Pharmacokinetic Study of Enrofloxacin in Healthy and Febrile Goats

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

(BIHAR)

In partial fulfilment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

IN

PHARMACOLOGY & TOXICOLOGY

(Minor Subject – Vety. Medicine & Biochemistry)

By

Uday Kumar

Department of Pharmacology & Toxicology

BIHAR VETERINARY COLLEGE

PATNA

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Dedicated to
My Benevolent
Parent

CERTIFICATE – II

We, the undersigned members of the Advisory Committee of **Dr. Uday Kumar**, a candidate for the degree of Master of Veterinary Science with major in Veterinary Pharmacology & Toxicology, have gone through the manuscript of the thesis and agree that the thesis entitled "**PHARMACOKINETIC STUDY OF ENROFLOXACIN IN HEALTHY AND FEBRILE GOATS**" may be submitted by **Dr. Uday Kumar** in partial fulfilment of the requirements for the degree.

19/5/2000

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Chairman
Advisory Committee

Members of the Advisory Committee :

1. **Dr. H. N. Jha**
   Associate Professor
   Department of Pharmacology & Toxicology

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4. **Dr. Mani Mohan**
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19.5.2000

CERTIFICATE – III

This is to certify that the thesis entitled "**PHARMACOKINETIC STUDY OF ENROFLOXACIN IN HEALTHY AND FEBRILE GOATS**" submitted by **Dr. Uday Kumar** in partial fulfilment of the requirements for the degree of Master of Veterinary Science (Veterinary Pharmacology & Toxicology) of the faculty of Post-Graduate studies, Rajendra Agricultural University, Bihar, Pusa, was examined and approved on _____ 2000.

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DRI-cum-Dean P. G. Studies

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Patna

Date :

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Introduction

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Introduction

INTRODUCTION

Antimicrobial therapy constitutes a major component of modern medical & veterinary practices. Keeping in view the microbial resistance to various antimicrobial agents, there is always need to have a drug that would be highly effective in combating different infectious diseases.

Enrofloxacin, the latest among the fluoroquinolones class of antimicrobials, was first synthesised in 1983 by Bayer Research laboratory in Germany. It is a quinolone carboxylic acid derivative developed exclusively for veterinary use (Altreuther, 1987; Chu and Fernandes, 1989). It possesses broad spectrum activities and is effective against both gram-negative bacteria such as *Escherichia coli*, salmonella, Klebsiella, pasteurella, proteus, Haemophilus, campylobacter, & pseudomonas (Scheer, 1987) and gram positive bacteria like streptococcus, staphylococcus, clostridium, *Erysipelothrix rhusiopathiae* and mycoplasma (Bauditiz, 1990). Enrofloxacin possesses excellent distribution in different tissues and body fluids and of distribution pattern is almost similar in all species. Enrofloxacin is suitable for the treatment of septicemia, gonorrhaea, respiratory infection, soft tissue, bone and joint infections. It is also active against several organisms which are resistant to many other antimicrobials. The MICs values of enrofloxacin for different species of microorganisms ranges between 0.01 to 2.0 $\mu g.ml^{-1}$ (Scheer, 1987; Bauditz, 1990).

Goat is a versatile animal in India, goat is reared for meat and milk purposes apart from its valuable hides. Besides, pashmina

goat contributes better wool to garment industries. Goat is popularly known as "poor man's cow" in India and as "wet nurse of infants" in Europe. Goat's milk is cheap, wholesome easily digestible and nutritious. Goat can play a significant role by enhancing the socio-economic status of marginal farmers and landless labourers. Hence, it becomes necessary to provide a better health coverage to this valuable species by achieving the new dimensions through Enrofloxacin therapy. This is the one of the way for increasing the productivity in terms of its production and replication.

The most important question during antimicrobial therapy is what dose of the drug is effective so as to maintain the desired minimum plasma concentration for longer duration. This can be arrived at only through pharmacokinetic studies, (Anadon *et al*; 1995; Dowling, 1995).

The drug is well absorbed in domestic animals after either oral or parentral administration (Cabanes *et al*, 1992; Froyman *et al* ,1994; Anadon *et al*, 1995). The high bioavailability and the good tissue penetration of enrofloxacin results in tissue concentration equal to or significantly higher than those achieved in serum (Anadon *et al*, 1995). The drug is partially metabolized in liver to ciprofloxacin, a primary metabolite which itself a potent antimicrobial agent used commonly in human therapy (Bergan *et al* , 1988: Tyezkowska *et al* ., 1989). The metabolism of enrofloxacin to ciprofloxacin is beneficial since many gram-negative bacteria have lower MIC values for ciprofloxacin (Gedek 1987; Prescott & Yielding., 1990).

To be effective in diseased and febrile conditions, the drug should reach the target organ in effective concentration. The

quantitative estimation of antimicrobials like enrofloxacin in plasma, milk and urine following parenteral administration will be highly helpful and useful in achieving rational therapy for treating local (mastitis, urinary tract infections etc) and systemic infections. Contamination of milk with antimicrobials is a public health hazards and requires milk withdrawal for sufficient period of time after cessation of therapy.

It has been established that the pharmacokinetics and dosage schedule of many drug is altered during disease conditions (Lesar and Zaske, 1984). Fever which may be associated with many bacterial and viral diseases changes the various physiological parameters viz. heart rate, renal blood flow, hepatic and total splanchnic blood flow, diuresis, enzyme activities and endocrine functions (Kasting *et al.*, 1982). Fever also modify the pharmacokinetics of certain drugs (Chang *et al.*, 1978; Forsyth *et al.*, 1982). Significant alterations in pharmacokinetics and dosage regimen of cephalosporins (Choudhary, 1996; Yadav, 1997; Singh, 1994), Sulfadimidine (Riffat *et al.*, 1982) minocycline and oxytetracycline L.A. (Jay Chandran, 1994) and amikacin (Saini, 1995) have already been reported.

On the basis of available literature no such study has been carried out with enrofloxacin in goats. In view of the above stated facts, the present study proposes to proceed with the following specific aims and objectives :

1. Determination of plasma, milk and urine levels of enrofloxacin in healthy and febrile goats following its intravenous and intramuscular administration.

2. Calculation of kinetic parameters of enrofloxacin in healthy and febrile goats following its intravenous and intramuscular administration.

3. Calculation of dosage regimen of the drug in healthy and febrile goats.

The finding of this work would facilitate the recommendation of appropriate dosage regimen of enrofloxacin for the effective treatment of various bacterial infections, particularly in febrile state in goats.

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Chapter II

Review of Literature

REVIEW OF LITERATURE

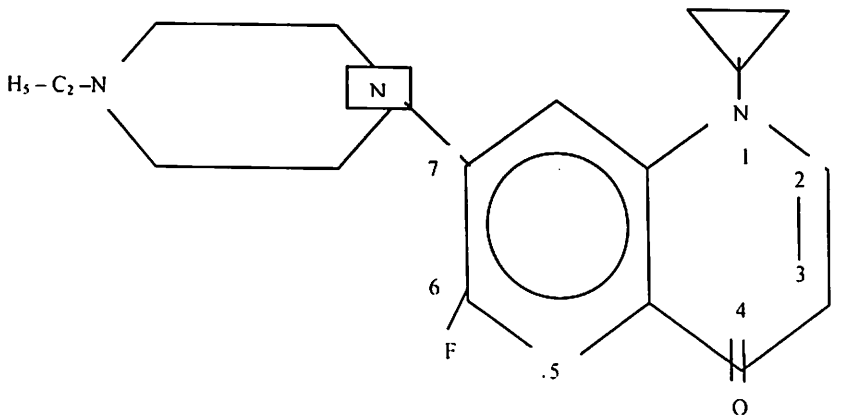
Quinolone carboxylic acid derivatives are synthetic antimicrobial agents that are becoming more popular in medical and veterinary practices. The first member of this group "Nalidixic acid" was introduced in clinical practice in 1963. Nalidixic acid possesses narrow spectrum activity (mostly gram-negative organisms) and mainly used for treating urinary tract infections caused by gram negative organisms. Due to rapid development of resistance and narrow spectrum activity of nalidixic acid, systemic search was carried out to synthesise agents possessing wide spectrum antimicrobial activity for systemic use.

Introduction of 6-fluorine atom into basic nucleus of quinolones in flumequine, produced a racemic mixture in which one isomer was more active than the other and extended gram-positive activity. Further advancement in the quinolone field came with the synthesis of norfloxacin which because of its 6-fluorine and 7-piperazine group, had enhanced antibacterial activity against gram-positive and gram-negative organisms. Since than a number of other newer quinolones have been synthesized; Viz.-Enrofloxacin, norfloxacin, ofloxacin, ciprofloxacin, pefloxacin, etc. (Harold, 1987), and some of then are effectively used in veterinary practice also for the treatment of various bacterial infections (Goldstein and citron, 1993). Enrofloxacin is one of the newly developed fluoroquinolones and used as a drug of choice for animal treatment.

ENROFLOXACIN

CHEMISTRY :

Enrofloxacin is a crystalline, slightly yellowish powder with a slight bitter taste. The chemical structure of enrofloxacin is as follows :



1-Cyclopropyl-7-(4-ethyl-1-pipera-jinyl)-6-fluro-

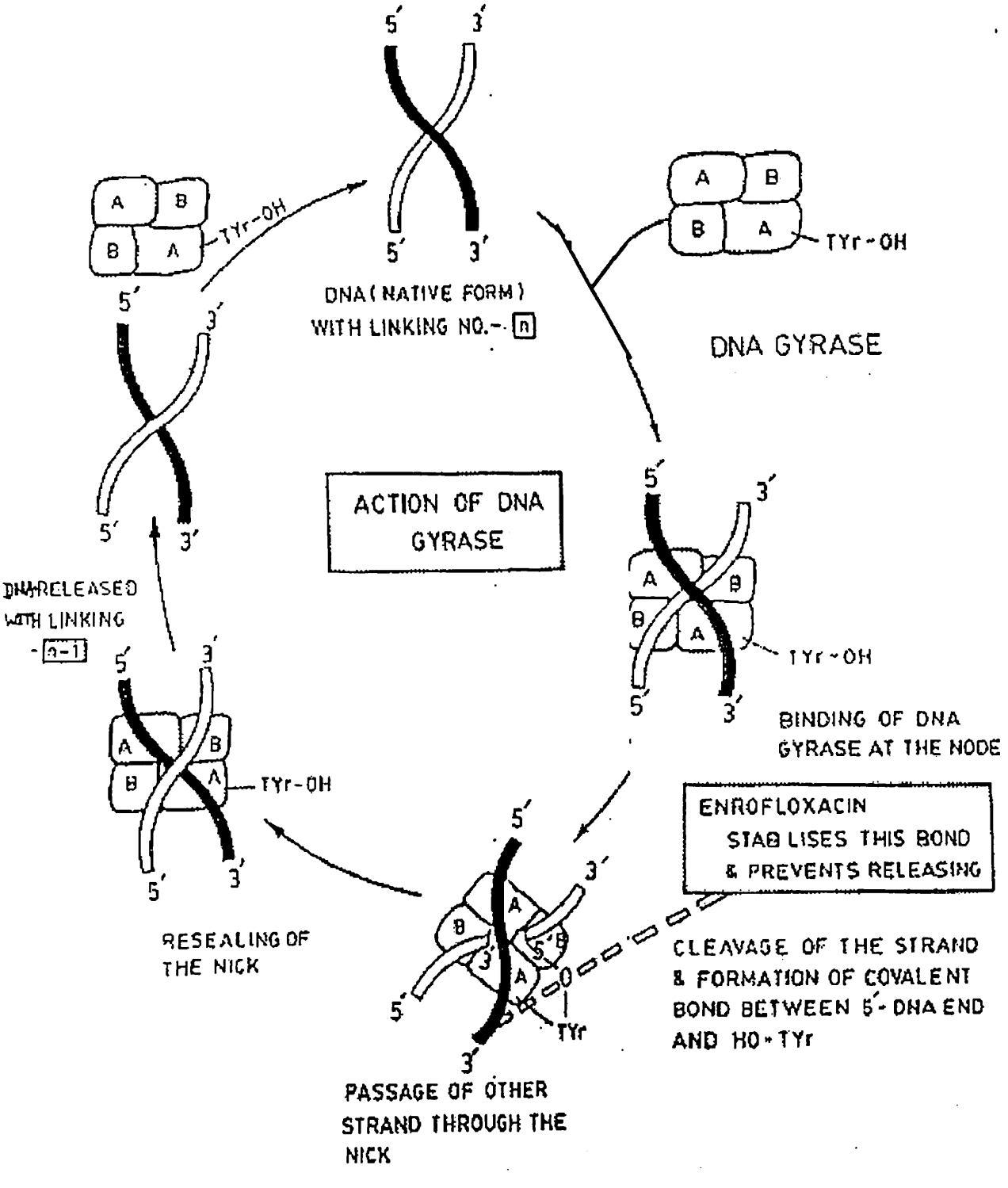
1, 4- dihydro-4-oxo-3-quinoline carboxylic acid.

Emperical formula – C_{19} H_{22} FN_2 O_3

Molecular weight – 359.40

MECHANISM OF ACTION :

Enrofloxacin is a bactericidal agent. The target site for bactericidal action is the enzyme "gyrase" the bacterial type-II-topoisomerase (Vancutsem *et al.*, 1990). Enrofloxacin penetrates the cell nucleus of bacteria and acts by inducing irreversible inhibition of DNA Gyrase, a bacterial enzyme responsible for vital functions of bacteria. The inhibition of gyrase by enrofloxacin stops the replication and supercoiling of DNA within a very short time and there by kills the bacteria (Crumplin *et al.*, 1984 and Bahri and Blouin 1991).



Mechanism of Action of Enrofloxacin

ANTIMICROBIAL ACTIVITY :

Enrofloxacin is a broad spectrum antimicrobial with bactericidal action. It is effective against both gram-negative and gram-positive bacteria as well as mycoplasma. In addition, some of the anaerobic pathogens are also susceptible. Development of resistance is low with other quinolone drugs. Hence it is effective against microorganisms that are resistant to β-lactum antibiotics, tetracyclines, aminoglycosides or macrolides and has a special role in the therapy of multi drug resistant infections. Further, it is not having immuno-suppressive properties, as it does not affect the DNA of the host cells. Thus it can be used simultaneously with vaccines without adversely affecting the immuno-response.

The MICs of enrofloxacin for most gram-negative organisms are generally less than 0.25 $\mu g.\ ml^{-1}$ and rarely exceeds 4.0 $\mu g.ml^{-1}$ (Neuman, 1988).

GENERAL PHARMACOKINETIC :

Wagner (1968) stated that the aim of pharmacokinetics is to study the time concentration course of drugs and their metabolites in various body fluids, tissues and excreta and interpretation of such data based on suitable pharmacokinetic models (compartment models). To study the pharmacokinetics of a drug, the body is subjected to different compartments. These compartments are mathematical entities having no physiological meaning. The disposition kinetics of a drug is described either by one compartment or multi compartment open models. An open compartment model

indicates free movement of drugs from one compartment to another compartment (i.e. blood to tissue and vice-versa.)

When the distribution of drug from central to peripheral compartment is very rapid, the drug is said to follow one compartment open model, like chloramphenical in dogs (Davis *et al.*, 1972) and salicylate in calves (Davis *et al.*, 1973). Any change in drug concentration in the blood reflects directly the quantitative change in its tissue levels. The rate of drug elimination from the body is proportional to the concentration of the drug in blood (Baggot, 1974). In one compartment open model if the plasma concentration time profile is plotted on a semilogrithmic scale, a straight line is obtained (Sams, 1978) as the plasma drug level decline according to equation;

$$C_p = Be^{-\beta t} \quad \text{..... Eq - 1}$$

Where 'C_p' is the concentration of drug in plasma, 'B' is the extrapolated zero time intercept of meno exponential curve, β is the over-all elimination rate constant, 't' is the time elapsed after drug administration and 'e' represents the base of natural logarithm.

The one compartment open model is particularly useful in describing the time course of most drugs in plasma after extravascular (oral/i.m./s.c.) administration (Baggot, 1977).

A two compartment open model accurately describes the pharmacokinetics of most drugs after i.v. administration. In this model, drug distribution is instantaneous and homogenous into the central compartment (blood and other readily accessible tissue like liver and kidney) and more slowly into the peripheral compartments, comprising of less perfused organs and tissues such as muscles and

fats (Baggot, 1974). In this model the distribution and elimination processes are assumed to follow first order kinetics. (The kinetic disposition of various antimicrobials, Khanikar *et al.*, 1986 a, b; Srivastava, 1987; Srivastava *et al.*, 1987 in buffalo species have been demonstrated to be best fitted in this model). Semilagrithmic plot of plasma drug concentration against time shows a biphasic curve. The initial steep decline in plasma drug concentration is mainly due to distribution of the drug from central to peripheral compartment. The latter slow decline is mainly because of irreversible elimination of drug from the central compartment. In this case, the plasma concentration of drug is expressed according to the biexponential equation.

$$C_p = A_e^{-\alpha t} + B_e^{-\beta t} \quad \text{..... Eq - 2}$$

Where 'C_p' represents the plasma concentration of the drug, 'A' and 'B' are the Zero time intercept of distribution and elimination phases, 'α' and 'β' are the distribution and elimination rate constants, respectively, 'e' is the base of natural logarithm and 't' is the time elapsed after drug administration.

To calculate the other Kinetic rate const constants (K_{12} and Kel) associated with two compartment open model, the values of A,B,α and β are essential. The values of these rate constant give an idea of relative contribution of distribution and elimination processes to the drug concentration time data (Baggot, 1977).

In three compartment open model initial sharp decline in plasma concentration against time is due to distribution of drug from blood to highly perfused tissue compartmt. The gradual decline is

because of distribution of drug from central to moderately perfused organs. Kinetics of cefotaxime (Sharma *et al.*, 1995) follow this model. The semilogrithmic plot of plasma drug concentration against time shows a triphasic curve. In this case, the plasma concentration of the drug after a single i.v. administration is estimated by using triexponential equation.

$$C_p = A_e^{-\alpha t} + B_e^{-\beta t} + C_e^{-\gamma t} \text{ -------- Eq} - 3$$

The residual methods are employed to estimate the additional constant γ and C. Gibaldi and Perrier (1975) stated that above constants can be used to calculate K_{13} and K_{31}.

RELEVANCE OF KINETIC PARAMETERS TO CLINICAL PRACTICE :

The clinical application of pharmacokinetic studies comprises of determination of different kinetic parameters of a drug following different routes of administration, calculation of dosage regimen of a drug in a particular species and estimation of drug withdrawl period for drug residues in milk and tissues of food producing animals.

The distribution constant (α) and distribution half life ($t_{1/2}$ α) indicate the rate of distribution (faster or slower) of a drug from plasma to body fluids and tissues following i.v. administration. The absorption constant (Ka) and absorption half life ($t_{1/2}$ka) denote the rate of absorption of a drug from its site after extravascular (i.m./s.c or oral) administration.

Baggot (1977) reported that the overall elimination rate constant (β) is the most important kinetic parameter as it is used to

calculate the half life ($t_{1/2}$ β), volume of distribution by area method (Vd_{area}) and total body clearance (Cl_B). It is also used to predict the with drawl period for drug residue in milk and tissues of food producing animals (Mercer *et al.*, 1977). Gibaldi and Weintraub (1971) described the elimination half life as time required to reduce the drug concentration to its half during the elimination phase of the drug concentration time profile. The half life is inversely proportional to the over all elimination rate constant. Half life is of prime importance in determining the duration of a drug in the body. The half life of a first order process is independent of the route of administration and the dose. This means that doubling the dose, does not double the duration of action of drug but increases it by one half life. Knowledge of the half life of a drug is extremely useful in predicting the design of rational dosage regimen.

The apparent volume of distribution (Vd) is an important parameter in the pharmacokinetic characterization of drugs. The apparent volume of distributionis a hypothetical volume of body-fluid that would be required to dissolve the total amount of drug to attain the same concentration as that found in the blood. Baggot (1977) stated that apparent volume of distribution of a drug without providing any clue whether the drug is uniformly distributed or restricted to certain tissues. A large volume of distribution indicates wide distribution through out the body or extensive tissue binding or rapid excretion of a drug or combination of all the above. The small volume of distribution means, that the drug is restricted to certain

fluid compartments, namely plasma water, extracellular fluid etc. This is due to the low lipid solubility or high protein binding of a drug.

Another important parameter, total body clearance (Cl_B) indicates the sum of the clearance of each eliminating organ, mainly liver and kidney. For most of the drugs, the half life is a complex function which depends upon the process of drug distribution, biotransformation, and renal excretion. The parameter, body clearance on the other hand is independent of these processes and gives a proper expression of the rate of drug removal from the body. Unlike β and $t_{1/2}\ \beta$ which are hybrid constants and depend upon K_{12}, K_{21} and Kel, the body clearance changes exactly in proportion to Kel (Jusko and Gibaldi, 1972; Rowland *et al.*, 1973).

Jusko and Gibaldi (1972) noted that the various constants α, β, A, B, $t_{1/2}\ \alpha$, $t_{1/2}\ \beta$, Vd_{area} etc, change disproportionally with the magnitude of the elimination rate constant (Kel) and therefor, should not be used individually as a direct or safe measure of a change in drug elimination or distribution.

Dose is a quantitative term estimating the amount of drug which must be administered to produce a particular biological response, is to establish a certain effective concentration of a drug in the body requires the administration of maintenance dose at a particular dose interval after administering the priming or loading dose, so that concentration must be above a minimum effective level and below a level producing excessive side effects and toxicity. Thus the objective of a multiple dosage regimen is to maintain the plasma

concentration of the drug within the limits of the maximum safe concentration and the minimum effective levels.

The dose of an antimicrobial is not constant but has to be adjusted to the microbiological pattern and to other factor like altered pathophysiological status of the body. The dose recommended by the manufactures is often based on a relatively high bacterial sensitivity and the variation in the minimum inhibitory concentrations are great (Ziv, 1980). Also they are based on the in vitro tests while as different conditions prevail in vivo where in natural defense works synergistically with an antimicrobial agent (Ziv, 1980 b). Thus in face of all these uncertainties the only scientific approach to recommend a suitable dose of the drug is based on its pharmacokinetic parameters.

PHARMACOKINETIC STUDIES OF ENROFLOXACIN IN HEALTHY STATE :

Enrofloxacin, a member of fluoroquinolone has been exclusively introduced in veterinary practice recently. Pharmacokinetic studies of enrofloxacin were carried out in different species of animals in healthy state on the basis of available literature it seems that no such study has been conducted in goat in febrile state so far. Literature on kinetics of enrofloxacin in various species of animals are stated 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. (5mg. Kg^{-1}). 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 minutes). The values of volume of distribution for enrofloxacin was 0.6 L/kg. The maximum concentration is 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 drug in the milk was achieved 3.3-8 h after injection (Gardorfer, 1991).

Gardorfer (1991) also reported that enrofloxacin by i.v., i.m. or s.c. routes at the dose rate of 2.5 and 5 mg/kg in cows persisted in milk three times as long as in serum. S.c. injection produced half life of 10-18 hr in milk, which is 2-4 times longer than i.v. injection. Bacteriostatic concentration in milk lasted up to 36 hours.

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. They noted that enrofloxacin penetrates into the blood, milk barrier easily and concentrations of the drug in milk were much higher and persisted longer as compared to that of blood.

Tras *et al*; (1993) noted the mean enrofloxacin concentration in the milk samples of dairy cow after i.m. injection of enrofloxacin (2.5 mg/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 120 hr.

Kaartinen *et al*; (1994) estimated elimination half life of 44 minutes after i.v. administration (5 mg. Kg^{-1}) of enrofloxacin. After

extravascular administration (i.m/s.c) at the dose rate of 5 $mg.kg^{-1}$ noted delayed absorption and extended elimination half life (350-457 minutes). Apparent volume of distribution was noted to be 0.6 L/kg.

