INFLUENCE OF EXPERIMENTAL *PASTEURELLA HAEMOLYTICA* PNEUMONIA IN GOATS ON THE PHARMACOKINETICS OF DOXYCYCLINE

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted for examination with our approval as university supervisors

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DEDICATION

This thesis is dedicated to
my parents, Jotham Ibrahim
Ole Mapenay and Sarah Naneu.

All for love and nothing for reward.
(Edmund Spencer 1552 - 1599).

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CHAPTER ONE

INTRODUCTION

Doxycycline, most often available as hydrochloride hemiethanolate hemihydrate (hyclate) is a tetracycline derivative of recent vintage. Because of lipophilicity it is characterized by comparatively better tissue penetration which is reflected by a large volume of distribution (Barza et al., 1975) and enhanced *in vivo* and *in vitro* antimicrobial activity (English, 1966; Rosenblatt et al.,1966).

Widespread use of doxycycline (DOTC) in human medicine has confirmed the therapeutic efficacy and safety of the drug (Cunha et al., 1982). However, few studies have been conducted in food animals (Ziv & Sulman, 1974; Van Gool et al., 1986; Riond & Riviere, 1988). In addition, reports on the efficacy of doxycycline in the treatment of infectious diseases in food animals are sparse. Favourable response to a single intramuscular injection was reported in sheep with induced heart water disease (Immelman & Dreyer, 1982) and in splenectomized calves with induced anaplasmosis (Kutler & Simpson, 1978). In addition, doxycycline efficacy has been documented in *Pasteurella haemolytica* infection in calves (Van Gool et al., 1986).

Many studies have already been done on antibiotic concentrations in blood following various routes of administration. Whereas such studies were initially done from a clinical point of view in human medicine (Stillman, 1960), they were later persued from pharmacological and public health points of view (Aronson, 1976). A number of bacteria isolated from domestic animals had shown varying sensitivities to antibiotics (Morse et al, 1950; Turner, 1959), and hence there was a desire to control dosage rates and also use practical routes

of administration in order to attain required therapeutic blood concentrations for prolonged intervals of time.

In most pharmacokinetic investigations, determination of serum concentrations has been extensively used as an appropriate tool to determine dosage regimens and disposition kinetics. However, it is well known that disease can alter drug absorption and disposition (Ladefoged, 1979; Ames et al., 1983; Groothius et al., 1987; Pijpers et al., 1990). Therefore it is desirable that drugs are evaluated in diseased as well as healthy animals. Minimum inhibitory concentration (MIC) values against bacterial respiratory tract pathogens have been described for tetracyclines (Shimizu et al., 1981; Pijpers et al., 1989). Moreover, some information is available on the pharmacokinetics of oxytetracycline (OTC) in healthy and diseased goats. Several studies have been carried out in diseased calves and pigs following intravenous administration of OTC (Clark et al., 1976; Pijpers et al., 1990). Anika et al. (1986b) studied the pharmacokinetics of OTC in dwarf goats experimentally infected with *Ehrlichia phagocytophila*. On the other hand, no published information is available on the pharmacokinetics of doxycycline in healthy and diseased goats; it is not known whether disposition in healthy and diseased subjects is the same. Hence it is important to study the pharmacokinetics of doxycycline in goats with induced respiratory tract disease. Since currently accepted dosage regimens in antimicrobial therapy are generally based on empiricism or microbiological and physiological considerations taken in conjunction with pharmacokinetic data derived from healthy animals; justifiably or otherwise, marked alterations in disease may have profound implications on clinical efficacy and \ or toxicity. Thus identification of changes in antibiotic

disposition during the course of pneumonia may allow for the development of more rational therapeutic approaches to this complex disease process.

The overall objective of the study was to evaluate the influence of *Pasteurella haemolytica*. infection on the pharmacokinetics of doxycycline in goats. The specific aims of this study were to :

1. Develop a respiratory tract infection model in the goat to be used in pharmacokinetic studies.

2. Determine serum concentrations of DOTC in both normal and experimentally *P. haemolytica* infected goats.

3. Determine whether the infection has any marked effect on the pharmacokinetics and serum concentrations of the drug, and whether this effect may necessitate alteration of the dosage regimen during disease.

