treatment. However, the results are inconsistent with these treatments (Gupta *et al.*, 1983) since the rational dosage regimen for these drugs are not fully established. Success with parentral administration could be achieved only when a particular antimicrobial agent is used in effective dose rate and repeated at appropriate time interval to maintain its effective therapeutic level at the site of infection.

The goal of antimicrobial therapy is to produce therapeutically effective concentration of the drug at the site of infection in order to get the desired result.

Jayachandran *et al.* (1987, 1988 and 1995), Sinha *et al.* (1994) and Sood *et al.* (1999) reported distribution of antimicrobial agents in uterine tissue of healthy animals. But little work has been done with regard to diseased uterus or animals suffering from endometritis. The reports on disposition of gentamicin and enrofloxacin in genital tissues subsequent to parentral route of administration are scanty. A very few studies have been done with regards to diseased uterus of animals suffering from endometritis.

Enrofloxacin has a wide range of antimicrobial spectrum and has been used in several clinical trials in cows and buffaloes with endometritis, metritis and other bacterial infections of the uterus (Rong-Roqiang *et al.*, 1997 and Anjaneyeulu *et al.*, 1999) However, the disposition studies to determine the dose, route and duration of the minimum inhibitory concentration (MIC) in the uterine tissues are scanty.

Gentamicin, a broad spectrum aminoglycoside antibiotic, is effective against most microorganism associated with infections of the bovine reproductive tract (Hennessey *et al.*, 1971; Panagala and Barum, 1978; Ensley and Hennessey 1979; Mohanty *et al.*, 1992 and Jacob *et al.*, 1995). The later authors have also postulated that parentral route of treatment would be sufficient in cattle with severe case of septic endometritis because of good penetrating and systemic absorption.

Perusal of literature revealed that such studies are scanty in cows. A series of experiments were therefore, designed with the following objectives,

1. Cases of endometritis will be identified
2. Uterine discharges will be collected to isolate the microorganisms and to conduct sensitivity test.
3. Determination of plasma and uterine fluid levels of enrofloxacin and gentamicin in healthy cows and in cases of endometritis in cows following its parentral administration.
4. Based on the above studies treatment of endometritis in cows will be carried out with enrofloxacin and gentamicin.
5. Efficacy of the treatment will be judge on the basis of bacteriological report and conception rate.

Chapter-II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

I. ISOLATION, IDENTIFICATION AND SENSITIVITY OF MICROFLORA IN ENDOMETRITIS

Large numbers of studies were carried out by different scientists on bacteriological aspect of endometritis along with their antibiotics sensitivity pattern. Various reports on isolation of microbes and their sensitivity pattern are described below.

Gunter *et al.* (1955) observed that *Streptococcus, Micrococcus, Corynebacterium* and other diptheroids were shown to be predominant saprophytic organisms in normal animals but proved to be pathogenic in different breeds.

Prasad (1967) isolated at least 30 species of bacteria including three strains of *Vibrio foetus* and two of *Brucella melitensis* out of 1109 uterine samples of buffaloes.

Baptista *et al.* (1971) carried out bacteriological study of 119 cases of bovine endometritis. They isolated *Corynebacterium pyogenes* from 39 cases of purulent endometritis and there were 5 cases of mixed infections with *Staphylococcus aureus* or *Pseudomonas aeruginosa*.

Koleff *et al.* (1973) performed antibiotic sensitivity test of mixed bacterial flora, cultured from the uterine content of over 100 cows with endometritis. Ampicillin was found to be the most effective antibiotic followed by chloramphenicol and tetracycline.

Fivaz and Swanepoel (1978) on their study on four dairy herd from parturition to reconception period concluded that endometritis associated with *Corynebacterium pyogenes* infection was the major causes of delayed conception.

Studer and Morrow (1978) evaluated genital tract of 106 cows after parturition. The most frequent bacteria isolated were *Corynebacterium pyogenes, Coliform* or *Streptococci* or combination of these. The purulent discharges particularly were highly correlated with infections caused by *Corynebacterium pyogenes*.

Namboothripad *et al.* (1978) obtained *Corynebacterium pyogenes, Staphylococcus aureus, Klebsiella pneumoniae* and *Salmonelleae arizona* isolates from subacute endometritis cases. *Staphylococcus aureus* and *Escherichia coli* isolates from chronic endometritis cases and *Staphylococcus aureus*, *Corynebacterium. pyogenes* and *C. treundi* from metritis cases in buffaloes.

Kodagali *et al.* (1980) carried out culture and sensitivity trial for the genital discharges collected from endometritic buffaloes. The isolates commonly found were *Corynebacterium pyogenes*, *Streptococci* and *Staphylococcus aureus* and they were commonly sensitive to nitrofurazone, oxytetracycline, streptomycin and penicillin. They also investigated slaughter house materials for genital infections and found isolates that were common to that of the genital discharge of live animals.

Luginbuhl and Kupfer (1980) found delay in involution of the uterus, which was associated with a higher incidence of infection with a higher number of bacteria. Further, they divided cervical mucous samples in the following groups viz., normal lochia, catarrhal, purulent and foetid. As the degree of change increased there was an increase in *Corynebacterium pyogenes* and *Haemophilus sumnus* and decreases in *Staphylococcus aureus* and *Streptococcus*.

Singh *et al.* (1983) observed that 95% repeaters were positive for uterine infections. During bacteriological examination of the samples of uterine biopsy materials they found the presence of *Corynebacterium, Streptococci, Staphylococci,* Gram negative bacilli, *Anthracoides* and gram positive bacilli.

Lalvani *et al.*(1984) isolated *Escherichia coli* in 14 isolates followed by *Corynebacterium pyogenes* in 9 isolates and *Staphylococcus aureus* in 8 isolates from 68 cows with fertility disorders including 32 with endometritis while Steffan *et al.* (1984) isolated *Corynebacterium pyogenes* in 51% of uterine swab form 59 cows with clinical metritis.

Ahmad *et al.* (1985) isolated *Staphylococcus* (18 isolates), *Streptococcal pyogenes* (2) *Corynebacterium pyogenes* (2) *Escherichia coli* (9) and *Proteus vulgaris* from the uterine fluid samples of 20 post partum buffaloes.

Deka *et al.* (1985) carried out bacteriological examination of 30 puerperal cows. They isolated *Staphylococcus aureus* from 9 (39.3%) cows, *Escherichia coli* form 2 (8.69%) cows, *Listeria monocytogenes* from 1(4.35) cow. Mixed infections of *Staphylococcus aureus* with *Escherichia coli* and *Listeria monocytogenes* with *Candida albicans* were recorded in 1(4.35%) case each. *Listeria murrayi* and *Staphylococcus epidermidie* were the non-pathogenic organisms isolated from 3(13.04%) samples each.

David (1986) diagnosed 383 cases of chronic mucopurulent endometritis or cervicitis from 421 dairy herd during 1980-1984 and isolated *Streptococcus acidominious* in 175 cases, *Corynebacterium pyogenes* in 82 cases and mixed infections in cases containing *Pseudomonas, Pasteurella, Salmonella, Actinobactor, Moraxella,* and *Absidia*. Similarly Stephen *et al.* (1986) also reported single and mixed infections in cows suffering from metritis.

David and Bonnier (1987) observed that the commonest bacteria associated with endometritis were *Streptococci* and *Corynebacterium pyogenes*.

Wang (1987) reported that lactobiogen prepared from a *Lactobacillus* species isolated from the vagina of healthy cows, had *in-vitro* inhibitory effect on *Staphylococcus aureus, Escherichia coli, Bacillus, Aeruginosa, Klebsiella,* Spp. and *Proteus vulgaris*.

Rose (1987) isolated *Actinomyces pyogenes* from 126 of 297 samples (Vaginal swabs) of cows with endometritis taken between Oct. 1985 and April 1986 in Britany (France).

Koruchiski *et al.* (1988) observed that ten bovine strains of *Staphylococcus aureus* isolated from cases of endometritis were resistant to many antibiotics viz., Penicillin, tetracycline, oleandomycin, cholramphenicol, neomycin, kanamycin, and streptomycin and five strains were found to be resistant to tylosin. These isolates were found to be resistant to antibiotics when cultured in the presence of acridine orange.

Williams *et al.* (1988) cultured bacteria and assessed cytological evidence of inflammation from samples of uterine swabs taken during estrus in 85 cows and found 65% of the samples were positive for bacterial isolates. A negative correlation between uterine inflammation and conception was observed. The commonest organisms identified were *Corynebacterium* Spp., *Moraxella* Spp., *Streptococcus* Spp., *Staphylococcus* Spp., and *Escherichia coli.*

Ambrose and Pattabiraman (1989) found aerobic spore bearing baccili in all the cases of purulent uterine discharges in bovine with puerperal infection .

Vicek *et al.* (1989) carried out study on 73 cows with the history of laboured calving and complicated puerperium for observation on the presence of bacteria in the cervical mucus. The

most frequently identified bacteria were *Actinomyces pyogenes*, *Staphylococcus aureus* and β–*Streptococci*.

Sirohi *et al.* (1989) conducted a study at 120 cows and buffaloes having reproductive problem in which 73 with endometritis and repeat breeding and 40 with metritis. The common isolates from the vaginal or uterine discharge samples were *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus* Spp., *Corynebacterium* Spp., *Escherichia coli, Klebsiella* Spp., *Enterobacter* Spp., *Pseudomonas* Spp., *Proteus vulgaris*., *Citrobacter* Spp. and some unidentified *Coccobacillary* rods. *In-vitro* sensitivity of these isolates indicated that the highest number of isolates were sensitive to gentamicin and least to penicillin. The sensitivities of other antibiotics were in between these two.

Char *et al.* (1990) isolated 210 strains of *Staphylococcus aureus* form the cases of bovine endometritis and tabulated their *in-vitro* antibiogram pattern to ten antibiotics.

Khan *et al.* (1990) cultured the uterine samples of 140 cows and 160 buffaloes. The main bacterial isolates were *Staphylococcus aureus* (26.9%), *Streptococcus pyogenes* (14.1%), *Strept. bovis* (3.3%), *Corynebacterium pyogenes* (13.2%), *C. bovis* (2.9%), *Escherichia coli* (6.9%), *Proteus vulgaris* (8.2%), *Pseudomonas aeruginosa* (7.2%), *Bacillus aureus* (10.8%) and *Bacillus megaterium* (6.5%).

Takacs *et al.*. (1990) observed that 30% of severe infections were caused by *Streptococcus, Escherichia coli* and *Corynebacterium* Spp.. in uterine of 150 cows 10-20 days after parturition.

Kudryavtsev *et al.* (1991) isolated 284 bacterial strains from 63 cows showing post-puerperal endometritis. Among them 42.6% were *Escherichia, Proteus* and *Salmonella*, 10.92% *Pseudomonas*, 27.82% *Bacillus* and *Mycobacterium*, 17.6% *Micrococcus*, *Staphylococcus* and *Streptococcus* Spp.

Verma *et al.* (1991) collected cervical mucus samples from infected cattle and they found 100% sensitive to enrofloxacin, 80% to ampicillin, 84.6% to chloramphenicol and 86.6% to erythromycin.

Ambrose and Pattabiraman (1993) found *Escherichia Coli* and *Pseudomonas aeruginosa,* the most frequent isolates (25 and 11 respectively) out of 77 infected uterine discharges collected from bovine suffering from puerperal uterine infections.

Gupte and Deopurkar (1993) studied the samples collected in the form of uterine tampoons. From five cows showing gynaecological problem, 13 isolates were identified as *Staphylococcus aureus, Escherichia coli, Pseudomonas* and *Salmonella*. Most of which were mixed type and *Staphylococus aureus*, and *Escherichia coli* were detected in all the cases. *In-vitro* sensitivity revealed maximum sensitivity to chloramphenicol (8 of 13), streptomycin (7 of 13) and 12 of these isolates were found to be resistant to penicillins.

Mulei and Gitau (1993) isolated mainly *Crynebacterium pyogenes* and *Escherichia coli* form uterine samples of cows in early puerperium. These isolates were mainly sensitive to oxytetracycline and resistant to streptomycin and neomycin.

Slimane *et al.* (1994) on their study in about 250 cows suffering from PPM, isolated most oftenly *Corynebacterium* from the vulvae (39.5%) and uterine cavity (52.5%), respectively.

Cohen *et al.* (1995) observed a profused growth of *Actinomyces pyogenes* from 72% of the 54 cows samples suffering from P.P.M. and retained foetal membrane.

Jacob *et al.* (1995) studied uterine discharge from 21 cows of clinical endometritis and subjected to bacterial isolation. The isolates were identified as coagulase negative *Staph.* Spp. (9.52%), *Staphylococcus aureus* (14.28), *Bacillus* Spp. (23.84), *Corynebacterium* Spp. (9.52), *Pseudomonas* Spp. (14.28%) , *Citrobacter* Spp. (23.84%) and *Candida guillermondii* (4.76%). Antibiotic sensitivity test revealed that maximum percent of the animals were sensitive to gentamicin followed by chloramphenicol, oxytetrocycline, sulphadiazine and nitrofurantoin whereas maximum resistance was to penicillins and streptomycin.

Cohen *et al.* (1996) isolated different bacteria like *Escherichia coli*, *Streptococcus* Spp., *Actinomyces pyogenes* and *Bacteroid* Spp. from uterine secretion of post-parturient dairy cows

with retained foetal membrane and endometritis. They isolated *Actinomyces pyogenes,* the predominant aerobic species in 70% of 83 uteri of post-parturient Israeli Holstein cows whereas *Bacterioids melaniogenicus* was the most frequent isolate of anaerobic species.

Ziv *et al.* (1996) compared the minimum inhibitory concentration of eighteen antimicrobial agents against *E. coli* and nine antimicrobial agents for 17 *Streptococci* spp. isolated form uterine samples. The MIC value of ampicillin, amoxycillin, streptomycin, neomycin, OTC (Oxytetracycline) and sulphadimidine for 90% of the *E.coli* isolates were more than 100 µg /ml whereas norfloxacin, enrofloxacin and cefotaxime showed the best *in-vitro* sensitivity against *E. coli* isolates with MIC_{90} value of less than 0.25 µg/ml. The majority (90%) of the *Streptococci* isolates were sensitive (MIC less than 5 µg/ml) to penicillin G, amoxycillin, cephalothin, cefotaxime and enrofloxacin.

Bekana *et al.* (1997) examined cows during first 8 weeks after calving. *Actinomyces pyogenes*, *Fusobacterium necrophorum* and *Bacterioids* spp. predominated in mixed cultures. High growth rates were seen during first 3 weeks. Number declining and disappearing between weak 3 and 4 in most of the cows.

Kumar (1997) tested anitimicrobial gents to different isolates on his study on cattle suffering from repeat breeding problems. Ciprofloxacin was found to be the most sensitive (94.73%)

followed by gentamicin (73.68%) cephalexin (57.89) and neomycin (52.63). Septran and ampicillin had limited effect of 52.64 and 31.57% respectively, whereas tetracycline was sensitive only in 27.90% of the isolates.

Sadhasiva Rao and Seshagiri (1997) studied antibiotic sensitivity pattern of endometritis in cows and sensitivity test revealed that maximum sensitivity to chloramphenicol and least with penicillin.

Baishy *et al.* (1998) studied the antibiogram of bacteria that is isolated form uterine discharges of repeat breading cattle. Out of 36 uterine samples collected from repeat breeder cattle, 31 (86.11%) were culturally positive for one or other type of bacteria. Six (16.67%) samples yielded mixed type of bacteria. Most common isolate were *Escherichia coli* (29.73%) followed by *Staphylococcus* spp. (24.32%), *Sreptococcus* spp. (18.92%), *Baccilus* spp. (18.22%) and *Corynebacterium* spp. (8-10%). The sensitivity pattern of antimicrobial agents revealed that 97.27% organism were sensitive to ciprofloxacin, 83.78% to gentamicin, 78.37% to kanamycin, 72.29% to erythromycin, 43.24% coloxacillin, 40.54% to ampicillim and 18.91% each to nitrofurazon and oxytetracycline. Ciprofloxacin was found to be most effective drug *in-vitro*.

Anjaneyulu *et al.* (1999) studied the antibiogram in bovine endometritis. Enrofloxacin was found to be highly effective

(+++) in 93% of the (360) isolates. Gentamicin and chloromphenicol were found to be effective (+++) in 62% of the (240) isolates.

Arora *et al.* (2000) bacteriological studies on the genital infections in repeat breeder bovines. Out of 225 samples processed, 190 (84.44%) yielded a total of 305 different isolated while the remaining 35 (15.56%) were negative for any bacteria growth. *Escherichia coli* was the predominent organism comporising 25.25% of the total isolates, followed by *Staphylococcus aureus* (17.05%), *Proteus* spp.(13.44), *Bhacurilytic Streptococci* (12.79%), *Bacillus spp.* (8.52), *Epidermis* (7.86), *Klebsiella peneumoniae* (6.89%), *Pseudomonas aeruginosa* (6.56%) and *Corynebacterium pyogenes* (1.64%). In mixed bacterial cultures the most common combinations were *Escherichia coli* and *Staphylococcus aureus*. The antimicrobial sensitivity of these isolates revealed that gentamicin (94.43%) is the most effective drug closely followed by pefloxacin (93.11%). A fairly high degree of sensitivity was also observed against nitrofurantoin (87.87%), chloramphenicol (74.43%) and neomycin (68.52%). However, a large number of isolates were resistant to oxytetracycline, co-trimoxazole, erythromycin, streptomycin, ampicillin and penicillin.

Seh *et al.* (2000) tested antimicrobial agents of different isolate on their study on cows suffering form repeat breeding problems. The sensitivity pattern of the isolates revealed that 98.48% organisms were sensitive to gentamicin, 92.42% to ciprofloxacin and

chloramphenicol, 71.21% to norfloxacin, 59.09% and 56.06% to chlortetracycline and nitrofurontoin, respectively. Erythromycin was the only antibiotic that showed least effective (3.03%).

II. DISTRIBUTION OF ANTIMICROBIAL AGENTS IN UTERINE TISSUE

Distribution studies are aimed at determining the therapeutic dose and concentration, route as well as frequency of administration of a drug. To be effective, the drug must reach the site of infection in concentration high enough to destroy or inhibit the infecting organisms. The largest organ involved in genital tract infection is the uterus where the drug should reach in sufficient amount in order to eliminate the infection. Primarily, two methods of administration are used for the treatment of genital tract infections with antimicrobials.

(1) Systemic (i.v./i.m.)

(2) Local (intrauterine)

Following systemic administration, an antimicrobial is absorbed in the blood and thereafter transported to the genital tissues where its concentration may be guided by several factors like plasma protein and tissue binding of the drugs, blood flow to the uterus, lipid solubility and diffusion characteristics of the drug etc.

Systemic route is a convenient alternative to intrauterine (i.u.) infusion if this could yield sufficient amount of the drug in the uterine tissue and the uterine lumen. Apart from this, animals with uterine infections may also suffer from systemic infections, which can be taken care of if drug is given by systemic route. Therefore a number of studies have been conducted to explore its feasibility in the treatment of uterine infections.

Masera *et al.* (1980 a) noted that on i.m. administration of oxytetracycline resulted in more rapid and complete absorption of the drug in to the blood than that following intrauterine (i.u.) infusion. Detectable concentrations of the drug were present in all tissues of the reproductive tract 24 h after i.m. injection.

Masera *et al.* (1980 b) also studied the peak blood concentration of sodium penicillin G after i.m. injection and they found a high peak blood concentration occurred more quickly and therapeutic concentration lasted longer than those achieved after i.v. infusion. Endometrial concentrations were present for 8 h but were considerably less than those achieved following i.u. administration.

Bretzlaff *et al.* (1983) found mean plasma to tissue ratio of 1.33 and 1.88 in the endometrium and uterine wall, respectively, following continuous i.v. infusion of oxytetracycline for 8 hr at rates predicted to approach a steady state plasma concentration of 5 μg /ml.

Caudle *et al.*(1983) observed the endometrial levels of amikacin in the mare for three consecutive days. Four mare were infused with 2.0 gms once a day and four others were infused with 3.0 gms a day. Biopsy result indicates that amikacin is readily absorbed into the equine endometrium. The 3.0 gms dose has no therapeutic advantage over the 2.0 gms dose.