Kartinen *et al*; (1995) also noted elimination half life 1.7, 5.9 and 5.6 h after i.v., i.m. and s.c. administration of enrofloxacin (5 mg. Kg^{-1}). Mean absorption times 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 peaks.

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 within 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 was 1.756 ± 0.346 $\mu g.ml^{-1}$, while after s.c it was 0.210 ± 0.037 $\mu g.ml^{-1}$ 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 :

The phamacokinetics of enrofloxacin was determined in horses, through i.v. route with 5 mg/kg at 24 hr interval in 5 horses. The half life averaged 6.5 h and volume of distribution 2 $L.kg^{-1}$ with the peak concentration of 9.4 $\mu g.ml^{-1}$ after 10 minutes, falling to 1.9 $\mu g.ml^{-1}$ after 24 h. After oral administration of 25 percent aqueous solution to 5 horses at 5 $mg.kg^{-1}$, 65 percent was absorbed when given at feeding time. An initial i.v. injection was recommended, continued by oral administration to provide a constant serum concentration around 0.5 $\mu g.ml^{-1}$ (Zehe, 1990).

Gigure et al., (1996) noted mean absorption half life of 0.68 and 0.63 h and elimination half life of 5.94 and 6.09 hr for the post i.v. doses of 2.5 and 5 $mg.kg^{-1}$ body weight, respectively. The apparent volume distribution of 1.22 ± 0.07 and 0.77 ± 0.11 L/kg, respectively for i.v. doses of 2.5 and 5 $mg.kg^{-1}$. The total body clearance (Cl_B) values of 0.14 ± 0.01 and 0.09 ± 0.01 L/kg/h for i.v. doses of 2.5 and 5 mg/kg, respectively. After intragastric administration, the bioavailability was noted to be 57.39 ± 8.45 and 62.52 ± 19.65% for the dosage of 2.5 and 5 $mg.kg^{-1}$, respectively. The above workers suggested a single daily i.v. dose of 5.5 mg/kg or orally administered doses of 7.5 $mg.kg^{-1}$ every 24 hr or 4 $mg.kg^{-1}$ every 12 hr would be effective in horses.

Sheep :

In sheep; Pugliese et al., (1991) detected enrofloxacin in serum for up to 4 hr after i.v. and 8 hr after i.m injection at the dose rate of 2.5 $mg.kg^{-1}$. With the i.m. route, the maximum serum

concentration was reached in 1 hr. With both routes, enrofloxacin was detected in milk in 1 hr and persisted for 8 hr.

In Sheep, Mengozzi et al., (1996) noted a rapid distribution phase and a slower elimination phase with a half life ($t_{1/2}\beta$) of 3.73 ± 0.44 h after i.v. dose of 2.5 mg. Kg^{-1}. When the same dose was administered i.m. the drug was rapidly absorbed, reaching mean peak plasma concentration in 1.2 ± 0.11h; after that time it appeared to decrease, with a half life of 3.65 ± 0.31 h. The bioavailability (F) of enrofloxacin by i.m. route was calculated to be 85.28 ± 3.40% volume distribution (Vd_{ss}) was noted to be 3.02 ± 0.22 and 3.03 ± 0.31 $L.kg^{-1}$ for i.v. and i.m route. The total body clearance (Cl_B) values of 0.55 ± 0.14 and 0.62 ± 0.33 $L.kg^{-1}$ hr^{-1} for i.v. and i.m. administration, respectively.

Goat :

Sudha Kumari (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^{-1} body weight. They noted mean absorption half life ($t_{1/2}$ ka) and distribution half life ($t_{1/2}$ α) of 0.60 ± 0.01 and 0.20 ± 0.03 hr in goat. Elimination half life ($t_{1/2}$ β) were also observed as 2.82 ± 0.33 and 1.42 ± 0.15 for i.v. and s.c. administration, respectively. The rate constant of drug transfer from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central compartment (Kel) were noted to be 0.436 ± 0.133, 0.639 ± 0.087 and 0.577 ± 0.137 hr^{-1} respectively, for i.v route. Vd_{area} of 2.34 ± 0.54 & 5.26 ± 1.23 $L.kg^{-1}$ and the total body clearance (Cl_B) 9.40 ± 1.36 & 43.3 ± 9.10 ml. Kg^{-1} min^{-1} have been found for i.v. and s.c. administration, respectively.

concentration was reached in 1 hr. With both routes, enrofloxacin was detected in milk in 1 hr and persisted for 8 hr.

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Pig :

Kuhn (1993) reported that following single i.v. injection of enrofloxacin in pig at the dose rate of 2.5 mg. Kg^{-1}, the peak plasma concentration of 0.68 μg. ml^{-1} was achieved at 225 minutes of injection. He also reported that since the amount in urine exceeded 4 mg. L^{-1} during 12 hr after injection, the drug may be suitable for treating urinary tract infection.

Dog :

In dog, mean $t_{1/2}$ β of 2.4 h, mean total body clearance of 27.1 ml. min^{-1}. Kg^{-1} and mean Vd_{ss} of 7 L/kg were obtained after i.v. administration of enrofloxacin at the dose rate of 5 mg. Kg^{-1} (Kung et. al., 1993). In the same species the half life of enrofloxacin at the dose rate of 5 mg. Kg^{-1} body weight, was noted to be 3hr and in vitro protein binding was 32.6% (Kanemaki et al., 1995).

Camel :

Enrofloxacin was administered i.v., i.m. and s.c. to normal camels and to camels deprived of water for 14 days. Camels lost an average 12.5% of body weight at the end of the water deprivation period. The disposition kinetics of i.v. administered drug in normal and water-deprived camels were similar. After s.c. administration, the mean absorption half-life in the water-deprived camels was significantly longer than in the normal camels (0.92 compared with 0.65 in water-deprived camels) after s.c. treatment. In normal camels, urinary recovery at 12 hr after i.v. and s.c. dosing was 25% and 15%, respectively, and the extent of serum protein binding ranged between 1.7% at 1.8 μg. ml^{-1} and 24% at 0.33 $\mu g.ml^{-1}$. Serum drug concentrations were consistently higher than in the milk. The

AUC milk/AUC serum ratios were 0.27 and 0.39 after i.v. and i.m. drug administration respectively. An i.m. or s.c. treatment regimen of 2.5 mg. Kg^{-1} at 12 hr is suggested for clinical and bacteriological efficacy trials with enrofloxacin in normal and dehydrated camels. (Gavrielli et al., 1995).

Rabbit :

MIC of enrofloxacin against rabbit isolates of *E.Coli*, Bordetalla, Yersinia and staphylococci ranged from 0.3-0.6 μg. $Liter^{-1}$ oral administration at 5 mg. Kg^{-1} have blood concentration of 0.5-0.6 $\mu g.L^{-1}$ (0.3-0.6 μg. L^{-1} after administration in drinking water). While s.c. injection produced 1.3 $\mu g.L^{-1}$ after 30-60 min. Tissue concentration were higher than serum concentrations (Scheer et al., 1990).

Broom et al., (1991) noted over all elimination half lives for i.v., s.c and oral routes of administration of enrofloxacin in rabbit were 2.5, 1.71 and 2.41 hrs, respectively. The half life of absorption for oral dosing was 26 times the half life of absorption after s.c. dosing (7.73 hr V_s 0.3 h). The observed time to maximum serum concentration was 0.9 hr after s.c. dosing and 2.3 hrs after oral administration. The observed serum concentrations at these times were 2.07 and 0.452 $\mu g^{-1}.ml^{-1}$, respectively. Mean residence times were 1.55 hrs for i.v. injections, 1.46 hrs for s.c. dosing and 8.46 hrs for oral administration Enrofloxacin was widely distributed in rabbit as suggested by the volume of distribution being 2.12 liters/kg. The volume of distribution at steady state was 0.93 litres/kg. Compared with i.v. administration, bioavailability was 77% after s.c. dosing and 61% for gastro intestinal absorption.

Cabanes et al (1992) conducted pharmacokinetics and bioavailability studies of enrofloxacin after i.v. and i.m. administration of 5 mg. Kg^{-1} in six healthy adult rabbits. After i.v. administration, rapid distribution phase was followed by a slower elimination phase, with a half-life of 131.5 ± 17.6 minutes. The mean value of total body clearance was 22.0 ± 6.8 $ml.min^{-1}.kg^{-1}$. Its large volume of distribution and the K_{12}/K_{21} ratio close to 1, indicated that enrofloxacin was widely distributed in the body, but not retained in the tissues. After a brief lag period (6.2 ± 2.86 min), i.m. absorption was rapid (4.1 ± 1.3 min) and almost complete. The mean extent of i.m. absorption was 92 ± 11 percent and maximum plasma concentration 10 minutes after administration.

Chicken :

In chicken Anadon et al., (1995) noted shorter distribution half life of 0.070 ± 0.001 hr and longer elimination half life of 10.29 ± 0.45 h after i.v. administration (10 mg. Kg^{-1}). In similar dose after oral administration a comparatively longer absorption half life of 1.43 ± 0.1 hr and a longer elimination half life of 14.23 ± 0.46 h and the bioavailability of 64.0 ± 0.2% were noted. Volume distribution (Vd_{area}) of 4.31 ± 0.15 and 5.94 ± 0.20 $L.kg^{-1}$ and total body clearance (Cl_B) of 0.29 ± 0.02 and 0.288 ± 0.02 $L.\ hr^{-1}.\ Kg^{-1}$ were obtained after i.v and oral administration of enrofloxacin, respectively. The values of rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (Kel) compartment were noted to be 6.13 ± 0.21, 0.19 ± 0.01, 3.46 ± 0.09 hr^{-1} after i.v administration of enrofloxacin.

IMPORTANT KINETIC PARAMETERS OF ENROFLOXACIN IN DIFFERENT SPECIES :

Species	Kinetic Parameters				
	Absorption half life (hr)	Dostributi on half life (hr)	Elimination half-life (hr)	Volume of distribution (L/kg))	Total body clearance
COW	-	-	1.7(i.v)[1] 5.9 (i.m)[1], 5.6 (s.c)[1]	1[1]	-
HORSE	-	0.63-0.68[2]	5.94-6.09[2] 6.5[3]	0.77-1.22[2] 2.0[3]	0.09-0.14[2] ($L.kg^{-1}$,hr^{-1})
SHEEP	-	-	3.73 ± 0.44[4]	3.02 ± 0.22[4]	0.55 ±0.144[4] (i.v.) ($L.kg^{-1}.hr^{-1}$) 0.62 ± 0.33 [4] (i.m) ($L.kg^{-1}.hr^{-1}$)
GOAT	0.60 ± 0.01[5]	0.20 ± 0.03[5]	2.82 ± 0.33[5] (i.v) 1.42± 0.15[5](s.c)	2.34 ± 0.54[5](i.v) 5.26 ±1.23[5] (s.c)	9.40±1.36[5](i.v) 43.3±9.10[5](s.c $ml.kg^{-1}.Min^{-1}$
DOG	-	-	2.4[6] 3[7]	7.0[6]	27[6] ($ml.min^{-1}$ kg^{-1})
RABBIT	-	-	2.18 ±0.29[8] 2.5 h (i.v)[9] 1.71 (s.c)[9] 2.41 (oral)[9]	3.4 ± 0.9[8]	22.8 ± 6.8[8] $ml.kg^{-1}$, min^{-1}
CHICKEN	1.43 ± 0.1[10] (oral)	0.07 ± 0.001[10]	10.29±0.45[10] (i.v 14.23 ± 0.46 (oral)[10].	4.31 ± 0.15[10]	0.29 ± 0.02[10] $L.kg^{-1}$, hr^{-1}

[1] Kaartinen *et al.* 1995 ; [2] Giguere *et al.* 1996 ; [3] Zehe, 1988,

[4] Mengozzi *et al.* 1996 ; [5] Sudha Kumari, 1998 ; [6] Kung *et al.*, 1993;

[7] Kanemaki *et al.*, 1995 ; [8] Cabanes *et al.*, 1992 ; [9] Broome *et al.*, 1991;

[10] Anadon *et al.*, 1995.

ALTERATION IN DISPOSITION KINETICS OF FLUOROQUINOLONE IN FEBRILE CONDITIONS :

Fluoroquinolone class of antimicrobial agents are currently widely used in human medicine and some of them in velerinary practice also. The studies dealing with the alteration in their disposition behaviour in fever or other diseases has not been investigated to full potential though some studies on febrile condition were connected with some agents of this class. Hence some important studies on alteration in disposition behaviour of other antimicrobial agents in physilogical disorders have also been reviewed.

Febrile state is produced by most of the infectious diseases and fever has been reported to alter the metabolism and excretion of drugs (Song *et al.*, 1972). Pharmacokinetic profiles of drugs may differ significantly between healthy and febrile animals and thus adjustment of dosage regimen may be needed in febrile condition.

Jha *et al.*, 1996 have performed kinetic study of norfloxacin and its interaction with probenacid in healthy and febril goats. They found the peak norfloxacin plasma level of 90.2 ± 3.18 $\mu g.ml^{-1}$ attained in the probenecid pretreated febrile goats was higher than in the febrile (75.46 ± 0.72 $\mu g.ml^{-1}$ or afebrile goats (62.25 ± 1.23 ± $\mu g.ml^{-1}$). The values of Cl_B and Kel were significantly ($P<0.01$) decreased in febrile animals as compared to afebrile one. These values were further reduced in febrile goats pretreated with probenecid. However, the values of $t_{1/2}$ β was not affected by the fever probenecid

interaction. On the basis of above findings, it was recommended that norfloxacin may be used as an infusion with probenecid in caprine diseases where very high plasma levels are required.

Ansari (1977) noted no change in the value of absorption half life ($t_{1/2}$ ka) and distribution half life ($t_{1/2}$ α) while elimination half life ($t_{1/2}$ β) was found to be significantly lower after i.v and i.m administration of pefloxacin in febrile goats as compared to afebrile goats.

Singh (1998) reported lower values of $t_{1/2}$ ka, $t_{1/2}$ α and $t_{1/2}$ β of ciprofloxacin in febrile goats as compared to healthy goats which denote that the drug is rapidly absorbed, distributed and also eliminated faster under febrile condition after its i.v and i.m administration. Due to its faster elimination the drug requires higher loading (D^*) and maintenance (D_o) doses to in febrile goats as compared to afebrile goats.

In contrast, Ahangar (1998) showed no change in various kinetic parameters such as $t_{1/2}$ α , $t_{1/2}$ β , Cl_B, Vd_{area} etc between afebrile and febrile cow calves which let to no change in calculated dosage regimen.

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Chapter III

Materials & Methods

INDUCTION OF FEVER :

Fever was induced in goats following single I.V. administration of *E Coli* endotoxim at the dose rate of 1 μg. kg^{-1} body weight (Singh *et al.*, 1997). Normal rectal temperature was noted in each goat at a particular time for three consecutive days. When the temperature was noted to be similar for all days, the initial trial was carried out. Lipoplysaccharide of *E coli.* was dissolved in sterile distilled water to make a solution of 1 $\mu g.ml^{-1}$. This was injected at a dose of 1 $\mu g.kg^{-1}$ body wt in goat, and rise of temperature of 1.5 – 2.5^0c was noted after ½ - 1 hour post injection. The temperature was maintained for about 6-8 hours. The drug was administered after the rise of temperature i.e. ½ -1 hr after the injection of *E.Coli* toxin. The temperature was recorded at every ½ hour upto 8 hour post injection of drug.

DRUG(s)/ CHEMICALS USED :

Enrofloxacin (Enrocin®) was used in the present experiment. Enrocin® (10%), an injectable commercial preparation containing enrofloxacin in concentration of 100 $mg.ml^{-1}$ was obtained as gift samples from Ranbaxy India Ltd. The dose rate of enrofloxacin was 5 $mg.kg^{-1}$ body weight. Antibiotic assay media was prepared from chemicals. The endotoxin of *E. Coli* (Lipopalysaccharide, Serotype 055: B1,5) obtained from Difco laboratories U.S.A was used. All other chemicals used in the present investigation were of analytical grade and extrapure quality.

COLLECTION AND PROCESSING OF BIOLOGICAL SAMPLES :

The samples of various biological fluids were collected after iv and i.m administration of drugs in healthy goats. The samples of plasma, urine and milk were collected at 2.5, 5, 10, 15, 20, 30 & 45 min and 1, 1.5, 2, 3, 4, 5, 6, 8, 10 & 12 hr. Samples of urine and milk were also collected beyond 12 hr at 24, 30 and 36 hr post i.v and i.m administration of enrofloxacin. Same time intervals had been kept for collection of the samples of various biological fluids in febrile condition also.

(A) BLOOD :

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 sterlized prior to each collection with rectified spirit. Blood samples were collected from jugular vein by venipuncture at the above noted various time intervals following drug administration in sterlized 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 then kept in a refrigerator until assay was carried out. For the preparation of standards, normal plasma was also collected prior to drug administration.

(B) MILK :

The samples of milk were collected after washing the udder with soap water and dried with clean soft towel. The milk

samples were collected manually in sterile test tubes by hand milking. The samples were collected at various time intervals as noted above after drug administration. Milk was also collected prior to drug administration for preparation of standards. The collected samples were kept in a refrigerator and the drug concentrations were measured on the following days.

(C) URINE :

The urine samples were collected by introducing a sterile Foley's balloon catheter (No.12) lubricated with glycerine through urethra into the bladder of the experimental goat with the aid of a flexible metal probe. The balloon of the catheter was inflated by injecting 30 ml of sterile water through a syringe so as to keep the catheter in position. The opening of the catheter was blocked with a pressure clip to check dripping of urine. Prior to drug administration, urine samples were collected in a sterile test tubes for the preparation of standards. After administration of the drug, the urine samples were collected in sterile test tubes at various time intervals. The samples were kept in a refrigerator and were analysed in successive days.

PROCEDURE ADOPTED FOR THE MICROBIOLOGICAL ASSAY :

The concentration of enrofloxacin in plasma, milk and urine was determined by employing the standard cylinder plate bioassay technique (Arret *et al.*, 1971). The details of the estimation method are noted below :

(A) STERLIZATION OF GLASS WARES, NEEDLES AND PORCELIN ASSAY CYLINDERS :

All glasswares, needles and porcelin assay cylinders were washed with detergent solution in running tap water, rinsed with glass distilled water and than air dried. Test tubes, centrifuge tubes, vials porcelain assay cylinders placed in vials and needles put in test tubes were wrapped by papers. The materials were sterilized in hot air woven at 160^0C for an hour.

(B) PREPARATION OF MEDIA :

(i) Assay Agar :

Special enrofloxacin assay agar media with the following composition was used for estimation of enrofloxacin in biological fluids after i.v. and i.m. injection in healthy and febrile goats.

Ingredients	Amount in gm per liter
1. Tryptone	15
2. Soya peptone	5
3. Sodium chloride	5
4. Agar	15

PH = 7.3 ± 0.2

The above ingredients were suspended in 1000 ml of glass distilled water. The media was heated to dissolve and the solution was transferred into a conical flask which was plugged with cotton wool. The sterlization of media was done by autoclaving at 15 pound pressure (121^0c) for 20 minutes.

(ii) *Nutrient broth :*

Nutrient broth was prepared with following ingredients :

Ingredients	Amount in gm per liter
1. Sodium chloride	5.0
2. Peptone	5.0
3. Beef extract	1.5
4. Yeast extract	1.5

These ingradients were suspended in 1000 ml of glass distilled water. The media was heated to dissolve and PH was adjusted to 7.4 (approx.). Sterlization of the broth was done in an autoclave at 15 pound pressure for 20 minutes.

(C) PREPARATION OF ASSAY AGAR PLATES :

Melted enrofloxacin antibiotic assay media (20 ml) was poured gently with the aid of a sterile measuring cylinder into each of the sterile special assay plate (Borocil) kept on a horizontally plane surface to get uniform thickness of media. The plates were kept inside the incubator at 37^0c for 24 hr to ascertain any microbial contamination. The plates were then stored in a refrigerator until assay was carried out.

(D) PREPARATION OF ORGANISM :

The test organism *Escherichia Coli* (ATCC 25922) was grown on the slant of culture tube containing enrofloxacin assay agar at 37^0c for over night. Then it was stored under refrigeration. The

organism was transfered weekly to fresh media to maintain its normal activities.

(E) PREPARATION OF STANDARDS OF ENROFLOXACIN IN BIOLOGICAL SAMPLES :

The drug (Enrofloxacin) was dissolved and diluted in glass distilled water to make different strengths, viz.: 80, 40, 20, 10, 5, 2, 1 and 0.5 $\mu g.ml^{-1}$. From each standard solution 0.1 ml was added to a sterile vial containing 0.9 ml of plasma, milk and urine collected prior to drug administration. This yielded drug standards of 8, 4, 2, 1, 0.5, 0.2, 0.1 and 0.05 $\mu g.ml^{-1}$ in the above noted biological samples. These standards were used simultaneously with test samples in the assay plates for determination of the drug concentration in the test samples.

(F) ASSAY PROCEDURE :

The quantitative estimation of enrofloxacin in biological samples was done by microbiological assay method (cylinder plate diffusion method) using *Escherichia Coli.* (ATCC 25922) as the test organism.

The test organism was grown in nutrient broth for 1 to 3 hr at 37^0c until the growth was seen (turbid by naked eye). Enrofloxacin assay plates were flooded with the broth containing the organism and excess broth was drained out. The plates were then dried in the incubator at 37^0c for a period about an hour. Sterile porcelain assay cylinders of uniform size were placed at appropriate

distance along the circumference in the inoculated assay plates. Fifty microlitre of standard solution of various strengths of the drug was poured in separate porcelin cylinder kept on the assay plates. The plates were left on the table for about 2 hr and than kept in the incubator at 37^0c for overnight to allow the growth of organism. The mean diameter of the bacterial zone of inhibition produced by the standards of the drug was measured. The standard curve was plotted from the zone of inhibition versus concentration of the drug on a semi log scale. The test samples were also analysed by using the same method and the concentration of the drug in different test samples of biological fluids were read with the help of standard curve.

CALCULATION OF THE PHARMACOKINETIC PARAMETERS :

The following pharmacokinetic parameters of enrofloxacin after a single iv and i.m administration were calculated from semilog plot of plasma drug concentration versus time curve. The experimental data was analysed using one compartment (for i.m route) or two compartment for (i.v route) open model as described by Gibaldi and Perrier (1975) and Notari (1980)

The concentration of drug in plasma at any time is obtained by the following formula :-

(i) $C_p = B_e^{\beta t}$ ----------(1- compartment model)

(ii) $C_P = A_e^{-\alpha t} + B_e^{-\beta t}$ ----------(2-compartment model)

Where

C_p = plasma drug concentration at time 't'

A = Zero time concentration of the drug in plasma.

α = Regression co-efficient for distribution phase (distribution rate constant) was calculated by the method or residual yields (Appendix-I)

B = Zero time concentration of the drug in plasma.

β = Regression co-efficient for elimination phase (elimination rate constant) was calculated by the method of least squares (Appendix-I).

e = Base of natural logarithm.

K_a = Regression co-efficient for absorption phase (absorption rate constant) after i.m administration of the drug was calculated by the method of residual yields.

(a) Zero time theoretical plasma concentration of drug : C_o^P

$C_o^P = A + B$ (2- compartment model)

Where

C_o^P= Zero time plasma concentration of drug/theoretical plasma concentration of drug at Zero time.

A = Zero time concentration of drug in plasma for distribution phase.

B = Zero time concentration of drug in plasma for elimination phase.

(b) $t_{1/2}$ Ka = 0.693 / Ka

$t_{1/2}\ \alpha$ = 0.693 / α

$t_{1/2}\,\beta = 0.693 / \beta$

Where,

$t_{1/2}$ Ka = Absorption half life

$t_{1/2}\,\alpha$ = Distribution half- life

$t_{1/2}\,\beta$ = Elimination half- life

K_a , α and β are described above.