4. Determine the MIC of DOTC against the *P. haemolytica* isolate used in this study.

CHAPTER TWO

2. LITERATURE REVIEW.

2.1. TETRACYCLINES

The discovery of the tetracycline came as a result of a systematic screening of soil specimens collected from many parts of the world for antibiotic producing microorganisms. Chlortetracycline was the first tetracycline to be introduced. It was first described by Duggar (1948). Two years later, Finlay et al (1950) announced the discovery of oxytetracycline. Tetracycline is a semi-synthetic product which is made by the catalytic reduction of chlortetracycline or by fermentation with one of the actinomycetes (Minieri et al., 1953). In 1957, demeclocycline was developed and became available in 1959. These four tetracyclines are all natural products that have been isolated from species of *streptomyces*. For example chlortetracycline is obtained from *S. aureofaciens* while oxytetracycline is obtained from *S.rimosus*.

The generic term tetracycline is used to describe the whole group with a common basic structure but it is also the name of a specific compound. Although there are specific and useful differences between tetracyclines, they are generally alike.

2.1.1: ANTIBACTERIAL SPECTRUM AND PHARMACODYNAMICS

Tetracyclines are effective against both gram positive and gram negative bacteria. In addition, they are active against *rickettsiae* (Raoult et al., 1987) *mycoplasma* spp (Turner, 1959) and *chlamydia* spp (Bowie et al., 1978; Kuo et al.,1977; Segreti et al., 1987). The clinically useful activity of tetracyclines extend to many protozoal parasites including *Plasmodium spp., Entamoeba histolytica, Balantidium coli, Babesia spp., Theileria spp., Giardia lamblia, Toxoplasma gondii,*

Ehrlichia spp., Cowdria ruminantium and *Anaplasma spp.* (Van Heerden & Immelman, 1979; Kutler & Simpson, 1978; Immelman & Dreyer, 1982; Chang et al., 1990). They have no activity against yeast or other higher fungi. Soon after their development, the lipophilic derivatives doxycycline and minocycline were demonstrated to be equally effective at lower doses than conventional tetracyclines (Clyde et al., 1971; Willerson et al., 1972; Divo et al., 1985).

The mode of their bacterial action is interference with RNA and bacterial protein synthesis by affecting protein induction at the ribosomes by messenger RNA (Bywater, 1982; Chopra, 1985). The tetracyclines also inhibit bacterial cellular metabolism by blocking attachment of amino acyl transfer RNA to ribosomes, which interferes with protein synthesis. The drug blocks protein synthesis by binding to the 50s ribosomal subunit of the 70s bacterial ribosome (Hash, 1972; Bywater, 1982). A molecular target of this protein synthesis inhibitor was recently identified within the small ribosomal subunit RNA of *Escherichia coli* (Moazed & Noller, 1987). The broad spectrum activity of tetracyclines may be explained by the universal conservation of this rRNA target (Gutell et al., 1985). It has also been previously suggested that tetracyclines at therapeutic concentrations may inhibit protein synthesis in mitochondria of eucaryotic cells without impairing the respective cytosolic protein synthesis (Gijzel & Kroon, 1978; Van den Borgert & Kroon, 1981).

The antibacterial activity is only affected to a small extent by the presence of bacterial debris, blood or serum. Shortening of blood coagulation time following the administration of chlortetracycline to some animals had been reported (Bywater, 1982). Among the bacteria relatively susceptible to tetracyclines are: *E. coli, Pasteurella* spp

Salmonella spp and *B. anthrasis*. The most susceptible include: *Clostridia* spp, *Klebsiella* spp, *Hemophilus* spp and *streptococcus* spp.

2.1.2: PHYSICAL AND CHEMICAL PROPERTIES

Tetracyclines are amphoteric compounds forming salts with acids or bases. The bases are yellow crystalline compounds which are odourless and slightly bitter. Oxytetracycline when dissolved in a propylene glycol-water solution is more stable compared to chlortetracycline (Huber, 1988). Aqueous solutions show appreciable loss of activity within 24-48 hours, especially when the pH is elevated (Thompson, 1976). Hydrochloride salts are commonly used for oral administration and are usually encapsulated. The compounds also form stable chelate complexes with divalent ions such as calcium, magnessium and iron. Tetracyclines are available as capsules, powders, feed additives, parenterals, boluses and ointments for use in veterinary medicine and animal production.