Chaudhary (1985) induced metritis with *Corynebacterium pyogenes* in buffaloes. The levels of trimethoprim (TMP) and sulfamethoxazole (SMZ), particularly the latter, were found to be generally lower in plasma and uterine tissue as compared to the animals with normal genitalia. The drugs were detectable in effective (above MIC) concentration for up to 12 h after intrauterine infusion at the dose rate of 0.4g TMP and 2.0 g SMZ in buffaloes with normal genitalia and in metritis. At double the dose the duration of effective tissue concentrations of the drugs was increased in normal animal and no such advantage was seen in metritis cases, thereby implying that the drug combination should be repeated at 12 h intervals, irrespective of the dose for the treatment of genital infections in buffaloes.

Jayachandran *et al.* (1987) studied distribution of streptomycin in uterine fluid of she buffaloes after i.m. injection of streptomycin (10 mg/kg). They observed that the drug was detectable up to 12 h in uterine fluid but therapeutic level was not achieved.

Jayachandran *et al.* (1988) also noted non-attainment of therapeutic level when sulphadimethoxine (100 mg/kg) was administered orally in buffaloes. The authors suggested that this drug could not be used by oral route for the treatment of uterine infections. In contrast, Singh *et al.* (1988) showed the maintenance of therapeutic concentration for a period of 12 h in uterine fluid when sulphadimidine was given by i. v route @ 200 mg/kg.

Boyd and Edward Allen (1988) studied the absorption of neomycin from equine uterus. Normal mare in estrus absorbed 6% while luteal mare absorbed 56%. In infected mare, the peak plasma concentration occurred two hours after neomycin infusion which is earlier than in healthy mare. In luteal phase, the absorption of neomycin in mare may have been maximal.

Sinha *et al.* (1994) observed the disposition of gentamicin in endometrial tissues of crossbreed cows after i.m. and i.u. administration. On i.m. administration (10mg/kg), the drug was not able to reach in sufficient quantities in uterine tissues while on i.u. administration (5mg/kg), the drug was detectable up to 6 hours.

Jayachandran *et al.* (1995) showed maintenance of therapeutic concentration of oxytetracycline for a period of 0.5-24 h when administered at the dose rate of 5mg/kg, i.v and the drug can be effectively used by systemic route for the treatment of endometritis in buffaloes.

Sood *et al.* (1999) studied the disposition kinetics and uterine tissue levels of neomycin in six reproductive healthy and normal cycling female buffaloes after i.v. administration during estrus period at the dose of 5 mg/kg body weight At 10, 30 and 720 min the levels of neomycin in uterine tissues were 42.43 ± 2.84, 26.72 ± 1.28 and 2.13±0.43 μg/g, respectively. They suggested that dosage regimen of neomycin in buffalo should be 5 mg/kg followed by 3 mg/kg at 12 h interval.

II.A. Distribution Study on Enrofloxacin

Enrofloxacin is a broad spectrum antimicrobial with bactericidal action. It is effective against both gram negative and gram positive bacteria as well as mycoplasma. Development of resistant is low. It is effective against microorganisms that are resistance to β-lactum antibiotics, tetracyclines, aminoglycosides or macrolides and has a special role in the therapy of multidrug resistant infections. Although enrofloxacin is being extensively used in cows and buffaloes for the treatment of genital infections literature on its disposition studies in the reproductive tract is particularly scanty. However few studies on kinetic of enrofloxacin in animals are described below.

Cow -

Pharmacokinetic properties of enrofloxacin and its antimicrobial active metabolite ciprofloxacin were studied in 5 cows.

Enrofloxacin was given i.v., i.m. and s.c. (5 mg/kg). After i.v. administration, the mean elimination half lives of enrofloxacin and ciprofloxacin were 44 and 56 minutes, respectively. Extravascular administration was associated with delayed absorption and extended elimination half lives (352-457 minuets) The value of volume of distribution for enrofloxacin was 0.6 L/kg. The maximum concentration in serum after i.m. injection was 0.70 μg/ml and 0.14 μg/ml for enrofloxacin and ciprofloxacin, respectively. After i.v. injection, C_{max} for enrofloxacin in milk was 1.3-2.1 μg/ml and for ciprofloxacin 0.8-1.2 μg/ml. The maximum concentration of the drug in milk was achieved 3.3-8 h after injection (Gardorfer, 1991).

Walser *et al.* (1993) conducted kinetic study of enrofloxacin after i.v. i.m. and s.c. administration of 2.5 mg/kg body weight in cows. They noted that enrofloxacin penetrated into milk in higher concentrations and persisted longer as compared to that of blood.

Tras *et al.* (1993) noted the mean enrofloxacin concentration in the milk samples of daisy cow after i.m. injection of enrofloxacin (2.5mg/kg) to be 0.035 ± 0.005 ,0.025 ± 0.009 and 0.005 ± 0.003 μg/ml at 24,48 and 72 hr, respectively. They also noted that enrofloxacin could not be detected at 96 and 72 hours.

Kaartinen *et al.* (1995) also noted elimination half life of 1.7,5.9 and 5.6 after i.v., i.m. and s.c. administration of enrofloxacin

(5 mg/kg). Mean absorption time were 6.2 and 6.9 hr after i.m. and s.c. administration. The bioavailability after i.m. administration was 82% and 137 % after s.c. administration. They noted volume distribution over 1 L/Kg for enrofloxacin. After i.v. injection, the peak concentration of enrofloxacin in milk was reached between 0.7 and 1.3 h. After i.m. and s.c. administration, the concentration time curves for enrofloxacin in milk were shallow and there were no obvious peak.

Buffalo -

Luna *et al.* (1991) administered enrofloxacin by uterine infusion (3 $mg.kg^{-1}$) and noted rapid absorption through uterine mucosa and rapidly excreted in the milk with in 12 h.

Amorena *et al.* (1992) administered enrofloxacin s.c. and i.v. to 6 buffaloes at the dose rate of 2.5 $mg.kg^{-1}$ after i.v administration. They observed the peak plasma concentration to be 1.756±0.346 μg/ml while after s.c. it was to be 0.210 ± 0.037 μg/ml after 70 minutes. The elimination half lives were similar for both the routes. They have recommended that enrofloxacin should be administered at the dose rate of 2.5 $mg.kg^{-1}$ body weight repeated at every 8 h interval.

Horse -

Gigure *et al.* (1996) conducted pharmacokinetic studies of enrofloxacin in adult horse and estimated the concentrations of the

drug in serum, body fluid and endometrial tissue after repeated intagastrically administered dosage. They noted mean absorption half life of 0.68 and 0.3 h and elimination half life of 5.94 and 6.09 for the post i.v. dosage of 2.5 and 5 mg/kg body weight, respectively. Endometrial tissue concentrations exceeded plasma concentration by as much as 3 fold. For the 5 mg/kg dosage, mean endometrial concentrations (10.19 μg/g at 74 hours and 6.56 μg/g at 84 hours) exceeded the MIC for most gram-positive and gram-negative aerobes, including *Pseudomonas* spp., *S. zooepidemicous*, *Klebsiella*, spp. and *E. coli* which are the agents most frequently isolated from mares with endometritis.

Goat -

Kumari Sudha (1998) conducted kinetic study of enrofloxacin after single i.v. and s.c. administration of enrofloxacin in healthy lactating goat at the dose rate of 5 mg/kg body weight. They noted mean absorption half life ($t_{1/2}$ Ka) and distribution half life ($t_{1/2}\alpha$) of 0.60 ± 0.01 and 0.20±0.03 hr in goat. Elimination half life ($t_{1/2}\beta$) were also observed as 2.82 ± 0.33 and 1.42±0.15 h for i.v and s.c. administration, respectively. Vd_{area} of 2.34±0.54 & 5.26 ± 1.23 L/kg were reports for i.v and s.c. administration, respectively.

Sheep -

Mengozzi *et al.* (1996) noted a rapid distribution phase and a slower elimination phase with a half life ($t_{1/2}\beta$) of 3.78 ±0.44 h after i.v. dose of 2.5 mg/kg. When the same dose was administered

i.m. the drug was rapidly absorbed, reaching mean peak plasma concentration in 1.2±0.11 h; after that time it appeared to decrease, with a half life of 3.65±0.31h. The bioavailability (F) of enrofloxacin by i.m. route was calculated to be 85.28±3.40% and volume distribution (Vd_{SS}) was noted to be 3.02 ± 0.22 and 3.03 ± 0.31 L/kg for i.v and i.m. routes, respectively.

Pig -

Kuhn (1993) reported that following single i.v injection of enrofloxacin in pig at the dose rate of 2.5mg/kg, the peak plasma concentration of 0.68 μg/ml was achieved at 225 minutes of injection. He also reported that since the amount in urine exceeded 4mg/L during 12 h after injection the drug might be suitable for treating urinary tract infections.

II. B. Distribution Study on Gentamicin

Gentamicin is an important broad spectrum aminoglycoside antibiotic effective against most microorganism associated with infections of the bovine reproductive tract (Panagala and Barnum, 1978; Ensley and Hennessey, 1979). The minimum concentration of gentamicin required for the *in-vitro* inhibition of a large majority of gram-negative aerobes is 3 to 5 μg/ml (Conzelman, 1980). Its biological half life is noted to be around 2 h (Ziv, 1980) Although gentamicin has been used in the treatment of a wide variety of infectious diseases in animals, the work with regard to distribution of gentamicin in uterine tissues are very few.

Al-guedway *et al.* (1983) studied the distribution of gentamicin between plasma and uterine content following i.m. and i.u. administration. A close system technique using the Foley's catheter inflated into the middle portion of one uterine horn was developed for collection of sample from the uterine lumen following i.m. or i.u. drug administration. A fixed volume (50 ml) of isotonic solution containing the PSP dye was infused in the uterus in both i.m. and i.u. group. The dose of gentamicin for i.u. infusion was 225 to 275 mg, and 4 mg /kg body weight for i.m. injection Serial samples of blood and infusion solution (2ml) were collected and assayed by RIA technique for gentamicin. The result indicates that gentamicin was rapidly absorbed from i.m. site reaching mean peak plasma concentration 17.50±7.280 μg/ml after 30 minutes. Gentamicin first appeared in the uterine lumen as early as 15 minutes after i.m. injection at the rate of 4 mg/kg body weight, but appeared only at 120 minutes after the lower i.m. dose (2 mg/kg body weight). The maximum gentamicin concentration in the uterine lumen of cow 4.7 ±2.0 μg/ml achieved at 6 after i.m. injection of 4mg /kg body weight dose. The concentrations of gentamicin in plasma during the six hour following i.u. Infusions were insignificant from the therapeutic point of view. At 6 h after i.u. infusion there was reduction at 13% in gentamicin concentration, 29% in its amount in uterus and 18% reduction in original infusate volume.

Haddad *et al.* (1986) conducted studies in six healthy mature cows infused i.u. with 2.5 g 50 ml of a 5% aqueous injection daily for 3 consecutive days. The serum and endometrial tissue concentrations of gentamicin were measured by radioimmunoassay. The mean peak concentration of gentamicin in endometrial tissue (639.16±307.22 μg/g) was measured at post infusion hour 6, decreasingly to 9.64 ±3.55 μg/g before the next i.u. dose. Gentamicin was still detectable in the endometrial tissues (0.86 ± 0.43 mcg/g) at 71h after the third i.u. infusion. The highest mean serum concentration of gentamicin occurred during the 3 h after each injection (2.49±1.46,6.60 ±5.47 and 4.98±2.76 mcg/ml, respectively).

Haddad *et al.* (1987) studied after a single i.m. injection of gentamicin at the dose rate of 5 mg/kg body weight. Peak serum concentrations averaged 15.39±6.19 μg/ml at 45 minutes post injection and were available up to 12 h (0.63 ±0.33 μg/ml) when i.m. injections were given every 8 h at the dose rate of 3.5 or 5.0 mg/kg body weight for 10 days. The serum concentrations were significantly higher at the higher dose level. The endometrial tissue levels ranged from 17.54 to 56 to 56.11 μg/g and 12.64 to 41.40 μg/g during the treatment with 5.0 mg/kg and 3.5 mg/kg dose, respectively.

Sinha *et al.* (1994) observed the disposition of gentamicin in endometrial tissues of crossbred cows after i.m. and i.u. administration. On i.m. administration (10 mg/kg), the drug was not

able to reach in sufficient quantities in uterine tissue while on i.u. administration (5 mg/kg), the drug was detectable up to 6 hour.

III. TREATMENT OF ENDOMETRITIS

III. A. Antimicrobial therapy of genital infections :

Pre-requisites of any rational therapy include a correct diagnosis, selection of proper and judicious use of drugs and thereby achieving the therapeutic objectives. Establishment of an aetiological diagnosis is essential to the selection of the most effective drugs with which to treat the infections.

Uterine infections commonly lead to infertility in cattle and buffaloes by causing denudation of the uterine mucosa and altering the p^H of the uterine content and thereby adversely affecting the survival of spermatozoa in the female genital tracts or by causing inflammation of the uterus and thus affecting implantation of the ova. For fertility point of view, important genital infections are cervicitis, endometritis, metritis and pyometra.

Several studies were conducted for isolation of the cervicovaginal and uterine microflora in diseased conditions and in repeat breeder. These studies implicate mainly the *Staphylococcus aureus*, *Streptococcus* spp. *Klebsiella* spp., *Proteus spp.* and *Micrococcus* spp. for these conditions in cows (Verma and Tyagi, 1974; Studer and Morrow, 1978; Takacs *et al.* 1990; Kudryavtsev *et al.*

1991) and buffaloes (Nambothripad *et al.*, 1978; Shah and Dholakia., 1983; Char *et al.* 1990 ; Khan *et al.* 1990). Amongst these bacterial isolates *Corynebacterium pyogenes* has bean blamed for causing endometritis in dairy herds (Baptista *et al.*, 1971; Studer and Morrow, 1978; David and Bonnier, 1987).

Antibiotics and other antimicrobial are employed in treatment of uterine infections in bovine by parentral route. The occurrence of a favourable result from antimicrobials therapy varies depending upon several factors including the sensitivity of the offending organism to the antimicrobial selected, dosage and duration of the treatment, route of administration, the time at which the treatment was initiated during the course of the disease, presence of concurrent maladies, nutritional, status and stress resulting from environmental or managemental factors and others (Ott, 1986).

Commonly antibiotic therapy is instituted without identifying the causative uterine microflora and carrying-out their *in-vitro* susceptibility trial (Gupta *et al.*, 1983; Singh *et al.*, 1986). At times, however, cultures of uterine microflora were made and their sensitivity to various antibiotics were only assessed before administering the appropriate therapy (Benjamin *et al*; 1982; Venkateswarlu *et al.*, 1983; Koruchisiki *et al.*, 1988; Khan *et al.*, 1991; Gupte and Deopurkar. 1993; Ziv *et al.*, 1996). In many of such rational trials, a majority of the common uterine pathogens were

demonstrated to be sensitive to gentamicin (El- Nagar *et al.* 1983; Venkateswarlu *et al.*, 1983; Rakesh sharda *et al.*,1991; Mohanty *et al.*, 1992 Kumar,1997) and enrofloxacin (Verma *et al.*,1991; Rong-Roqiang *et al.*, 1997; Anjaneyulu *et al.*, 1999). Consequently these are the antimicrobials commonly used with satisfactory results though the dosage regimen and their administration for this purpose is largely empirical rather than rational. As a results several dosage regimen of these drugs are in practice. For example Rong-Roqiang *et al.*, (1997) treated endometritis with 200 mg of enrofloxacin (injected in ovary), where as Venkateswarlu *et al.* (1983) treated endometritis with 160 mg gentamicin by intrauterine route, Ensley and Hennessey (1979) while suggested a dose of 200 mg gentamicin to be effective for the treatment of bovine metritis as single intrauterine infusion. However, the results with these entire treatment regimens are not always encouraging, since these are not based on proper disposition studies of these drugs in the genital tract.

III.B.Cure and conception with the use of antimicrobial

Several workers and clinicians treated endometritis in their own ways. Therapeutic measures adopted by some of the workers and clinician with relation to cure rate and conception rate are given below.

Koleff *et al.* (1973) treated 53 case of bovine endometritis with antibiotic based on their in-vitro sensitivity and found recovery

more rapid than after empirical therapy and also required fewer treatments.

Oxender and Seguin (1976) classified different types of metritis of cows on the basis of discharge from cervix cows having no abnormal discharge from the cervix was termed free from metritis, excessive mucid discharge indicated mild metritis and discharge of cloudy mucous was taken to be sever metritis. Conception rates at first service were 68.7,54.7 and 7.7% respectively, in above classified cases. In a mixed batch of 320 cows, uterine infusion with antibacterial preparations (penicillin, chloramphenicol or sulfonamide), with or without 60000 I.U., vitamin 'A' increased the first service conception rate and the advantage persisted in the cumulative rate after two or three services.

Sinha *et al.* (1977) observed that 87.8 % of the cases were cured from the 19 cows with second degree of endometritis after treating them on the basis of antibiotic sensitivity report by using penicillin, strepromycin, ledermycin, teramycin and chloromycetin.

Murty and Rao (1978) reported 100% cure rate and 66.6% conception rate with penicillin and 58.3 % cure rate and conception rate with streptomycin in non specific endometritis in buffaloes under field condition.

According to Studer and Morrow (1978), bacterial infections significantly increased the number of services required for

conception and significantly increased the number of cows culled because of infertility. The development of one or more post partum diseases significantly reduced first services conception rate.

Georgiev *et al.* (1980) composed three different method of treatment of endometritis in cows (a) with intrauterine vitamin plus antibiotic (b) by application of ultra short wave and (c) combination of both. They found the combination of ultra-short wave therapy and chemotherapy more superior.

Jovanovic *et al.* (1980) treated 63 cow suffering from endometritis with intrauterine infusion of erythromycin powder and geomycin (oxytetracycline). A total of 90% (51) cows were conceived, 21 of them after first insemination and rest 38 were conceived after two treatments.

Kodagali *et al.* (1980) carried out clinical trial on 92 endometritis cows in Surti buffaloes with furea bolus. Two to three intrauterine infusion were able to clear endometritis from 70% of suffering buffaloes which were found to be fit for insemination by an average of 32 days after commencement of the treatment.

Chaffaux *et al.* (1981) carried out study of type and timing of treatment of chronic endometritis involving ten dairy herds. The affected cows, when treated late showed the interval between calving and conception was much longer (84 days) whereas those

treated early the interval was slightly (only 6 days) longer than normal.

Chauhan and Takkar (1983) in their study on cows and buffaloes concluded that the intrauterine antibiotic treatment was found to be superior to prostaglandin treatment in case of chronic endometritis.

Venkateshwarlu *et al.* (1983) treated 155 cows and buffaloes having second degree of endometritis with 3 to 5 intrauterine administration of the drug of choice, based on *in-vitro* sensitivity. The over all conception rate after treatment in cows was 71.93%. The highest percentage of conception (88%) was obtained in animals treated with furacin. The other drugs in order of conception rate were tetracycline (77.78%), Chloramphenicol (72%), Streptomycin (68.75%), ampicillin and gentamicin (61.54% each).

Singh et al. (1983) observed cases of chronic endometritis in cows showed poor conception rate where as acute cases had better conception rate following treatment.

Edqvist *et al.* (1984) observed that the post-partum uterine infections may be partly responsible for the post-partum prostaglandin release for 10-20 days and this bacteriological endocrine inter-relationship represent, a way in which the uterus eliminates infective agents particularly gram-negative bacteria.

Ahmad *et al.* (1985) made a study on bacterial causes of delayed uterine involution in post partum buffaloes. They found streptopenicillin to be 94% effective against various isolates. After treatment with this antibiotic, uterine involution was completed on or before 42 days of post partum.

Bohme *et al.* (1986) studied the efficacy of various treatments in late puerperium. Conception rate after first insemination of each of 66 cows treated with 100 ml of 5% lugol's solution, 50 ml sulfanilamide were 68.8 and 39.7%, respectively. Intervals between pregnancy were 85.6 and 119 days and intervals between first insemination and conception were 15.3 & 45.5 days.

Sudhakar *et al.* (1986) showed correlation between *in-vitro* antibiotic sensitivity test and conception rate. A total of 106 animals (59 cows and 47 heifers) of endometritis were treated with an appropriate antibiotic through intrauterine route and insemination after two normal estrus in which 63% were conceived. All of 87 samples were highly or moderately sensitive to gentamicin and out 25 animals treated with gentamicin 19 (76%) were conceived. This correlation between *in-vitro* sensitivity and conception were not apparent with other antibiotics.