(c) The total area under the curve : AUC

(i) For 2- Compartment model

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta}$$

(ii) For 1- Compartment model

$$AUC = \frac{B}{\beta} - \frac{A}{\alpha}$$ (Ritschell, 1976)

(d) Total area under the first moment of plasma drug concentration time curve : AUMC

(i) For 2- Compartment model

$$AUMC = \frac{A}{\alpha^2} + \frac{B}{\beta^2}$$

(ii) For 1- Compartment model

$$AUMC = \frac{B}{\beta^2} - \frac{A}{K_a^2}$$

(e) Mean residential time : MRT

$$MRT = \frac{AUMC}{AUC}$$

(f) Rate constant of transfer of drug from peripheral (tissue) compartment to the central (blood) compartment : K_{21} : (hr^{-1})

$$K_{21} = \frac{A.\ \beta\ +\ B.\ \alpha}{A + B}$$

(g) The elimination rate constant of drug from central compartment : K_{el} (hr^{-1})

$$K_{el} = \frac{\alpha \times \beta}{K_{21}}$$

(h) The rate constant of transfer of drug from central to peripheral compartment K_{12} : (hr^{-1})

$$K_{12} = \alpha + \beta - kel - k_{21}$$

(i) The fraction of drug available for elimination from central compartment : F_C

$$T \approx P = \frac{K_{12}}{K_{21} - \beta}$$

(j) The approximate tissue to plasma concentration ratio : T ~ P

$$F_c = \frac{\beta}{K_{cl}}$$

(k)The volume of distribution, based on distribution and elimination

Vd ($L.\ kg^{-1}$)

$$Vd = \frac{D}{C_o^p} \qquad \text{where D = Dose rate}\left(mg.kg^{-1}\right)$$

(l) The volume of distribution based on elimination : Vd_B : ($L.kg^{-1}$)

$$Vd_B = \frac{D}{B}$$

(m) The volume of distribution based on total area under curve : Vd_{area} : $(L.kg^{-1})$

$$Vd_{area} = \frac{D}{(AUC).\beta}$$

(n) The volume of distribution at steady state : Vd_{ss} : $(L.kg^{-1})$

$$Vd_{s.s} = \frac{K_{12} + K_{21}}{K_{21}} \times Vd$$

(o) The total body clearance : Cl_B : $(L.kg^{-1}.\ hr^{-1}$ or $ml.kg^{-1}.min^{-1})$

$$Cl_B = Vd_{area} \times \beta$$

CALCULATION OF DOSAGE REGIMEN:

Dose is a quantitative term estimating the amount of drug which must be administered to produce a particular biological response i.e. to attain optimum effective concentration of drug in the body fluids. Maintenance of therapeutic concentration of a drug in the body requires the administration of maintenance dose at a particular dose interval after administering the priming or loading dose, so that plasma drug concentration must be above a minimum effective level and below a level producing excessive side effects and toxicity. Thus, the objective of a multiple dosage regimen is to maintain the plasma concentration of the drug within the limits of the maximum safe concentration and the minimum effective levels. The minimum inhibitory concentration (MIC) values of enrofloxacin for different species of bacteria isolated from animals ranged between 0.001 to 1.0 $\mu g.ml^{-1}$ (Mevius et al., 1990; Prescott and Yielding 1990). The sensitivity or resistance of enrofloxacin is more or less similar to its

Chapter IV

Results

RESULTS

PHARMACOKINETIC STUDY OF ENROFLOXACIN IN HEALTHY GOATS AFTER I.V. ADMINISTRATION :

1. PLASMA LEVELS :

Plasma levels of enrofloxacin at various time intervals after a single intravenous administration (5 mg. kg^{-1}) in healthy goats have been shown in Table-1 and Fig-1. The mean plasma concentration of the drug at 2.5 min was estimated as 11.19 ± 0.94 $\mu g.ml^{-1}$. The mean therapeutic concentration of 0.12 $\mu g.ml^{-1}$ of enrofloxacin was maintained up to 10 hr in plasma. The drug was present upto 10 hr in all animals (0.13 ± 0.03 $\mu g.ml^{-1}$) and only in one animal at 12 hr.

2. MILK LEVELS :

The concentrations of enrofloxacin at different time intervals in milk following i.v. administration (5 $mg.kg^{-1}$) are depicted in Table-2 and Fig-2. The drug appeared in four out of six goats at 2.5 min and the mean concentration was found to be 0.08 ± 0.03 $\mu g.ml^{-1}$. The mean peak concentration was attained at 1hr with the value of 5.92 ± 0.74 $\mu g.ml^{-1}$. The drug was detectable up to 30 hr in all goats with mean concentration of 0.17 ± 0.03 $\mu g.ml^{-1}$. The mean therapeutic concentration (≥ 0.12 $\mu g.ml^{-1}$) was attained at 5 min and maintained up to 30 hr.

3. URINE LEVELS :

The concentrations of drug in urine (5$mg.kg^{-1}$) post i.v. administration have been presented in Table-3 and Fig-3. The drug

appeared in effective concentration (≥ 0.12 $\mu g.ml^{-1}$) at 2.5 min and was maintained even beyond 36 hr. The mean peak urine concentration of 1008 ± 157.4 $\mu g.ml^{-1}$ was observed at 45 min. The drug was detectable in all six animals at 36 hr (0.40 ± 0.09 $\mu g.ml^{-1}$).

4. KINETIC PARAMETERS :

The log plasma drug concentration versus time profile has confirmed the 2-compartment open model as depicted in Fig-4. Table-4 shows the values of different kinetic parameters calculated by the above noted compartment model.

The mean extrapolated zero time concentration of the drug in plasma during distribution phase (A), elimination phase (B) and the theoretical zero time concentration (C^P_0 =A + B) were noted to be 5.68 ± 0.46, 1.38 ± 0.27 and 7.07 ± 0.51 $\mu g.ml^{-1}$, respectively. The distribution rate constant (α) ranged from 1.015 to 2.110 hr^{-1} with a mean value of 1.741 ± 0.171 hr^{-1}, while its elimination rate constant (β) ranged from 0.188 to 0.315 hr^{-1} with a mean value of 0.246 ± 0.019 hr^{-1}. The mean distribution half life ($t_{1/2}$ α) and elimination half life ($t_{1/2}$ β) were calculated to be 0.42 ± 0.05 and 2.90 ± 0.22 hr, respectively. The mean of the area under curve in plasma (AUC) was noted to be 9.05 ± 0.79 mg. L^{-1} hr and the mean residential time (MRT) was noted to be 2.80 ± 0.24 hr. The average rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central compartment (Kel) were

calculated to be 0.640 ± 0.116, 0.555 ± 0.084 and 0.792 ± 0.050 hr^{-1}, respectively. The mean value of the fraction of drug available for elimination from central compartment (F_c)and approximate tissue to plama concentration ratio (T ≈P) were noted to be 0.319 ± 0.033 and 2.36 ± 0.44, respectively. The various values of volume distribution calculated by different methods are shown in Table-4. The mean volume of distribution (Vd_{area}) was calculated to be 2.43 ± 0.32 $L.kg^{-1}$. The total body clearance (Cl_B)ranged from 6.98 to 11.40 with a mean value of 9.55 ± 0.76 $ml.kg^{-1}. min^{-1}$.

5. DOSAGE REGIMEN:

The dosage regimen required to maintain the fifferent levels of therapeutic concentrations (C_p^{α} min= 0.12 and 0.25 $\mu g.ml^{-1}$) in plasma for i.v. administration in healthy goats at different dosage intervals of 8 and 12 hr has been depicted in Table-5. For maintaining C_p^{α} min of 0.12 $\mu g.ml^{-1}$, the mean value of loading doses (D^*) were calculated to be 2.03 ± 0.17 and 5.58 ± 0.75 mg. kg^1, while average of maintenance doses (D_o) were calculated to be 1.74 ± 0.17 and 5.29 ± 0.77 $mg.kg^{-1}$ at the dosage intervals (υ) of 8 and 12 hr, respectively. The D^*_s were calculated to be 4.23 ± 0.35 and 11.63 ± 1.57 $mg.kg^{-1}$, while D_os were found to be 3.62 ± 0.35 and 11.02 ± 1.61 $mg.kg^{-1}$ at 8 hr and 12 hr, respectively, for maintaining C_p^{α} min of 0.25 $\mu g.ml^{-1}$.

Table-1

Plasma concentrations ($\mu g.ml^{-1}$) of enrofloxacin following single intravenous dose of 5 $mg.kg^{-1}$ in healthy goats.

Time	Animal Number						Mean±S.E
↓	1	2	3	4	5	6	↓
2.5 min	10.30	9.63	13.7	9.87	9.15	14.53	11.19 ± 0.94
5 min	8.30	6.16	9.26	6.51	7.99	9.65	7.98 ± 0.58
10 min	6.00	4.13	6.27	5.67	5.13	5.35	5.43 ± 0.30
15 min	5.80	3.37	3.50	4.94	4.72	3.78	4.35 ± 0.39
20 min	5.23	2.26	2.87	4.30	4.37	2.63	3.61 ± 0.48
30 min	4.90	1.85	2.36	3.74	3.76	2.25	3.14 ± 0.48
45 min	3.75	1.52	1.94	3.26	3.20	1.89	2.59 ± 0.38
1 hr	1.63	1.24	1.60	2.84	1.85	1.37	1.76 ± 0.23
1.5 hr	1.38	1.02	1.20	2.15	1.52	0.95	1.37 ± 0.18
2 hr	1.20	0.92	1.0	0.71	1.24	0.68	0.96 ± 0.10
3 hr	0.84	0.63	0.53	0.40	1.02	0.47	0.65 ± 0.10
4 hr	0.60	0.56	0.42	0.31	0.83	0.37	0.52 ± 0.08
5 hr	0.42	0.45	0.24	0.23	0.68	0.28	0.38 ± 0.07
6 hr	0.34	0.32	0.22	0.17	0.45	0.20	0.28 ± 0.04
8 hr	0.16	0.15	0.20	0.12	0.37	0.18	0.20 ± 0.04
10 hr	0.10	0.11	0.10	0.08	0.25	0.12	0.13 ± 0.03
12 hr	0.00	0.00	0.00	0.00	0.13	0.00	0.02 ± 0.02
24 hr	0.00	0.00	0.00	0.00	0.00	0.00	0.00 ± 0.00

Table-2

Milk concentrations ($\mu g.ml^{-1}$) of enrofloxacin following single intravenous dose of 5 $mg.kg^{-1}$ healthy goats.

Time	Animal Number						Mean±S.E
↓	1	2	3	4	5	6	↓
2.5 min	0.16	0.14	0.00	0.10	0.08	0.00	0.08 ± 0.03
5 min	0.24	0.28	0.10	0.12	0.25	0.12	0.17 ± 0.04
10 min	1.40	0.71	0.20	0.46	0.60	0.19	0.59 ± 0.18
15 min	2.60	0.84	0.75	1.10	1.10	0.49	1.15 ±0.31
20 min	4.30	1.50	1.90	2.08	1.80	0.64	2.04 ± 0.50
30 min	6.90	2.62	3.40	3.85	2.90	0.82	3.42 ± 0.82
45 min	7.20	3.10	4.20	4.70	3.26	1.40	3.98 ± 0.79
1 hr	8.00	5.60	5.10	6.80	7.10	2.90	5.92 ± 0.74
1.5 hr	3.70	2.70	2.65	3.00	3.40	1.68	2.86 ± 0.29
2 hr	2.20	1.62	2.78	1.85	1.90	1.42	1.96 ± 0.20
3 hr	1.78	1.32	1.42	1.50	1.60	1.26	1.48 ± 0.08
4 hr	1.50	1.24	1.10	1.30	1.42	1.14	1.28 ± 0.06
5 hr	1.32	1.14	1.02	1.10	1.24	0.98	1.13 ± 0.05
6 hr	1.18	0.96	0.84	1.02	1.12	0.92	1.01 ± 0.05
8 hr	1.12	0.78	0.70	0.95	0.96	0.82	0.89 ± 0.04
10 hr	0.90	0.64	0.62	0.78	0.72	0.74	0.73 ± 0.04
12 hr	0.78	0.42	0.40	0.66	0.60	0.62	0.58 ± 0.04
24 hr	0.44	0.18	0.28	0.30	0.32	0.20	0.29 ± 0.04
30 hr	0.30	0.08	0.14	0.16	0.18	0.14	0.17 ± 0.03
36 hr	0.18	0.00	0.00	0.08	0.10	0.08	0.07 ± 0.03

Table-3

Urine concentrations ($\mu g.ml^{-1}$) of enrofloxacin following single intravenous dose of 5 $mg.kg^{-1}$ in healthy goats

Time	Animal Number						Mean± S.E
↓	1	2	3	4	5	6	↓
2.5 min	3.28	3.25	2.45	3.65	3.10	1.80	2.92±0.28
5 min	3.90	3.60	3.10	4.70	3.50	2.50	3.55 ±0.30
10 min	41.0	28.0	20.80	45.0	27.0	16.30	29.68 ±4.59
15 min	52.0	42.10	34.05	62.0	42.0	24.45	42.77 ±5.38
20 min	272.0	223.1	140.2	290.0	168.2	118.0	201.9 ±28.9
30 min	436.0	395.0	294.0	474.5	389.2	282	378.5±31.3
45 min	1225	1080	748.6	1492	1110	392.1	1008±157.4
1 hr	552	478.2	369	690	482.5	210.2	463.7±66.5
1.5 hr	410	304	265.4	489	305.5	169	323.8±45.8
2 hr	282.5	186.5	182	308	196.4	130.3	214.3±27.5
3 hr	106	102.1	110	116.6	96.0	94	104.1±3.49
4 hr	83.50	79.0	68.0	97.50	90.0	65.06	80.51±5.12
5 hr	42.0	36.0	22.45	52.0	44.70	20.20	36.23±5.17
6 hr	34.50	21.20	19.50	41.0	27.80	12.85	26.14±4.24
8 hr	20.80	15.0	11.20	32.0	18.40	9.15	17.76±3.35
10 hr	10.40	6.20	5.90	15.50	10.80	6.20	9.17±1.56
12hr	6.72	4.70	2.80	7.25	5.20	3.40	5.01±0.72
24 hr	2.82	2.60	1.93	3.84	2.30	2.20	2.62±0.28
30 hr	1.74	1.65	0.85	2.05	1.45	1.10	1.47±0.18
36 hr	0.41	0.58	0.12	0.72	0.32	0.24	0.40±0.09

Table-4

Kinetic parameters of enrofloxacin folowing single intravenous dose of 5 mg.kg^{-1} in healthy goats.

Parameters (unit)	Animal Number						Mean± S.E
↓	1	2	3	4	5	6	↓
A(μg.ml^{-1})	6.58	3.55	5.67	5.92	5.80	6.57	5.68±0.46
B(μg.ml^{-1})	2.19	1.58	0.91	0.77	2.07	0.75	1.38±0.27
C_o^p (μg.ml^{-1})	8.77	5.13	6.58	6.69	7.91	7.32	7.07±0.51
α (hr^{-1})	1.691	2.096	1.568	1.015	2.110	1.968	1.741±0.171
$t_{1/2}$ α (hr)	0.41	0.33	0.44	0.68	0.33	0.35	0.42±0.05
β (hr^{-1})	0.315	0.273	0.216	0.258	0.225	0.188	0.246 ± 0.019
$t_{1/2}$ β(hr)	2.20	2.54	3.21	2.69	3.08	3.69	2.90±0.22
AUC (mg.L^{-1}.hr^{-1})	10.84	7.48	7.84	8.83	11.95	7.33	9.05±0.79
AUMC (mg.L^{-1}.hr^2)	24.2	23.37	21.81	17.36	42.38	22.92	25.34±3.55
MRT (hr)	2.23	3.12	2.78	1.97	3.55	3.13	2.80±0.24
K_{12} (hr^{-1})	0.539	0.849	0.541	0.170	0.956	0.786	0.640±0.116
K_{21} (hr^{-1})	0.658	0.834	0.403	0.346	0.717	0.370	0.555±0.084
Kel (hr^{-1})	0.809	0.686	0.840	0.757	0.662	1.000	0.792±0.050
F_C	0.389	0.398	0.257	0.341	0.340	0.188	0.319±0.033
T=P	1.57	1.51	2.89	1.93	1.94	4.32	2.36±0.44
Vd(L.kg^{-1})	0.57	0.98	0.76	0.75	0.63	0.68	0.73±0.06
Vd_B(L.kg^{-1})	2.28	3.16	5.49	6.47	2.42	6.67	4.42±0.83
Vd_{area}(L.kg^{-1})	1.46	2.45	2.96	2.19	1.86	3.63	2.43±0.32
Vd_{ss}(L.kg^{-1})	1.04	1.97	1.78	1.12	1.97	2.13	1.67±0.19
Cl_B (ml.kg^{-1} min^{-1})	7.69	11.13	10.66	9.42	6.98	11.4	9.55±0.76

Table-5

Dosage regimens of enrofloxacin when given through intravenous route in healthy goats.

$C_p\alpha$ min($\mu g.ml^{-1}$)	γ (hr)	Dose ($mg.kg^{-1}$)	Animal Number						Mean ± S.E
↓	↓	↓	1	2	3	4	5	6	↓
0.12	8	D*	2.18	2.61	2.00	2.07	1.35	1.96	2.03 ± 0.17
		D_o	2.00	2.32	1.64	1.81	1.13	1.52	1.74 ± 0.17
	12	D*	7.68	7.78	4.74	5.81	3.32	4.16	5.58 ± 0.75
		D_o	7.50	7.49	4.39	5.54	3.10	3.72	5.29±0.77
0.25	8	D*	4.54	5.44	4.17	4.31	2.81	4.08	4.23 ± 0.35
		D_o	4.17	4.83	3.43	3.77	2.35	3.18	3.62 ± 0.35
	12	D*	15.99	16.21	9.88	12.10	6.92	8.66	11.63 ± 1.57
		D_o	15.63	15.60	9.14	11.56	6.45	7.75	11.02 ± 1.61

II. PHARMACOKINETIC STUDY OF ENROFLOXACIN IN FEBRILE GOAT AFTER I.V. ADMINISTRATION

1. PLASMA LEVELS :

The plasma drug concentration versus time profile after i.v. administration (5mg Kg^{-1}) of enrofloxacin in febrile goats has been presented in Table - 6 and Fig. - 1. The mean plasma concentration at 2.5 min was found to be 8.18 ± 0.60 μg. ml^1. The drug was detectable in plasma samples of all animals up to 10hr with a mean concentration of 0.11 ± 0.26 μg. ml^{-1}. The mean

therapeutic, concentration (≥ 0.12 μg.ml^{-1}) was maintained in plasma from 2.5 min to 8hr.

2. MILK LEVELS :

The concentrations of enrofloxacin in milk following i.v. administration (5mg. Kg^{-1}) in febrile goats are shown in Table - 7 and Fig. - 2. the drug appeared in milk samples of five out of six goats at 2.5 min and the mean concentration was observed to be 0.11 ± 0.3 μg.ml^{-1}. The drug reached its meanpeak concentrationof 6.14 ± 0.76 μg.ml^{-1} at 1 hr. The drug was detectable up to 30 hr in all goats with a mean concentration of 0.20 ± 0.03 μg.ml^{-1}. In four out of six goats, it was detectable even at 36 hr. The therapeutic concentration (≥ 0.12 μg.ml^{-1}) was maintained from 5 min to 30 hr.

3. URINE LEVELS :

The concentations of enrofloxacin in urine post i.v. administration (5mg.Kg^{-1}) in febrile goats are shwn in Table - 8 and Fig.- 3. The therapeutic concentration (≥ 0.12 μg.ml^{-1}) was achieved in all animals even at 2.5 min with a mean concentration of 3.25 ± 0.31 μg.ml^{-1} which was maintaind even beyond 36 hr. The drug was detectable in all the six animals at 36 hr with mean concentration of 0.89 ± 0.13 μg.ml^{-1}. The drug reached its mean peak concentration of 1050 ± 162.6 μg.ml^{-1} in urine at 45 min.

4. KINETIC PARAMETERS :

The log plasma drug-concentration versus time profile has confirmed the two-compartment open model as depictd in Fig.-4. Different kinetic parameters calcuated by the above mentioned model have been pesented in Table - 9.

The mean extropolated zero time concentration during distribution phase (A), elimination phase (B) and the theoretical zero time cocentration (C_o^p = A + B) were calculated to be 4.77 ± 0.32, 1.32 ± 0.24 and 6.08 ± 0.33 $\mu g.ml^{-1}$, respectively. The distribution rate constant (α) ranged from 1.054 to 1.730 hr^{-1} with a mean value of 1.409 ± 0.106 hr^{-1}. The range of elimination rate constant (β) varied from 0.223 to 0.327 hr^{-1} with an average of 0.258 ± 0.02 hr^{-1}. The distribution half life ($t_{1/2}\alpha$) and elimination half-life ($t_{1/2}\beta$) were noted to be 0.51 ± 0.04 and 2.74 ± 0.16 hr, respectively. The mean value of the area under curve (AUC) was found to be 8.58 ± 0.76 $mg.L^{-1}.hr$ and the mean residential time (MRT) was calculated to be 2.60 ± 0.24 hr. The average rate constant of drug transfer from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central compartment (K_{el}) were estimated to be 0.431 ± 0.071, 0.507 ± 0.049 and 0.729 ± 0.055 hr^{-1} respectively. The fraction of drug available for elimination from central compartment (F_c) and approximate tissue to plasma concentration ratio (T≈P) were noted

to be 0.364 ± 0.035 and 1.87 ± 0.28, respectively. The various values of volume distribution calculated by different methods are presented in Table-9. The mean volum of distribution (Vd_{area}) was calculated to be 2.42 ± 0.31 $L.kg^{-1}$ with the total body clearance (Cl_B) of 10.09 ± 0.87 $ml.kg^{-1}$ min^{-1}.

5. DOSAGE REGIMEN :

Table 10 exhibits the dosage regimen required to maintain different levels of therapeutic concentrations (C_p^{α} min = 0.12 and 0.25 $\mu g.ml^{-1}$) in plasma for i.v. route in febrile goat at different dosage intervals (γ) of 8 and 12 hr. For maintaining C_p^{α} min of 0.12 $\mu g.ml^{-1}$ the average loading doses (D^*) were calculated to be 2.23 ± 0.16 and 6.39 ± 0.73 $mg.kg^{-1}$, while maintenance doses (D_o) were calculated to be 1.94 ± 0.15 and 6.10 ± 0.74 $mg.kg^{-1}$ at the dosage intervals (ν) of 8 and 12 hr respectively. The D^*S were calculated to be 4.65 ± 0.33 and 13.31 ± 1.52 $mg.kg^{-1}$, while Dos were noted to be 4.05 ± 0.31 and 12.70 ± 1.55 $mg.kg^{-1}$ at the γ of 8 and 12 hr, respectively, for maintaining C_p^{α} of 0.25 $\mu g.ml^{-1}$.

Table-6

Plasma concentrations ($\mu g.ml^{-1}$) of enrofloxacin following single intravenous dose of 5 $mg.kg^{-1}$ in febrile goats.