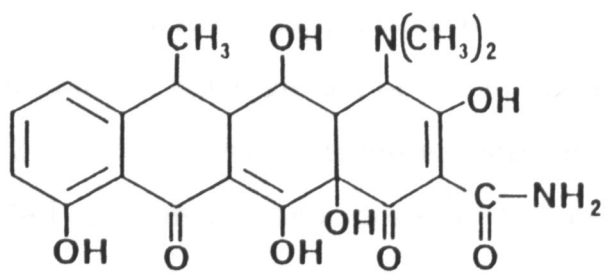


Fig. 1: The molecular structure of doxycycline

2.1.3: ROUTES OF ADMINISTRATION

Tetracyclines can be administered parenterally or orally.

2.1.3.1: Parenteral

Intravenous(IV) and IM injections are common routes of administration of some of the tetracycline compounds in veterinary medicine. IV injection is usually given once daily but in cases of acute illnesses, it can be given twice a day, in divided doses. This is said to reduce the chances of shock or toxaemia from bacterial toxins (Huber, 1988). Chlortetracycline cannot be administered intramuscularly because it causes severe tissue irritation. Oxytetracycline can be given as a deep IM injection but it has to be combined with 5% magnessium chloride and 2% procaine to minimize post injection discomfort at the site. The suggested dose for IV and IM injection of tetracycline antibiotic is 4.4-11 mg\kg daily (Huber, 1988).

2.1.3.2: Oral

In herbivores, tetracyclines are orally administered in subtherapeutic doses. Following oral administration, normal bacterial fermentation of plant fibre is initially suppressed. In small animals, the antibiotics are given orally at a dosage rate of 33-110 mg/kg/day, preferably divided into 2 or 3 doses. Due to its more complete absorption, DOTC has been applied to humans and dogs at the recommended oral dosage approximately one-tenth that of the other three tetracyclines (Aronson, 1980). The recommended dosages via drinking water in poultry are 0.05- 0.5 g/l.

2.1.3.3: Topical

Topically an opthalmic ointment containing 1 mg tetracycline per gramme ointment may be used on conjuctival membranes. The eye may also be treated with a buffered aqueous solution of tetracycline containing 5 mg/ml (Huber, 1988).

2.1.3.4: Intramammary

Tetracyclines are available as intramammary infusions for the treatment of mastitis in the cow, doe and ewe. In the cow, mastitis is effectively eliminated with a 440 mg hydrochloride preparation.

2.1.4: PHARMACOKINETICS

2.1.4.1: Absorption

Many variations on the basic tetracycline structure have been isolated or synthesized. These variant structures have fairly similar antibacterial activities but differ in their pharmacokinetics (Huber, 1988). DOTC and minocycline are more lipid soluble than the others, and hence their intestinal absorption and tissue penetration are more complete. After oral administration, tetracyclines are absorbed readily but not completely from the stomach to give a maximum plasma concentration within 2-4 hours. Hardly any drug is detectable in plasma after 24 hours. An aqueous vehicle reduces the amount of time for peak plasma concentrations of oxytetracycline to be achieved, while an oil suspension vehicle prolongs the time of absorption. In a study of the absorption of tetracycline from loops of the dog small intestines, Pindell et al.(1959), showed that only about 3% of an administered dose of the drug was absorbed in 1.5 hours. Since the rate of absorption remained constant throughout this period, and the amount of drug

absorbed was directly proportional to the concentration over a ten-fold range, it was concluded that absorption was by passive diffusion.

The absorption is greatly retarded in the presence of calcium and magnessium ions since the divalent metals form insoluble chelates with the drug. Absorption is also impaired to a variable degree by milk and milk products (Maritim, 1985). The percentage of an oral dose that is absorbed on an empty stomach in humans has been found to be high for minocycline (100%) and doxycycline (95%);intermediate for oxytetracycline, demeclocycline and tetracycline (60-80%) and lowest for chlortetracycline (30%) (Barza and Scheife, 1977). Doxycycline and minocycline differ in that they are nearly ten times more lipid soluble (Colaizzi & Klink, 1969). Both doxycycline (Rosenblatt et al., 1966) and methacycline show enhanced absorption over tetracycline, and are effective in lower doses. Plasma concentrations of tetracycline and oxytetracycline administered IM are detectable within 15 minutes, reach a maximum within one hour, maintain significant blood concentrations (about 0.5ug/ml) for about 12 hours, and then decline to trace amounts approximately 24 hours after injection. For maximum blood concentrations in food producing animals, not more than 10 ml should be injected in a single IM site (Huber, 1988).