Dhami *et al.*(1986) treated 123 buffaloes with 0.5 % metronidazole intrauterine 3 days regularly which were previously refractory to the usual antibiotic treatment for reproductive problem.

Metronidazole was found to be effective in 40% buffaloes with endometritis, with a mean interval of 63.75 days of fertile estrus.

Hopfner (1987) compared a glucose-electrolyte-vitamin combination with lugol's solution solupront by intrauterine infusion in 50 cows with endometritis of differing severity and found equal effective result to those recorded with the two other standard preparations. Further in next year, he tried 1400 cows with therapeutic for endometritis using preparation free from antibiotic and sulfonamides with good results.

Varshney *et al.*(1987) treated 45 cows and buffaloes suffering from post partum-metritis with ampicillin (1 gm dissolved in 50/ml distilled water daily for 6-8 dose intrauterine. About 80.77 cows and 78.94% buffaloes responded very well with the treatment.

Korudhiiski et al. (1988) treated 16 cows after with acute catarrhal purulent endometritis once every third day with foam generating formulation of oxyteracycline or gentamicin or a combination of equal part of kanamycin and carbencillin. Isolates of bacteria obtained after 5, 8, 11 and 14 days after treatment were studied for antibiotic sensitivity and concluded that inadequate and indiscriminate chemotherapy lead to increased drug resistance.

Mates and Cosma (1988) treated 240 cows suffering from endometritis with 2-4 bougies of foaming oxytetracycline (OTC) into the uterus, together with i.m injection of tripedin on day 2 after

parturition and 2 more bougies of OTC on day 3. Endometritis was treated in a further 120 cows by implanting three OTC bougies after expulsion of the placenta. The two treatments were about equally effective in endometritis, suggesting that the parentral treatment with antibiotic could be dispensed with.

Murray *et al.* (1990) made a comparative study of the treatment of 306 severe moderate or mild cases of bovine endometritis in two calving seasons. The cases were treated with alfaprostol or an intrauterine antibacterial preparation or with a combination of both therapies. They showed that there was no significant difference between the efficacy of these treatments but a single injection of alfaprostol was effective in 74% cases. They conclude that the effectiveness of the treatment was related to the degree of self cure of endometritis after parturition, luteal activity at the time of treatment and farm management were the factors the affecting the health and condition of the cows.

Abdullah Rehman *et al.* (1991) found that post A.I. intrauterine infusion of gentamicin significantly increased the conception rates in endometritic buffaloes. Among buffaloes suffering from first degree of endometritis, the conception rate improved from 31.81 % (control) to 49.38% (treated).

Pateria *et al.* (1992) treated 72 buffaloes with endometritis and observed 100% recovery rate in the animals treated

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Pateria *et al.* (1992) treated 72 buffaloes with endometritis and observed 100% recovery rate in the animals treated

with oxytetracycline and nitrofurozone with urea, followed by Lugol's iodine (91.66), sulfonamide (75%) and hormonal preparation (58.33%) whereas conception rate after insemination at first estrous after treatment was highest in lugol's iodine treated animals (33.33%) followed by oxytetracycline, nitrofurazone with urea treated group (25%). However, no animal was found pregnant in the hormone treated group.

Mohanty *et al.*(1992) treated the endometritis in cows and heifers based on sensitivity test. Among five antibiotics viz., gentamicin, chloramphenicol, oxytetracycline, streptomycin and furazolidone, gentamicin was the most sensitive antimicrobial drug.

Ambrose and Pattabiraman (1993) studied *in-vitro* susceptibility of 77 bacterial isolates puerperal uterine infection of bovine and found that sensitivity was highest to cotrimoxazole (75.32%) and nitrofurantoin (62.34%) and lowest to penicillin to which 94.80% of the isolates were resistant. Further they observed that out of 47 animals treated with antibiotic, 68% recovered completely, 14.9% improved and 8.5% did not responded to treatment.

Brauner *et al.* (1993) given mysolfan (20 mg tergaride citrate) in a single i.m to 28 cows with acute endometritis (up to 20 days post-partum) and 50 with chronic endometritis. Treatment showed recovery in 10 days of the 68 subsequently inseminated, 40 became pregnant, 21 to the first insemination.

Koujan et al. (1996) obtained better recovery and conception rate with i.u. betadine treatment than that with septocid of 112 repeat breeder cows with the sign of endometritis or cervicitis.

Money (1996) treated the endometritis in cross-bred cows with pefloxacin 400 mg daily i.v. from 18.3.95 to 24.3.95 on the basis of culture sensitivity test of uterine discharge. He observe cure of endometritis with this treatment.

Rong-Roqiang *et al.* (1997) treated endometritis with enrofloxacin of 200 mg (injected in ovary of cows) and 90-100% was cured.

Singh (1997) tested antimicrobial agent to different isolates on his study on cows suffering from endometritis problem and treated endometritis by intrauterine route. Gentamicin found to be most sensitive (76.47%) followed by neomycin (52.94%), cephalexin (48.04%), ampicillin and cloxacillin (42.16%), tetracycline (24.50%) and septran (31.37%) and conception rate was highest in lixen treated group (69.23%). Overall conception rate was 63.86%.

Kotowski *et al.* (1998) treated endometritis with Metrisan (AN) in cows. Metrisan AN (Ampicillin + Neomycin) was used in the treatment of endometritis in 62 polish low land black and white cows. 12 cows in group I had purulent endometritis, while 20 cows in group II had endometritis without systemic changes. 30 cows in group III were healthy controls. Metrisan was used twice in cows in group I and

once in group II. Cows. Group I cows were also synchronized with prostaglandin. Pregnancy rate was 66.66%, 85 and 83.33% in the 3 group, respectively.

Misra *et al.* (1999) studied the efficacy of various available drugs for suboestrus in buffaloes of infectious origin. Intrauterine administration of enrocin (Enrofloxacin) enhanced the conception rate by 83.33% in such cases.

Bhaskar and Agrawal (2001) observed the therapeutic efficacy of enrofloxacin and metronidazole against endometritis by intrauterine route. The experiment was carried out in 15 recently calved buffaloes. The buffaloes were divided into three group A, B and C with 5 animals in each group. In group A, enrofloxacin 2.5% - 30ml was used similarly in group B, metronidazole 0.5% –30 ml was used and in group C, enrofloxacin (10%) -20ml + metroniadzole (0.5%)-25 ml was used. The conception rate in A, B, and C was 60, 40 and 80 percent,. respectively. So, they concluded that enrofloxacin is an effective therapy, followed by metronidazole. Usage of enrofloxacin and metronidazole combination is proposed for an effective therapy of endometritis.

Chapter-III

MATERIALS AND METHODS

MATERIALS AND METHODS

Experiments were carried out on cows which came for gynaecological check-up at the Department of Animal Reproduction, Gynaecology and Obstetrics of Bihar Veterinary College, Patna and from organised Khatals in and around Patna. In each case, history of the animal was recorded in relation to number of calving, number of artificial insemination or natural service done, oestrus cycle, whether regular or irregular. After obtaining the history of the animals, they were subjected to detailed gynaecological examinations to diagnose endometritis. Only those cases were taken up for the purpose of study which were having a continuous mucopurulent uterine discharge resembling second degree of endometritis.

In the present study, attempts were made for isolation of etiological agents, their antibiograms as well as treatment and conception rate in case of endometritis. Further, concentrations of enrofloxacin and gentamicin in plasma and uterine fluid at specific time intervals were estimated in few cases of endometritis as well as in case of healthy animals weighing between 265-410 kg. In order to achieve the above noted aims, studies were conducted in a total of 76 cases. Out of 76 cases, a total of 68 cases were of endometritis and rest 8 cases were of normal healthy animals. A total of 16 cases of

endometritis out of 68 cases of endometritis and 8 healthy animals were selected for the study of distribution of the above said antimicrobials.

For carrying out the present study in case of healthy (normal) cows and cows with endometritis, the following proforma were used.

Proforma - I

Anamnesis and case record of animals

1. Case No.
2. Date
3. Name of the owner
4. Address
5. Species
6. Breed
7. No. of calving
8. Last calving
9. Estrus cycle
10. Number of service
    (i) Natural
    (ii) Artificial
11. Time of heat
12. General Condition

Proforma -II

Special examination of animals

1. Vagina
2. Os Cervix
3. Discharge (colour, consistency, odour etc)
4. Cervix
5. Uterine horn
    (a) Left
    (b) Right
6. Oviduct
    (a) Left
    (b) Right
7. Ovaries
    (a) Left
    (b) Right
8. Diagnosis

Healthy animals – Healthy animals (Normal) were differentiated from endometritic cows on the basis of rectovaginal examination and history of the animals.

(i) **History** – Owner's were enquired regarding the last calving, whether it was a normal or assisted delivery; regarding regularity of oestrus cycle, regarding number of insemination or natural service tried and whether conceived or not.

(ii) **Rectal examination** – It was carried out to manipulate the genitalia. Tonic uterus, horns were coiled, normal thickness of uterine wall, uterine wall consistency was meaty normal, uterine discharge was clear transparent mucus, soft follicle & CL was regressed totally, loin/tail reflex was seen, clitoris massage reflex was seen and did not flate when milked etc.

Endometritic Cows - Endometritic cows were confirmed on the basis of rectovaginal examination and relevant history pertaining to diseases.

(i) **History** : Owner's were enquired regarding the last calving, whether it was a normal or assisted delivery; regarding regularity of oestrus cycle; regarding number of insemination or natural service tried; conceived or not conceived after A.I.; if the abnormal uterine discharge was apparent and had attracted the attention of owner's, they were asked regarding the time when they first noticed it.

(ii) **Speculum examination** : The vulva and perinium of the animal was washed and dried. The vaginal examination was carried out with help of a well sterilized speculum. In almost all the cases when the speculum was applied, a mucopurulent discharge was seen. The side wall of the vagina was examined for the presence of granules, inflammation or any other abnormality. The os-uteri were examined and found to be partially opened, enlarged and inflamed in most of the cases.

(iii) **Rectal examination** : It was carried out to manipulate the genitalia. Inflammation of os-uteri was confirmed by detectable enlargement in the size and induration of the organ. The horns of the uterus were examined after feeling the body. The horns were manipulated for any enlargement in their size – either unilateral or bilateral, tone and thickness of the wall. The fallopian tubes were then examined for any enlargement in their size, strictures and salpingitis.

Manipulation of the ovary was done for any persistent corpus luteum (CL) or cyst, their size and shape, and adhesion if any due to ovarian bursitis.

Cervicitis either in moderate or severe form was found invariably present along with endometritis. Both the cornua, in cases of endometritis, were generally found to be enlarged, doughy and thick walled.

Cases of chronic cervicitis with marked induration, salpingitis and adhesions of ovaries were not taken-up for the purpose of treatment to maintain uniformity.

Method of collection of samples

Uterine samples -

Uterine samples were collected in healthy cows and cows suffering from endometritis by the method as described by Dabas and Maurya (1988) with some minor modifications. The equipments

needed were an A.I. Gun with its sheath and a 20 ml glass syringe. The inseminating guns were wrapped in a craft papper and sterilized in hot air oven at 160^{0}C for one hour and were kept in dry cabinet till use. Factory sterilized polythene bag containing sheaths was kept in a clean, dry tray covered with a clean dry towel. The sheath was withdrawn one by one through a small opening at the time of use.

The animal from which sample to be collected was restrained properly in a travis. The vulva and perineum were washed with soap water and dried with a piece of sterilized gauze. Then rectified sprit was applied on the area with a cotton swab and allowed to dry. Taking other sterile routine precautions, the vulvar lips were spread by an assistant while inseminating gun along with sheath was passed through the vagina in rotating movement. By rectal palpation, the cervix and inseminating gun was manipulated until the tip of sheath was introduced in to the body of the uterus. The inseminating gun was then withdrawn leaving the sheath in the uterus. The glass syringe was fitted to the back end of the sheath so that it could become air tight. A negative pressure was applied by retracting the syringe plunger to withdraw the uterine content into the sheath. Since the uterine mucus was quite viscid, the syringe plunger was slowly and maximally retracted and held in position with slight movement in sheath for about 20 second.

The sheath with aspirated fluid was withdrawn along with fitted syringe again by spreading the vulvar lips and immediately the content was poured into sterilized test tubes over the flame of a burner. The samples was marked and brought to laboratory for further microbiological studies, identification of bacteria and sensitivity test. In few animals distribution studies of antimicrobials were also done on uterine samples collected at specific time interval after single i.v. administration of two drugs i.e. enrofloxacin and gentamicin. The dose rate for both the drugs were 5 mg/kg.

Blood samples (for distribution study)

Before collection of blood, hairs around the jugular vein on either side of neck of the animals were shaved and the area was cleaned with ether. The site was sterilized prior to each collection with rectified sprit. Blood samples were collected from jugular vein by venipuncture at the specific time intervals following drug administration in sterilized centrifuge tubes containing appropriate amount of sodium oxalate as anti-coagulant. The blood samples were centrifuged at 2500 rpm for 10 minutes for the separation of plasma. The plasma samples were kept in a refrigerator until assay was carried out. For the preparation of standards, normal plasma was also collected prior to drug administration.

Collection and processing of biological samples (for distribution study) –

The sample of biological fluids (plasma & uterine fluid) of healthy cows & cows suffering from endometritis were collected after single i.v. administration of enrofloxacin (5 mg/kg) and gentamicin (5 mg/kg). The sample of biological fluids (plasma & uterine fluid) of enrofloxacin group were collected at 0.5, 1, 2, 3, 4, 5, 6, 8, 12 & 24 h. While in case of gentamicin groups the sample of biological fluid were collected at 0.5, 1, 2, 4, 8, 12 & 24 h.

Media used and their preparation

1. Nutrient broth :

Nutrient broth was prepared from the readymade media manufactured by Hi-media, Mumbai (Appendix-I). The prepared broth was poured into sterilized test tubes and incubated at 37^0C for 24 hours to test the sterility of the media. Then the tubes were stored at 4^0C in a refrigerator for further use.

2. Nutrient Agar :

Nutrient agar plates and slants were prepared from the readymade media obtained from Hi-media, Mumbai (Appendix-II). The prepared media was then incubated at 37^0C for 24 hours to test the sterility and then stored in a refrigerator at 4^0C for further use.

3. MacConkey agar :

MacConkey agar plates were prepared from the readymade media obtained from Hi-media, Mumbai (Appendix-III). The plates were incubated at 37^{0}C for 24 hours to test the sterility and then stored in a refrigerator at 4^{0}C for further use.

4. Blood Agar :

Blood agar plates were prepared by adding 10% defibrinated sterile sheep blood to the nutrient agar media at 45^{0}C at the time of pouring. The plates were incubated at 37^{0}C for 24 hrs to test the sterility and then stored in a refrigerator at 4^{0}C for further use.

I. BACTERIOLOGICAL STUDIES

Processing of the samples

Samples collected were inoculated on blood agar plates and MacConkey's agar plates by streak method and incubated at 37^{0}C for 24 hours. After incubation, plates were examined for any types of colonial growth.

Organisms having colonial growth on blood agar plate were taken in pure form on nutrient agar slant and were incubated again at 37^{0}C for 24 hours for further study.

MacConkey's agar plates were examined for the presence of lactose fermenter (LF) and non-lactose fermenter (NLF) colonies. All the representative LF and NLF colonies were obtained in pure form on nutrient agar slants and were kept for further studies.

The mixed cultures were also purified and obtained in pure form by conventional method i.e. inoculated in nutrient broth and then streaked on nutrient agar plate. Pure representative colonies were then taken on nutrient agar slants and were incubated at 37^0C for 24 hrs. Growths obtained cultures were kept at 4^0C in a refrigerator for further study. Various types of organisms growing in different media, colonial morphology on different media were examined for their morphological characters and gram staining reactions.

Gram-positive cocci found in cluster, formed smooth, glistening, opaque, convex golden and/or white colour colonies on nutrient agar. These cocci resembling to *Staphylococci* were differentiated from genus *Micrococcus* using Hugh and Leifson test. Differentiations of pathogenic cocci to that of non-pathogenic cocci were performed by coagulase test. All coagulase positive *Staphylococci* were designated as *Stapphylococcus aureus* irrespective of pigment productions whereas coagulase negative were *Staphylococcus epidermidis*.

Gram-positive cocci were arranged in chains resembling to *Streptococci* and were formed pinhead colonies on blood agar plates. Clear zone of haemolysis was indicative of *Streptococcus pyogenes*, partial haemolysis indicated *Streptococcus viridens* and non-haemolysis was *Streptococcus salivaris*.

Gram-negative organisms both from pink colony (lactose fermenter) and yellow colony (non-lactose fermenter) on MacConkey's agar plates were studied for IMVC (indol, methyl red, voges-proskauer, citrate utilization test) and sugar fermentation tests using lactose, maltose, glucose, sucrose and mannitol to identify the organisms. Cultures were identified according to the classification of *Enterobacteriaceae* described by Edward and Ewing (1972).

Gram positive rods found in an overall plate resembling to Chinese letter appearance and clubbing at both poles (beaded appearance) were considered to be *Corynebacterium* spp.

Gram-negative, non-spore forming and motile form, smooth mucoid blue green colonies with musty smell, both oxidase and catalase positive but negative for Indol, M.R. and V.P. test were treated as *Pseudomonas aeruginosa.*

Test for identification of isolates :-

To identify the organisms some specific bacterial tests were performed as described by Cruickshank *et al.* (1975) which are as follows. The isolates were identified based on morphological and biochemical properties as described under Table-1.

1. Oxidase activity test : It was done by dropping oxidase reagent (1% aquous solution of tetra-methyl-p-phenylene diamine) on the filter

paper and rubbing the isolate on this moist surface. Development of dark purple colour on the paper within few second confirmed positive.

2. Catalase activity test : 4-5 drops of 18 hours broth culture was taken on a slide and a drop of 3% H_2O_2 was added over it. Production of gas bubbles indicates a positive reaction.

3. Coagulase test : 0.1 ml of 24 hours broth culture was added to 3 ml of diluted rabbit plasma (1 in 10) and incubated at 37°C for one to six hours. The tubes were observed for coagulase production at one, three and six hours interval. Presence of coagulase indicates positive result.

4. Haemolysis : *Streptococci*, grown on 10% sheep blood agar plate incubated at 37°C for 24 hours, were examined for haemolysis. A clear zone of haemolysis around the colonies was indicative of β–haemolysis whereas partial haemolysis immediately surrounding the colonies was called as α-haemolysis. No haemolysis was termed as σ–haemolysis.

5. Indol production test : 0.5 ml of Kovac's reagent was added in peptone water, inoculated and incubated at 37°C for 48 hours and shaken gently. Development of red colour in alcohol layer indicates the presence of indole.

6. Methyl red (M.R.) test : Glucose-phosphate-peptone-water medium was inoculated and incubated at 37°C for 48 hours. Few drops of 0.4% methyl red reagent was added. Red colour due to acidity confirms positive whereas in negative case it remains yellow.

7. Voges-Proskauer (V.P.) test : 1-2 drops of inoculum was dropped in tube containing glucose-phosphate-peptone-water and incubated at 37°C for 48 hours. Then 1 ml of 40% KOH and 3 ml of 5% Naphthol were added. A pinch of creatinine powder was added too and shaken well. A positive reaction was indicated by development of a pink colur in 2-5 minutes becoming crimson in 30 minutes.

8. Citrate utilization test : One drop of 24 hours broth culture was added to 5 ml of Koser's medium and incubated at 37°C for 48 hours. Utilization of citrate was indicated by growth and consequent turbidity of the medium. A control non-inoculated tube was incubated for comparison.

9. Sugar fermentation test : Test of culture for the liberation of acid and gas from sugars were performed in peptone water with a particular sugar added in such a way that the concentration of sugar became 1%. pH was determined with the help of Andrade's indicator. A Durham's tube was included to detect the presence of gas. Un-inoculated control tubes for each sugar were also incubated along with inoculated tubes to trace out any type of contamination. The tubes containing sugars like lactose, maltose, glucose, sucrose and manitol were arranged. A drop of 18 hours incubated peptone water was inoculated to each tube. The inoculated tubes were incubated for 24-72 hours and the production of acid and gas was noted on every

24 hours. The tube showing pink colours was considered positive for acid and presence of air bubbles in Durham's tubes was indicative for presence of gas.