Time	Animal Number						Mean± S.E
↓	1	2	3	4	5	6	↓
2.5 min	7.40	6.96	9.07	8.78	6.51	10.35	8.18 ± 0.60
5 min	6.35	5.94	5.62	5.15	5.0	7.56	5.94 ± 0.38
10 min	5.85	5.62	5.72	4.98	4.31	5.13	5.27 ± 0.24
15 min	5.60	4.88	3.08	4.49	4.27	3.57	4.32 ± 0.37
20 min	5.00	4.15	2.50	4.03	4.15	2.36	3.70 ± 0.43
30 min	4.65	3.18	2.17	3.47	3.60	2.02	3.18 ± 0.40
45 min	3.57	2.95	1.74	3.02	3.05	1.75	2.68 ± 0.31
1 hr	1.40	1.20	1.46	2.48	1.76	1.27	1.60± 0.19
1.5 hr	1.22	1.05	1.15	1.90	1.45	0.90	1.28 ± 0.15
2 hr	1.00	0.89	1.05	1.00	1.15	0.79	0.98 ± 0.05
3 hr	0.70	0.69	0.50	0.33	1.00	0.40	0.60 ± 0.10
4 hr	0.45	0.52	0.40	0.21	0.88	0.30	0.46 ± 0.10
5 hr	0.36	0.40	0.28	0.20	0.57	0.25	0.34 ± 0.05
6 hr	0.28	0.30	0.26	0.13	0.48	0.19	0.27 ± 0.05
8 hr	0.12	0.17	0.16	0.08	0.30	0.13	0.16 ± 0.03
10 hr	0.08	0.10	0.10	0.05	0.23	0.08	0.11 ± 0.26
12hr	0.00	0.00	0.00	0.00	0.12	0.00	0.02 ± 0.02
24 hr	0.00	0.00	0.00	0.00	0.00	0.00	0.00 ± 0.00

Table-7

Milk concentrations ($\mu g.ml^{-1}$) of enrofloxacin following single intravenous dose of 5 $mg.kg^{-1}$ in febrile goats.

Time	Animal Number						Mean± S.E
↓	1	2	3	4	5	6	↓
2.5 min	0.20	0.16	0.00	0.12	0.10	0.08	0.11 ± 0.03
5 min	0.30	0.32	0.10	0.20	0.30	0.16	0.23 ± 0.03
10 min	1.46	0.78	0.24	0.50	0.72	0.26	0.66 ± 0.18
15 min	2.70	0.86	0.80	1.25	1.28	0.60	1.25 ± 0.31
20 min	4.36	1.80	2.00	2.30	1.92	0.75	2.19 ± 0.48
30 min	7.02	3.00	3.56	4.00	3.00	0.90	3.58 ± 0.81
45 min	7.30	3.28	4.50	4.75	3.35	1.50	4.11 ± 0.79
1 hr	8.40	5.84	5.36	6.96	7.26	3.04	6.14 ± 0.76
1.5 hr	3.85	2.82	2.88	3.16	3.50	1.75	2.99 ± 0.29
2 hr	2.26	1.75	2.94	2.02	1.96	1.46	2.07 ± 0.21
3 hr	1.84	1.36	1.50	1.65	1.72	1.35	1.57 ± 0.08
4 hr	1.60	1.30	1.24	1.36	1.54	1.20	1.37 ± 0.07
5 hr	1.48	1.22	1.10	1.18	1.35	1.05	1.23 ± 0.07
6 hr	1.26	1.04	1.00	1.10	1.20	1.00	1.10 ± 0.05
8 hr	1.20	0.86	0.90	1.02	1.10	0.90	1.00 ± 0.05
10 hr	1.00	0.70	0.72	0.80	0.85	0.78	0.81 ± 0.04
12hr	0.85	0.54	0.50	0.70	0.65	0.64	0.65 ± 0.05
24 hr	0.50	0.24	0.35	0.35	0.36	0.25	0.34 ±0.04
30 hr	0.32	0.10	0.20	0.20	0.24	0.16	0.24 ± 0.30
36 hr	0.20	0.08	0.00	0.00	0.12	0.10	0.08 ± 0.03

Table-9

Kinetic parameters of enrofloxacin following single intravenous dose of 5 mg.kg^{-1} in febrile goats.

Parameters (Unit)	Animal Number						Mean± S.E
↓	1	2	3	4	5	6	↓
A(μg.ml^{-1})	5.26	5.23	3.99	5.54	3.62	4.97	4.77 ± 0.32
B(μg.ml^{-1})	1.89	1.58	0.96	0.68	2.02	0.76	1.32 ± 0.24
C_o^p (μg.ml^{-1})	7.15	6.80	4.95	6.22	5.64	5.73	6.08 ± 0.33
α (hr^{-1})	1.217	1.730	1.382	1.054	1.398	1.671	1.409 ±0.106
$t_{1/2}$ α (hr)	0.57	0.40	0.50	0.66	0.50	0.41	0.51 ±0.04
β (hr^{-1})	0.327	0.276	0.226	0.264	0.232	0.223	0.258±0.02
$t_{1/2}$ β(hr)	2.12	2.51	3.07	2.63	2.98	3.11	2.74 ± 0.16
AUC (mg.L^{-1}.hr^{-1})	10.10	8.73	7.14	7.84	11.30	6.38	8.58 ± 0.76
AUMC (mg.L^{-1}.hr^2)	20.73	22.36	20.89	14.75	39.38	17.06	22.53 ± 3.56
MRT (hr)	2.05	2.56	2.93	1.88	3.49	2.67	2.60±0.24
K_{12} (hr^{-1})	0.274	0.615	0.464	0.173	0.481	0.579	0.431±0.071
K_{21} (hr^{-1})	0.562	0.613	0.450	0.350	0.649	0.415	0.507±0.049
kel (hr^{-1})	0.708	0.778	0.694	0.795	0.500	0.900	0.729±0.055
F_C	0.462	0.350	0.326	0.332	0.464	0.247	0.364±0.035
T≈P	1.16	1.82	2.07	2.01	1.15	3.02	1.87±0.28
Vd(L.kg^{-1})	0.70	0.74	1.01	0.80	0.89	0.87	0.84±0.05
Vd_B(L.kg^{-1})	2.65	3.17	5.21	7.35	2.48	6.58	4.57±0.86
Vd_{area}(L.kg^{-1})	1.51	2.07	3.11	2.42	1.91	3.51	2.42 ± 0.31
Vd_{ss}(L.kg^{-1})	1.04	1.48	2.05	1.20	1.55	2.08	1.57± 0.18
cl_B(ml.kg^{-1} min^{-1})	8.23	9.52	11.71	10.65	7.39	13.05	10.09± 0.87

Table-8

Urine concentrations ($\mu g.ml^{-1}$) of enrofloxacin follwing single intravenous dose of 5 $mg.kg^{-1}$ in febrile goats.

Time	Animal Number						Mean± S.E
↓	1	2	3	4	5	6	↓
2.5 min	4.0	3.40	2.60	4.10	3.20	2.20	3.25 ± 0.31
5 min	4.60	4.0	3.24	5.0	3.68	2.80	3.89 ± 0.34
10 min	50.0	36.2	26.4	48.16	32.0	22.0	35.79± 4.65
15 min	64.0	54.0	42.8	68.0	46.36	36.89	52.01±5.00
20 min	304.1	242	152.4	298.1	176	132.3	217.5±30.45
30 min	450	420.4	308.2	480	395.5	310.2	394.1±29.22
45 min	1336.5	1120	764.2	1520	1140	420.8	1050 ±162.6
1 hr	580	450.4	380.5	712.3	502.4	226.2	475.3±68.20
1.5 hr	440.6	326	278	402.5	322	184	325.5±37.18
2 hr	300.2	208	194.1	316	204.2	148.1	228.4±26.74
3 hr	130	110.6	116	128.5	102.2	110	116.2±4.50
4 hr	104.1	90	80.50	104	98.08	78.3	92.5 ± 4.66
5 hr	60	41.50	32	60.24	52.10	30.84	46.11±5.42
6 hr	44.5	38	28.4	54.5	36.6	27.5	38.25±4.16
8 hr	38.6	28.5	18.50	35.0	34.6	20.8	29.33±3.35
10 hr	20.20	14.40	12.10	24.0	16.20	10.0	16.15±2.12
12hr	12.80	8.90	5.60	13.20	9.40	6.20	9.35 ± 1.30
24 hr	5.60	4.80	2.25	6.10	5.10	3.0	4.48 ± 0.62
30 hr	2.80	2.9	1.18	2.46	2.35	2.40	2.35 ± 0.25
36 hr	1.20	1.24	0.40	0.98	0.70	0.80	0.89 ± 0.13

Table-10

Dosage regimens of enrofloxacin when given through intravenous route in febrile goats.

$C_p\alpha$ min($\mu g.ml^{-1}$)	γ (hr)	Dose ($mg.kg^{-1}$)	Animal Number						Mean ± S.E
↓	↓	↓	1	2	3	4	5	6	↓
0.12	8	D*	2.48	2.26	2.28	2.40	1.47	2.51	2.23 ±0.16
		D_o	2.30	2.01	1.90	2.11	1.24	2.09	1.94 ± 0.15
	12	D*	9.17	6.82	5.62	6.90	3.71	6.12	6.39±0.73
		D_o	8.99	6.57	5.25	6.61	3.48	5.70	6.10±0.74
0.25	8	D*	5.16	4.71	4.74	5.00	3.06	5.22	4.65±0.33
		D_o	4.79	4.19	3.96	4.40	2.58	4.35	4.05±0.31
	12	D*	19.10	14.20	11.71	14.37	7.73	12.75	13.31 ± 1.52
		D_o	18.72	13.68	10.93	13.77	7.25	11.87	12.70±1.55

III. COMPARISION OF PHARMACOKINETICS OF ENROFLOXACIN BETWEEN HEALTHY AND FEBRILE GOATS AFTER I.V ADMINISTRATION.

1. PLASMA LEVELS :

Comparative plasma concentration of enrofloxacin between healthy and febrile goats after its i.v. administration (5 $mg.kg^{-1}$) have been shown in Table-11 and Fig-1. The drug was detectable up to 12 hr in plasma of both healthy and febrile goats.

Plasma drug concentrations were noted to be significantly lower et 2.5 and 5 min in febrile goats as compared to healthy goats. There was no significant difference at other time intervals. The mean therapeutic concentration (≥ 0.12 $\mu g.ml^{-1}$) was maintained from 2.5 min to around 10 hr in both healthy and febrile goats.

2. MILK LEVELS :

Table 11 and Fig-2 reveal that the drug appeared in milk of both healthy and febrile goats at 2.5 min in very low concentration of 5.92 ± 0.74 and 6.14 ± 0.76 $\mu g.ml^{-1}$ in healthy and febrile goat, respectively were attained at 1hr. The therapeutic concentration (≥ 0.12 $\mu g.ml^{-1}$) was maintained from 5 min to 30 hr in both healthy and febrile goats. The drug was detected up to 36 hr in both healthy and febrile goats.

3. URINE LEVELS :

Concentration of enrofloxacin in urine have been shown in Table-11 and Fig-3, significantly higher urine concentration of the drug was observed from 10 hr to 36 hr in febrile goats as compared to healthy goats. From 2.5 min to 8 hr, no significant difference was observed in urinary drug concentration between both the groups of goats. The therapeutic concentration (≥ 0.12 $\mu g.ml^{-1}$) was maintained from 2.5 min to even beyond 36 hr in both groups. The mean peak

urine concentration of 1008 ± 157.4 and 1050 ± 162.6 $\mu g.ml^{-1}$ in healthy and febrile goat, respectively were attained at 45 min.

4. KINETIC PARAMETERS :

Table 12 infers the statistical comparison of different kinetic parameters of enrofloxacin betwen healthy and febrile goats. All the kinetic parameters calculated by two compartment open model after i.v administration in healthy and febrile goats did not differ significantly which denote that pattern of distribution elimination and amount of drug in different body tisues and fluids may be more or less similar in both healthy and febrile goats. Hence the concentration obtained in plasma, milk and urine at most of the time intervals did not show significant difference (Table-11).

5. DOSAGE REGIMEN :

Comparison of dosage regimen between healthy and febrile goats for different therapeutic levels (C_p^{α} min = 0.12 and 0.25 $\mu g.ml^{1}$) and at different dosage intervals (γ) 8 and 12 hr have been shown in Table-13. Loading dose. (D^{*}) and maintenance dose (D_o) were found to be non significantly ($P>0.05$) lower for healthy goats at all therapeutic levles and for all dosage intervals.

Table-11

Comparison of concentrations ($\mu g.ml^{-1}$) of enrofloxacin in various biological fluids between healthy and febrile goat after single intravenous administration at a dose rate of 5 $mg.kg^{-1}$.

TIME	HEALTHY GOAT (n = 6)			FEBRILE GOAT (n = 6)		
↓	Plasma	Milk	Urine	Plasma	Milk	Urine
2.5 min	11.19 ± 0.94	0.08 ± 0.03	2.92 ± 0.28	8.18 ± 0.60*	0.11 ± 0.03 +	3.25 ± 0.31 +
5 min	7.98 ± 0.58	0.17 ± 0.04	3.55 ± 0.30	5.94 ± 0.38*	0.23 ± 0.03 +	3.89 ± 0.34 +
10 min	5.43 ± 0.30	0.59 ± 0.18	29.68 ± 4.59	5.27 ± 0.24 +	0.66 ± 0.18 +	35.79 ± 4.65 +
15 min	4.35 ± 0.39	1.15 ± 0.31	42.77 ± 5.38	4.32 ± 0.37 +	1.25 ± 0.31+	52.01 ± 5.00 +
20 min	3.61 ± 0.48	2.04 ± 0.50	201.9 ± 28.93	3.70 ± 0.43 +	2.19 ± 0.48 +	217.5 ± 30.45 +
30 min	3.14 ± 0.48	3.42 ± 0.82	378.5 ± 31.28	3.18 ± 0.40+	3.58 ± 0.81 +	394.1 ± 29.22 +
45 min	2.59 ± 0.38	3.98 ± 0.79	1008 ± 157.4	2.68 ± 0.31+	4.11 ± 0.79 +	1050 ± 162.6 +
1 hr	1.76 ± 0.23	5.92 ± 0.74	463.7 ± 66.53	1.60 ± 0.19 +	6.14 ± 0.76 +	475.3 ± 68.20 +
1.5 hr	1.37 ± 0.18	2.86 ± 0.29	323.8 ± 45.76	1.28 ± 0.15+	2.99 ± 0.29 +	325.5 ± 37.18+
2 hr	0.96 ± 0.10	1.96 ± 0.20	214.3 ± 27.46	0.98 ± 0.05+	2.07 ± 0.21+	228.4±26.74+
3 hr	0.65 ± 0.10	1.48 ± 0.08	104.1 ± 3.49	0.60 ± 0.10 +	1.57 ± 0.08+	116.2 ± 4.50+
4 hr	0.52 ± 0.08	1.28 ± 0.06	80.51 ± 5.12	0.46 ± 0.10+	1.37 ± 0.07+	92.5 ± 4.66+
5 hr	0.38 ± 0.07	1.13 ± 0.05	36.23 ± 5.17	0.34 ± 0.05+	1.23 ± 0.07+	46.11 ± 5.42+
6 hr	0.28 ± 0.04	1.01 ± 0.05	26.14 ± 4.24	0.27 ± 0.05+	1.10 ± 0.00+	38.25 ± 4.16+
8 hr	0.20 ± 0.04	0.89 ± 0.06	17.76 ± 3.35	0.16 ± 0.03+	1.00± 0.05+	29.33 ± 3.35+
10 hr	0.13 ± 0.03	0.73 ± 0.04	9.17 ± 1.56	0.11 ± 0.26+	0.81 ± 0.04+	16.15 ± 2.12*
12 hr	0.02 ± 0.02	0.58 ± 0.06	5.01 ± 0.72	0.02 ± 0.02+	0.65± 0.05+	9.35 ± 1.30 *
24 hr	0.00 ± 0.00	0.29 ± 0.04	2.62 ± 0.28	0.00 ± 0.00+	0.34 ± 0.04+	4.48 ± 0.62 *
30 hr	N.D.	0.17 ± 0.03	1.47 ± 0.18	N.D.	0.20 ± 0.03+	2.35 ± 0.25*
36 hr	N.D.	0.07 ± 0.03	0.40 ± 0.09	N.D.	0.08 ± 0.03+	0.89 ± 0.13*

+ = Non-singnificant, * $p < 0.05$, N.D. = Not Done

Table-12

Comparison of pharmacokinetic parameters of enrofloxacin between healthy and febrile goats after single intravenous administration of 5 mg.kg^{-1}.

Parameters & Unit	Healthy goat (n=6)	Febrile goat (n=6)
A(μg.ml^{-1})	5.68 ± 0.46	4.77 ± 0.32^{+}
B(μg.ml^{-1})	1.38 ± 0.27	1.32 ± 0.24^{+}
C_o^p (μg.ml^{-1})	7.07 ± 0.51	6.08 ± 0.33^{+}
α (hr^{-1})	1.741 ± 0.171	1.409 ± 0.106^{+}
$t_{1/2}$ α (hr)	0.42 ± 0.05	0.51 ± 0.04^{+}
β (hr^{-1})	0.246 ± 0.019	0.258 ± 0.02^{+}
$t_{1/2}$ β(hr)	2.90 ± 0.22	2.74 ± 0.16^{+}
AUC (mg.L^{-1}.hr^{-1})	9.05 ± 0.79	8.58 ± 0.76 +
AUMC (mg.L^{-1}.hr^{2})	25.34 ± 3.55	22.53 ±3.56^{+}
MRT (hr)	2.80 ± 0.24	2.60 ± 0.24^{+}
K_{12} (hr^{-1})	0.640 ± 0.116	0.431 ± 0.071^{+}
K_{21} (hr^{-1})	0.555 ± 0.084	0.507 ± 0.049^{+}
kel (hr^{-1})	0.792 ± 0.050	0.729 ±0.055^{+}
F_C	0.319 ± 0.033	0.364 ± 0.035^{+}
T≈P	2.36 ± 0.44	1.87 ± 0.28^{+}
Vd(L.kg^{-1})	0.73 ± 0.06	0.84 ±0.05^{+}
Vd_B(L.kg^{-1})	4.42 ± 0.83	4.57 ± 0.86^{+}
Vd_{area}(L.kg^{-1})	2.43 ± 0.32	2.42 ± 0.31^{+}
Vd_{ss}(L.kg^{-1})	1.67 ± 0.19	1.57 ± 0.18^{+}
Cl_B(ml.kg^{-1} min^{-1})	9.55 ± 0.76	10.09 ± 0.87^{+}

+ = Non - significant.

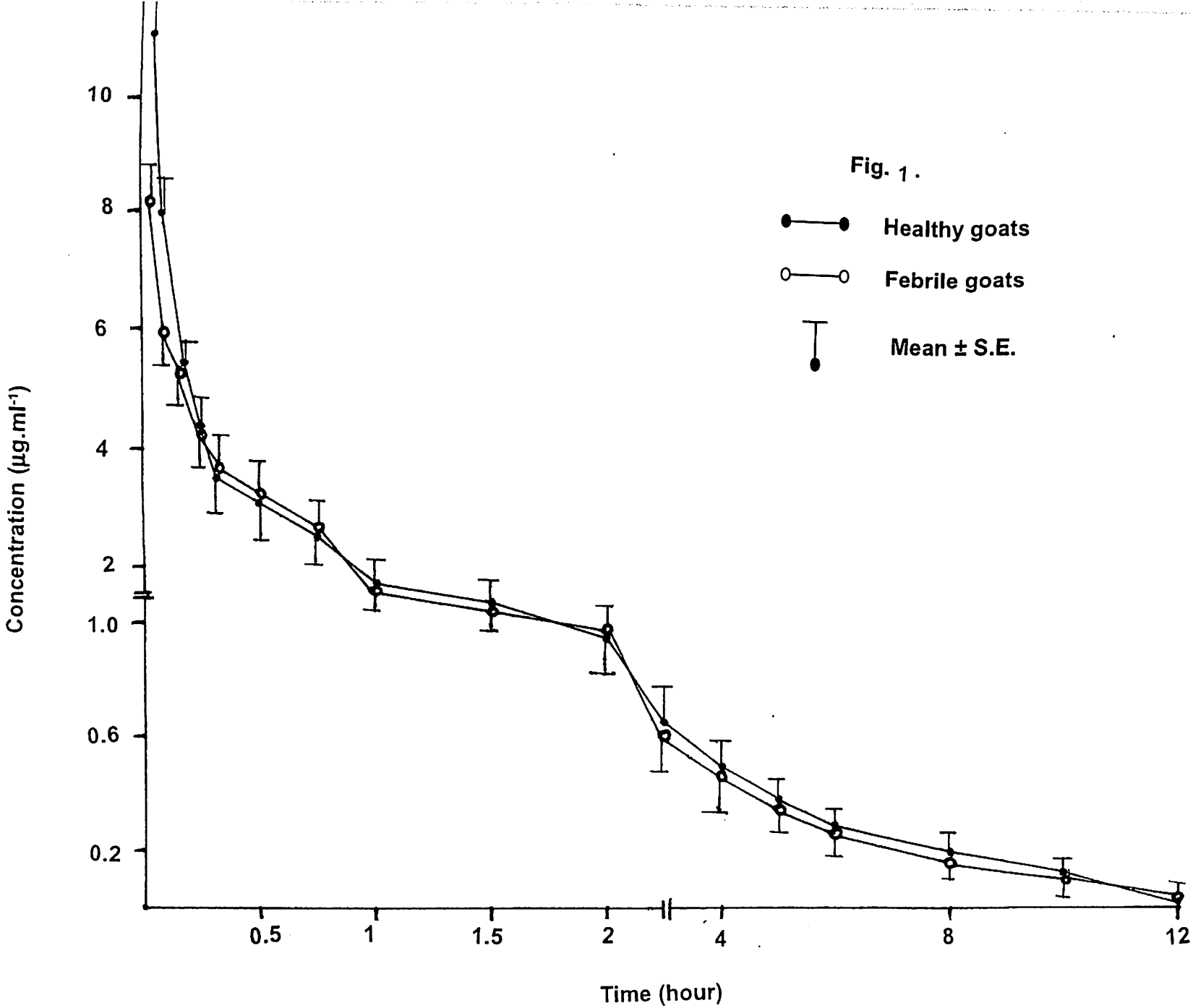

Concentration (µg.ml-1)
10
8
6
4
2
1.0
0.6
0.2
0.5
1
1.5
2
4
8
12
Time (hour)
Healthy goats
Febrile goats
Mean ± S.E.

Fig. 1.