Preparations with local anaesthetics are intended for IM use and should not be administered intravenously because of the undesirable effect local anaesthetics have on cardiac conduction mechanisms. Oxytetracycline and chlortetracycline are also absorbed, to a slight extent into the bloodstream after intramammary infusion.

2.1.4.2: Distribution

The volume of distribution of tetracyclines is larger than that for total body water (TBW), due to binding to plasma proteins. Tetracyclines are reversibly bound to plasma proteins and widely distributed. Within the therapeutic range, the extent of binding to plasma proteins is different among the different tetracyclines, and slight species differences have been reported. They are removed from the blood by the liver and high concentrations are observed in the parenchymatous organs (such as the spleen and lungs) and in the bile. All the tetracyclines are concentrated by the liver and then excreted in way of bile, into the intestine from which they are partially reabsorbed. About 73% of tetracycline excreted in bile is reabsorbed in the intestinal lumen, indicating enterohepatic circulation (Adir, 1975). Tetracycline is also deposited in bones at the active ossification sites. It has been found that the concentration of the drug in fetal blood is approximately one-half that in maternal blood (Huber, 1988). Of the three common tetracyclines (oxytetracycline, chlortetracycline and tetracycline) only tetracycline can diffuse into the cerebrospinal fluid (CSF) with ease. Some tetracyclines also find their way into the prostatic fluid and this can be helpful in the treatment of prostatis. Tetracyclines accumulate in teeth, appear in the synovial fluid and the mucosa of the maxillary sinus where the concentrations are close to those of plasma (Parker and Schmidt, 1971; Lunderberg et al., 1974). In tissues and body fluids of dogs the concentration of DOTC was several times higher than that of OTC (Barza et al., 1975). Nogawa et al(1981) compared tetracycline (TC), OTC and chlortettracycline (CTC) with regard to their residues in eggs after medication to laying hens in drinking water (0.5 g/l) for 7 consecutive days. The mean maximum concentration of OTC in the

yolk was 0.77 μg/g, which occurred 2 days after withdrawal. Yoshimura et al. (1991) noted that OTC was detected for 9 days after withdrawal. The depletion period in the albumin was 6 and 27 days for OTC and DOTC respectively (Yoshimura et al., 1991). In a similar study Omija et al. (1994) detected OTC residues in the yolk and albumen upto 13 and 10 days respectively. The mean values in yolk and albumen were 0.526 and 0.280 mg/kg.

Table 1. Some pharmacokinetic parameters of tetracyclines

Drug	Serum half-life hr	Volume of distribution L/Kg	Clearance ml/kg/hr
Oxytetracycline			
Horse	10.5	1.4	89
Cattle	9.70	0.8	57
Dog and Cat	6.0	2.1	254
Human	9.5	-	-
Tetracycline			
Dog and Cat	5.5	1.2	59
Human	10.6	1.5	102
Minocycline			
Dog	6.9	1.9	198
Human	17.5	0.4	18
Chlortetracycline			
Human	5.5	-	-

Data adapted from Huber (1988).

2.1.4.3. Elimination

It is generally accepted that tetracyclines are not metabolized in the body (Buyske and Kelly, 1960; Riond et al., 1989; Riond & Riviere, 1990). The most frequently identified substances in urine, faeces and tissues is the parent tetracycline However, conflicting observations exist in literature. With the exception of minocycline, members of the tetracycline family are metabolically inert (Aronson, 1980). In a study to determine the metabolic inertness of DOTC in ruminants (Riond et al., 1989), doxycycline metabolites were not detected in urine and serum of calves. This is in agreement with previous reports for human beings, dogs and cats (Fourtillan et al., 1980; Riond et al., 1990).

Tetracyclines are eliminated either by urinary or feacal route. It has been found that approximately 25-30% of a single dose can be found in urine during the first 24 hours. Following oral administration, the highest urine concentration is obtained between 2-8 hours, but antibacterial activity can be detected for three days or more after therapy is discontinued (Huber, 1988). Tetracyclines are also excreted via the bile (Adir, 1975)) and in milk. Slee and Brightling (1981) reported oxytetracycline residues in milk from cows with pueperal endometritis or retained placenta following treatment with OTC boluses placed in the uterus.