10. Hugh and Leifson test : It was done to separate the two genera of *Staphylococcus* and *Micrococcus* on the ability of former genus to grow and to produce acid anaerobically from glucose. Hugh and Leifson medium was prepared. Duplicate sterilized tubes filled with medium to a depth of 2 cm were taken for each organism to be tested. The tubes were inoculated with a heavy inoculum by Stab method with the help of a straight plantinum wire. After inoculation, surface of each tube was covered with 1-2 cm layer of sterile liquid paraffin. All the tubes were incubated for 5-10 days and examined for acid production. *Staphylococcus* grew and formed acid throughout in both open and sealed tubes whereas *Micrococcus* produced acid in open tubes only and failed to grow and produce acid in the closed tubes.

11. Nitrate reduction test : The test strain was grown in broth medium containing 2% potassium nitrate for 96 hour at 37^0C. Presence of nitrite was tested by adding a few drops of test solution 'A' (containing 8 gm of 0.5% sulphanic acid in dilute sulphuric acid) and test solution 'B' (containing 6 ml of dimethyl α–naphthylamine in one litre of acetic acid) to about 2 ml of culture. Production of red colour denotes the presence of nitrites.

Table - 1

Properties of bacterial isolates for identification

Isolates	Basis of identification	
	Marphological characters	**Biochemical properties**
Escherichia coli	Gram-negative bacilli formed white glistening colonies on nutrient agar and pink colonies on MacConkey's agar	Lactose fermenter, production of gas from glucose, positive for indol and M.R. tests, negative for V.P and did not produce H_2S
Klebsiella pneumoniae	Gram-negative bacilli with pink mucoid culture growth similar to *Esch. coli*	Lactose fermenter, produced gas form glucose, positive for M.R, negative for indol, V.P. and H_2S
Staphylococcus aureus	Gram-positive cocci arranged in grapes like clusters. Produced cream or golden coloured, circular smooth colonies on blood agar.	Produced acid from glucose aerobically and anaerobically. Positive for V.P., Coagulate positive, Ferments lactose, maltose and mannitol.
Streptococcus pyogenes	Gram-positive cocci arranged in chains of varied length. Colonies were small smooth and haemolysis on blood agar.	Acidify the litmus but no coagulation. Ferments sugars with production of acid only.
Corynebacterium pyogenes	Gram-positive rods appeared as picture likened to Chinese lettering.	Produced acid from carbohydrates (Glucose, Maltose and Sucrose), negative to catalase, oxidase, urease, indol and nitrate reduction.
Proteus vulagris	Gram-negative rods with swarming growth on nutrient agar.	Urease positive, Indol positive, V.P, negative, fermenting maltose and produced H_2S.
Pseudomonas aeruginosa	Gram-negative with bluish pigment colonies gave a ground grass appearance	Oxidase positive, negative for indol, M.R., V.P. and not produced H_2S

***In-vitro* sensitivity test :**

In vitro antimicrobial sensitivity tests were performed by disc diffusion method as per Bauer *et al.* (1966). The organisms isolated were tested against three antimicrobial agents viz., cephalexin, gentamicin and enrofloxacin. These antimicrobial discs supplied by Hi-Media Laboratories Ltd. (India) were used (symbols assigned to each disc along with their concentrations was given in Table-2). The pure colony obtained from each sample was inoculated in a nutrient broth tube and incubated at 37^{0}C for 6 to 8 hours to obtain growth in log phase. The content of the tube was poured on nutrient agar plate and thoroughly spread over the entire plate to prepare lawn. The excess fluid was discarded. The plate was allowed to dry in incubator for 10 minutes. Then antimicrobial discs were placed into the plate accordingly to the symbol marked on bottom of the plate. The plates were incubated at 37^{0}C for 24 hours. The zone of inhibition was noted thereafter. In case of mixed culture, isolates were used separately for the antibiogram after obtaining into its single form. The result of the sensitivity was explained on the basis of the size of zone of inhibition.

Table-2

Antimicrobial disc used for in-vitro sensitivity along with concentration

Sl. No.	Name of Antimicrobials	Symbol	Concentration per disc (μg)
1.	Cephalexin	'Cs'	30
2.	Gentamicin	'G'	10
3.	Enrofloxacin	'En'	50

II. DISTRIBUTION STUDY OF ANTIMICROBIALS IN PLASMA AND UTERINE FLUID

Drug(s) / chemical used :-

Enrofloxacin (Enrodac-10®) and Gentamicin (Gentamicin®) were used in the present experiment. Enrodac-10 and Gentamicin injectable commercial preparations containing enrofloxacin in concentration of 100 $mg.ml^{-1}$ and gentamicin 40 $mg.ml^{-1}$ obtained as gift sample from Sarabhai Zydus Animal Health Care Limited and Karnattaka Antibiotic Pharmaceutical Enterprises, respectively were used.

The dose of enrofloxacin and gentamicin was 5 $mg.\ kg^{-1}$ body weight.

Animals :

The number of animals used for distribution study of enrofloxacin and gentamicin are given in Table-3.

Table-3

Number of animals and drugs used for distribution study

Name of drugs	Cows suffering from endometritis	Healthy cows
Enrofloxacin	8	4
Gentamicin	8	4
Total	16	8

Procedures adopted for the microbiological assay :

The concentrations of enrofloxacin and gentamicin in plasma and uterine fluid were determined by employing the standard cylinder plate bio-assay technique (Arret *et al.*, 1971). The details of the estimation methods are noted below.

Sterilization of glasswares, needle & porcelin assay cylinders :

All glassewares, needles and porcelin assay cylinders were washed with detergent solution in running tap water, rinsed with glass distilled water and then air dried. Test tubes, centrifuge tubes, vials, porcelain assay cylinders placed in vials and needles put in test tubes were plugged with cotton wool. Assay plates, pipettes and syringes were wrapped by paper. All these materials were sterilized in hot air oven at 160^{0}C for an hour.

Preparation of assay agar plates :

For estimation of concentrations of enrofloxacin and gentamicin in biological fluids, readymade antibiotic assay media of Hi-Media Bombay was used (Appendix-IV). Melted enrofloxacin & gentamicin antibiotic assay media (20 ml) was poured separately with aid of a sterile measuring cylinder into each of the separate sterile special assay plate kept on a horizontally plane surface to get uniform thickness of media. The plates were kept inside the incubator at 37^{0}C for 24 hr to ascertain any microbial contamination. The plates were then stored in a refrigerator until assay was carried out.

Preparation of organism :

The two test organisms, *E. coli* (ATCC 25922) for assay of enrofloxacin and *B. subtilis* (ATCC 6633) for assay of gentamicin were grown separately on the slants of culture tubes containing nutrient agar at 37^0C for overnight. Then it was stored under refrigeration. The organisms were transferred weekly to fresh media to maintain their normal activities.

Preparation of standard of enrofloxacin and gentamicin in biological samples :

The drug (enrofloxacin/gentamicin) was dissolved and diluted in sterile glass distilled water to have different strength viz., 80 µg/ml, 40 µg/ml, 20 µg/ml, 10 µg/ml, 5 µg/ml, 2 µg/ml, 1 µg/ml and 0.5 µg/ml. From each standard solution 0.1 ml was added to a sterile vial containing 0.9 ml of plasma / uterine fluid (collected from healthy animals before drug administration). This yield drug standards of 8 µg/ml and 4 µg/ml, 2 µg/ml, 1 µg/ml, 0.5 µg/ml, 0.2 µg/ml, 0.1 µg/ml and 0.05 µg/ml in the above noted biological fluid. These standards were used simultaneously with test samples in the assay plates for determination of the drug concentrations in test samples.

Assay procedure –

The quantitative estimation of enrofloxacin and gentamicin in biological samples were done by microbiological assay method (cylinder plate diffusion method) using *E. coli* (ATCC 25922) and *B. subtilis* (ATCC 6633) as the test organism, respectively.

The test organisms were grown in nutrient broth for 1 to 3 hours at 37°C until the growth was seen (turbid by naked eye). Enrofloxacin and gentamicin assay plates were flooded with the broth containing the organisms and excess broth was drained out. The plates were then dried in the incubator at 37°C for a period of about an hour. Sterile porcelain assay cylinders of uniform size were placed at appropriate distance along the circumference in the inoculated assay plates. Fifty microliters of standard solution of various strengths of the drug as well as test samples (biological fluid) viz. plasma and uterine fluids collected after drug administration were was poured in separate porcelin cylinder kept on the assay plate. The plates were left on the table for about 2 hr and than kept in the incubator at 37°C for overnight to allow the growth of organism. The mean diameter of the bacterial zone of inhibition produced by the standard of the drug was measured.

The concentrations of the drugs in different test samples of a biological fluid were estimated from the standard curve plotted from the zone of inhibition versus concentrations of the drug standards in semilog scale.

III. TREATMENT OF ENDOMETRITIS

A total of three chemotherapeutic agents were used for *in vitro* sensitivity against the isolates obtained from the sample. The animals were treated with the drug selected on the basis of sensitivity report. The medicines were given by two different route i.e. intrauterine (i.u.) given one dose alternate days for six days and

intravenous (i.v.) twice daily in case of gentamicin and once daily in case of enrofloxacin for five days. The dose and dosage interval of the above drugs for i.v. route was selected based on the distribution study of these antimicrobials. Cows were categorized in three treatment (T_1, T_2 & T_3) groups. Beside this, the control group comprising of 6 cows was given no treatment. Furthermore treatment groups (T_1 & T_2) subdivided in two treatment groups on the basis of route of administration of drugs i.e. T_1a, T_1b & T_2a, T_2b. No subdivision in T_3 group where only one route i.e. i.u. infusion alone was done. The drug along with their composition, route of administration and dose were noted below (Table-4).

Table-4

Composition, route of administration and dose of antimicrobials

Group	Drug	Route	Dose & Dosage schedule
Control	----	----	----
Treatment (T_1a)	Enrofloxacin 10% w/v (Enrodac-10® Sarabhai zydus)	i.v.	5 mg/kg body weight given once daily for five days.
(T_1b)		i.u.	5 ml (500mg) dissolved in 15 ml distilled water and given alternate days (six doses)
Treatment T_2a	Gentamicin sulphate 40 mg/ml (Gentamicin®) - (Karnataka Antibiotic)	i.v.	5 mg/kg body weight given twice daily for five days
T_2b		i.u.	5 ml (200mg) dissolved in 15 ml distilled water and given alternate day (six doses)
Treatment T_3	Cephalexin 7.5% W/W (Lixen®-Agrivet India)	i.u.	10 gm desolved in 20 ml distilled water and given alternate day (six doses)

DOSE SCHEDULE :

Intrauterine (i.u.) therapy was administered alternate day for 6 treatment. Parenteral (i.v.) therapy was administered twice daily for five days in case of gentamicin and once daily for five days in case of enrofloxacin.

Table-5 shows the number of animals kept in each group. The treatment was started from the date of the sensitivity report.

Table-5

Showing group-wise number of animals for the treatment

Treatment group (No. of animal)	No of animal treated	route of administration of drug	Drug used	Retreatment
C(6).	No treatment	------	-----	-----
T_1(36)	16 (T_1a)	i.v.	Enrofloxacin	1
	20 (T_1b)	i.u.	-do-	4
T_2(20)	8 (T_2a)	i.v.	Gentamicin	1
	12 (T_2b)	i.u.	-do-	3
T_3(6)	6	i.u.	Cephalexin	2
Total	62			11

During the course of the treatment, the drugs were given in strict sterile condition. For i.u. therapy, disposable sterile polythene catheter was used and introduced into the uterus with the help of sterilized metal gun.

For parentral therapy, hairs around the jugular vein on either side of the neck of the animals were shaved. The area was cleaned with ether and the site was sterilized prior to each administration with rectified sprit.

The animals were re-examined in subsequent estrus. Discharge was examined and samples were again taken for bacteriological study. Animals found clinically diseased free after treatment were then inseminated with frozen semen. Owners of the animals were cautioned to restrict their animals for natural service and were advised further to bring their animals again after 45-60 days for pregnancy diagnosis. The animals free from bacteria and even failed to conceive on first insemination and exhibited symptom of estrus in next cycle were observed. Such animals were followed up to 3rd insemination and diagnosed for being pregnant or not.

The animals in control group (cows suffering from endometritis), given no treatment were inseminated in three subsequent estrus and examined for conception. The data of the results were subjected to statistical analysis as per the method described by Snedcor and Cochran (1967).

Chapter-IV

RESULTS

RESULT

I. BACTERIOLOGICAL STUDIES OF ENDOMETRITIS :

Animals brought to the out door clinic of the Department of Animal Reproduction, Gynaecology and Obstetrics, Bihar Veterinary College, Patna-14 having problems of infertility and abnormal vaginal discharge were examined.

An effort was made to isolate, identify and characterize the different species of bacteria which were found in uterine discharge of cows suffering from endometritis. *In-vitro* sensitivity test was performed against each bacterial isolate to know the pattern of sensitivity towards the antimicrobials to be used.

I.A. Isolation of bacteria :

The studies were conducted on 68 cows suffering from endometritis. Bacterial isolates were recorded in 68 cases. Out of 68 samples, 58 sample were single isolates and rest 10 samples were mixed culture and bacteria from 68 samples were isolated and identified as per the method described under the chapter "Materials and Methods". Different bacteria obtained from uterine samples collected from the animals suffering from endometritis have been presented in Table-6 and Fig. 1.

Table-6

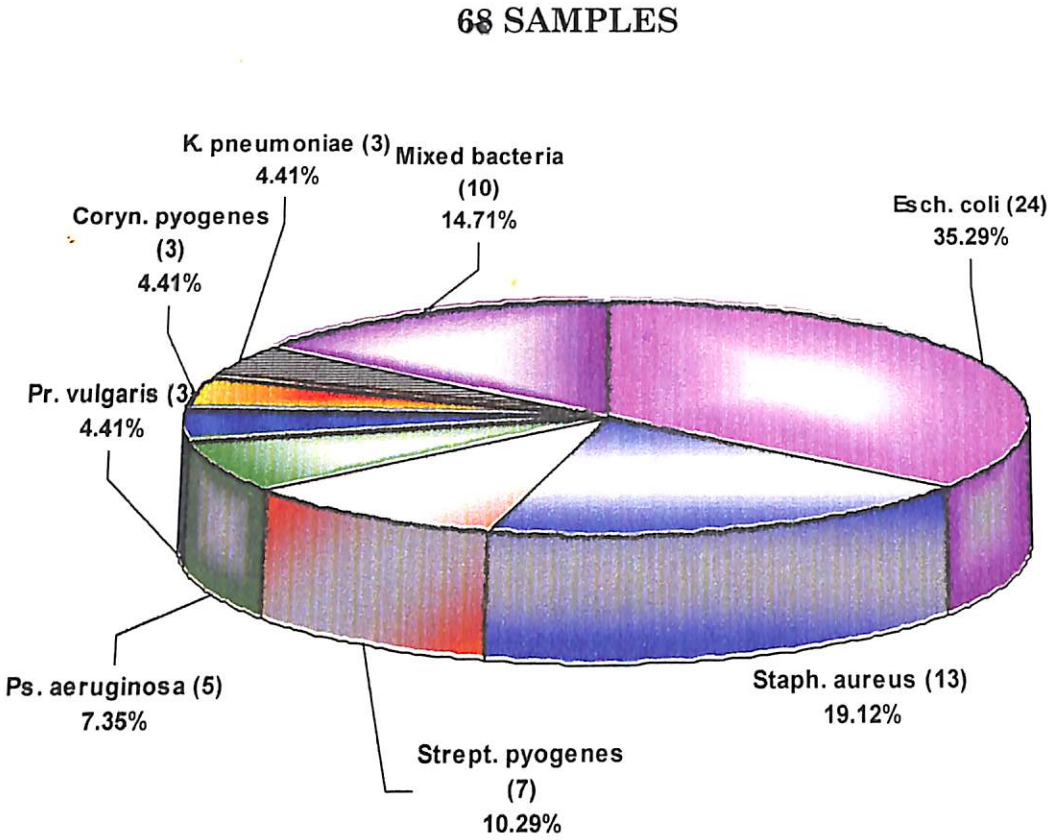
Different isolates obtained from uterine samples of cow suffering from endometritis.

Name of isolate	No. of Samples with pure and mixed isolates	Percentage	x^2 at 7 d.f
Esch. coli	24	35.29	43.54**
Staph. aureus	13	19.12	
Strep. pyogenes	7	10.29	
Ps. aeruginosa	5	7.35	
Pr. vulgaris	3	4.41	
Coryn. pyogenes	3	4.41	
K. pneumoniae	3	4.41	
Mixed bacteria	10	14.71	
Total	68		

**= Significant, $p<0.01$

It is evident from Table-6 and Fig-1 that 58 (85.29%) samples had single type of organism whereas 10 (14.71%) of the samples were of mixed type. *Esch. coli* alone was found maximally in 24 (35.29%) samples followed by *Staph. aureus* in 13 (19.12%), *Strep. pyogenes* in 7 (10.29%), *Ps. aeruginosa* 5 (7.35%) and *Pr. vulgaris*, *Coryne. pyogenes* and *K. pneumoniae* were least present i.e. in 3 cases (4.41%) each. Chi-square test reveals that there is a significant difference in the percentage of occurrence of bacteria in cases of endometritis and *Esch. coli* was the predominant organism followed by *Staph. aureus*.

Fig.1. *Different isolates obtained from uterine samples of cows suffering from endometritis*



Note : Figures in parantheses denote number of isolates

Bacterial isolates found in mixed culture were separated in single form and grouped there after on the basis of their combination. Only two types of isolates were obtained from each of the mixed culture, which have been shown in Table-7.

Table-7

Mixed isolates obtained from uterine samples of cows suffering from endometritis.

Combination of isolates	**No. of Samples**	**Percentage**	x^2at3d.f.
Esch. coli + *Staph. aureus*	4	40	2^{NS}
Esch. coli + *Strept. pyogenes*	3	30	
Staph. aureus + *Ps. aeruginosa*	2	20	
Staph. aureus + *Pr. vulgaris*	1	10	
Total	10	100%	

NS = Non-significant.

Combination of *Esch. coli* and *Staph. aureus* was predominant and found in 4 (40%) cases. *Esch. coli* with *Strept. Pyogenes*, *Staph. aureus* with *Ps. aeruginosa* and *Staph. aureus* with *Pr. vulgaris* combinations were found in 3 (30%), 2 (20%) and 1 (10%) cases, respectively. Chi-square test reveals non-significant difference among the combination of bacteria in mixed isolates in cases of endometritis.

A total of 78 isolates (58 from single and 20 from mixed culture) were obtained from 68 samples of cows, which has been

depicted in Table-8 and Fig.2. Numbers of different isolates in decreasing order were noted to be *Esch. coli* (31), *Staph. aureus* (20), *Strept. pyogenes,* (10), *Ps. aeruginosa* (7), *Pr. vulgaris* (4) *Coryn. pyogenes* (3) and *K. pneumoniae* (3) as shown in Table-8 and Fig.2.

I.B. Sensitivity Test

In-vitro sensitivity tests were performed by using different antimicrobial agents such as cephalexin, gentamicin and enrofloxacin. The antimicrobial discs were obtained from Hi-media, Mumbai. The concentration of the drug per disc was presented in Table-2 under 'Materials and Methods'. The result of antimicrobials sensitivity tests of different isolates from cows against the various antimicrobial agents has been depicted in Table-9 and Fig-3. It is evident from the table that most of the isolates were found sensitive to more than one drug and none of them were found completely resistant to all the drugs used. Enrofloxacin (73.07%) was found to be the most effective drug followed by gentamicin (67.94%) and cephalexin (60.25%).