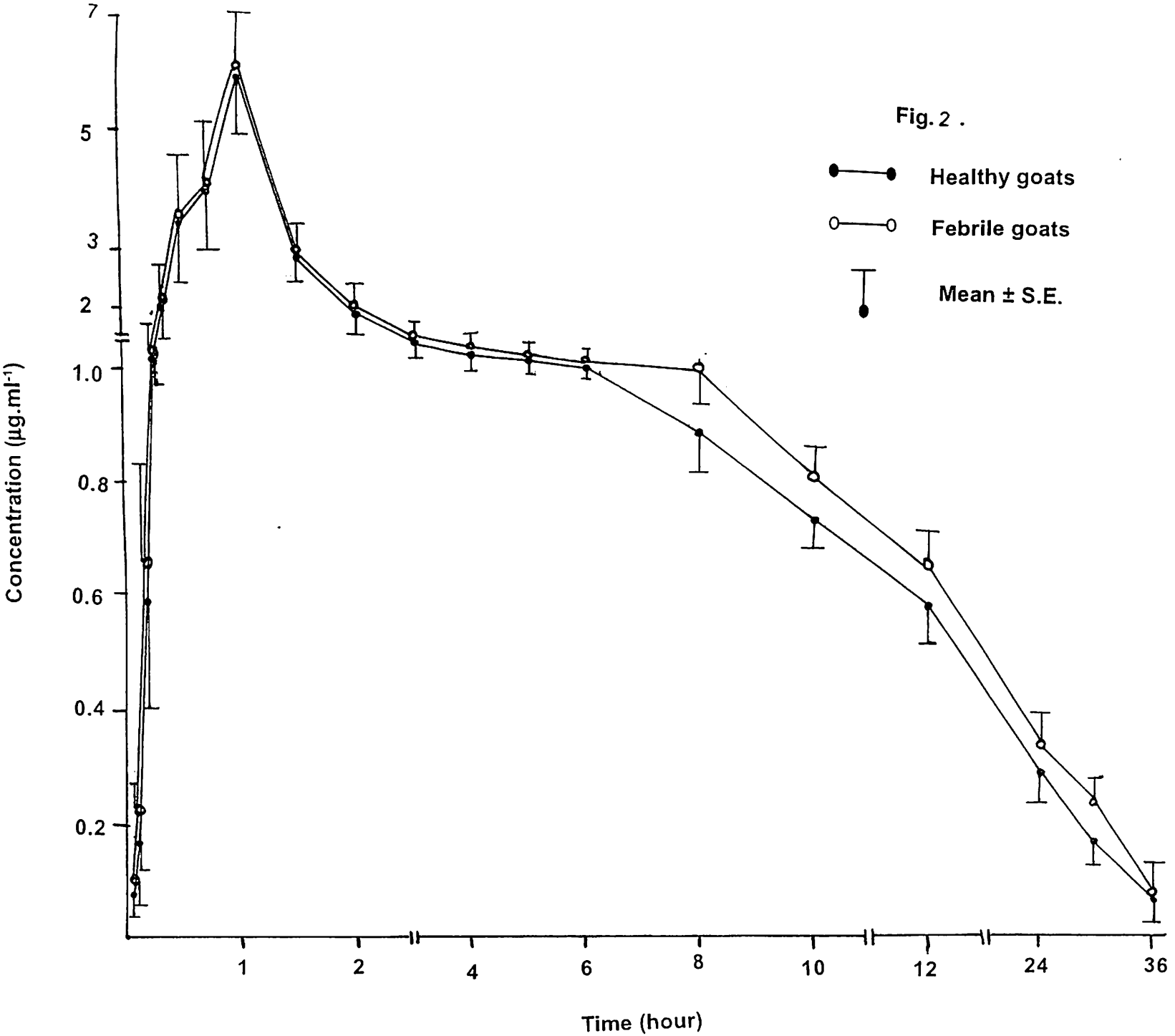


Fig. 2 .
Healthy goats
Febrile goats
Mean ± S.E.
Concentration (µg.ml-1)
7
5
3
2
1.0
0.8
0.6
0.4
0.2
1
2
4
6
8
10
12
24
36
Time (hour)

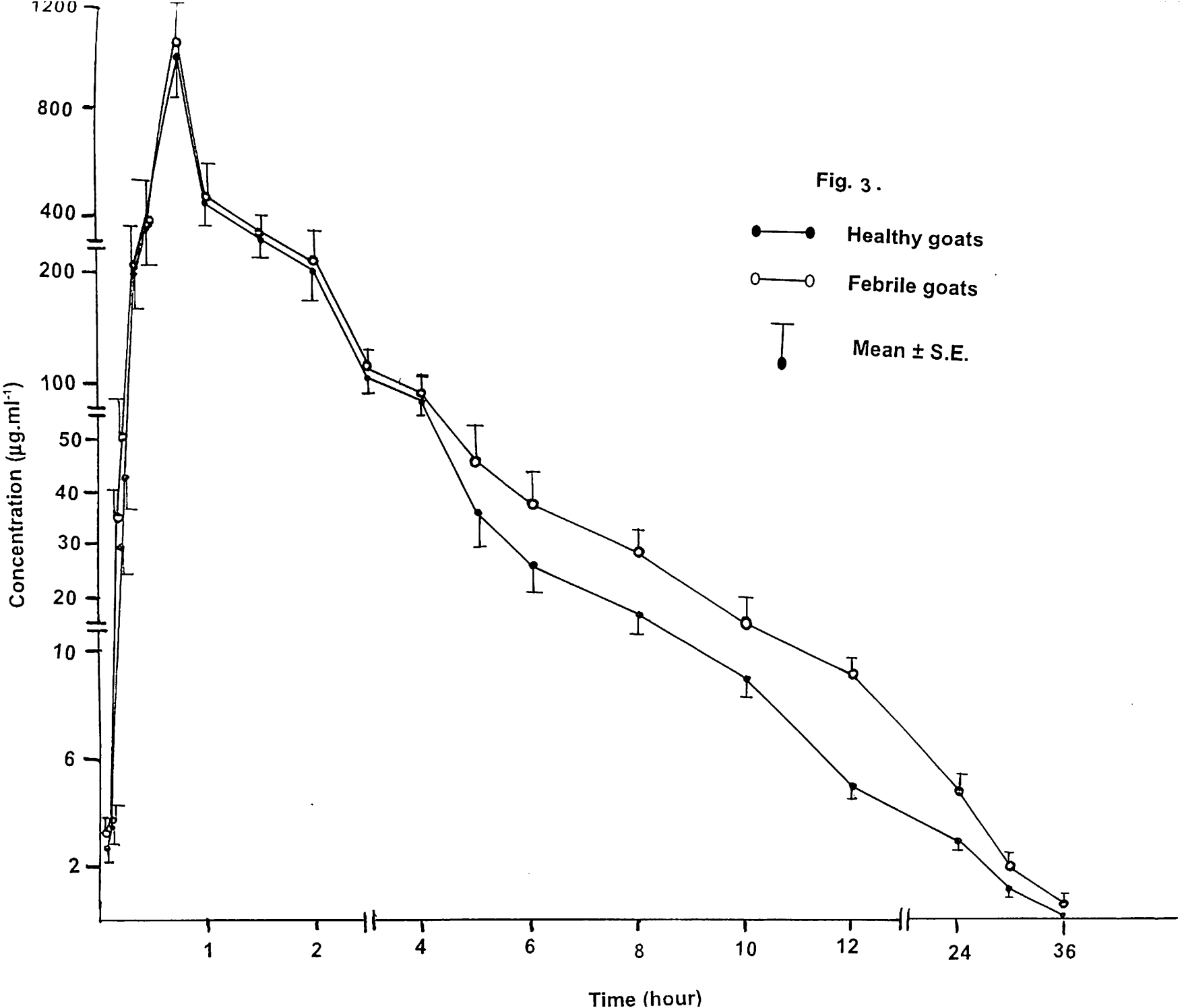


Fig. 3.
Healthy goats
Febrile goats
Mean ± S.E.
Concentration (µg.ml-1)
1200
800
400
200
100
50
40
30
20
10
6
2
1
2
4
6
8
10
12
24
36
Time (hour)

Fig. 4

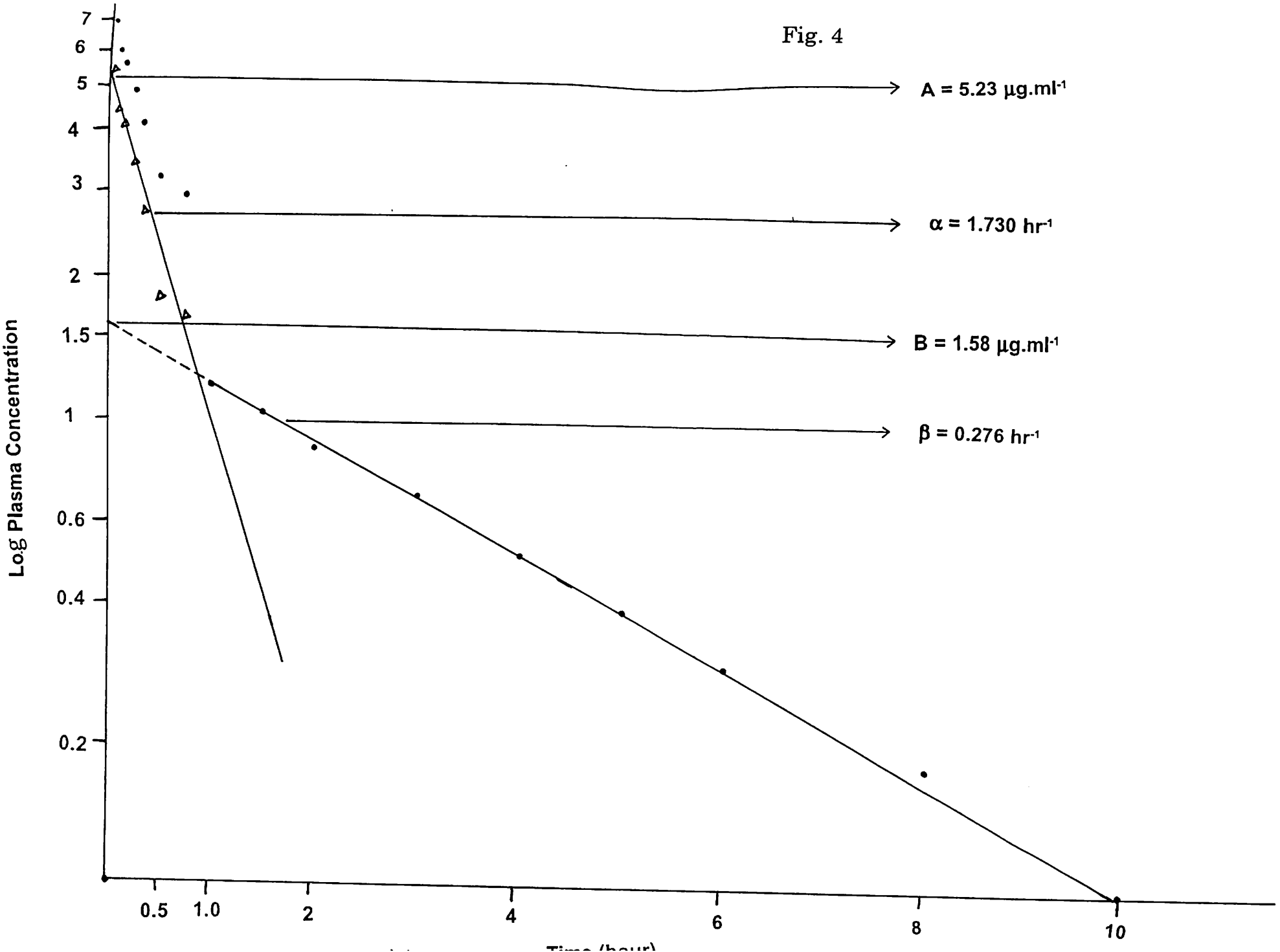

A = 5.23 µg.ml-1
α = 1.730 hr-1
B = 1.58 µg.ml-1
β = 0.276 hr-1
Log Plasma Concentration
7
6
5
4
3
2
1.5
1
0.6
0.4
0.2
0.5
1.0
2
4
6
8
10

Table-13

Comparison of calculated dosage regimens of enrofloxacin for intravenous route between healthy and febrile goats.

C_p^{α}(min) (μg.ml^{-1})	γ (hr)	Dose (mg.kg^{-1})	Healthy Goat (n=6)	Febrile Goat (n =6)
0.12	8	D*	2.03 ± 0.17	2.23 ± 0.16+
		D_o	1.74 ± 0,17	1.94 ± 0.15+
	12	D*	5.58 ± 0.75	6.39 ± 0.73+
		D_o	5.29 ± 0.77	6.10 ± 0.74+
0.25	8	D*	4.23 ± 0.35	4.65 ± 0.33+
		D_o	3.62 ± 0.35	4.05 ± 0.31+
	12	D*	11.63 ± 1.57	13.31 ± 1.52+
		D_o	11.02 ± 1.61	12.70 ± 1.55+

+ = Non- Significant

IV. PHARMACOKINETIC STUDY OF ENROFLOXACIN IN HEALTHY GOATS AFTER I.M. ADMINISTRATION

1. PLASMA LEVELS :

Table 14 and Fig-5 depict the plasma drug concentration at various time intervals in healthy goats after single i.m. administration (5 mg.kg^{-1}). The drug appeared at 5 min in plasma of all the animals with a mean value of 0.19 ± 0.02 μg.ml^{-1} and persisted up to 12 hr (0.15 ± 0.01μg.ml^{-1}). The mean peak concentration of 5.91 ± 0.38 μg.ml^{-1} was achieved at 1 hr. The mean therapeutic concentration (≥ 0.12 μg.ml^{-1}) was maintained from 5 min to 12 hr.

2. MILK LEVELS :

The concentrations of enrofloxacin in milk following single i.m. administration (5 $mg.kg^{-1}$) have been presented in Table 15 and Fig-6. The drug appeared at 2.5 min in two and at 5 min in four out of six goats in low concentrations. The drug was present from 10 min to 24 hr in all animls. The drug reached its mean peak concentration (4.92 ± 0.44 $\mu g.ml^{-1}$) at 1 hr. The mean therapeutic concentration (≥ 0.12 $\mu g.ml^{-1}$) was maintained from 10 min to 24 hr.

3. URINE LEVELS :

Tabel 16 and Fig-7 represent the concentrations of enrofloxacin in urine at different time intervals after a single i.m. dose of 5 mg. kg^{-1}. The drug appeared in effective therapeutic concentration (≥ 0.12 $\mu g.ml^{-1}$) even at 2.5 min with the mean value 0.57 ± 0.13 $\mu g.ml^{-1}$, and it ws maintained up to 36 hr (0.14 ± 0.02 $\mu g.ml^{-1}$). Mean peak concentration of 288.4 ± 36.14 $\mu g.ml^{-1}$ was attained at 45 min.

4. KINETIC PARAMETERS :

The log plasma drug concentration versus time profile has shown monophasic curve as depicted in Fig-8. Hence, the kinetic parameters were calculated using one-compartment open model. Table-17 presents the various kinetic parameters obtained for enrofloxacin following i.m. administration (5 $mg.kg^{-1}$).

The mean extrapolated zero time concentration of the drug in plasma during absorption phase (A) and elimination phase (B) were calculated to be 10.04 ± 0.73 and 9.30 ± 0.59 $\mu g.ml^{-1}$,

respectively. The mean value of absorption rate constant (Ka) and elimination rate constant (β) were noted to be 1.05 ± 0.083 and 0.331 ± 0.012 hr^{-1}, respectively. The absorption half life ($t_{1/2}$ K_a) varied from 0.50 to 0.83 hr with a mean of 0.68 ± 0.05 hr, while elimination half life. ($t_{1/2}$ β) ranged from 1.89 to 2.37 hr with a mean of 2.11 ± 0.08 hr. The total area under curve (AUC) ws found to be 18.64 ± 0.67 $mg.L^{-1}$ hr. The mean of total area under the first moment of plasma drug concentration time curve (AUMC) was calculated to be 75.25 ± 3.51 $mg.L^{-1}hr^2$, while the mean residential time (MRT) was found to be 4.05 ± 0.17 hr. The mean values of volume distribution Vd_B and Vd_{area} were observed to be 0.55 ± 0.04 and 0.84 ± 0.05 $L.kg^{-1}$, respectively. The total body clearance (Cl_B) ranged from 4.06 to 4.97 $ml.kg^{-1}.min^{-1}$ with a mean value of 4.50 ± 0.16 ml. kg^{-1} min^{-1}.

5. DOSAGE REGIMEN :

Table 18 shows the dosage regimen of enrofloxacin for i.m. route in healthy goats at various therapeutic concentrations (C_p^{α} min) and dosage intervals (γ). For maintaining C_p^{α} (min) of 0.12 $\mu g.ml^{-1}$, the everage loading doses (D^*) were calculated to be 1.43 ± 0.12 and 5.46 ± 0.70 $mg.kg^{-1}$, while maintenance doses (D_o) were calculated to be 1.32 ± 0.12 and 5.35 ± 0.70 $mg.kg^{-1}$ at the dosage intervals (γ) of 8 and 12 hr, respectively. The D^*s were calculated to be 2.97 ± 0.25 and 11.37 ± 1.46 $mg.kg^{-1}$, while Dos were noted to be 2.76 ± 0.25 and 11.15 ± 1.46 at the ν of 8 and 12 hr, respectively for maintaing C_p^{α} min of 0.25 $\mu g.ml^{-1}$

Table-14

Plasma concentrations ($\mu g.ml^{-1}$) of enrofloxacin following single intramuscular dose of 5 $mg.kg^{1}$ in healthy goats.

Time	Animal Number						Mean± S.E
↓	1	2	3	4	5	6	↓
2.5 min	0.09	0.00	0.00	0.10	0.00	0.00	0.03±0.02
5 min	0.17	0.15	0.22	0.24	0.12	0.20	0.19±0.02
10 min	0.44	0.28	0.48	0.52	0.26	0.50	0.41±0.05
15 min	0.60	0.45	0.78	0.85	0.50	0.82	0.67±0.07
20 min	0.82	0.70	1.25	1.00	0.75	1.15	0.95±0.11
30 min	1.55	1.05	6.25	1.70	1.15	2.65	2.39±0.81
45 min	7.50	2.45	5.90	3.15	2.38	3.85	4.21±0.84
1 hr	6.50	5.68	5.30	7.20	4.58	6.20	5.91±0.38
1.5 hr	5.40	5.10	4.40	6.00	5.80	5.20	5.32±0.23
2 hr	4.90	4.25	4.00	5.50	5.00	4.75	4.73±0.22
3 hr	3.40	3.10	3.20	3.80	3.40	3.25	3.36±0.10
4 hr	2.15	2.40	2.40	2.70	2.50	2.60	2.46±0.08
5 hr	1.50	1.90	1.80	2.35	2.25	1.90	1.95±0.13
6 hr	1.05	1.30	1.25	1.50	1.40	1.40	1.32±0.06
8 hr	0.55	0.80	0.75	0.78	0.74	0.72	0.72±0.04
10 hr	0.25	0.45	0.40	0.40	0.35	0.38	0.37±0.03
12 hr	0.12	0.18	0.20	0.14	0.12	0.15	0.15±0.01
24 hr	0.00	0.00	0.00	0.00	0.00	0.00	0.00 ± 0.00

Table-15

Milk concentrations ($\mu g.ml^{-1}$) of enrofloxacin following single intramuscular dose of 5 $mg.kg^{-1}$ in healthy goats.

Time	Animal Number						Mean± S.E
↓	1	2	3	4	5	6	↓
2.5 min	0.06	0.00	0.00	0.08	0.00	0.00	0.02±0.01
5 min	0.12	0.08	0.00	0.10	0.07	0.00	0.06±0.02
10 min	0.26	0.38	0.20	0.42	0.30	0.18	0.29±0.04
15 min	0.60	0.62	0.54	0.80	0.75	0.26	0.60±0.08
20 min	0.88	0.96	1.10	1.32	1.20	0.60	1.01±0.10
30 min	2.60	2.85	3.00	3.12	2.98	0.82	2.56±0.36
45 min	3.75	3.90	3.50	4.45	4.10	2.05	3.63±0.34
1 hr	4.62	5.40	3.90	6.30	5.72	3.58	4.92±0.44
1.5 hr	3.24	3.70	2.86	4.00	3.08	2.70	3.26±0.20
2 hr	1.98	2.10	1.64	2.36	1.92	1.42	1.90±0.14
3 hr	1.60	1.62	1.50	1.70	1.55	1.25	1.54±0.06
4 hr	1.12	1.25	1.08	1.36	1.10	1.04	1.16±0.05
5 hr	0.96	1.05	0.90	1.12	0.95	0.86	0.97±0.04
6 hr	0.75	0.84	0.70	0.98	0.82	0.62	0.79±0.05
8 hr	0.65	0.70	0.62	0.84	0.66	0.55	0.67±0.04
10 hr	0.42	0.50	0.40	0.68	0.44	0.38	0.47±0.05
12hr	0.34	0.39	0.30	0.50	0.36	0.24	0.36±0.04
24 hr	0.14	0.18	0.10	0.24	0.16	0.08	0.15±0.02
30 hr	0.10	0.14	0.00	0.16	0.08	0.00	0.08±0.03
36 hr	0.05	0.08	0.00	0.10	0.00	0.00	0.04 ±0.02

Table-16

Urine concentrations ($\mu g.ml^{-1}$) of enrofloxacin following single intramuscular dose of 5 $mg.kg^{-1}$ in helathy goats.

Time	Animal Number						Mean± S.E
↓	1	2	3	4	5	6	↓
2.5 min	0.88	0.52	0.26	0.95	0.64	0.14	0.57±0.13
5 min	6.18	5.40	4.68	7.05	5.80	0.65	4.96±0.92
10 min	18.10	12.25	10.33	18.90	16.50	1.08	12.86±2.72
15 min	30.00	18.0	22.60	42.00	25.0	6.54	24.02±4.84
20 min	94.20	41.50	54.0	123.4	64.78	32.0	68.31±14.09
30 min	224.5	108.2	68.5	248.0	155.0	88.75	148.8±30.17
45 min	256.0	238.4	248.0	428.0	364.8	195.2	288.4±36.14
1 hr	248.4	192.8	156.0	402.4	250.0	204.5	242.4±35.15
1.5 hr	145.8	68.0	90.3	332.5	83.1	92.4	135.4±40.87
2 hr	105.2	54.9	67.8	181.2	75.6	70.0	92.45±19.01
3 hr	68.0	48.0	53.6	94.34	60.0	42.8	61.12±7.56
4 hr	48.1	40.0	38.9	60.52	49.4	32.0	44.82±4.09
5 hr	32.82	26.6	22.0	34.0	30.0	17.5	29.45±2.67
6 hr	25.46	16.2	15.0	27.90	26.0	13.9	20.74±2.59
8 hr	12.50	10.0	11.25	18.62	14.5	9.85	12.79±1.36
10 hr	8.23	5.05	6.45	9.45	8.75	7.10	7.51±0.66
12hr	4.16	2.00	3.80	4.85	4.24	3.70	3.79±0.39
24 hr	1.05	0.60	0.78	1.48	0.84	0.90	0.94±0.12
30 hr	0.45	0.20	0.28	0.53	0.36	0.25	0.35±0.05
36 hr	0.19	0.06	0.12	0.20	0.19	0.10	0.14±0.02

Table - 17

Kinetic parameters of enrofloxacin following single intramuscular dose of 5 mg.kg^{-1} in healthy goats.

Parameters (Unit)	Animal Number						Mean± S.E
↓	1	2	3	4	5	6	↓
A(μg.ml^{1})	10.02	8.54	7.59	11.58	12.32	10.19	10.04± 0.73
B(μg.ml^{1})	9.65	7.99	7.34	10.96	10.76	9.12	9.30±0.59
Ka (hr^{-1})	0.840	0.939	0.975	0.935	1.202	1.381	1.05±0.083
$t_{1/2}$ Ka (hr)	0.83	0.74	0.71	0.74	0.58	0.50	0.68±0.05
β (hr^{-1})	0.366	0.301	0.293	0.343	0.353	0.327	0.331±0.012
$t_{1/2}$ β(hr)	1.89	2.30	2.37	2.02	1.96	2.12	2.11±0.08
AUC (mg.L^{-1}.hr^{-1})	16.79	17.45	17.27	19.56	20.23	20.51	18.64±0.67
AUMC (mg.L^{-1}.hr^{2})	57.84	78.5	77.51	79.91	77.82	79.95	75.26±3.51
MRT (hr)	3.44	4.50	4.49	4.09	3.85	3.90	4.05±0.17
Vd_B(L.kg^{-1})	0.52	0.63	0.68	0.46	0.47	0.55	0.55±0.04
Vd_{area}(L.kg^{-1})	0.89	0.95	0.99	0.75	0.70	0.75	0.84±0.05
Cl_B (ml.kg^{-1} min^{-1})	4.97	4.77	4.83	4.36	4.12	4.06	4.50±0.16

Table-18

Dosage regimen of enrofloxacin when given through intramuscular route in healthy goats.