2.1.5: UNDESIRABLE EFFECTS OF TETRACYCLINES

Over the years, there has been much concern about the possible hazards to human health posed by the use of tetracyclines for animal husbandry purposes (Watanabe, 1966: Huber,1988). The main undesirable effects associated with tetracyclines include:

(a) Hypersensitivity

Hypersensitivity reactions have been reported for many antibiotics including tetracyclines (Huber, 1988). However, they are not as common as those due to penicillins. Hypersensitivity to tetracyclines have been described as allergic manifestations of mucosal tissues.(Sande & Mandell, 1990).

(b) Resistance factors

It has been shown that resistance to tetracyclines by bacteria develops very readily and persists for a long time. This resistance was first detected in 1950 in Staphylococci (Lowbury et al., 1952). The resistance genes are located on plasmids. R-factor tetracycline resistance is caused by defective drug uptake by bacteria. A mutant of *E.coli* has been shown to contain a defective drug uptake and binding system (Huber, 1988). Tetracycline R-factor resistance can be transferred to a non-resistant organism from a resistant one through transfer of extrachromosomal portions of DNA (Watanabe, 1966). Several studies have confirmed that tetracycline-resistant strains of *E. coli* are abudant in animals reared under intensive conditions (Richmond and Linton, 1980). Although antibiotic-resistant strains of enteric bacteria had been isolated more frequently from cattle given low concentrations of tetracyclines than those given drug-free rations, little had been reported on the development of bacterial resistance in livestock given injectable antibiotics (Stabler et al., 1982). However, extensive use of tetracyclines in livestock industry for growth promotion purposes had a significant role to play in promoting bacterial resistance in man, thus in 1976, tetracyclines were removed from the list of approved feed additives in EC countries (Mitema, 1985).

(c) Immunosuppression

Recently, it had been reported that tetracyclines can cause immunosuppression in domestic animals. Smith et al.(1983) reported immunosuppression in calves vaccinated with *Brucella abortus* strain 19. Chlortetracycline, when added to poultry feed at concentrations of 50-200 g/kg had reduced the immune response to *Mycoplasma synoviae* (Olson and Sahu, 1976).

(d) Hepatotoxicity

Tetracyclines have been shown to cause liver damage in pregnant women (Schultz et al., 1963). Postmortem examinations revealed characteristic severe diffuse fatty infiltration of the liver. Among the tetracyclines, oxytetracycline and tetracycline appear to be less hepatotoxic.

(e) Nephrotoxicity

Oxytetracycline had been implicated in nephrotoxicoses of feedlot calves (Laimore et al., 1984), although excessive doses were involved. Histopathological changes include cortical tubular nephrosis.

(f) Gastrointestinal disturbances

In humans, oral administration of tetracyclines can cause irritation of the gastrointestinal tract although the severity depends on the dosage, type of tetracycline as well as individual variation. Clinical manifestations include nausea, vomiting, abdominal discomfort and epigastric burning. In domestic animals, the most susceptible species are cats and horses where clinical signs include diarrhoea, colic, fever and anorexia (Wilkinson, 1968; Cook, 1973). In horse, the situation can be fatal. In ruminants, the main effect is the alteration of the normal

microflora leading to anorexia and diarrhoea. Tetracyclines can also lead to superinfections within the gut. They depress the growth of the normal gut microflora, thus enhancing the proliferation of antibiotic-resistant microorganisms such as *Candida albicans*, various strains of *Proteus* and *Staphylococci* (Sande & Mandell,1990).

(g) Miscellaneous effects

Tetracyclines, mainly oxytetracycline, have been reported to cause yellowing or browning of teeth as well as dental hypoplasia (Weyman, 1965; Moffit et al., 1974). Tetracycline injections are painful and there can be tissue damage at the site of injection (Immelman et al., 1978; Maritim, 1985). When a single vein is used repeatedly over a long time, for IV administration, this can lead to the development of thrombophlebitis which can be fatal. Rapid IV injection of oxytetracycline in cattle can produce acute cardiovascular collapse (Gross et al.,1979). This may be due to the ability of the tetracycline to chelate divalent ions, especially calcium (Cohen et al., 1970). Tetracyclines given to infants can cause an increase in intracranial pressure and may cause bulging of the fontanelles (Fields, 1961). Reports by Krejci (1980) that tetracyclines cause corneal discolourations and lens opacities have been discounted by Maritim (1985).