Cephalexin showed the maximum sensitivity (75%) to *Pr. vulgaris* but resistant to *C. pyogenes* while the minimum sensitivity (33.33%) was against the *K. peneumonaie.* In case of gentamicin, maximum sensitivity (74.19%) against *Esch. coli* and minimum (50%) to both *Strep. pyogenes* and *Pr. vulgaris* were noted. In case of enrofloxacin, maximum sensitivity (77.41%) was seen against *Esch. coli* while minimum sensitivity (66.67%) was seen against both *K. pneumoniae* and *C. pyogenes.*

Fig.2. *Different isolates obtained from uterine samples of cows suffering from endometritis*

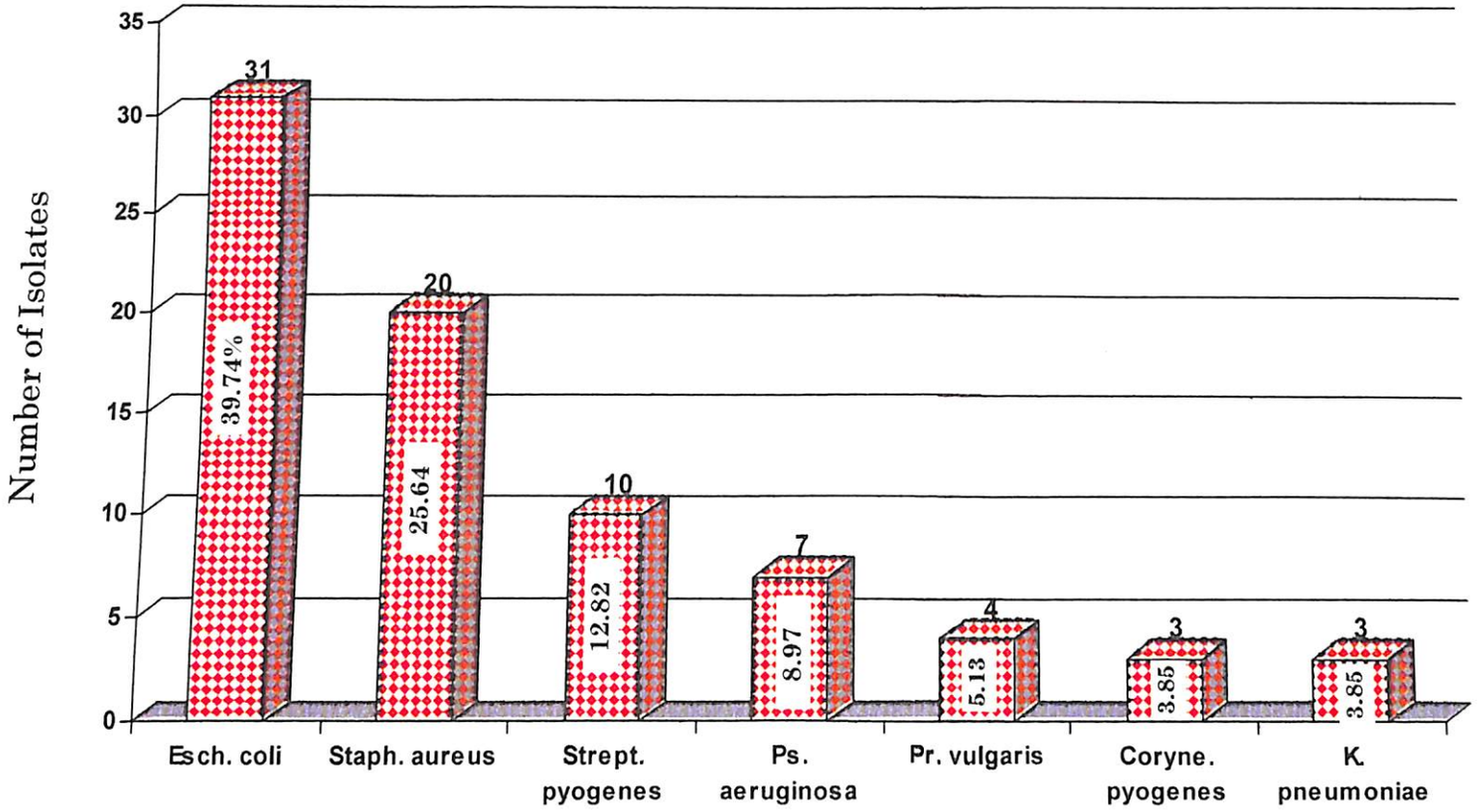


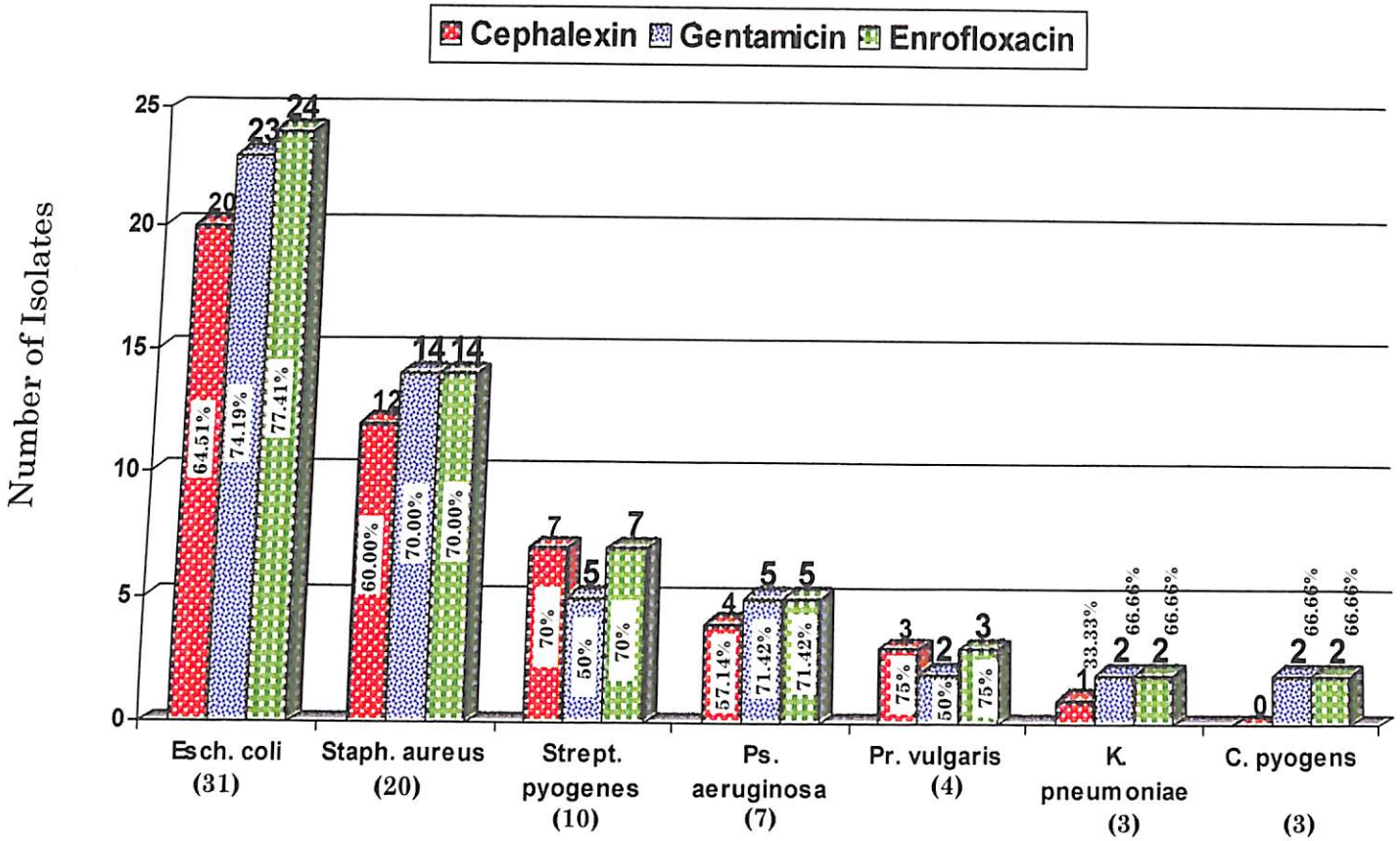
Table – 8

No. of isolates obtained from uterine samples of cow suffering from endometritis

of samples xamined	No. of samples of single isolates	No. of samples of mixed isolates	Name of organism	Sample with single isolate (No.)	Sample with mixed isolates (No.)	Total No. of isolates both from single & mixed (No.) (%)	X^2 at 6.d.
68	58	10	*Esch. Coli*	24	7	31 (39.74)	60.62**
			Staph. aureus	13	7	20 (25.64)	
			Strept. pyogenes	7	3	10 (12.82)	
			Ps. aeruginosa	5	2	7 (8.97)	
			Pr. vulgaris	3	1	4 (5.13)	
			Coryne. pyogenes	3	---	3 (3.85)	
			K. pneumoniae	3	---	3 (3.85)	
			Total	58	20	78	

** p<0.01

Fig.3. *In vitro sensitivity of isolates from uterine samples of cow*



* Figure in parenthesis denote number of isolates

Table – 9

In-vitro sensitivity test

Name of isolate	No. of isolates tested	NO. OF ISOLATES SENSITIVE TO ANTIMICROBIALS		
		Cephalexin	Gentamicin	Enrofloxacin
Esch. Coli	31	20 (64.51%)	23 (74.19%)	24 (77.41%)
Staph. aureus.	20	12 (60%)	14 (70%)	14 (70%)
Strept. pyogenes	10	7 (70%)	5 (50%)	7 (70%)
Ps. aeruginosa	7	4(57.14%)	5 (71.42%)	5 (71.42%)
Pr. vulgaris	4	3 (75%)	2 (50%)	3 (75%)
K. pneumoniae	3	1 (33.33%)	2 (66.66%)	2 (66.66%)
C. pyogenes	3	0 (()%)	2 (66.66%)	2 (66.66%)
Total	78	47 (60.25%)	53 (67.94%)	57 (73.07%)

II. DISTRIBUTION STUDY OF ANTIMICROBIALS IN PLASMA AND UTERINE FLUID

A. Distribution study on healthy animal

1. Enrofloxacin

(a) Plasma levels

The plasma drug concentrations of enrofloxacin in healthy cows after i.v. administration (5 mg/kg) are presented in Table 10 and Fig. 4. The drug was present in plasma with a mean of 4.67±0.23 μg/ml at 0.5 h, which was declined with time and was present in all animals up to 12 h (0.17±0.05 μg/ml). The therapeutic concentration (≥ 0.12μg/ml) was maintained up to 12 h in all animals.

Table - 10

Concentrations of enrofloxacin (μg/ml) in plasma of healthy cows after its iv. administration @ 5 mg/Kg.

Time (hour)	Animal Number				Mean±S.E.M.
	1	2	3	4	
0.5	5.15	4.41	4.98	4.13	4.67±0.23
1	4.60	3.98	4.02	3.12	3.93±0.30
2	3.40	2.67	2.89	2.12	2.77±0.27
3	2.46	2.05	2.22	1.69	2.11±0.16
4	1.71	1.59	1.28	0.93	1.38±0.17
5	1.06	0.92	0.87	0.69	0.89±0.08
6	0.87	0.64	0.60	0.52	0.66±0.08
8	0.68	0.59	0.41	0.33	0.50±0.08
12	0.32	0.14	0.11	0.10	0.17±0.05
24	ND	ND	ND	ND	---

ND = Non Detectable.

(b) Uterine fluid levels

Enrofloxacin appeared in uterine fluid only two out of four animals at 0.5 h whereas it appeared in all animals at 1 h with a mean of 0.11±0.02 µg/ml (Table 11 and Fig. 4). The drug reached its mean peak concentration of 2.20±0.08 µg/ml at 4 h. The drug was present in all animal up to 12 h and mean concentration was noted to be 0.23±0.05 µg/ml. The drug was detectable only in two out of 4 animals at 24 h. The therapeutic concentration (≥ 0.12µg/ml) was maintained from 2 to 12 h.

Table - 11

Concentrations of enrofloxacin (µg/ml) in uterine fluid of healthy cows after its i.v. administration @ 5 mg/Kg.

Time (hour)	Animal Number				Mean±S.E.M.
	1	2	3	4	
0.5	0.00	0.05	0.00	0.07	0.03±0.02
1	0.08	0.13	0.07	0.14	0.11 ± 0.02
2	0.31	0.39	0.28	0.42	0.35±0.03
3	0.99	1.32	1.12	1.96	1.35±0.22
4	2.02	2.35	2.31	2.12	2.20±0.08
5	1.31	1.41	1.51	1.10	1.33±0.09
6	0.98	0.99	1.02	0.89	0.97±0.03
8	0.66	0.52	0.67	0.47	0.58±0.05
12	0.35	0.27	0.15	0.13	0.23±0.05
24	0.05	0.06	ND	ND	0.03±0.02

ND = Non Detectable.

(c) Uterine fluid to plasma ratio

Table-12 presents the uterine fluid to plasma ratio of enrofloxacin in healthy cows. The data of this table shows >1 from 4 to 12 h which denotes that the drug penetrates into a greater amount in uterine tissues in healthy cows. Thus, the drug crosses the uterine barrier easily in healthy animals.

Table - 12

Uterine fluid to plasma ratio of enrofloxacin in healthy cows after its i.v. administration @ 5 mg/Kg

Time (hour)	Animal Number				Mean±S.E.M.
	1	2	3	4	
0.5	0.00	0.01	0.00	0.02	0.008±0.005
1	0.02	0.03	0.02	0.04	0.03±0.005
2	0.09	0.15	0.10	0.20	0.14±0.03
3	0.40	0.64	0.50	1.16	0.68±0.17
4	1.18	1.48	1.80	2.28	1.69±0.24
5	1.24	1.53	1.74	1.59	1.53±0.10
6	1.12	1.55	1.70	1.71	1.52±0.14
8	0.97	0.88	1.63	1.42	1.23±0.18
12	1.09	1.57	1.36	1.30	1.33±0.10
24	ND	ND	ND	ND	---

ND = Non Detectable.

2. *Gentamicin*

(a) Plasma levels

The concentrations of gentamicin in plasma obtained at various time intervals are shown in Table-13 and Fig-5. The mean concentration of gentamicin in plasma at 0.5 h was noted to be 8.94±0.31μg/ml. The drug was present only up to 12 h in all animals with a mean of 0.71±0.05 μg/ml at 12 h. The mean therapeutic concentration of >4 μg/ml was maintained up to 2 h only.

Table - 13

Concentrations of gentamicin (μg/ml) in plasma of healthy cows after its iv. Administration @ 5 mg/Kg.

Time (hour)	Animal Number				Mean±S.E.M.
	1	2	3	4	
0.5	8.12	9.58	8.85	9.21	8.94±0.31
1	5.98	7.76	6.79	7.31	6.96±0.38
2	4.71	4.31	5.12	5.31	4.86±0.22
4	2.61	3.04	2.74	2.98	2.84±0.10
8	1.21	1.73	1.34	1.75	1.51±0.14
12	0.61	0.78	0.65	0.80	0.71±0.05
24	ND	ND	ND	ND	---

ND = Non Detectable.

(b) Uterine fluid levels

The concentrations of gentamicin in uterine fluid of healthy cows after i.v. administration of 5mg/kg are presented in Table-14 and Fig-5. The drug was present with a mean concentration of 1.16±0.16 μg/ml at 0.5h. The drug reached its mean peak concentration of 6.25 ± 0.44 μg/ml at 4h. The drug was detectable in all animals up to 24 h with a mean of 0.60 ± 0.19 μg/ml. The mean therapeutic concentration of ≥ 4 μg/ml was maintained from 4 to 8 h in healthy cows.

Table - 14

Concentrations of gentamicin (μg/ml) in uterine fluid of healthy cows after its i.v. administration @ 5 mg/Kg

Time (hour)	Animal Number				Mean±S.E.M.
	1	2	3	4	
0.5	0.98	1.58	1.22	0.84	1.16±0.16
1	1.96	2.74	2.32	1.72	2.19±0.22
2	2.34	3.96	3.52	3.02	3.21±0.35
4	4.96	6.89	6.52	6.62	6.25±0.44
8	3.92	4.73	4.25	4.03	4.23±0.18
12	1.72	2.96	2.02	2.90	2.40±0.31
24	0.14	0.92	**0.45**	0.90	0.60±0.19

(c) Uterine fluid to plasma ratio

Table -15 presents the values of uterine fluid to plasma ratio of gentamicin in healthy cows at various time intervals. A mean ratio of 0.12 ± 0.01 was obtained at 0.5 h, which increased with time and the maximum ratio of 3.33 ± 0.22 was noted at 12 h.

Table – 15

Uterine fluid to plasma ratio of gentamicin in healthy cows after its i.v. administration @ 5 mg/Kg

Time (hour)	Animal Number				Mean±S.E.M.
	1	2	3	4	
0.5	0.12	0.16	0.14	0.09	0.12±0.01
1	0.32	0.35	0.34	0.24	0.31±0.02
2	0.50	0.91	0.69	0.57	0.66±0.08
4	1.90	2.27	2.38	2.22	2.19±0.10
8	3.23	2.73	3.17	2.30	2.86±0.21
12	2.81	3.80	3.10	3.62	3.33±0.22
24	--	---	---	---	---

B. Distribution study in endometritis cases

1. Enrofloxacin

(a) Plasma levels

The plasma drug concentrations of enrofloxacin in endometritic cows after i.v. administration (5mg/ kg) have been presented in Table 16 and Fig-4. The drug was present in plasma with a mean of 3.47±0.21 μg/ml at 0.5 h, which was declined with time and was present in all animals up to 8 h (0.31 ±0.04 μg/ml). The mean therapeutic concentration (≥ 0.12 μg/ml) was maintained up to 12 h.

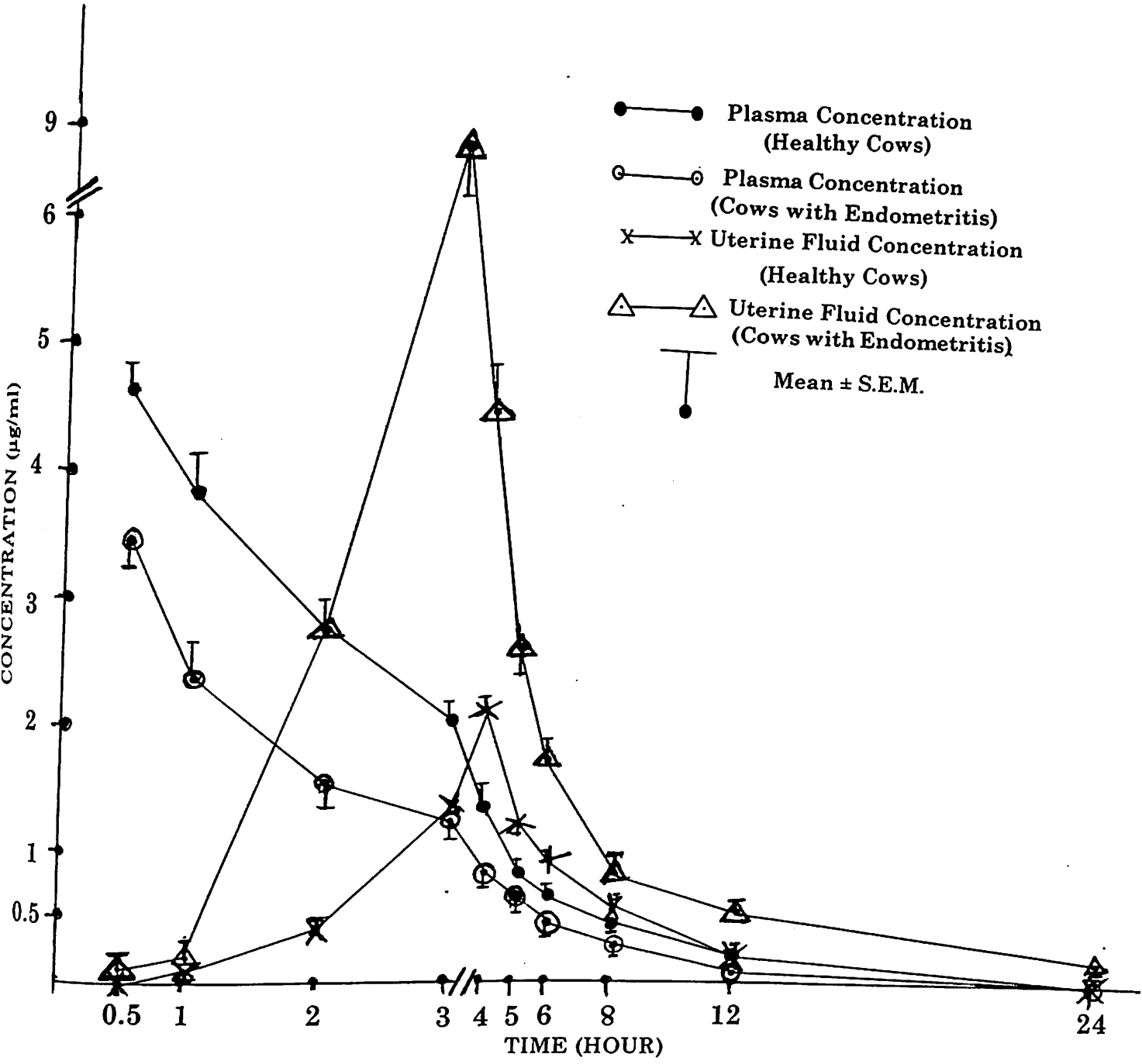
Table - 16

Concentrations of enrofloxacin (μg/ml) in plasma of cows suffering from endometritis after its i.v. administration @ 5 mg/Kg.