$C_p\alpha$ min(μg.ml^{-1})	γ (hr)	Dose (mg.kg^{-1})	Animal Number						Mean ± S.E
↓	↓	↓	1	2	3	4	5	6	↓
0.12	8	D*	2.00	1.27	1.24	1.40	1.41	1.23	1.43±0.12
		D_o	1.89	1.15	1.12	1.31	1.33	1.14	1.32±0.12
	12	D*	8.63	4.22	4.00	5.52	5.81	4.55	5.46±0.70
		D_o	8.52	4.11	3.88	5.43	5.72	4.46	5.35±0.70
0.25	8	D*	4.16	2.64	2.58	2.92	2.95	2.57	2.97±0.25
		D_o	3.94	2.40	2.33	2.73	2.77	2.38	2.76±0.25
	12	D*	17.98	8.80	8.33	11.50	12.10	9.49	11.37±1.46
		D_o	17.76	8.56	8.08	11.30	11.92	9.30	11.15±1.46

V. PHARMACO KINETIC STUDY OF ENROFLOXACIN AFTER I.M. ADMINISTRATIONS IN FEBRILE GOATS.

1. PLASMA LEVELS :

Table 19 and Fig-5 show the plasma concentration profile of enrofloxacin at different time intervals after a single i.m. dose (5 mg.kg^{-1}) in febrile goats. At 2.5 min enrofloxacin ws detected in two goats, while at 5 min in all goats with mean concentration 0.04 ± 0.02 and 0.17 ± 0.02 μg.ml^{-1}, respetively. The peak plasma drug concentration of 5.61 ± 0.26 μg.ml^{-1} was ained at 1 hr. The mean

therapeutic concentration (≥ 0.12 $\mu g.ml^{-1}$) in plasma was maintained up to 12 hr, and the mean was observed to be 0.25 ± 0.02 $\mu g.ml^{-1}$.

2. MILK LEVELS :

Table-20 and Fig-6 represents the distribution of enrofloxacin in milk of febrile goats after i.m. dose of 5 $mg.kg^{-1}$. The drug appeared at 2.5 min in two, at 5 min in five goats respectively. The mean theraeutic concentration (≥ 0.12 $\mu g.ml^{-1}$) was attained at 10 min (0.31 ± 0.04 $\mu g.ml^{-1}$) and maintained up to 24 hr (0.17 ± 0.02 $\mu g.ml^{-1}$). The drug was detected in milk of four animals up to 30 hr and in three animals at 36 hr. The peak concentration in milk was achieved at 1 hr (5.06 ± 0.44 $\mu g.ml^{-1}$)

3. URINE LEVELS :

Table 21 and Fig-7 reveal the concentrations of enrofloxacin in urine of febrile goats following signle i.m. administration (5 $mg.kg^{-1}$). The drug appeared at 2.5 min in urine of all the animals with a mean concentration of 0.61 ± 0.14 $\mu g.ml^{-1}$. The mean therapeutic concentration (≥ 0.12 $\mu g.ml^{-1}$) was maintained from 2.5 min and even beyond 36 hr (0.16 ± 0.02 $\mu g.ml^{-1}$). The mean peak concentration was attained at 45 min which was noted to be 291.2 ± 36.12 $\mu g.ml^{-1}$.

4. KINETIC PARAMETERS :

Table 22 reveals the various kinetic parameters after single i.m. administration (5 $mg.kg^{-1}$) in febrile goats based on the calculation using one-compartment open model.

The Mean extrapolated zero time plasma drug concentration during absorption phase (A) and elimination phase (B) were found to be 9.12 ± 0.45 and 8.38 ± 0.49 $\mu g.ml^{-1}$, respectively. Ranged from 0.945 to 1.770 hr^{-1} the absorption rate constant (Ka) had a mean value of 0.963 ± 0.168 hr^{-1}, while the elimination rate constant (β) varied from 0.322 to 0.379 hr^{-1} with mean value of 0.343 ± 0.009 hr^{-1}. The mean absorption half life ($t_{1/2}$ Ka) and mean elimination half life ($t_{1/2}$ β) were found to be 0.59 ± 0.05 and 2.03 ± 0.05 hr, respectively. The total area under curve (AUC) was noted to be 16.48 ± 0.73 $mg.L^{-1}$ hr. while the total area under the first moment of plasma drug concentration time curve (AUMC) was calculated to be 64.20 ± 2.95 $mg.L^{-1}$ hr^2. The mean residential time (MRT) was found to be 3.78 ± 0.13 hr. The mean values of volume distribution, Vd_B and Vd_{area} were calculated to be 0.61 ± 0.03 and 0.89 ± 0.04 $L.kg^{-1}$, respectively. The total body clearance (Cl_B) ranged from 4.63 to 5.93 $ml.kg^{-1}$ min^{-1} with a mean value of 5.12 ± 0.23 $ml.kg^{-1}.min^{-1}$.

5. DOSAGE REGIMEN :

Table 26 presents the dosage regimen required to maintain different levels of therapeutic concentration (C^{α}_{p} min) in plasma for i.m. route in febrile goats at different dosage intervals (γ). for maintaining (C^{α}_{p} min) of 0.12 $\mu g.ml^{-1}$. The average loading doses (D^*) were calculated to be 1.43 ± 0.12 and 5.46 ± 0.70 $mg.kg^{-1}$, while maintenance doses (D_o) were calculated to be 1.32 ± 0.12 and 5.35 ± 0.70 $mg.kg^{-1}$ at the dosage intervals (ν) of 8 and 12 hr, respectively. The D^*s were calculated to be 2.97 ± 0.25 and 11.37 ± 1.46 $mg.kg^{-1}$,

while Dos were noted to be 2.76 ± 0.25 and 11.15 ± 1.46 $mg.kg^{-1}$ at the ν of 8 and 12 hr, respectively for maintaining C^{α}_{p} (min) of 0.25 $\mu g.ml^{-1}$.

Table-19

Plasma concentrations ($\mu g.ml^{-1}$) of enrofloxacin following single intramuscular dose of 5 $mg.kg^{-1}$ in febrile goats.

Time	Animal Number						Mean± S.E
↓	1	2	3	4	5	6	↓
2.5 min	0.00	0.00	0.00	0.10	0.12	0.00	0.04±0.02
5 min	0.14	0.10	0.18	0.20	0.22	0.15	0.17±0.02
10 min	0.35	0.20	0.42	0.48	0.46	0.38	0.38±0.04
15 min	0.50	0.38	0.70	0.72	0.65	0.62	0.60±0.05
20 min	0.85	0.65	1.15	0.86	0.95	0.95	0.90±0.07
30 min	2.25	1.00	3.25	1.54	1.25	2.12	1.90±0.33
45 min	6.20	2.15	5.40	2.92	2.88	3.00	3.76±0.67
1 hr	5.65	5.40	4.98	6.80	5.60	5.20	5.61±0.26
1.5 hr	4.8	4.65	4.28	5.65	4.70	4.50	4.76±0.19
2 hr	4.14	3.60	3.90	5.10	4.05	3.72	4.09±0.22
3 hr	2.62	2.85	3.00	3.48	2.90	3.26	3.02±0.13
4 hr	2.11	1.98	2.26	2.60	2.00	2.48	2.24±0.11
5 hr	1.42	1.46	1.62	2.08	1.45	1.56	1.60±0.10
6 hr	0.96	0.92	1.22	1.36	1.05	1.25	1.13±0.07
8 hr	0.46	0.58	0.56	0.75	0.52	0.68	0.59±0.04
10 hr	0.20	0.25	0.36	0.34	0.26	0.32	0.29±0.03
12hr	0.08	0.14	0.12	0.11	0.12	0.14	0.12±0.01

Tabel-20

Milk concentrartions ($\mu g.ml^{-1}$) of enrofloxocin following single intramuscular dose of 5 mg. kg^{-1} in febrile goats.

Time	Animal Number						Mean± S.E
↓	1	2	3	4	5	6	↓
2.5 min	0.08	0.00	0.00	0.09	0.00	0.00	0.03±0.02
5 min	0.14	0.10	0.08	0.12	0.10	0.00	0.09±0.02
10 min	0.28	0.39	0.24	0.44	0.32	0.19	0.31±0.04
15 min	0.64	0.65	0.56	0.81	0.76	0.28	0.62±0.08
20 min	0.90	0.99	1.16	1.34	1.25	0.66	1.05±0.10
30 min	2.82	3.0	3.15	3.28	3.05	1.0	2.72±0.35
45 min	3.96	4.10	3.62	4.56	4.12	2.18	3.76±0.34
1 hr	4.70	5.48	3.96	6.42	6.0	3.82	5.06±0.44
1.5 hr	3.26	3.75	2.98	4.20	3.18	2.84	3.37±0.21
2 hr	2.08	2.25	1.68	2.44	1.94	1.48	1.98±0.15
3 hr	1.72	1.68	1.52	1.74	1.60	1.34	1.60±0.06
4 hr	1.25	1.27	1.19	1.38	1.15	1.12	1.23±0.04
5 hr	1.0	1.10	0.95	1.20	0.98	0.90	1.02±0.04
6 hr	0.78	0.85	0.72	1.0	0.85	0.64	0.81±0.05
8 hr	0.66	0.74	0.65	0.85	0.69	0.55	0.69±0.04
10 hr	0.45	0.52	0.43	0.70	0.46	0.39	0.49±0.05
12hr	0.36	0.40	0.34	0.52	0.39	0.25	0.38±0.04
24 hr	0.15	0.20	0.12	0.26	0.17	0.11	0.17±0.02
30 hr	0.12	0.15	0.00	0.17	0.10	0.00	0.09±0.03
36 hr	0.06	0.09	0.00	0.11	0.00	0.00	0.04±0.02

Tabel-21

Urine concentrartions ($\mu g.ml^{-1}$) of enrofloxocin following single intramuscular dose of 5 mg. kg^{-1} in febrile goats.

Time	Animal Number						Mean± S.E
↓	1	2	3	4	5	6	↓
2.5 min	0.94	0.56	0.30	1.0	0.70	0.15	0.61 ± 0.14
5 min	6.50	6.0	4.82	7.20	5.96	0.70	5.20 ±0.95
10 min	18.68	12.40	10.45	17.80	17.0	1.25	12.93 ±2.68
15 min	34.05	18.24	23.0	44.14	26.18	7.0	25.44 ±5.24
20 min	90.5	43.08	54.62	130.0	65.33	35.20	70.62 ±14.65
30 min	226.8	110.3	69.1	252.1	156.4	90.04	150.79 ± 30.59
45 min	258.2	240.6	252.3	430.0	368.5	197.7	291.2 ±36.12
1 hr	251.0	194.2	160.1	405.0	254.0	208.7	245.5 ±35.03
1.5 hr	146.0	72.81	95.45	340.7	84.25	94.60	138.97 ± 41.62
2 hr	109.3	55.20	70.30	183.0	78.62	72.54	94.83 ± 19.07
3 hr	70.0	51.43	55.0	95.28	64.75	44.12	63.43 ± 7.41
4 hr	50.25	41.65	40.16	62.0	52.34	35.36	46.96 ± 3.98
5 hr	36.70	30.10	29.5	38.25	33.04	26.0	33.77 ± 2.66
6 hr	27.85	17.42	18.2	28.15	28.22	15.0	22.47 ± 2.54
8 hr	13.62	12.10	12.0	19.83	16.50	10.24	14.05 ± 1.44
10 hr	10.14	8.25	8.85	12.74	10.96	8.92	9.98 ± 0.68
12hr	6.32	4.13	4.50	6.96	5.89	4.65	5.41 ± 0.47
24 hr	2.18	0.96	1.25	3.04	2.10	1.24	1.80 ± 0.32
30 hr	0.48	0.25	0.30	0.55	0.37	0.26	0.37 ± 0.05
36 hr	0.20	0.10	0.14	0.22	0.20	0.10	0.16 ± 0.02

Tabel-22

Kinetic parameters of enrofloxacin following single intramuscular dose of 5 mg. kg^{-1} in febrile goats.

Parameters (Unit)	Animal Number						Mean± S.E
↓	1	2	3	4	5	6	↓
A(μg.ml^{-1})	9.39	8.00	8.61	11.18	8.90	8.65	9.12±0.45
B(μg.ml^{-1})	8.74	7.47	7.50	10.62	8.09	7.85	8.38 ± 0.49
Ka (hr^{-1})	1.135	0.955	1.770	0.945	1.285	1.278	0.963±0.168
$t_{1/2}$ Ka (hr)	0.61	0.73	0.39	0.73	0.54	0.54	0.59±0.05
β (hr^{-1})	0.379	0.333	0.323	0.356	0.346	0.322	0.343±0.009
$t_{1/2}$ β(hr)	1.83	2.08	2.15	1.95	2.00	2.15	2.03±0.05
AUC (mg.L^{-1}.hr^{-1})	14.79	14.05	18.36	18.0	16.05	17.61	16.48±0.73
AUMC (mg.L^{-1}.hr^{2})	53.56	58.59	69.14	71.28	62.19	70.41	64.20±2.95
MRT (hr)	3.62	4.17	3.77	3.27	3.87	4.00	3.78±0.13
Vd_B(L.kg^{-1})	0.57	0.67	0.67	0.47	0.62	0.64	0.61±0.03
Vd_{area}(L.kg^{-1})	0.89	1.07	0.84	0.78	0.90	0.88	0.89±0.04
Cl_B (ml.kg^{-1} min^{-1})	5.62	5.93	4.63	4.63	5.19	4.72	5.12±0.23

Table-23

Dosage regimens of enrofloxacin when given through intramuscular route in febrile goats.

$C_p\alpha$ min($\mu g.ml^{-1}$)	γ (hr)	Dose ($mg.kg^{-1}$)	Animal Number						Mean ± S.E
↓	↓	↓	1	2	3	4	5	6	↓
0.12	8	D*	2.21	1.84	1.34	1.61	1.72	1.39	1.69±0.13
		D_o	2.11	1.71	1.23	1.52	1.61	1.28	1.58±0.13
	12	D*	10.01	6.98	4.86	6.71	6.86	5.03	6.74±0.76
		D_o	9.98	6.85	4.76	6.61	6.76	4.93	6.65±0.77
0.25	8	D*	4.61	3.84	2.78	3.36	3.58	2.89	3.51±0.27
		D_o	4.39	3.57	2.57	3.17	3.36	2.67	3.29±0.27
	12	D*	21.01	14.55	10.13	13.97	14.30	10.48	14.07±1.60
		D_o	20.79	14.28	9.92	13.78	14.08	10.26	13.85±1.60

VI. COMPARISON OF PHARMACOKINETICS OF ENROFLOXACIN BETWEEN HEALTHY AND FEBRILE GOATS AFTER I.M. ADMINISTRATION

1. PLASMA LEVELS :

Table-24 and Fig.5 depict the comparative plasma concentrations of enrofloxacin obtained at various time intervals between healthy and febrile goats after single i.m. administration (5 mg. kg^{-1}). The drug maintained its therapeutic concentration ($\geq 0.12\mu g.ml^{-1}$) from 5 min to 12 hr in both healthy and febrile goats. the mean peak concentration of drug were found to be 5.91 ±0.38 and

5.61 ±0.26 μg.ml^{-1} at 1 hr in both healthy and febrile goats, respectively. No significant difference in plasma concentration of drug was observed at any time interval.

2. MILK LEVELS:

Table-24 and Fig-6 reveal the milk concentrations of enrofloxacin post i.m. administration (5 mg.kg^{-1}) in healthy and in febrile goats. In both the groups drug was detectable from 2.5 min to 36 hr. The drug reached its peak concentration in in both the groups and the concentrations were noted to be 4.92 ± 0.44 and 5.06 ± 0.44 μg.ml^{-1} in healthy and febrile goats, respectively. There was non-significant difference in milk levels of the drug between both the groups. The drug maintained its therapeutic concentration (≥ 0.12 μg.ml^{-1}) from 10 min to 24 hr. in both the groups.

3. URINE LEVELS:

Table 24 and Fig-7 show the urine concentrations of enrofloxacin in healthy and febrile goats after i.m. administration (5 mg.kg^{-1}). Significantly higher concentrations of the drug were observed from 10 to 24 hr only in febrile goats as compared to healthy goat. Peak concentrations were obtained in both groups at 45 min with mean values of 288.4 ± 36.14 and 291.2 ± 36.12 μg.ml^{-1} in healthy and febrile goats, respectively. The theraputic concentration

(≥ 0.12 μg.ml^{-1}) was maintained from 2.5 min to even beyond 36 hr in both the groups.

4. KINETIC PARAMETERS :

Table 25 depicts the comparison of kinetic parameters of enrofloxacin administered in healthy and febrile goats through i.m. route. Non significant variation was observed for all kinetic parameters except area under mean plasma concentration time curve (AUMC), which was become significantly lower in febrile condition as compared to healthy one. Since the kinetic parameters did not differ between healthy and febrile goats, more or less similar pattern of distribution, elimination and amount of drug in different body tissues and fluids would be expected to occur in healthy as well as febrile goats. This may be the reason for obtaining non-significant difference in concentration of plasma and milk at all time intervals and most of the time in urine post i.m. administration in healthy and febrile goats.

5. DOSAGE REGIMEN :

Table-26 reveal the comparison of dosage regimen between healthy and febrile goats for different therapeutic levels (C_p^{α} min = 0.12 and 0.25 μg.ml^{-1}) and at different dosage intervals (v) of 8 and 12 hrs. Loading doses (D^*) and maintenance doses (D_o) were found to be non significant lower for healthy goats at all therapeutic levels and for all dosage intervals.

Table-24

Comparison of concentrations (μg.ml^{-1}) of enrofloxacin in various biological fluids between healthy and febrile goat after single intramuscular administration at a dose rate of 5 mg.kg^{-1}.

TIME	HEALTHY GOAT (n = 6)			FEBRILE GOAT (n = 6)		
↓	Plasma	Milk	Urine	Plasma	Milk	Urine
2.5 min	0.03 ± 0.02	0.02 ± 0.01	0.57 ± 0.13	0.04 ± 0.02^{+}	0.03 ± 0.02^{+}	0.61 ± 0.14^{+}
5 min	0.19 ± 0.02	0.06 ± 0.02	4.96 ± 0.92	0.17 ± 0.02^{+}	0.09 ± 0.02 $^{+}$	5.20 ± 0.95^{+}
10 min	0.41 ± 0.05	0.29 ± 0.04	12.86 ± 2.72	0.38 ± 0.04^{+}	0.31 ± 0.04^{+}	12.93 ± 2.68 $^{+}$
15 min	0.67 ± 0.07	0.60 ± 0.08	24.02 ± 4.84	0.60 ± 0.05^{+}	0.62 ± 0.08^{+}	25.44 ± 5.24^{+}
20 min	0.95 ± 0.11	1.01 ± 0.10	68.31 ± 14.09	0.90 ± 0.07^{+}	1.05 ± 0.10^{+}	70.62 ± 14.65^{+}
30 min	2.39 ± 0.81	2.56 ± 0.36	148.8 ± 30.17	1.90 ± 0.33^{+}	2.72 ± 0.35^{+}	150.79± 30.59^{+}
45 min	4.21 ± 0.84	3.63 ± 0.34	288.4± 36.14	3.76 ± 0.67^{+}	3.76 ± 0.34^{+}	291.2 ± 36.12^{+}
1 hr	5.91 ± 0.38	4.92± 0.44	242 .4 ±·35.15	5.61 ± 0.26^{+}	5.06 ± 0.44^{+}	245.5± 35.03^{+}
1.5 hr	5.32 ± 0.23	3.26± 0.20	135.4± 40.87	4.76± 0.19^{+}	3.37± 0.21^{+}	138.97± 41.62^{+}
2 hr	4.73± 0.22	1.90± 0.14	92.45± 19.01	4.09± 0.22^{+}	1.98± 0.15^{+}	94.83± 19.07^{+}
3 hr	3.36± 0.10	1.54± 0.06	61.12± 7.56	3.02± 0.13^{+}	1.60± 0.06^{+}	63.43± 7.41^{+}
4 hr	2.46± 0.08	1.16 ± 0.05	44.82± 4.09	2.24± 0.11^{+}	1.23± 0.04^{+}	46.96± 3.98^{+}
5 hr	1.95± 0.13	0.97± 0.04	29.45± 2.67	1.60± 0.10^{+}	1.02 ± 0.04 $^{+}$	33.77± 2.66^{+}
6 hr	1.32± 0.06	0.79± 0.05	20.74± 2.59	1.13± 0.07^{+}	0.81± 0.05^{+}	22.47± 2.54^{+}
8 hr	0.72± 0.04	0.67± 0.04	12.79± 1.36	0.59± 0.04^{+}	0.69± 0.04^{+}	14.05±1.44^{+}
10 hr	0.37± 0.03	0.47± 0.05	7.51± 0.66	0.29± 0.03^{+}	0.49± 0.05^{+}	9.98± 0.68*
12 hr	0.15± 0.01	0.36± 0.04	3.79± 0.39	0.12± 0.01^{+}	0.38± 0.04^{+}	5.41± 0.47*
24 hr	0.00 ± 0.00	0.15± 0.02	0.94± 0.12	0.00 ± 0.00	0.17± 0.02^{+}	1.80± 0.32*
30 hr	N.D.	0.08± 0.03	0.35± 0.05	N.D.	0.09± 0.03^{+}	0.37± 0.05^{+}
36 hr	N.D.	0.04± 0.02	0.14± 0.02	N.D.	0.04± 0.02^{+}	0.16± 0.02^{+}

$^{+}$ = Non-singnificant, * $p < 0.05$, N.D. = Not Done

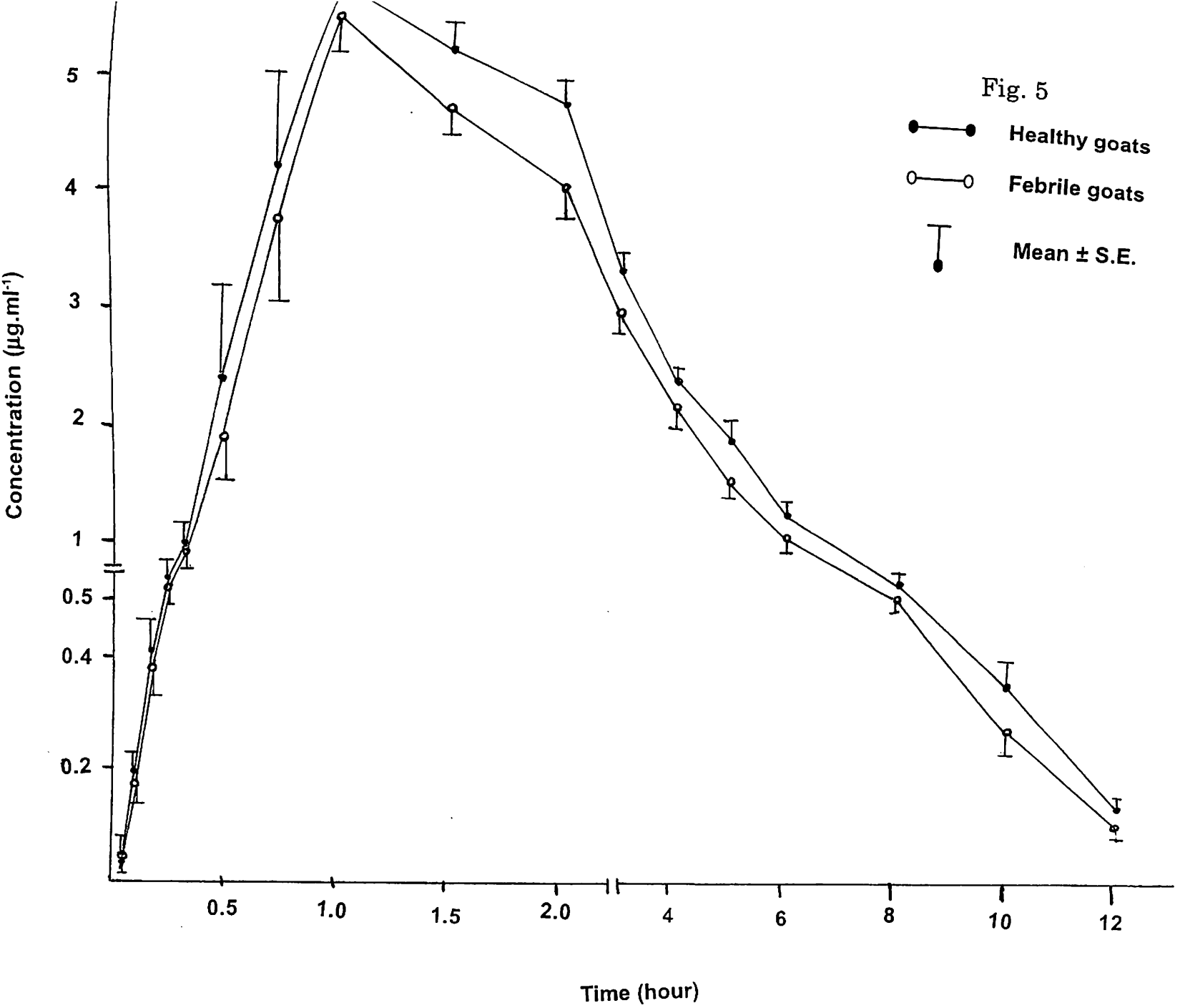


Fig. 5
Healthy goats
Febrile goats
Mean ± S.E.
Concentration (µg.ml⁻¹)
5
4
3
2
1
0.5
0.4
0.2
0.5
1.0
1.5
2.0
4
6
8
10
12
Time (hour)