2.1.6: USES OF TETRACYCLINES

In veterinary medicine, tetracyclines have been used for the treatment of mastitis, coliform infections, blue comb of turkeys, coryza, pasteurellosis, leptospirosis, fusiformis infections, actinomycosis, actinobacillosis and anaplasmosis among others. In human medicine, tetracyclines are used for the treatment of bacterial infections such as

brucellosis, cholera, chancroid and granuloma inguinale. They are also drugs of choice for rickettsial infections-typhus, scrub typhus, spotted fever and Q -fever endocarditis. They are also used for the treatment of chlamydial infections such as psittacosis, *Lymphogranuloma venereum,* trachoma, inclusion conjuctivitis. and in case of non-gonococcal urethritis. Tetracycline can be used as an alternative drug in patients allergic to penicillin although often, another antibiotic may be specifically indicated.

In the last few years, tetracyclines have found clinical usage in the following diseases; canine ehrlichiosis and canine pancytopenia, bovine hoof disease, porcine atrophic rhinitis and porcine balantidiosis. They are currently being used in the chemotherapy of ruminants against anaplasmosis , babesiosis and cowdriosis.

2.1.7: ASSAY METHODS

2.1.7.1: Microbiological

This method is most commonly used, especially for routine determination of antibiotic concentrations in clinical practice. It involves the use of a micro-organism (usually *Staphylococcus aureus* or *Bacillus subtilis)* and measuring the diameter of the zones of inhibition. The principle here is that the concentration of the antibiotic is exponentially proportional to the inhibition of bacterial growth. Kirshbaum et al., (1967) and Kramer et al., (1968) used the microbiological method for detection and quantitation of tetracycline residues in animal tissues and dairy products.

2.1.7.2 Analytical methods

2.1.7.2.1 Fluorimetry

This method has been employed, but it is only effective after extraction of chelates formed with calcium, magnessium or aluminium ions has been carried out (Kohn, 1961; Wilson et al., 1972). It is based on the fact that when the complex aromatic structure of the tetracycline is exposed to UV-light, it emits a golden-yellow light. This method ,like microbiological assay, does not discriminate between the individual tetracyclines.

2.1.7.3: Chromatography

This method depends on the fact that different compounds exhibit different affinities for the mobile and stationary phases in a chromatographic process.This method allows analysis of the antibiotic as well as its metabolites from biological material (Bocker and Estler, 1979). During the past twenty years, planar chromatographic methods for the separation of tetracyclines have appeared in literature. Wagman and Weinstein (1973) and Ryan and Dupont (1974) have used Paper and Thin-Layer Chromatography for separation of tetracyclines. Since then a large number of liquid chromatographic methods have been described. However, these methods are usually insufficient for determination of tetracycline residue levels below 10 µg/kg. A rapid and relatively accurate assay method employing High Perfomance Liquid Chromatography (HPLC) has been used by Lin (1985). Recently, Weng Naidong et al. (1990) described a Thin Layer Chromatography (TLC) method using densitometry for the assay and purity control of OTC and DOTC. This method was fast, accurate and easy to perform.

2.1.7.4: Radioimmunoassay

This method which is sensitive and specific, has been described by Faraj and Ali (1981). The Whole Body Autoradiography technique has been used for studying the general distribution patterns of radioisotope-labelled tetracyclines (Blomquist and Hanngren, 1966). Tritium-labelled tetracycline is used as described by Ullberg (1977).

2.2: CAPRINE PNEUMONIC PASTEURELLOSIS

2.2.1: Introduction

Pasteurellosis in goats occurs principally in two forms: a pneumonic form caused by *P.haemolytica* biotype A and a systemic form caused by biotype T. Within biotype A there are serotypes 1, 2, 5, 6, 7, 8, 9, 11 and 12 while biotype T comprises serotype 3, 4 and 10. These serotypes differ in their geographic distribution. The disease has been reproduced experimentally by the aerosol administration of *P.haemolytica* biotype A (Gilmour et al., 1982b).