Time (hours)	Animal Number								Mean±S.E.M.
	1	2	3	4	5	6	7	8	
0.5	4.51	3.41	3.73	3.17	4.01	2.98	2.74	3.21	3.47±0.21
1	3.62	2.98	2.39	2.12	3.34	1.21	1.31	2.56	2.44±0.31
2	2.21	2.03	1.81	1.75	2.52	0.85	0.92	1.04	1.64±0.22
3	1.74	1.52	1.42	1.34	1.75	0.71	0.79	0.75	1.25±0.16
4	1.01	0.98	1.12	1.03	1.31	0.59	0.63	0.65	0.92±0.09
5	0.74	0.65	0.85	0.74	0.97	0.43	0.49	0.51	0.67±0.07
6	0.63	0.54	0.59	0.52	0.62	0.24	0.31	0.35	0.48±0.05
8	0.45	0.42	0.38	0.32	0.39	0.11	0.19	0.22	0.31±0.04
12	0.19	0.18	0.17	0.11	0.21	0.00	0.07	0.11	0.13±0.03
24	0.00	0.09	0.00	0.05	0.10	0.00	0.00	0.06	0.04±0.02

Figure 4 : Showing concentrations of enrofloxacin in plasma and uterine fluid of healthy cows and cows suffering from endometritis

Figure- 4


Plasma Concentration
(Healthy Cows)
Plasma Concentration
(Cows with Endometritis)
Uterine Fluid Concentration
(Healthy Cows)
Uterine Fluid Concentration
(Cows with Endometritis)
Mean ± S.E.M.
CONCENTRATION (µg/ml)
9
6
5
4
3
2
1
0.5
0.5
1
2
3
4
5
6
8
12
24
TIME (HOUR)

(b) Uterine fluid levels

Table-17 and Fig.-4 reveal the concentrations of enrofloxacin in endometritic cows after i.v. administration of 5mg/kg. The drug was detectable even at 0.5 h in all animals with a mean concentration of 0.11 ± 0.01 µg/ml. The drug reached its mean peak concentrate of 9.00 ± 0.73 µg/ml at 3h. The drug was present in all animals up to 24 h and the mean concentration at 24 h was noted to be 0.15 ± 0.02 µg/ml. The therapeutic concentration (≥ 0.12µg/ml) was maintained from 1 h to 24 h.

Table - 17

Concentrations of enrofloxacin (µg/ml) in uterine fluid of cows suffering from endometritis after its i.v. administration @ 5 mg/Kg.

Time (hours)	Animals Number								Mean±S.E.M
	1	2	3	4	5	6	7	8	
0.5	0.06	0.09	0.12	0.13	0.10	0.11	0.11	0.12	0.11±0.01
1	0.12	0.16	0.27	0.38	0.17	0.17	0.18	0.30	0.22±0.03
2	1.96	2.21	3.12	3.92	2.43	2.61	2.96	3.05	2.78±0.22
3	6.73	7.41	9.62	10.96	11.01	8.62	11.42	6.25	9.00±0.73
4	4.25	4.51	5.12	5.02	4.75	4.36	6.12	2.32	4.56±0.38
5	3.01	2.96	3.12	3.41	2.25	2.31	2.96	1.92	2.74±0.18
6	1.93	1.86	2.01	2.17	1.65	1.67	1.93	1.01	1.78±0.13
8	0.98	1.01	1.04	0.99	0.87	0.91	0.99	0.55	0.92±0.06
12	0.72	0.82	0.75	0.65	0.55	0.49	0.56	0.32	0.61±0.06
24	0.12	0.21	0.09	0.25	0.14	0.16	0.18	0.05	0.15±0.02

(c) Uterine fluid to plasma ratio

The uterine fluid to plasma ratio of enrofloxacin in endometritic cows is shown in Table-18. A ratio >1 was noted from 2 to 24h which denotes that the drug penetrates into uterine tissues in greater amount in cows suffering from endometritis. Thus, the drug is expected to cross the uterine barrier easily in endometritic cows.

Table – 18

Uterine fluid to plasma ratio of enrofloxacin in endemetritis suffering cows.

Time (hours)	Animal Number								Mean±S.E.M
	1	2	3	4	5	6	7	8	
0.5	0.02	0.03	0.04	0.04	0.03	0.04	0.04	0.03	0.03±0.003
1	0.03	0.05	0.12	0.17	0.05	0.15	0.14	0.12	0.10±0.02
2	0.89	1.09	1.73	2.24	0.97	3.07	3.21	2.93	2.02±0.35
3	3.87	4.88	6.78	8.17	6.30	12.15	14.46	8.33	8.12±1.28
4	4.21	4.61	4.58	4.87	3.62	7.39	9.72	3.57	5.32±0.76
5	4.07	4.56	3.67	4.60	2.32	5.37	3.94	3.77	4.03±0.32
6	3.06	3.44	3.41	4.18	2.66	6.95	3.19	2.88	3.72±0.49
8	2.17	2.40	2.73	3.10	2.23	8.27	5.21	2.50	3.58±0.76
12	3.79	4.56	4.42	5.91	2.62	0.00	8.00	2.91	4.03±0.83
24	0.00	2.33	0.00	5.00	1.40	0.00	0.00	0.83	1.19±0.62

2. Gentamicin

(a) Plasma levels

Table-19 and Fig.-5 show the mean concentrations of gentamicin in plasma after its i.v. administration (5mg/kg). The drug at 0.5 h was noted to be 8.51 ± 1.19 μg/ml. The drug was present only up to 12 h in all animals with a mean of 0.30 ± 0.04 μg/ml. The mean therapeutic concentration of ≥ 4μg/ml was maintained up to 1 h only.

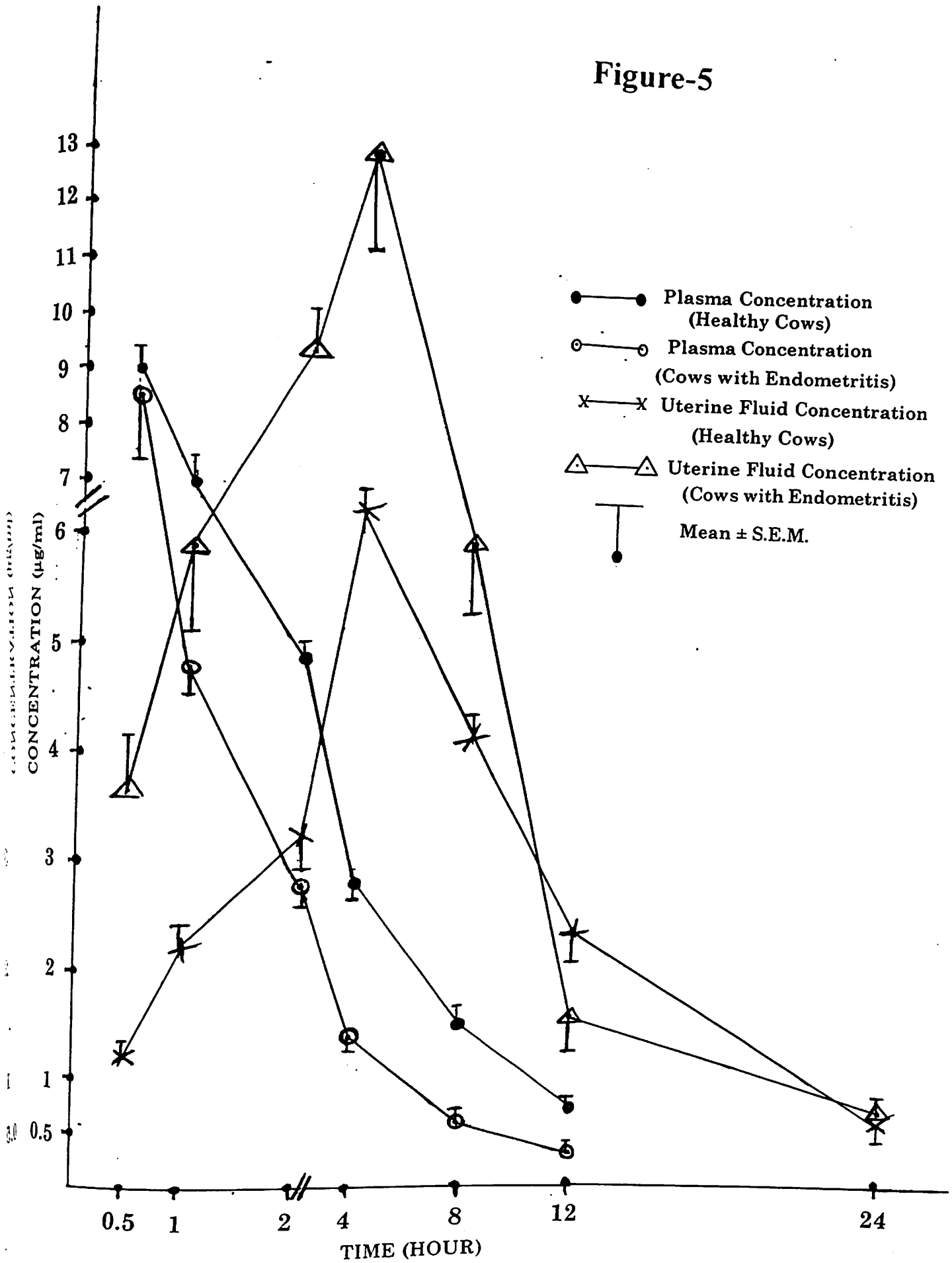
Table - 19

Concentrations of gentamicin(μg/ml) in plasma of cows suffering from endometritis after its i.v. administration @ 5 mg/Kg.

Time (hours)	Animals Number								Mean±S.E.M.
	1	2	3	4	5	6	7	8	
0.5	5.62	14.55	8.15	9.00	12.35	6.12	5.15	7.12	8.51±1.19
1	4.90	5.78	4.22	5.15	5.42	4.12	3.98	4.41	4.75±0.23
2	2.49	3.74	2.86	3.00	3.65	2.12	2.12	2.51	2.81±0.22
4	1.02	1.88	1.26	1.65	1.78	1.02	0.98	1.35	1.37±0.13
8	0.42	0.82	0.52	0.57	0.72	0.39	0.25	0.71	0.55±0.07
12	0.22	0.45	0.32	0.36	0.40	0.16	0.12	0.38	0.30±0.04
24	ND	ND	ND	ND	ND	ND	ND	ND	-----

Figure 5 : Showing concentrations of gentamicin in plasma and uterine fluid of healthy cows and cows suffering from endometritis

Figure-5


13
12
11
10
9
8
7
6
5
4
3
2
1
0.5
CONCENTRATION (µg/ml)
0.5
1
2
4
8
12
24
TIME (HOUR)
Plasma Concentration (Healthy Cows)
Plasma Concentration (Cows with Endometritis)
Uterine Fluid Concentration (Healthy Cows)
Uterine Fluid Concentration (Cows with Endometritis)
Mean ± S.E.M.

(b) Uterine fluid levels

The concentrations of gentamicin in uterine fluid of cows with endometritis after i.v. administration of 5 mg/kg are presented in Table-20 and Fig-5. The drug was present with a mean concentration of 3.66 ± 0.59 μg/ml at 0.5 h. The drug reached its mean peak concentration of 13.17 ± 1.93 μg/ml at 4 h. The drug was detectable in all animals up to 24 h with a mean 0.68 ± 0.14 μg/ml. The mean therapeutic concentration of ≥ 4μg/ml was maintained from 1 to 8 h in cows with endometritis.

Table - 20

Concentrations of gentamicin (μg/ml) in uterine fluid of cows suffering from endometritis after its i.v. administration @ 5 mg/Kg.

Time (hours)	Animals Number								Mean±S.E.M
	1	2	3	4	5	6	7	8	
0.5	5.62	6.85	2.82	3.16	2.12	2.85	3.02	2.80	3.66±0.59
1	8.52	10.50	4.32	5.86	3.14	4.76	5.75	4.56	5.93±0.86
2	10.28	14.22	8.16	9.12	7.13	8.01	9.62	9.09	9.45±0.77
4	14.55	26.04	10.25	12.28	9.86	10.21	12.15	10.01	13.17±1.93
8	6.44	10.52	4.85	5.12	3.92	4.75	6.96	5.72	6.04±0.73
12	1.26	4.10	0.78	1.05	0.52	0.96	2.32	1.92	1.61±0.41
24	0.73	1.25	0.22	0.82	0.31	0.21	1.00	0.91	0.68±0.14

(c) Uterine fluid to plasma ratio

Table-21 shows the values of uterine fluid to plasma ratio of gentamicin in cows suffering from endometritis. A mean ratio of 0.47 ± 0.09 was obtained at 0.5 h that increases with time up to 8 h and the maximum ratio of 12.50 ± 2.45 was noted at 8 h.

Table – 21

Uterine fluid to plasma ratio of gentamicin in cows suffering from endometritis.

Time (hours)	Animals Number								Mean±S.E.M
	1	2	3	4	5	6	7	8	
0.5	1.00	0.47	0.35	0.35	0.17	0.47	0.59	0.39	0.47±0.09
1	1.74	1.82	1.02	1.14	0.58	1.16	1.44	1.03	1.24±0.14
2	4.13	3.80	2.85	3.04	1.95	3.78	4.54	3.62	3.46±0.29
4	14.26	13.85	8.13	7.44	5.54	10.01	12.40	7.41	9.88±1.16
8	15.33	12.83	9.33	8.98	5.44	12.18	27.84	8.06	12.50±2.45
12	5.73	9.11	2.44	2.92	1.30	6.00	19.33	5.05	6.49±2.03

C. **Comparison of distribution of antimicrobials between healthy and cows suffering from endometritis after its i.v. administration.**

1. *Enrofloxacin*

(A) Plasma levels

Comparative plasma concentrations of enrofloxacin in healthy cows and cows suffering from endometritis after its i.v administration of 5mg/kg are shown in Table 22 and Fig. 4. The drug was present in plasma up to 12 h in healthy cows while up to 24 h in cows suffering from endometritis. The mean therapeutic concentration (0.12 µg/ml) of enrofloxacin was maintained up to 12 h in both healthy cows and cows suffering from endometritis. Significantly higher plasma drug concentrations were maintained from 0.5 to 12 h in healthy cows as compared to cows suffering from endometritis.

Table –22

Comparison of distribution of enrofloxacin between healthy cows and cows suffering from endometritis after a single i.v. dose of 5 mg/kg.

Time (hours)	HEALTHY COWS			COWS SUFFERING FROM ENDOMETRITIS		
	Mean ± S.E.M.			Mean ± S.E.M.		
	PLASMA LEVELS (µg/ml)	UTERINE FLUID / TISSUE LEVELS (µg/ml)	UTERINE FLUID TO PLASMA RATIO	PLASMA LEVELS (µg/ml)	UTERINE FLUID / TISSUE LEVELS(µg/ml)	UTERINE FLUID TO PLASMA RATIO
0.5	4.67±0.23	0.03±0.02	0.008±0.005	3.47±0.21**	0.11±0.01**	0.03±0.003**
1.	3.93±0.30	0.11±0.02	0.03±0.005	2.44±0.31**	0.22±0.03**	0.10±0.02**
2.	2.77±0.27	0.35±0.03	0.14±0.03	1.64±0.22**	2.78±0.22**	2.02±0.35**
3	2.11±0.16	1.35±0.22	0.68±0.17	1.25±0.16**	9.00±0.73**	8.12±1.28**
4.	1.38±0.17	2.20±0.08	1.69±0.24	0.92±0.09*	4.56±0.38**	5.32±0.76**
5.	0.89±0.08	1.33±0.09	1.53±0.10	0.67±0.07NS	2.74±0.18**	4.03±0.32**
6.	0.66±0.08	0.97±0.03	1.52±0.14	0.48±0.05NS	1.78±0.13**	3.72±0.49**
8	0.50±0.08	0.58±0.05	1.23±0.18	0.31±0.04*	0.92±0.06**	3.58±0.76**
12.	0.17±0.05	0.23±0.05	1.33±0.10	0.13±0.03NS	0.61±0.06**	4.03±0.83**
24	ND	0.03±0.02	+	0.04±0.02NS	0.15±0.02**	1.19±0.62 NS

ND=Non- detectable *p<0.05 NS = Non-significant **p<0.01

(b) Uterine fluid levels

Table-22 and Fig.-4 reveal the uterine fluid concentrations of enrofloxacin after its i.v. administration (5mg/kg) in healthy cows and in cows suffering from endometritis. In both the groups, the drug was detectable from 0.5 to 24 h. The drug reached its peak concentration of 2.20 ± 0.08 μg/ml at 4 h in healthy cows while peak concentration 4.56 ± 0.38 μg/ml was noted in cows suffering from endometritis. Highly significant ($p<0.01$) higher drug concentrations were noted at all time intervals in cows suffering from endometritis as compared to healthy cows. The drug maintained its therapeutic concentration (≥ 0.12 μg/ml) from 2 to 12 h in healthy cows whereas from 1 to 24 h in cows suffering from endometritis.

(c) Uterine fluid to plasma ratio

Table-22 presents the uterine fluid to plasma ratio of enrofloxacin after its i.v. administration of 5mg/kg in healthy cows and cows suffering from endometritis. The ratio obtained at 0.5 h in healthy cows and cows suffering from endometritis were 0.008 ± 0.005 and 0.03±0.003, respectively. This ratio in cows with endometritis were significantly differed form healthy cows from 0.5 to 12 h. The maximum ratio of 1.69 ± 0.24 at 4 h was obtained in case of healthy cows while 8.12 ± 1.28 at 3 h was noted in cows suffering from endometritis. Highly significant ($p<0.01$) increase in uterine fluid to plasma ration was noted at all time intervals (except 24 h) in cows suffering from endometritis as compared to healthy cows which denotes that enrofloxacin penetrates to a greater amount in uterus of cows suffering from endometritis.

2. *Gentamicin*

(a) Plasma levels

Table-23 Fig.-5 show the comparative plasma concentrations of gentamicin in healthy cows and cows suffering from endometritis after its i.v. administration of 5mg /kg. The drug was present in plasma from 0.5 to 12 h in healthy cows and cows suffering from endometritis. The mean therapeutic concentration (4 µg/ml) of gentamicin was maintained up to 2 h in healthy cows and upto 1 h in cows suffering from endometritis. Highly significant ($p<0.01$) lower drug concentrations in plasma were maintained from 1 to 12 h in cows suffering from endometritis.

Table –23

Comparison of distribution of gentamicin between healthy cows and cows suffering from endometritis after a single i.v. dose of 5 mg/kg.