Fig. 6

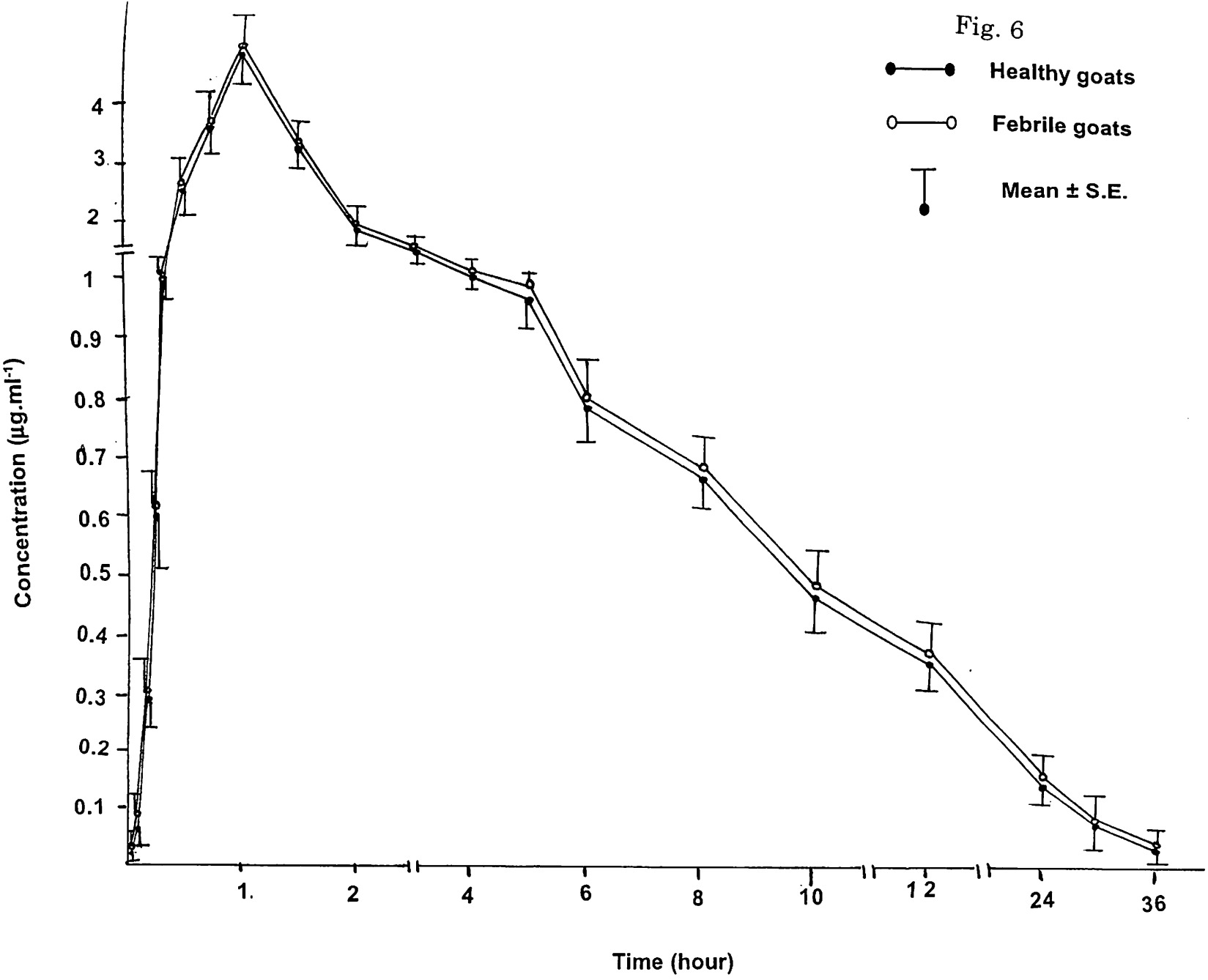
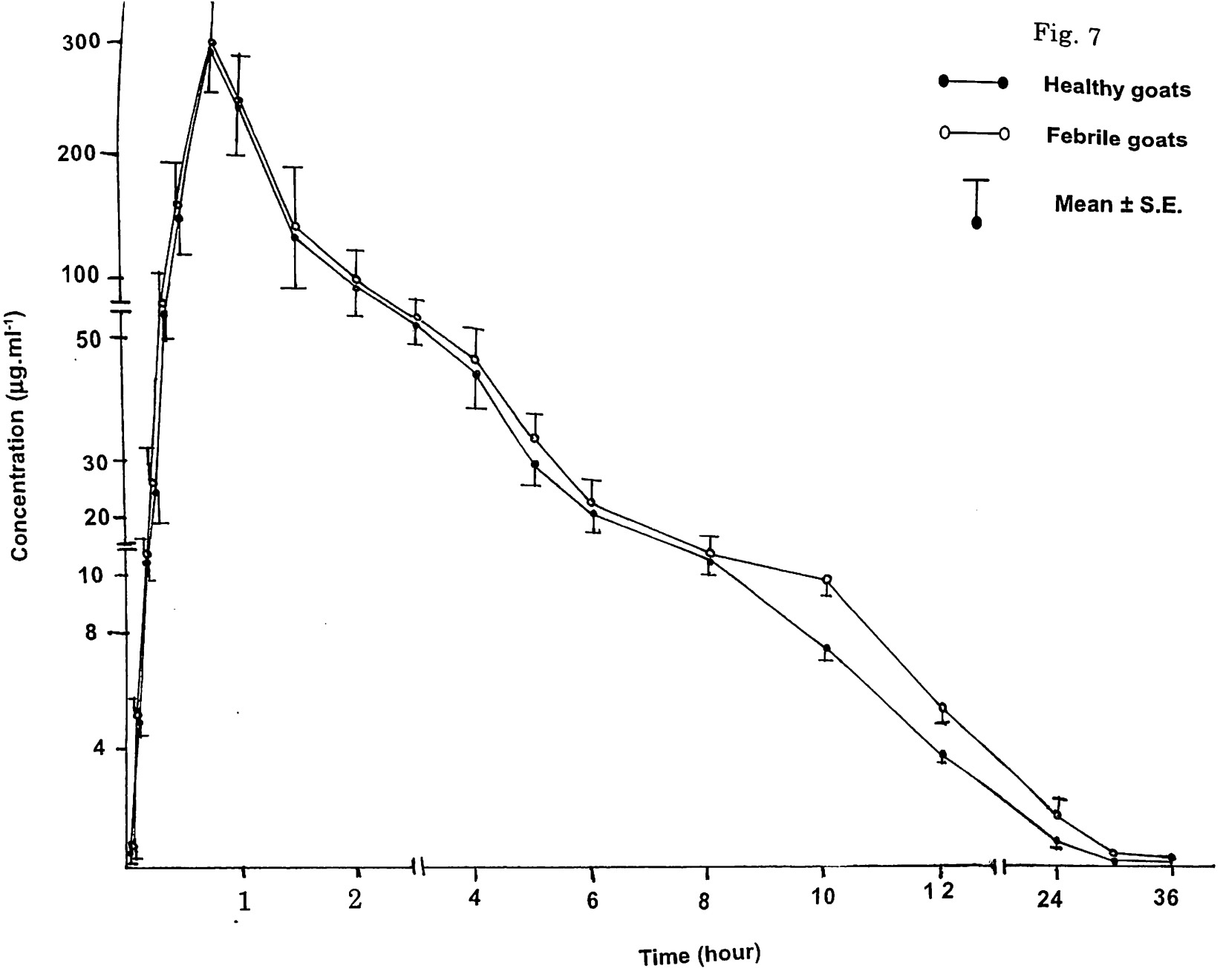

Healthy goats
Febrile goats
Mean ± S.E.
Concentration (µg.ml-1)
4
3
2
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
1.
2
4
6
8
10
12
24
36
Time (hour)

Fig. 7


Healthy goats
Febrile goats
Mean ± S.E.
Concentration (µg.ml⁻¹)
300
200
100
50
30
20
10
8
4
1
2
4
6
8
10
12
24
36
Time (hour)

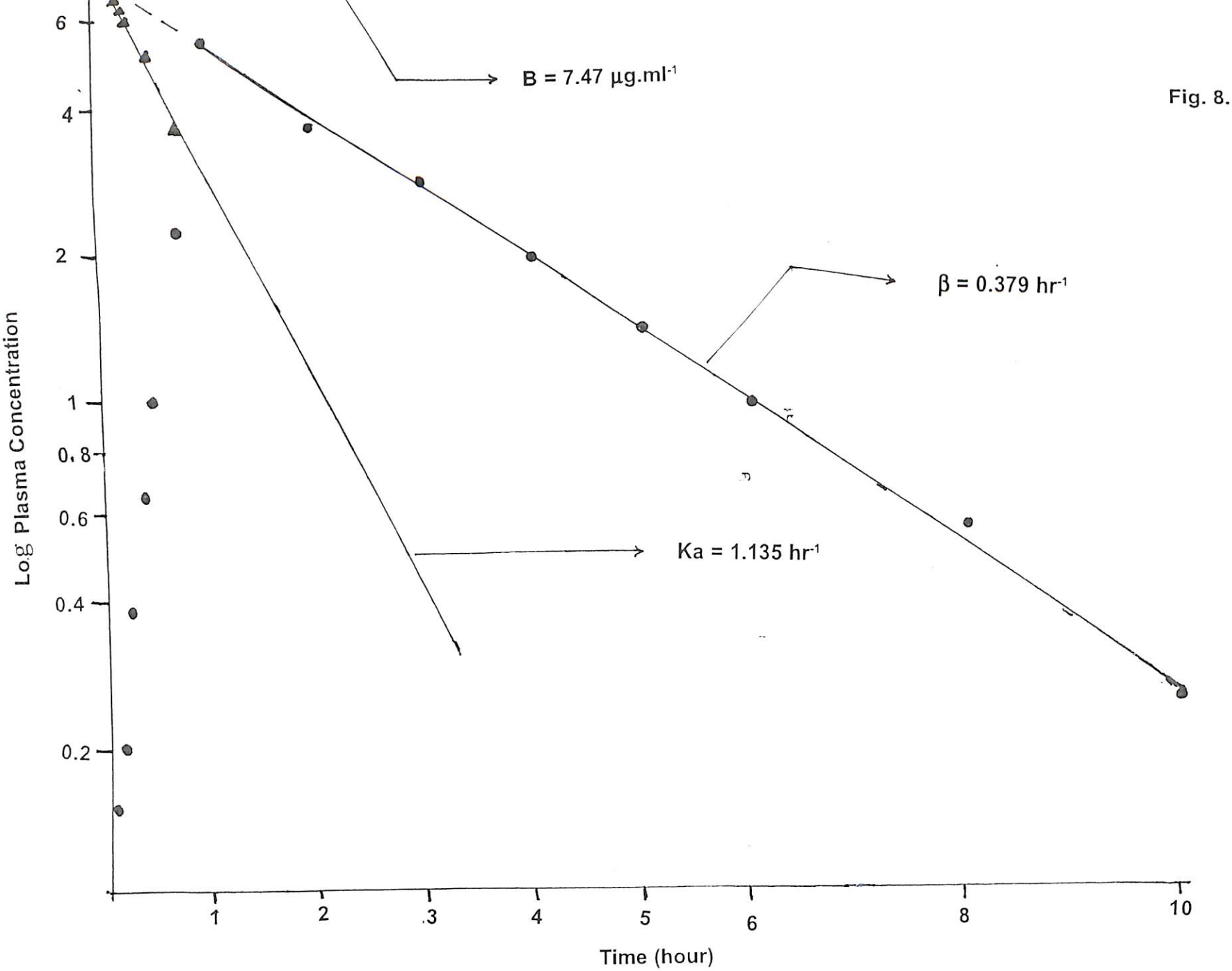

B = 7.47 µg.ml-1
β = 0.379 hr-1
Ka = 1.135 hr-1
Log Plasma Concentration
6
4
2
1
0.8
0.6
0.4
0.2
1
2
3
4
5
6
8
10
Time (hour)

Fig. 8.

Table-25

Comparison of pharmacokinetic parameters of enrofloxacin between healthy and febrile goat after single intramuscular administration at a dose rate of 5 mg.kg^{-1}.

Parameters & Unit	Healthy Goat (n=6)	Febrile Goat (n=6)
A(μg.ml^{-1})	10.04 ± 0.73	9.12 ± 0.45^{+}
B(μg.ml^{-1})	9.30 ± 0.59	8.38 ± 0.49^{+}
K_a (hr^{-1})	1.05 ± 0.083	0.963 ± 0.168^{+}
$t_{1/2}$ K_a (hr)	0.68 ± 0.05	0.59 ± 0.05^{+}
β (hr^{-1})	0.331 ± 0.012	0.343 ± 0.009^{+}
$t_{1/2}$ β(hr)	2.11 ± 0.08	2.03 ± 0.05^{+}
AUC (mg.L^{-1}.hr^{-1})	18.64 ± 0.67	16.48 ± 0.73^{+}
AUMC (mg.L^{-1}.hr^{2})	75.25 ± 3.51	64.20 ± 2.95*
MRT (hr)	4.05 ± 0.17	3.78 ± 0.13^{+}
Vd_B(L.kg^{-1})	0.55 ± 0.04	0.61 ± 0.03^{+}
Vd_{area}(L.kg^{-1})	0.84 ± 0.05	0.89 ± 0.04^{+}
Cl_B(ml.kg^{-1} min^{-1})	4.50 ± 0.16	5.12 ± 0.23^{+}

+ = Non-significant, * $p < 0.05$

Table- 26

Comarison of calculated dosage regimen of enrofloxacin for intramuscular route between healthy and febrile goat.

C^{α}_{p} (min) ($\mu g.ml^{-1}$)	y (hr)	Dose ($mg.kg^{-1}$)	Healthy Goat (n = 6)	Febrile Goat (n = 6)
0.12	8	D^{*}	1.43 ± 0.12	1.69 ± 0.13^{+}
		D_o	1.32 ± 0.12	1.58 ± 0.13^{+}
	12	D^{*}	5.46 ± 0.70	6.74 ± 0.76^{+}
		D_o	5.35 ± 0.70	6.65 ± 0.77^{+}
0.25	8	D^{*}	2.97 ± 0.25	3.51 ± 0.27^{+}
		D_o	2.76 ± 0.25	3.29 ± 0.27^{+}
	12	D^{*}	11.37 ± 1.46	14.07 ± 1.60^{+}
		D_o	11.15 ± 1.46	13.85 ± 1.60^{+}

+ = Non-significant

Chapter V

Discussion

DISCUSSION

Enrofloxacin, a recent member of fluoroquinolones is currently used exclusively in veterinary practice due to its broad spectrum of activity against various micro organisms (Scheer, 1987; Bauditiz, 1990). Apart from this, enrofloxacin possesses no cross-resistance with other class of chemotherapeutic agents and also distributed to a great extent in different organs, tissues and body fluids in various species of animals. Commonly febrile state is produced by most of the infectious diseases that atters the metabolism and excretion of drug (Song *et al.*, 1972), and thus variations in disposition kinetic behaviour of drugs, as well as change dosage regimen have been reported by many workers (Jayachandran, 1994; Ansari, 1997; Singh, 1998). Though pharmacokinetic studies of enrofloxacin were conducted in different species of animals, but not much work was done in goat particularly under febrile condition. Keeping this fact in view the pharmacokinetic study of enrofloxacin was under taken in healthy and febrile goats in order to assess the effects of fever on various kinetic parameters. So as to decide the dosage regimen for effective therapy of microbial infections in animals.

PHARMACOKINETIC STUDY OF ENROFLOXACIN IN GOATS :

(A) DISTRIBUTION IN BODY FLUIDS :

Concentrations of enrofloxacin were noted to be significantly higher ($P < 0.05$) in plasma initially (2.5 and 5 min) in

healthy goats as compared to febrile goats. While non-significant difference was noted at other time intervals (Table-11 and Fig-1) after its single i.v. administration (5 $mg.kg^{-1}$). After i.v. administration of the drug, therapeutic concentration of ≥ 0.12 $\mu g.ml^{-1}$ in plasma was maintained from 2.5 min to around 10 hr in both healthy and febrile goats. In milk, though the concentrations of enrofloxacin were found to be slightly higher at all time intervals in febrile goats, but they were noted to be non-significant. Peak concentration of drug in milk was achieved at 1hr in both the group (healthy & febrile) of goats. The therapeutic concentration (≥ 0.12 $\mu g.ml^{-1}$) in milk was maintained for a longer duration (5 min to 30 hr) both in healthy and febrile goats. Although the concentrations of enrofloxacin in urine were found to be slightly higher at most of the time intervals in febrile goats but significantly higher ($P<0.05$) urinary concentration were observed from 10 to 36 hr in febrile goats as compared to healthy one after i.v. administration. Peak concentrations of the drug in urine were observed at 45 min in both the groups of goats. The therapeutic concentrations in urine were maintained from 2.5 min to even beyond 36 hr in both healthy and febrile goats.

After i.m. administration, the concentrations of enrofloxacin in plasma and milk were found to differ non-significant between healthy and febrile goats when administered at the dose rate of 5 $mg.kg^{-1}$ (Table-24). In urine, the concentrations were found to be significantly ($P<0.05$) higher from 10 to 24 hr in febrile goats as compared to healthy goats. The therapeutic concentration (≥ 0.12

$\mu g.ml^{-1}$) was maintained from 5 min to 12 hr, 10 min to 24 and 2.5 min to even beyond 36 hr in plasma, milk and urine respectively, in both healthy and febrile goats. Peak concentrations were achieved at 1 hr, 1 hr and 45 min in plasma, milk and urine of both the groups of goats.

It is a well known fact that in febrile condition the peripheral circulation and the permeability of plasma membrane may increases, which in turn leads to increase in distribution of drugs into peripheral tissues. Thus this may results in the lower values of plasma levels of the drug in febrile goats as compared to healthy one in support of the above statement, Pennington *et al.*, (1975) reported lower plasma levels (25-40%) of gentamicin during fever in man. Similarly Ansari (1997) reported significant lower concentrations of pefloxacin (a fluoroquinolone) in plasma at most of the time intervals in febrile goats after its i.v. and i.m. administration. In contrast to the present study, Jha *et al.* (1996) reported higher plasma levels of norfloxacin, a fluoroquinolone during febrile state as compared to afebrile condition in goats. Singh (1998) reported initial higher concentration (2.5 to 30 min) and significantly ($p<0.05$) lower concentration (6 & 8 hr) of ciprofloxacin a close congener of enrofloxacin in plasma of febrile goats after its i.v. administration. On the other hand, Ahmed *et al* (1992) reported no significant difference in plasma levels of gentamicin between healthy and febrile goats. In the present investigation also plasma levels of enrofloxacin did not differ significantly between healthy and febrile goats after i.v. (except

2.5 & 5 min where significantly lower level were recorded in febrile goats) and i.m. administration.

The better distribution of the drug in milk can be expected in febrile goats which may be due to increase in blood circulation and possible increase in permeability of the cell membrane. An increase in concentrations of oxytetracyclline in lungs of pneumonic calves was explained by the increase in its permeability (Ames *et al.* 1983). As expected, increased milk levels of pefloxacin (Ansari, 1997) and ciprofloxacin (Singh, 1998) in febrile goats as compared to afebrile goats were demonstrated. On the contrary, in the present study, though the concentrations of enrofloxacin in milk were noted to be slightly higher in febrile goats at all time intervals but they were found non-significant.

Enrofloxacin is metabolized in the liver mainly to ciprofloxacin and both the compounds (enrofloxacin and ciprofloxacin) are excreted through bile and urine (Hooper *et al.*, 1985). The biliary excretion is an important route of elimination of ciprofloxacin (Fraser *et al.*, 1991). In the present study, increased urinary excretion of enrofloxacin was noted in febrile animals since significantly higher concentrations of the drug in urine were noted from 10 to 36 hr (i.v. route) and 10 to 24 hr (i.m. route) in febrile animals as compared to healthy ones. This may possible be due to increase in blood circulation and at the same time increase in permeability of cell membrance of the renal blood vessels.

(B) KINETIC PARAMETERS :

On comparing the disposition kinetics of enrofloxacin of febrile goats with that of healthy ones, it was observed that almost none of the kinetic parameter differed significantly. Lower but non-significant value for the extrapolated zero time concentration during distribution/absorption phase (A), elimination phase (B) and theoretical zero time concentration (C_o^P) were noted in febrile goats as compared to healthy goats after its i.v. and i.m. administration at the dose of 5mg.kg^{-1} (Table-12 & 25).

There was no significant difference in absorption rate constant (K_a) between healthy (1.05 ± 0.083 hr^{-1}) and febrile goats (0.963 ± 0.168 hr^{-1}). Similar values of absorption half life of 0.68 ± 0.05 hr and 0.59 ± 0.05 hr were noted in healthy and febrile goats, respectively. The above findings indicate that the route of absorption was similar in both the groups of goats after i.m administration (Table-25). This finding is supported by similar appearance of drug in plasma (2.5 min) and similar time to reach peak concentration (1hr) in both healthy and febrile goats (Table-24). After s.c. administration Sudha Kumari (1998) in goat and Cabanes *et al.* (1992) in rabbit reported lower $t_{1/2}$ Ka of 0.20 ± 0.03 and 0.3 hr respectively.

Distribution rate constant (α) of 1.741 ± 0.171 hr^{-1} and distribution half life ($t_{1/2}$ α) 0.42 ± 0.05 hr were noted in the present study in healthy goats (Table-12). More or less similar values of α and $t_{1/2}$ α of 1.380 ± 0.312 hr^{-1} and 0.60 ± 0.10 hr. respectively were reported in goat (Sudha Kumari, 1998). Similar $t_{1/2}$ α of 0.63 - 0.68 hr

in hors (Gigure *et al.*, 1996) and very low $t_{1/2}$ α of 0.04 ± 0.03 hr in cow calf (Ahangar, 1998) and 0.07 ± 0.001 hr in chicken (Anadon *et al.*, 1995) were reported. In the present study, no significant difference in the values of α and $t_{1/2}$ α were noted between healthy and febrile animals, which denote that the route of distribution of drug into different tissues and body fluids is more or less similar in healthy and febrile condition. This is supported by the similar appearance of the drug (2.5 min) and similar time to reach peak concentration (1 hr) in milk of both healthy and febrile goats after i.v. administration .