Time (hours)	HEALTHY COWS Mean ± S.E.M			COWS SUFFERING FROM ENDOMETRITIS Mean ± S.E.M		
	PLASMA LEVELS (µg/ml)	UTERINE FLUID / TISSUE LEVELS (µg/ml)	UTERINE FLUID TO PLASMA RATIO	PLASMA LEVELS (µg/ml)	UETRINE FLUID / TISSUE LEVELS (µg/ml)	UTERINE FLUID TO PLASMA RATIO
0.5	8.94±0.31	1.16±0.16	0.12±0.01	8.51±1.19^{NS}	3.66±0.59**	0.47±0.09**
1.	6.96±0.38	2.19±0.22	0.31±0.02	4.75±0.23**	5.93±0.86**	1.24±0.14**
2.	4.86±0.22	3.21±0.35	0.66±0.08	2.81±0.22**	9.45±0.77**	3.46±0.29**
4	2.84±0.10	6.25±0.44	2.19±0.10	1.37±0.13**	13.17±1.93**	9.88±1.16**
8.	1.51±0.14	4.23±0.18	2.86±0.21	0.55±0.07**	6.04±0.73*	12.50±2.45**
12.	0.71±0.05	2.40±0.31	3.33±0.22	0.30±0.04**	1.61±0.41*	6.49±2.03NS
24.	N.D	0.60 ± 0.19	------	N.D	0.68±0.14^{NS}	-

ND=Non- detectable *$p<0.05$ NS = Non-significant **$p<0.01$

(C) Uterine fluid levels

Comparative uterine fluid levels of gentamicin after its i.v. administration (5mg/kg) in healthy cows and cows suffering from endometritis are presented in Table 23 and Fig-5. In both the groups, the drug was detectable from 0.5 to 24 h. The drug reached its peak concentration at 4h in both the groups and concentration were noted to be 6.25 ± 0.44 and 13.17 ± 1.93 μg/ml in healthy cows and cows suffering form endometritis, respectively. There was significant difference in uterine fluid level of the drug between both the group form 0.5 to 12 h. The drug maintained its therapeutic concentration of 4 μg/ml from 4 to 8 h in healthy cows and 1 to 8 h in cows suffering from endometritis.

(c) Uterine fluid to plasma ratio

Table-23 presents the uterine fluid to plasma ratio of gentamicin after its i.v. administration (5mg/kg) in healthy cows and cows suffering from endometritis. The ratios in cows suffering from endometritis were significantly higher ($p<0.01$) at almost all time intervals as compared to healthy cows. The maximum ratio of 3.33 ± 0.22 at 12 h was noted in healthy cows while ratio of 12.50 ± 2.45 at 8h was observed in cows suffering from endometritis.

III. THERAPEUTIC TRIAL

Endometritis cases were treated with the drug of choice based on the *in-vitro* sensitivity. Most of the isolates were found sensitive to more than one antimicrobial agent used in sensitivity test. In such circumstances, where more than one drug was found to be effective, the choice of treatment was based on amount of medicine available in stock. The treatment was done by two different routes i.e intrauterine and intravenous. Intrauterine was given one dose alternate day for six days and i.v. treatment was done daily for five days in case of enrofloxacin and twice daily in case of gentamicin except cephalexin where administration was done intrauterine route only. In the animals, whose samples contained mixed isolates were tested in *in-vitro* with each isolate and the drug was chosen based on common sensitivity against both the isolates.

Cows suffering from endometritits were categorized in three treatment (T_1,T_2,&T_3) group, besides this one control group comprising of 6 cows were given no treatment. The cows of control group were inseminated without any treatment after collection of samples for bacteriological analysis. Further more, treatment group (T_1&T_2) subdivided into two treatment group on the basis of route of administration of drug i.e. T_1a, T_1b, & T_2a, T_2b. In T_3 group only one route i.e. i.u. infusion was done. The treated animals were examined during the next estrus after completion of treatment. Samples

(uterine discharge) were collected for bacteriological study after treatment and then the animals were inseminated with frozen semen. The animals failed to conceive and exhibited estrus symptom in next cycle were inseminated again with frozen semen.

The efficacies of the treatment of endometritis have been presented in Table-24 and Fig.-6. Out of 62 animals, 44 animals were found free from bacteria and the efficacies of the drugs were 70.97%, while 18 cows responded partial to the drug but their uterine samples were found positive for bacteria. A total of 42 animal conceived - 35 from bacterial free group and 7 from bacterial positive group. Non-pregnant bacterial positive group numbering 11 were subjected to retreatment as per culture and sensitivity report.

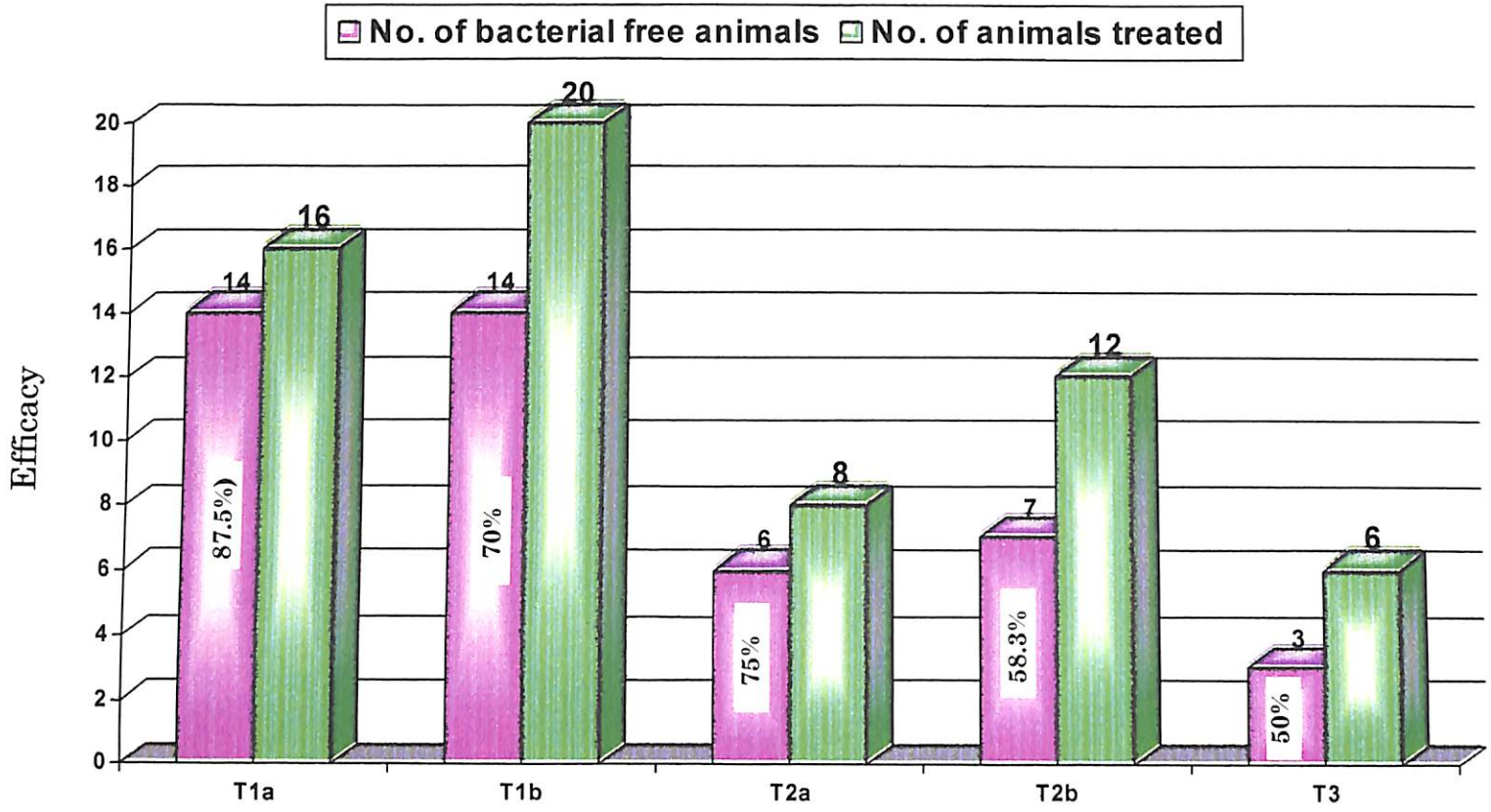
In T_1a group, (enrofloxacin, i.v.) out of 14 bacterial free animals 12 animals conceived. Out of 2 bacterial positive animals,1 conceived.

In T_1b (enrofloxacin, i.u.) group out of 14 bacteria free animals, 12 animals conceived. Out of 6 bacterial positive animals, 2 animals conceived.

In T_2a (gentamicin, i.v.) out of 6 bacterial free animals, 5 animals conceived, out of 2 bacterial positive animals 1 conceived.

In T_2b (gentamicin, i.u.) out of 7 bacterial free animals, 4 animals conceived. Out of 5 bacterial positive animals, 2 animals conceived.

Fig.6. *Efficacy of Treatment of Cows suffering from endometritis*



T_1a = Enrofloxacin (i.v.), T_1b = Enrofloxacin (i.u.), T_2a = Gentamicin (i.v.), T_2b=Gentamicin (i.u.), T_3 = Cephalexin (i.u.)

Table – 24

Efficacy of treatment of cows suffering from endometritis

Treatment group	No. of animal treated		Route of administration of drug	No. of animals after treatment		No. of animals conceived after treatment.			N. of animal found non-pregnant	
				Bacterial free group (B/F)	Bacterial positive group (B/P)	Bacterial free group (B/P)	Bacterial positive group (B/P)	Total	Bacterial free group (B/F)	Bacterial positive group (B/P)
Control (6)	No treatment		---	---	6	---	--	---	---	---
T_1	36	16 (T_1 a)	i.v.	14 (87.5%)	2	12	1	13	2	1
		20 (T_1b)	i.u.	14 (70%)	6	12	2	14	2	4
T_2	20	8 (T_2a)	i.v.	6 (75%)	2	5	1	6	1	1
		12 (T_2b)	i.u.	7 (58.3%)	5	4	2	6	3	3
T_3	6	6(T_3)	i.u.	3 (50%)	3	2	1	3	1	2
Total T_1 to T_3	62		---	44 (70.97%)	18 (29.03%)	35 (79.55%)	7 (38.89%)	42 (67.74%)	9 (20.45%)	11 (61.11%)

T_1 = Enrofloxacin, T_2 = Gentamicin, T_3 = Cephalexin i.v.= Intravenous, i.u.= Intrauterine

In T_3 (cephalexin, i.u.) out of 3 bacterial free animals, 2 animals conceived. Out of 3 bacterial positive animals, 1 animal conceived.

The result of treatment in relation to conception on different insemination has been depicted in Table-25 and Fig. 7. A total of 62 cows, which were, treated by different treatment groups were inseminated in estrus after taking swab for culture. A total of 42 (67.74%) cows conceived. Out of 42, 26 (41.94%) conceived on first insemination followed by 11(30.56%) and 5(20%) cows on second and third insemination, respectively.

In T_1 (a) group, all the 16 cows were inseminated, out of which 9 (56.25%) cows conceived. Seven animals exhibited estrus in second cycle and were re-inseminated, in which only 3 (42.86%) cows conceived, 4 cows again exhibited heat, were reinseminated (3rd insemination) in which 1 (25%) animal conceived. Altogether in T_1(a) group, out of 16 animals, a total of 13(81.25%) animals conceived. Likewise in T_1 (b) group, out of 20 animals, a total of 14(70%), in T_2(a) group out of 8 animals, a total of 6 (75%), in T_2 (b) group out of 12 animals, a total of 6 (50%) and in T_3 group out of 6 animals, a total of 3 (50%) conceived up to 3rd insemination. In control group conception rate was nil up to 3rd insemination. Chi-square test for r×c contingency table was done regarding conception in different treatment groups. The value of chi-square was found to be non-significant which suggest that all drugs are more or less equally effective.

Fig.7. *Conception rate in cows suffering from endometritis*

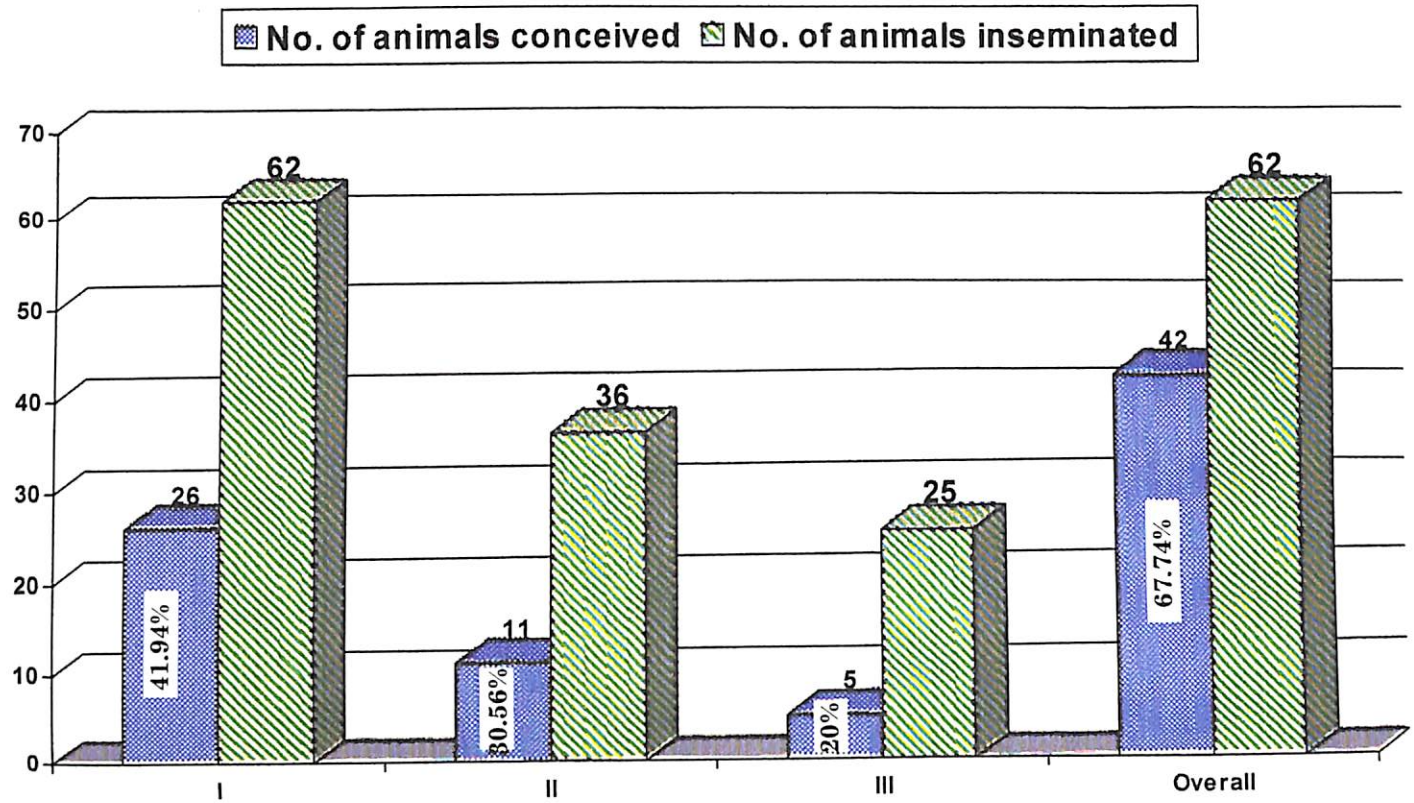


Table – 25

Conception rate in cows suffering from endometritis

Treatment group	No. of animal treated		No. of animals inseminated			No. of animal conceived after AI			Total Conception	Percentage (%)	x^2 at 4d.f.
			Ist AI	IInd AI	IIIrd AI	Ist	IInd	IIIrd			
Control (6)	No treatment		6	6	6	---	---	---	---	---	
T_1	36	16 (T_1 a)	16	7	4	9 (56.25%)	3 (42.86%)	1 (25.0%)	13	81.25	
		20 (T_1b)	20	10	8	10 (50.0%)	2 (20.0%)	2 (25.0%)	14	70.00	
T_2	20	8 (T_2a)	8	5	2	3 (37.50%)	3 (60.0%)	---	6	75.00	4.15^{NS}
		12 (T_2b)	12	9	7	3 (25.0%)	2 (22.22%)	1 (14.29%)	6	50.00	
T_3	6	6(T_3)	6	5	4	1 (16.66%)	1 (20.0%)	1 (25.0%)	3	50.00	
Total	62		62	36	25	26 (41.94%)	11 (30.56%)	5 (20.0%)	42	67.74	

T_{1a}=Enrofloxacin (i.v.), T_{1b}=Enrofloxacin (i.u.), T_{2a}=Gentamicin (i.v.), T_{2b}=Gentamicin (i.u.), T_3 = Cephalexin (i.u.),NS=Non-Significant

Retreatment group :-

The uterine samples of non-pregnant cows of bacterial positive group were subjected to isolation of microorganisms and *in-vitro* sensitivity test. Based on this, the retreatment was done and cows were inseminated when returned to estrus. The *in-vitro* sensitivity test with the antimicrobials against different isolates of microorganism is shown in table-26.

Table –26

In-Vitro sensitivity test of isolates from reculture of uterine sample of Cows

Name of isolate	No.	Cephalexin	Gentamicin	Enrofloxacin
Esch. coli	4	2 (50%)	3 (75%)	4 (100%)
Ps. aeruginosa	3	2 (66.66%)	2 (66.66%)	3 (100%)
Staph. aureus	3	2 (66.66%)	2 (66.66%)	2 (66.66%)
Coryne. pyogenes	1	1(100%)	1(100%)	1(100%)
Total	11	7 (63.63%)	8 (72.72%)	10 (90.90%)

It is evident from the table that out of 11 samples, 11 isolates were obtained maximum number of *Esch. coli* (4) were isolated followed by *Ps. aeruginosa* and *Staph. aureus* 3 each and only one isolate of *Coryne. pyogenes*.

According to sensitivity report, 100% *Esch. coli* was sensitive to enrofloxacin and 75% to gentamicin where as only 50% of *E. coli* was sensitive to cephalexin. Maximum *Ps. aeruginosa* (100%) was sensitive to enrofloxacin while 66.66% was sensitive to gentamicin and cephalexin. 66.66% of *S. aureus* was sensitive to all the antimicrobials used in sensitivity test. Likewise 100% *C. pyogenes* was sensitive to all the antimicrobials used in sensitivity test. Thus out of 11 isolates 10 (90.90%) isolates were sensitive to enrofloxacin followed by 8 (72.72%) to gentamicin and 7 (63.63%) to cephalexin.

As per sensitivity report (Table-26), the isolates were sensitive to more than one antimicrobial agent. Hence, two most sensitive antimicrobials were taken for treatment. Two treatment groups were made (Table-27). The animals were treated with the drug by intrauterine administration only. The results revealed that over all conception rate was 72.72%. Maximum conception rate occurred with enrofloxacin. 5 animals (83.33%) were conceived from 6 animals which is followed by gentamicin where 4 animals (60%) conceived from 5 animals.

Table – 27

Conception rate in retreatment group of cows suffering from endometritis

Treatment group	Total No. of animal	Total No. of animal conceived	Percentage
T_1	6	5	83.33%
T_2	5	3	60%
Total T_1 to T_2	11	8	72.72%

T_1 = Enrofloxacin T_2 = Gentamicin

Chapter-V

DISCUSSION

DISCUSSION

Endometritis is one of the common problems in dairy animals and among infectious causes of infertility in cattle. It ranks first both in heifers and cows (Sreeramulu, 1995). Endometritis leads to heavy economical losses in dairy industry. The present study deals with the isolation of the etiological agents as well as their antibiogram in order to carry out the effective treatment. Further, more studies were done to known the distribution of enrofloxacin and gentamicin in plasma and uterine fluid at specific time interval in a few cases of endometritis as well as in healthy animals. Based on the above information, treatments were carried out in cows suffering from endometritis. The efficacy of treatment was recorded based on the conception rate.

I. BACTERIOLOGICAL STUDIES

A. Isolation of Bacteria :

A total of 68 uterine samples of 68 cows suffering from endometritis were taken into consideration for isolation and identification of various microorganisms. Out of 68 samples, single isolates were obtained from 58 (85.29%) samples where as rest 10 (14.71%) of the samples revealed mixed isolates. Mixed infections revealed the association of only two types of bacterial isolates. The

results of present studies are in agreement with the findings of David (1986), Stephens *et al.* (1986), Baishy *et al.* (1998), Shankar Prasad (1998) and Arora *et al.* (2000) who also recorded single and mixed types of isolates in their studies.

It is evident from the results (Table – 8) that the highest percentage of isolates (39.74%) was *Escherichia coli* out of the total samples (including single & mixed isolates). The other organisms obtained were *Staphylococcus aureus* (25.64%), *Streptococcus pyogenes* (12.82%), *Pseudomonas aeruginosa* (8.97%), *Proteus vulgaris* (5.13%), *Corynebacterium pyogenes* (3.85%) and *Klebsiella pneumoniae* (3.85%). Similarly, the findings of Lalvani *et al.* (1984), Sharma *et al.* (1993) and Arora *et al.* (2000) also recorded the presence of *Escherichia coli* in highest percentage. However, Fivaz and Swanepoel (1978), Studer and Morrow (1978), Kodagali *et al.* (1980), Slimane *et al.* (1994) found *Coryne. pyogenes* as a major isolate.