The mean elimination rate constant (β) of 0.246 ± 0.019 and 0.331 ± 0.012 hr^{-1} and elimination half life ($t_{1/2}$ β) of 2.90 ± 0.22 and 2.11 ± 0.08 hr were obtained in healthy goats after i.v. and i.m. administration respectively (Table-12 & 25). More of less similar $t_{1/2}$ β of 2.82 ± 0.33 (Sudha Kumari, 1998) in goat, 2.19 hr (Cabanes *et al.*, 1992) and 2.5 hr (Broome *et al.*, 1991) in rabbits and 2.4 hr (Kung *et al.*, 1993) and 3 hr (Kanemaki *et al.*, 1995) in dogs were noted. Lower $t_{1/2}$ β of 1.7 hr (Kaartinen *et al.*, 1995) in cow and 0.96 ± 0.06 hr in cow calf (Ahangar, 1998) were noted after i.v. administration of enrofloxacin. On the other hand, higher $t_{1/2}$ β of 3.73 ± 0.44 hr in sheep (Mengozzi *et al.*, 1996), 5.94 –6.09 hr in horse (Giguere *et al.*, 1996) and 10.29 ± 0.45 hr in chicken (Anadon *et al.*, 1995) were observed after i.v. administration. A slightly lower $t_{1/2}$ β of 1.42 hr in goat (Sudha Kumari, 1998) and 1.71 hr in rabbit (Cabanes *et al.*, 1991) were noted for enrofloxacin after its s.c. administration. The

difference in observed half life values in present study as compared to other species may be due to difference in metabolism, dose, route of administration and species variation etc. Non-significant but slightly increase in β and decrease in $t_{1/2}$ β obtained in febrile goats (Table-12 & 25) denote that the drug is eliminated at a slightly faster rate in febrile goats than that of healthy goats after its i.v. and i.m. administration. This is supported by increased urinary excretion of enrofloxacin after i.v. & i.m. administration which is shown by higher urinary concentrations of enrofloxacin from 10 to 36 hr and 10 to 24 hr of i.v. and i.m. administration, respectively, in febrile goats as compared to healthy goats (Table 11 and 24).

There was no significant difference for the rate constant of drug transfer from central to peripheral compartment (K_{12}) between healthy (0.640 ± 0.116 hr^{-1}) and febrile (0.431 ± 0.071 hr^{-1}) goats, similarly non-significant difference was observed for the rate constant of drug transfer from peripheral to central compartment (K_{21}) between healthy (0.555 ± 0.084 hr^{-1}) and febrile goats (0.507 ± 0.049 hr^{-1}). Sudha Kumari (1998) observed the values of K_{12} and K_{21} in healthy goats as 0.436 ± 0.133 and 0.639 ± 0.087 hr^{-1} respectively. In horse K_{12} and K_{21} values of 0.45 ± 0.62 and 0.54 ± 0.55 hr^{-1} were noted (Giguere *et al.*, 1996) after i.v. dose of enrofloxacin (5 $mg.kg^{-1}$). However Cabanes *et al.*, (1992) noted higher values for K_{12} (Mean value of 0.0216 min^{-1}= 1.296 hr^{-1}) and K_{21} (0.021 min^{-1} = 1.26 hr^{-1}) in rabbit. In chicken, Anadon *et al.*, (1995) reported a very high K_{12} of 6.13 ± 0.21 hr^{-1} and low K_{21} value of 0.19 ± 0.01 hr^{-1} after i.v.

administration of enrofloxacin. Slightly lower value of route of elimination of drug from central compartment (K_{el}) was observed in febrile goats (0.729 ± 0.055 hr^{-1}) as compared to healthy goats (0.793 ± 0.050 hr^{-1}) though it was non-significant. K_{el} value 0.577 ± 0.137 hr^{-1} in goat (Sudha Kumari, 1998) and 0.22 ± 0.04 hr^{-1} in horse (Giguere *et al.*, 1996) were estimated.

The various other kinetic parameters viz., area under curve (AUC), total area under the first moment of plasma drug concentration curve (AUMC), Mean residential time (MRT) and approximate tissue to plasma concentration ratio (T≈P) were observed to be non-significantly lower in febrile goats as compared to healthy goats, after i.v. administration (Table-12). Similarly non-significant lower value of AUC and MRT were noted, while significantly ($p<0.05$) lower value of AUMC were observed after i.m. administration of enrofloxacin (Table-25).

The various values of volume distribution did not differ significantly between healthy and febrile condition after i.v. and i.m. administration of enrofloxacin (Table-12 & 25). Notari (1980) demonstrated that for a 2-compartment open model, the value of Vd_B > Vd_{area}> $Vd_{s.s}$ and Vd. He further mentioned that among these values of volume distribution only Vd_{area} correctly predicts the amount of drug in the body during elimination phase where as Vd_B over estimates and $Vd_{s.s.}$ & Vd under estimates the amount of drug in the body. The values of Vd_{area} of 2,43 ± 0.32 and 0.84 ± 0.05 L. Kg^{-1} were obtained for enrofloxacin in healthy goat after its i.v. and i.m.

administration, respectively in the present study. Vd_{area} of 2.34 ± 0.54 $L.kg^{-1}$ (i.v.) in goat (Sudha Kumari, 1998), was observed. Volume distribution of 1 $L.Kg^{-1}$ in cow (Giguere *et al.*, 1992) and 3.20 ± 0.22 $L.kg^{-1}$ in sheep (Mengozzi *et al.*, 1996). 7 $L.kg^{-1}$ in dog (Kung *et al.*, 1993), 3.4 ± 0.9 $L.kg^{-1}$ in rabbit (Cabanes *et al.*, 1992 and 4.31 ± 0.15 $L.kg^{-1}$ in chicken (Anadon *et al.*, 1995) were reported. The above findings denote that enrofloxacin is well distributed in different tissues and body fluids of all the above species of animals including goat. This statement is supported by higher concentrations of this drug observed in milk than that of plasma at different time intervals as noted in the present study. However, Ahangar (1998) noted a very low Vd_{area} of 0.40 ± 0.02 and 0.44 ± 0.02 $L.kg^{-1}$ in healthy and febrile cow calves respectively after i.v. administration of enrofloxacin.

In the present study, the total body clearance (Cl_B) did not differ significantly between healthy and febrile goats after i.v. and i.m. administration (Table-12 & 25). The values of Cl_B were noted to be 9.55 ± 0.76 and 4.50 ± 0.16 $ml.kg^{-1}$, min^{-1} in healthy goats after i.v. and i.m. administration, respectively. Sudha Kumari (1998) noted similar Cl_B value of 9.40 ± 1.36 ml. Kg^{-1} min^{-1} in goat after i.v. administration of enrofloxacin (5 $mg.kg^{-1}$). Cl_B values of 9.17 ± 2.4 ml. Kg^{-1} min^{-1} noted in sheep (Mengozzi *et al.*, 1996) was also similar to the present investigation. However, lower Cl_B values of 0.09 ± 0.14 $L.kg^{1}.min^{-1}$ (1.50 ± 2.33 $ml.kg^{-1}.min^{-1}$) in horse (Giguere *et al.*, 1996), 0.29 ± 0.02 $L.kg^{-1}.hr^{-1}$ (4.83 ±0.33$ml.kg^{1}.min^{-1}$) in chicken (Anadon *et al.*, 1995) and 0.28 ± 0.01 $L.kg^{-1}.hr^{-1}$ (4.67 ± 0.17 $ml.kg^{-1}.min^{-1}$) in

cow calves (Ahangar, 1998) were noted. A very high Cl_B value of 27 $ml.Kg^{-1}$ min^{-1} in dog (Kung *et al.*, 1993) and 22.8 ± 6.8 $ml.kg^{1}.min^{-1}$ in rabbit (Cabanes *et al.*, 1992) were obtained.

(C) DOSAGE REGIMEN :

The minimum inhibitory concentration concentration (MIC) values of enrofloxacin for different species of microcrganism ranged between 0.001 to 1.0 $\mu g.ml^{-1}$ in veterinary practice (Mevius *et al.*, 1990; Prescott and Yielding, 1990). The minimum therapeutic plasma concentration of enrofloxacin is reported to be in the range of approximately 0.02 – 0.05 $kg.ml^{-1}$ with a mean value of 0.25 $\mu g.ml^{-1}$ (Neuman, 1988). It is Known that sensitivity or resistant of micro-organisms to enrofloxacin is more or less similar to its close congener, ciprofloxacin. Various workers (Singh, 1998; Srivastava, 1987) have taken 0.12 $\mu g.ml^{-1}$ as MIC for calculating/suggesting dosage regimen of ciprofloxacin. Ahangar (1998) calculated the dosage regimen of enfofloxacin in cow calves at MIC of 0.05, 0.12 and 0.25 $\mu g.ml^{-1}$. Hence in the present investigations 0.12 $\mu g.ml^{-1}$ and 0.25 $\mu g.ml^{-1}$ have been taken as therapeutic concentration (MIC) for calculating dosage regimen.

In the present study, loading dose (D^*) and maintenance dose (D_o) were calculated for maintaining therapeutic concentration (MiC = C_p^{α} min) of 0.12 and 0.25 $\mu g.ml^{-1}$ at dosage intervals (ν) 8 and 12 hr for afebrile and febrile goats for i.v. & i.m. route (Table- 13 & 26). Non-significant differences were observed between healthy and febrile goats for D^* and D_o at ν of 8 & 12 hr for maintaining C_p^{α} min of

0.12 and 0.25 $\mu g.ml^{-1}$. However, slightly higher dosage were observed for febrile condition as compared to healthy state. With regard to enrofloxacin, the present study recommends an initial loading dose (D^*) of 5.61 ± 0.77 $mg.kg^{-1}$ and maintenance dose (D_o) of 5.32 ± 0.79 $mg.kg^{-1}$ at the dosage interval (ν) of 12 hr for maintaining C_p^{α} min = 0.12 $\mu g.ml^{-1}$, while D^* & D_o for febrile state as 6.39 ± 0.73 and 6.10 ± 0.74 $mg.kg^{-1}$, respectively for i.v. rout in order to treat systemic infections. For i.m. administration, the required D^* and D_o at 12 hr for C_p^{α} min 0.12 $\mu g.ml^{-1}$ are 5.46 ± 0.70 and 5.35 ± 0.70 $mg.kg^{-1}$ respectively, but in febrile state these were calculated as 6.74 ± 0.76 and 6.65 ± 0.77 $mg.kg^{-1}$, respectively. Similarly, Sudha Kumari (1998) suggested dosage regimen of enrofloxacin for treating septicaemia and other systemic infections is 5 $mg.kg^{-1}$ every 12 hourly by i.v. route and every 6-8 hourly by s.c. route.

Chapter VI

Summary

SUMMARY

A detailed pharmacokinetic study of enrofloxacin was undertaken in healthy and febrile goats weighing 20 to 25 Kg body weight. The influence of *E.Coli* endotoxin induced fever on disposition kinetics of enrofloxacin was investigated in goats. Attempts were made to calculate the various pharmacokinetic parameters and rational dosage regimen of this drug in both the groups of goats in the present study. The salient features of the present study are as follows :

1. With a single i.v. dose of enrofloxacin (5 $mg.kg^{-1}$), significantly ($p<0.05$) lower concentrations in plasma was noted at 2.5 & 5 min in febrile goats as compared to healthy one. The mean therapeutic concentration ($\geq$ 0.12 $\mu g.\ ml^{-1}$) in plasma was maintained form 2.5 min to around 10 hr in both healthy and febrile goats. Non-significant but slightly higher concentrations of the drug in milk of febrile goats were observed at all time intervals while peak milk concentration was attained at 1 hr in both the groups. Significantly ($p<0.05$) higher urinary concentrations of the drug was observed from 10 to 36 hr in febrile goats (as compared to healthy goats), and achieved its peak concentration at 45 min in both the groups of goats. Therapeutic concentration was maintained for longer duration in milk (5min to 30hr) as well as in urine (2.5 min to even beyond 36 hr) of bot healthy and febrile goats post i.v. administration of enrofloxacin (5$mg.kg^{-1}$).

2. Following i.m. administration of enrofloxacin (5mg. kg^{-1}), the concentrations of the drug in plasma were noted to be non-significantly lower in febrile goats as compared to healthy goats. Non-significantly higher concentrations of the drug were observed in milk during febrile condition while significantly ($P < 0.05$) higher concentrations of drug in urine were observed from 10 hr to 24 hr in febrile goats as compared to healthy goats. Peak concentrations were achieved at 1 hr, 1 hr and 45 min in plasma, milk and urine, respectively in both healthy and febrile goats. the therapeutic concentration in plasma, milk and urine were maintained from 5 min to 12 hr, 10 min to 24 hr & 2.5 min to 36 hr, respectively, in both healthy and febrile goats.

In coarse of fever, the peripheral circulation and the permeability of plasma membrane may increase, which in turn lead to wider distribution of the drug in different tissues, includig body fluids. This may results in lower level of drug concentration in plasma in febrile goats as compared to healthy goats, is supported by increased drug concentrations in milk of febrile goat after i.v. and i.m. administration.

3. More or less similar values of absorption half life ($t_{1/2}$ Ka) was noted in healthy goats (0.68 ± 0.05 hr) as compared to febrile goats (0.59 ± 0.05 hr). The above findings indicate that the rate of drug absorption is almost similar in both groups of goats after i.m. administration. Similar values of distributiion half life ($t_{1/2}$ α) were observed in healthy goats (0.42 ± 0.05 hr) as compared

administration. This is further supported by more elimination of drug in urine in febrile condition in respect of healthy condition. Fraction of drug available for elimination from central compartment (F_c), approximate tissue to plasma concentration ration ($T \approx P$) values do not differ significantly between healthy and febrile goats after i.v. administation. More or less similar values of area under curve (AUC) and mean residential time (MRT) for healthy and ferbrile goats were observed. Area under first moment of plasma cncentration time curve (AUMC) did not differ significantly between healthy and febrile goats for i.v. route but it became significantly ($p < 0.05$) lower in febrile goats in respect of healthy goats for i.m. administration.

6. The various values of volume distribution were observed to be non-significant between healthy and febrile goats after i.v. and i.m. administration. Volume distribution based on total area under curve (Vd_{area}) values in healthy goats (2.43 ± 0.32 and 0.84 ± 0.05 $L.kg^{-1}$) and in febrile goats (2.42 ± 0.31 and 0.89 ± 0.04 $L.kg^{-1}$) were found after i.v. and i.m. administration, respectively. the high value of Vd_{area} attained in the present study denotes that the drug is distributed well in tissues and body fluids of goat. This statement is supported by higher concentrations of drug obtained in milk and urine in the present study.

Early detection of enrofloxacin in milk of both healthy and febrile goats after i.v. and i.m. administrations and persistance of the drug in milk for a longer period after i.v (up to 30hr) and i.m. (upto 24 hr) in both groups of goats suggest better penetration of the drug into blood-milk barrier. Similarly, in urine also the drug was detected for a more longer period (even beyond 36hr.) after i.v. and i.m. administration in both healthy and febrile goats. This could prove that enrofloxacin is beneficial in the treatment of mammary gland and urinary tract infections in both non-febrile and febrile conditions of animals especially in goats.

DOSAGE REGIMEN

In the present investigation, calculated (D*) and maintenance (D_0) dose to maintain C_p^{∞} min (MIC) of 0.12 and 0.25 $\mu g.ml^{-1}$ in plasma at dosage intervals (γ) of 8 and 12 hr were observed to be non significantly higher in febrile goat for i.v. route. On the basis of present study for i.v. route and at (γ) 12 hr interval D* and D_0 were calculated as 5.61 ± 0.77 and 5.32 ± 0.79 $mg.kg^{-1}$ for non-febrile goats and 6.39 ± 0.73 and 6.10 ± 0.74 $mg.kg^{-1}$ for febrile goats, respectively to maintain C_p^{∞} min = 0.12 $\mu g.ml^{-1}$ for treating systemic infections. For i.m. route D* and D_0 of 5.46 ± 0.70 and 5.35 ± 0.70 $mg.kg^{-1}$, respectively for non-febrile goat and 6.74 ± 0.76 and 6.65 ± 0.77 $mg.kg^{-1}$, respectively for febrile goat at (γ) 12 hr. interval for

Early detection of enrofloxacin in milk of both healthy and febrile goats after i.v. and i.m. administrations and persistance of the drug in milk for a longer period after i.v (up to 30hr) and i.m. (upto 24 hr) in both groups of goats suggest better penetration of the drug into blood-milk barrier. Similarly, in urine also the drug was detected for a more longer period (even beyond 36hr.) after i.v. and i.m. administration in both healthy and febrile goats. This could prove that enrofloxacin is beneficial in the treatment of mammary gland and urinary tract infections in both non-febrile and febrile conditions of animals especially in goats.

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maintaing C_p^{∞} min = 0.12 μg. ml^{-1}, is recommended for its use in the treatment of drug sensitive bacteria. Since the drug maintained its therapeutic concentration in milk for a longer time in both afebrile and febrile goats (30 hr by i.v. and 24 hr by i.m. routes), the drug can be used with 5 $mg.kg^{-1}$ daily for treating mastitis. The drug can also be used with 5 mg. Kg^{-1} by i.v. and i.m. route in treating urinary tract infections daily or 36 hrly. Since the urinary concentrations were maintained above the therapeutic concentration for a period of > 36 hrs.

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Appendix

APPENDIX - 1

CALCULATION OF KINETIC PARAMETERS

Kinetic parameters were calculated from the plasma drug concentration versus time profile. An example is noted here from the data of goat no. 1 obtained after i.v. administration of enrofloxacin (5mg. kg^{-1}) in healthy goat. The data showed a biphasic curve and hence, fits well into a 2 compartment open model.

No. of observation (n)	Time in hour (X)	X^2	Plasma Concentration Y ($\mu g.ml^{-1}$)	Log Y	XY
1	1	1	1.63	0.2122	0.2122
2	1.5	2.25	1.38	0.1399	0.2098
3	2	4	1.20	0.0792	0.1584
4	3	9	0.84	$\bar{0}$.0757	$\bar{0}$.2272
5	4	16	0.60	$\bar{0}$.2218	$\bar{0}$.8874
6	5	25	0.42	$\bar{0}$.3768	$\bar{1}$.8838
7	6	36	0.34	0.4685	$\bar{2}$.8111
8	8	34	0.16	$\bar{0}$.7959	$\bar{6}$.3670
9	10	100	0.10	$\bar{1}$0.000	$\bar{10}$.00

$n = 9$ $\quad \Sigma x = 40.5$ $\quad \Sigma x^2 = 257.25$ $\quad \Sigma y = \bar{2}.5074$ $\quad \Sigma xy = \bar{21}.5961$

$\bar{x} = 4.5$ $\quad \bar{y} = \bar{0}.2786$ $\quad \Sigma x^2 = 257.25$ $\quad (\Sigma x)^2 = 1640.25$

$$\text{b, slope of line} = \frac{n\Sigma xy - \Sigma x.\Sigma y}{n\Sigma x^2 - (\Sigma x)^2}$$

$$= \frac{9 \times (\overline{21}.596) - 40.5 \times (\overline{2}.5074)}{9 \times 257.25 - 1640.25}$$

$$= \frac{-194.3649 - (+\overline{101}.5497)}{675}$$

$$= \frac{-92.8152}{675} = -0.1375$$

Where, x = Time (hr)

y = drug concentration ($\mu g.ml^{-1}$)

n = number of samples

β = elimination rate constant

$= b \times (-2.303)$

$= -0.1375 \times -2.303 = 0.3167$

B, zero time concentration during elimination phase can be obtained from the formula :

$\overline{Y}$ = Mean log concentration

$\overline{x}$ = Mean time

b = slope of line

a = zero time concentration

Therefore,

$a = \overline{Y} - b.\overline{X}$ = log - 0.2786 - (-0.1375 × 4.5)

= log - 0.2786 + 0.6188

= log 0.3402

Zero time concentration = antilog of 0.3402 = 2.1888 $\mu g.ml^{-1}$

Hence, zero time concentration (B) = 2.1888 $\mu g.ml^{-1}$ = 2.19 $\mu g.ml^{-1}$

Similarly, the theoretical plasma concentration (y) canbe calculated by putting the values of time (x) in the above equation during the time-intervals of distribution phase (y = a + bx).

Substracting the theoretical values from observed values, a series of residual concentrations were obtained and slope of line in natural log (distribution constant, α) and the zero time intercept (zero time concentration during distribution phase (A)) can be calculated as per the method adopted for calculation of B and β.

The calculated values are = 1.692 hr^{-1}

A = 6.58 $\mu g.ml^{-1}$

C_o^p, theoretical plasma concentration at zero time

C_o^p = A + B = 6.58 + 2.19 = 8.77 $\mu g.ml^{-1}$

$t_{1/2}\alpha$ distribution half life $= \frac{0.693}{\alpha} = \frac{0.693}{1.692} = 0.41$ hr

$t_{1/2}\beta$, elimination half life $= \frac{0.693}{\beta} = \frac{0.693}{0.317} = 2.19$ hr

AUC, area under curve $= \frac{A}{\alpha} + \frac{B}{\beta}$

$$= \frac{6.58}{1.692} + \frac{2.19}{0.317}$$

$$= 3.89 \pm 6.91$$

$$= 10.80 \text{ mg.L}^{-1}. \text{ hr}^{-1}$$

AUMC, area under first moment of plasma concentration time curve:

$$= \frac{A}{\alpha^2} + \frac{B}{\beta^2}$$

$$= 2.30 + 21.79 \qquad = 24.09$$

MRT, Mean residential time $= \frac{AUMC}{AUC}$

$$= \frac{24.09}{10.80} = 2.23$$

K_{21}, rate constant for drug transfer from peripheral to central compartment,

$$K_{21} = \frac{A.\beta + B.\alpha}{C_o^p}$$

$$= \frac{6.58 \times 0.317 + 2.19 \times 1.692}{8.77}$$

$$= \frac{2.086 + 3.705}{8.77} \qquad = 0.660 \text{ hr}^1$$

Kel, the elimination rate constant of the drug from central compartment,

$$\text{Kel} = \frac{\alpha.\beta}{K_{21}} = \frac{1.692 \times 0.317}{0.660} \qquad = \frac{0.536}{0.660} = 0.812 \text{ hr}^{-1}$$

K_{21}, rate constant of drug transfer from central to peripheral compartment,

$$= \frac{5}{10.80 \times 0.317}$$

$$= \frac{5}{3.424} = 1.46 \text{ L.kg}^{-1}$$

$Vd_{s.s.}$, the valume of distribution at study state.

$$Vd_{s.s.} = \frac{K_{12} + K_{21}}{K_{21}} \times Vd$$

$$= \frac{0.537 + 0.660}{0.660} \times 0.57$$

$$= \frac{0.682}{0.660} = 1.03 \text{ L.kg}^{-1}$$

Cl_B, the total body clearance

$$Cl_B = Vd_{area} \times \beta$$

$$= 1.46 \times 0.317$$

$$= 0.4628 \text{ L.kg}^{-1}. \text{ hr}^{-1}$$

$$= 7.71 \text{ ml. kg}^{-1}. \text{ min}^{-1}$$

APPENDIX -II

Dosage regimen were calculated to maintain the desired levels of therapeutic concentration (MIC) in plasma at desired dosage intervals using the formula of Baggot (1997) described by saini and srivastava (1997). The data of animal no 1 obtained after i.v. ijnection of enrofloxacin in healthy (afebrile) goat has been used as an example for calculation of dosage regimen for maintaining MIC (C_p^{∞} min) of 0.12 $\mu g.ml^{-1}$ at the dosage interval (ν) of 12 hr. The calculation is as follows :

CALCULATION OF LOADING DOSE (D^*) :-

For calculation of D^*, the following formula is used :-

$$D^* = C_p^{\infty}(min)Vd_{area}(e^{\beta\gamma})$$

Where, β = elimination rate constant

ν = dosage interval

For C_p^{∞} (min) of 0.12 $\mu g.ml^{-1}$ and ν of 12 hr

$$D^* = 0.12 \times 1.46 \times (e^{0.317 \times 12})$$

$$= 7.86\ mg.kg^{-1}$$

CALCULATION OF MAINTENANCE DOSE (D_o):-

For calculating D_o, the following formula is employed :

$$D_o = C_p^{\infty}(\min).Vd_{area}\left(e^{\beta v^{-1}}\right)$$

for $C_p^{\infty}(\min)$ of 0.12 $\mu g.ml^{-1}$ and v of 12 hr

$$D_o = 0.12 \times 1.46 \times (e^{0.317 \times 12} - 1)$$

$$= 7.68 \text{ mg. kg}^{-1}$$