Staphylococcus aureus was isolated from 25.64% of the total isolates and was assumed to be the major causes responsible for endometritis next to *E. coli*. Sadhasiva Rao and Seshagiri (1997) and Khan *et al.* (1990) reported *S. aureus* as a major isolate from the cases of cows suffering from metritis. The other isolates were *Streptococcus pyogenes, Pseudomonas aerueginosa, Proteus vulgaris, Corynebacterium pyogenes* and *Klebsiella pneumonia* which are

present in 12.82%, 8.97%, 5.13%, 3.85% and 3.85 percent, respectively. The incidence reported by Shankar Prasad (1998) and Singh (1997) were in accordance to the observation made in the present study with minor variations.

B. *In-vitro* sensitivity :

In the present work, an attempt was made to study the antibiogram of the isolates to institute an effective rational therapy by proper use of drugs. Indiscriminate and prolonged uses of antimicrobials in absence of drug sensitivity test were resulted in emergence of drug resistant strains of bacteria.

A total of three antimicrobials agents viz., cephalexin, gentamicin and enrofloxacin were tested against the 78 isolates obtained from 68 cows suffering from endometritis (Table-9). Enrofloxacin was found to be highly sensitive (73.07%) followed by gentamicin (67.94%) and cephalexin (60.25%). Verma *et al.* (1991) and Anjaneyulu *et al.* (1999) showed maximum sensitivity (100% & 93%, respectively) to enrofloxacin while Jacob *et al.* (1995) and Singh (1997) reported 80%, 76.47% sensitivity to gentamicin, respectively in case of endometritis. Percentage sensitivity to cephalexin (60.25%) noted in present studies is in close agreement with the findings of Kumar (1997) who noted sensitivity percentage of 57.85% to cephalexin in repeat breeding animals. Singh (1997) reported 48.04% sensitivity to cephalexin in cases of endometritis.

II. DISTRIBUTION STUDIES OF ANTIMICROBIAL AGENTS

An antimicrobial agent may become effective only when it reaches the site of infection. In endometritis, usually uterus may be infected with microorganisms, which may need therapy with antimicrobials. Therefore, it is essential to find out the distribution of antimicrobials in uterine tissues so that the infected organisms may be removed ultimately so that conception may be attained. In the present study, an attempt has been made to know the distribution of the following antimicrobials by the convenient i.v. route so that the effective doses regimen can be suggested for the treatment of endometritis apart from the conventional i.u. administration.

A. Enrofloxacin :

It is a recently introduced fluroquinolone, which is exclusively used in veterinary practice. It is rapidly acting bactericidal agent with a broad spectrum of activity against aerobic and facultative anaerobic bacteria including strains resistant to many other antimicrobials such as β–lactum antibiotics, aminoglycosides, tetracyclines, macrolides etc.

Distribution studies of enrofloxacin in plasma and uterine fluid were carried out in cows suffering from endometritis after a single i.v. administration at the dose of 5mg/kg. The data was

compared with healthy cows to know the pattern of distribution in endometritis. The study revealed that significant decrease in plasma concentrations at most of time intervals while significant increase in uterine levels at all the time interval were noted in cases of endometritis as compared to healthy animals (Table -22). It is well known that inflammatory changes may cause increase in higher permeability of a drug due to increase in pore size of the membrane. Generally infections of the uterus by microbes may cause endometritis which in turn may lead to the above inflammatory changes. Due to higher penetration of the drug in uterus caused by endometritis, the plasma levels were decreased in the present study. This has led to significant increase in uterine fluid to plasma ratio in endometritis as compared to healthy cows. Very little studies have been conducted with regard to distribution of enrofloxacin in cases of endometritis, particularly in cows. Similar is the observation of Gigure *et al.* (1996) who conducted parmacokinetic study of enrofloxacin in adult horses. They observed that endometrial tissue concentrations exceeded that of plasma concentration by as much as three folds. Ames *et al.* (1983) similarly noted an increase in concentrations of oxytetracycline in pneumonic lungs of cows as compared to that of healthy calves and they interpreted the increased in lung concentrations due to higher permeability of the drug.

In the present study, the mean therapeutic concentration of enrofloxacin 0.12 μg/ml was maintained for a period up to 12 h in uterine fluid of healthy cows while it was maintained up to 24 h cows suffering from endometritis after i.v. administration (5 mg/kg of drug). Thus, the present study clearly established that enrofloxacin can be effectively used at the usual recommended therapeutic dosage of 5 mg/kg daily by parental route for the treatment of cows suffering from endometritis.

B. Gentamicin

Gentamicin - an aminoglycoside antibiotic, since its availability for therapy of infections has been widely used in human as well as veterinary practice. It is a broad spectrum antibiotic which is highly effective against both gram-negative and gram-positive organisms. Various worker have reported that it is an effective against the most organism associated with bovine metritis (Hennessey *et al.*, 1971; Bachmann *et al.*, 1975).

Distribution of gentamicin in plasma and uterine fluid were studied in cows suffering from endometritis as well as in healthy cows after its single i.v. administration at the dose rate of 5mg/kg. The study revealed that significant decrease in plasma drug concentration while significant increase in uterine fluid levels at most of the time interval were noted in case of cows suffering from

endometritis as compared to healthy animals (Table 23). This has led to highly significant ($p<0.01$) increase in uterine fluid to plasma ratio in cows with endometritis as compared to healthy cows. The higher permeability of the drug in endometritis may be due to increase in permeability of the drug caused by inflammatory changes of the uterine membrane. It is well known that inflammation of any tissue may increase the size of the pores of the tissue membrane. Such increase in permeability was noted by Ames *et al.* (1983) who noted increase in the concentration of oxytetracycline in pneumonic lungs of cows as compared to healthy animals. The therapeutic concentration (4 μg/ml) was maintained from 4 to 8 h in healthy cows while it was maintained for a longer duration of 1 to 8 h in cases of cows suffering from endometiritis in uterine fluid. By going through the above facts, gentamicin can be administered by parentral route at the dose rate of 5 mg/kg every 12 hourly by considering the lag phase of bacteria. Only few studies have been done with regard to distribution of gentamicin in plasma and uterine fluid of healthy cows and cows suffering from endometritis. Salim *et al.* (2000) studied the plasma levels of gentamicin in cows suffering from endometritis after i.m. administration of gentamicin (5 mg/kg) along with oxytocin (50 I.U., i.m.). He also noted that the drug was detectable up to 12 h, which is similar to the finding of the present study. Al-Guedawy *et al.* (1983)

studied the distribution of gentamicin between plasma and uterine content following i.m. administration (4 mg/kg). They noted that gentamicin was rapidly absorbed from i.m. site and it first appeared in uterine lumen as early as 15 minute and peak uterine concentration of 4.7±2.0 μg/ml achieved at 6 h. More or less similar observation was noted in the present study and the drug was detected at 0.5 h (First sample) and a peak concentration (6.25 ±0.44 μg/ml) was attained little earlier at 4 h. This may be due to intravenous higher dose of 5 mg/kg by i.v. route while the above author used a lower dose of 4 mg/kg by i.m. route. In contrast to the above study, Sinha *et al.* (1994) observed that the concentration of gentamicin was not detected in majority of endometrial tissues sample, when the drug was given @ 10 mg /kg i.m.

III. THERAPEUTIC TRIAL

In the present study, therapeutic measures were adopted based on *in-vitro* sensitivity test as well as based on distribution studies of antimicrobials agents (enrofloxacin and gentamicin) conducted in cows suffering with endometritis noted above. The beneficial effect on the use of specific antimicrobial drug sensitivity test has been reported by various workers (Oxender and Seguin, 1976; Sinha *et al.* 1977; Steffan *et al.* 1984; Bohme *et al.*1986; Singh 1997; Baishya *et al.*1998 and Arora *et al.* 2000).

In the present investigation, drug trial was also conducted through i.u. route which was accordance with the recommendation of Chauhan and Takkar (1983), Abdullah-Rehman *et al.*(1991), and Ambrose and Pattabiraman (1993) while Money (1996) and Mates and Cosma (1988) adopted parentral route of antimicrobial therapy in cases of endometritis.

The present work, isolation of bacteria and their antibiogram have been recorded in cases of endometritis. A total of 68 cows suffering from endometritis were taken for the study. Out of 68 cows, 62 were divided into three treatment groups and six cases were kept without any treatment (control group). The treatment groups were further subdivided on the basis of route of administration of antimicrobials. The efficacy of the treatment with antimicrobial was judged on the basis of clearance of microorganisms and conception rate after artificial insemination.

In all the treatment groups, the treated animals responded well and improved as shown by uterine discharges return towards normalcy though in some animals the pathogens were not cleared altogether. The overall result of treated groups of cows indicated complete eradication of microorganisms in 44 (70.97%) cases whereas in rest of 18 (29.03%) cases were found positive for bacteria though with reduced intensity as shown by improvement in uterine discharges. Out of 44 cases of bacterial free animals 35

(79.55%) animals were conceived and rest of 9 (20.45%) were not conceived even after eradication of causative organisms. This may be due to the presence of any other type of infections other than bacterial as well as due to other factors such as hormonal imbalance, nutrition deficiency etc. Similar recovery rate of 70% and 68% were noted by Kodagali *et al.* (1980) and Ambrose and Pattabiraman (1993) by the use of antimicrobial agents.

In the present work, out of 18 bacterial positive group 7 (38.89%) were conceived. This may be due to partial recovery of the animals from infection as shown by lesser intensity of uterine discharge as well as the natural defense mechanism of the body against the bacteria. Endometritis, as other inflammatory process in the body tend to recover spontaneously with each repeated estrus cycle. The natural body defense and the changes produced within the uterus and the genital tract by the estrogen tend to aid recovery. This effect of estrus period on infection has been described by Rowson *et al.* (1953) and Hawk *et al.* (1964).

In the present study, attempts were made to treat the cases of endometritis with antimicrobials based on *in vitro* sensitivity on different bacteria isolated from cows with endometritis. Enrofloxacin by i.v. route showed the highest efficacy as noted by conception in 13 cases (81.25%) out of 16 cases which is followed by i.v. administration of gentamicin [6 (75%)out of 8 cases]. Next highest

efficacy was noted with i.u. administration with enrofloxacin in which 14 cases (70%) were conceived out of 20 cows with endometritis. On the other hand, gentamicin (6 conceived out of 12 cows with endometritis) and cephalexin (3 out of 6 cases) by i.u. administration showed conception rate of 50% only. This is well correlated with the bacterial sensitivity test in which enrofloxacin showed the overall highest sensitivity to enrofloxacin (73.07%) followed gentamicin (60.94%) and cephalexin (62.25%) as shown in Table-9. The overall conception rate by antimicrobials is noted to be 67.74%. More or less similar overall conception rate of 63.86% in cases of cows with endometritis was reported by Singh (1997) while higher conception rate of 71.93% and 80% were noted by Venkateshwarlu *et al.* (1983) and Bhaskar and Agrawal (2001).

In the present study, conception rate of 81.25% by i.v. route and 70% by i.u. route was noted for enrofloxacin. Bhaskar and Agarwal (2001) reported conception rate of 60% (i.u. route) in cases endometritis with enrofloxacin. In case of gentamicin, conception rate of 75% by i.v. route and 50% by i.u. route was noted. Venkateshwarlu *et al.* (1983) and Sudhakar *et al.* (1986) reported conception rate of 61.54% and 76%, respectively, with gentamicin by i.u. route in case of endometritis. Cephalexin achieved the conception rate of 50% by i.u. route in the present study. Singh (1997) however, reported a higher conception rate of 69.23% in case of endometritis with cephalexin by i.u. route.

Retreatment of bacterial positive animals were done by using the enrofloxacin and gentamicin based on sensitivity test. Enrofloxacin showed highest sensitivity against isolates (90.90%) while gentamicin showed sensitivity of 72.72%. Treatment with enrofloxacin showed higher percentage of conception rate 83.33% while with gentamicin the conception rate of 60% was noted. The above results showed that the conception rate closely correlate with the sensitivity test.

The results of the present study lead to the conclusion that the treatment of endometritis with enrofloxacin and gentamicin can be carried out by parental route apart from its conventional i.u. route. Similar such studies should be carried out with other newer antimicrobials agents for effective therapy of endometritis.

Chapter-VI

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Endometritis is one of the serious and common problems in dairy animals that may ultimately lead to infertility in cattle and thus, heavy economical losses may occur in dairy industry. The present study on endometritis deals with the isolation of etiological agents, their antibiogram as well as distribution studies of the two important antimicrobial agents namely enrofloxacin and gentamicin in uterine fluid. Based on the above noted studies, treatments were carried out with these antimicrobial agents by intravenous (i.v.) and intra-uterine (i.u.) routes as well as by using cephalexin (cephalosporin) by i.u. route. Efficacy of the treatment was judged on the basis of bacteriological report and conception rate.

Experiments were conducted in a total of 76 cows. Out of these cases, a total of 68 cases were of endometritis and rest 8 cases were of normal healthy animals. A total of 16 cases out of 68 cows with endometritis and 8 healthy animals weighing between 265-410 kg were selected for the study of distribution of enrofloxacin and gentamicin. In cases of endometritis, isolation of bacteria and their antibiogram as well as treatment with antimicrobials were carried out.

Bacteriological studies :

In the present study, a total of 68 uterine samples of 68 cows suffering from endometritis were taken into consideration for isolation and identification of various microorganisms. Out of 68 samples, single isolates were obtained from 58 (85.29%) samples whereas rest 10(14.71%) of the samples revealed mixed isolates of more than one organisms. Out of these bacterial isolates, the highest incidence was of *Escherichia coli* (39.74%) followed by *Staphylococcus aureus* (25.64%), *Streptococcus pyogenes* (12.82%), *Pseudomonas aeruginosa* (8.97%), *Proteus vulgaris* (5.13%), *Corynebactrium pyogenes* (3.85%) and *Klebsiella pneumoniae* (3.85%).

The bacterial isolates were subjected to antibiogram studies against the above noted three different antimicrobial agents. Out of total 78 bacterial isolates, the highest sensitivity was noted with enrofloxacin (73.07%) followed by gentamicin (67.94%) and cephalexin (60.25%). With regards to sensitivity to individual organisms *Escherichia coli* showed the highest sensitivity (77.41%) against enrofloxacin followed by gentamicin (74.19%) and cephalexin (64.51%). *Staphylococcus aureus* showed maximum sensitivity of 70% to both enrofloxacin and gentamicin while cephalexin showed 60% sensitivity to the organisms. On the other hand, *Streptococcus pyogenes* showed maximum sensitivity of 70% to both enrofloxacin and cephalexin while gentamicin showed a lower sensitivity of 50% to

this organism. In case of *Pseudomonas aeruginosa,* both enrofloxacin and gentamicin showed higher sensitivity of 71.42% whereas cephalexin showed sensitivity of 57.14% only. In case of *Proteus vulgaris* a higher sensitivity of 75% was noted with both enrofloxacin and cephaloxin while gentamcin showed a sensitivity of 50% only. *Klebsiella penumoniae* and *Coryne. pyogenes* showed a sensitivity of 66.66% to both gentamicin and enrofloxacin while lowest sensitivity of 33.33% and 0%, respectively was noted for cephalexin to the above organisms.

Distribution studies of antimicrobials agents :

Distribution studies of enrofloxacin and gentamicin (5 mg/kg, i.v.) revealed that significant decrease in plasma drug concentrations at most of the time intervals were noted for both the drugs while significant increase in uterine fluid levels of enrofloxacin and gentamicin were noted at all the time interval (except in case of gentamicin at 24 h where non-significant difference was noted) in cows with endometritis as compared to healthy animals. Further, therapeutic concentration of 0.12 μg/ml in uterine fluid was maintained for a longer time (1 to > 24 h) with enrofloxacin while therapeutic concentration of 4 μg/ml was maintained for a shorter time (1 to 8 h) with gentamicin. Hence, the study recommends the parentral administration of enrofloxacin @ 5 mg/kg once daily while dose of 5 mg/kg of gentamicin by parentral route twice daily for effective therapy of susceptible bacteria causing endometritis in cows.

Therapeutic trial :

In the presence study, therapy with enrofloxacin and gentamicin were carried out by i.v. and i.u. routes while treatment with cephalexin was done by only i.u. route The treatment was carried out in 62 cows based on bacterial sensitivity test. The efficacy of the treatment with antimicrobial was judged on the basis of bacteriological report and conception rate after artificial insemination.

In all the treatment groups, the animals responded well and improved as shown by uterine discharge returned towards normalcy. The overall result of treated groups of cows indicated complete eradication of micro-organisms in 44 (70.97%) cases whereas in rest of 18 (29.03%) cases were found positive for bacteria. Out of 44 cases of bacterial free animal 35 (79.55%) animals were conceived and rest 9 (20.45%) were not conceived even after eradication of microorganisms. This may possible due to the presence of any other types of infections other than bacteria as well as due to other factors such as hormonal imbalance, nutrition deficiency etc.

In the present study out of 18 bacterial positive groups 7(38.89%) were conceived. This may be due to partial recovery of the animals from infections as shown by lesser intensity of uterine discharge as well as the natural defence mechanisms of the body against the bacteria.

In the present work, attempts were made to treat the cases of endometritis with antimicrobial based on *in-vitro* sensitivity on different bacteria isolated from cows with endometritis. Enrofloxacin by i.v. route showed the highest efficacy as noted by conception rate of 81.25% followed by i.v. administration of gentamicin (75%). Next highest efficacy was noted with i.u. administration of enrofloxacin (70%) while gentamicin and cephaexin by i.u. administration showed conception rate of 50% each. This is well correlated with bacterial sensitivity test in which enrofloxacin showed the overall highest sensitivity to enrofloxacin (73.07%) followed by gentamicin (67.94%) and cephalexin (60.25%).

Retreatment of bacterial positive animals were done by using enrofloxacin and gentamicin based on sensitivity test. Enrofloxacin showed highest sensitivity (90.90%) while gentamicin showed sensitivity of 72.72%. Similarly a higher conception rate of 83.33% was achieved by treatment with enrofloxacin while with gentamicin conception rate of 60% was noted. The above results showed that the conception rate closely correlated with the sensitivity test.

The results of the present study lead to the conclusion that the treatment of endometritis with enrofloxacin and gentamicin can be carried out by parentral route apart from its conventional i.u. route. Similar such studies may be carried out with other newer antimicrobial agents for effective theraply of endometritis.

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APPENDIX

APPENDIX

INGREDIENTS OF

I. *Nutrient Broth* :

Beef extract	-	3 gm
Peptone	-	5 gm
Potassium nitrate	-	1 gm
Distilled water	-	1 lit
pH	-	6.8 ± 0.2

II. *Nutrient Agar* :

Beef extract	-	1.5 gm
Peptone	-	5 gm
Sodium Chloride	-	5 gm
Yeast extract	-	1.5 gm
Agar	-	15 gm
Distilled water	-	1 lit
pH	-	7.0 ± 0.2

III. *MacConkey's Agar* :

Peptone	-	17 gm
Proteose Peptone	-	3 gm
Lactose	-	10 gm
Sodium Chloride	-	5 gm

Bile Salt	-	1.5 gm
Agar	-	15 gm
Distilled water	-	1 lit
pH	-	7.1 ± 0.2

IV. ***Antibiotic assays media of enrofloxacin and gentamicin :***

Peptone	-	6 gm
Tryptone	-	4 gm
Yeast extract	-	3 gm
Beef extract	-	1.5 gm
Dextrose	-	1.0 gm
Agar	-	15 gm
Distilled water	-	1 lit
pH	-	7.9 ± 0.1

SYMBOLIC PRESENTATION

Conception rate	=	CR
Esch. coli	=	*Eschericihia coli*
Staph. aureus	=	*Staphylococcus aureus*
Strept. pyogenes	=	*Streptococcus pyogenes*
Coryn. pyogenes	=	*Corynebacterium pyogenes*
Ps. aeruginosa	=	*Pseudomonas aeruginosa*
K. pneumoniae	=	*Klebsiella pneumoniae*
B.F.	=	Bacterial free
B.P.	=	Bacterial positive