2.2.2: Aetiological agents

Gourlay & Barber (1966) reported the isolation of a strain of *P.haemolytica* associated with pneumonia and septicaemia in goats in Uganda. Mugera and Kramer (1967) also described an outbreak of an acute disease of goats associated with *P.haemolytica* in goats. The disease syndrome was pneumonic in character. Ojo (1975) characterized 200 isolates of *P.haemolytica* from goats in Nigeria. These were mainly of six serotypes while 25 isolates were untypeable. 43% of the isolates were serotype 2, 20% serotype 6, 10% serotype 7, 6.5% serotype 8, 3.5% serotype 10 and 5% serotype 11. This represents the first major work on the serological characterization of *P.haemolytica* of Caprine origin.

Biotypes A and T have been identified, and were biochemically similar to the ovine biotypes described by Smith (1959).

2.2.3: Pathology

Turner (1959) described caprine pneumonic pasteurellosis as crouppus and catarrhal in type and mostly confined to the lungs, the pleura and the epicardium. In addition, the lobes of the lungs show various degrees and stages of hepatization, together with dilatation of the interlobular septa to 2 or 3 mm coupled with severe bronchitis and pleurisy (Pillai, 1965; Cottew & Lloyd, 1965). Some affected alveoli contain only fibrinous exudate, others exudate with alveolar macrophages, although in some areas the cells in the alveoli are all neutrophils (Ojo, 1976). In many cases the thoracic cavity contained 1- 2 litres of fluid which varied from a uniformly cloudy grey to a blood-tinged colour (Ojo, 1976). Histologically, the lung tissue from natural cases show extensive areas of grey hepatization, but areas of red hepatization are also discernable where changes are more recent. In chronic cases, the pleura is covered by a thick fibrinous exudate undergoing various stages of organization. The inter lobular septa in continuity with the thickened pleura contain leucocytic infiltrations and engorged capillaries (Mugera and Kramer, 1967).

2.2.4: Incubation period and clinical signs

Ojo (1976) put the incubation period in experimental pasteurellosis of goats at 3- 7 days by the intravenous route. Clinically the main signs of *Pasteurella* infection include unwillingness to eat, high temperature (40.5- 42.2°C) serous or thick mucoid discharge from the nose. Heavy

breathing, mainly abdominal and frequent groaning are common. in acute cases.

2.2.5: Chemotherapy

The recommendations for the treatment of caprine pneumonic pasteurellosis, like in cattle and sheep, are based on clinical experience because there is no published information available based on clinical trials. There is some limited information on the efficacy of certain antimicrobials in the experimentally induced disease. Common antimicrobials such as oxytetracycline, trimethoprim-sulphonamides, chloramphenical,penicillin and sulphonamides have been used with success. One treatment is usually adequate and most economical for most cases but severly affected goats or those with relapse require teatment daily, or even two to three times daily depending upon the drug used, for up to 3 - 5 days. Penicillin is also a successful treatment in goats. However, not all strains of biotype A are sensitive to penicillin, but most strains are sensitive to OTC, which is the drug of choice as long - acting preparations are available (Gilmour et al., 1982a). Medication of the water supplies with OTC for 7 - 10 days is beneficial.

2.2.6: Effects of pneumonia on tissue drug concentrations.

The choice of antimicrobial agent will depend not only on the economics, microbial sensitivity but also on the concentrations of the drug which can be achieved in the lung tissues of the affected animals. The reported effects of disease on serum and tissue concentrations of antimicrobial agents are variable. In cattle the concentration of OTC are higher in pneumonic lung than in normal lung (Ames et al., 1983). Pharmacokinetic studies of OTC in experimental pneumonic

pasteurellosis indicate the need of observance of 12- hour dose intervals in calves (Burrows, et al., 1986). Pharmacokinetic studies for lincomycin and chloramphenicol indicate that the presence of pneumonia does not change the dosage regimen determined in normal animals (Burrows et al., 1986). Because of the increased rate of elimination of erythromycin it may be advisable to use shorter dosage intervals in animals with respiratory disease (Burrows, 1985). Similarly the kinetics of tylosin but not gentamicin are sufficiently altered in pneumonic animals to require increased frequency of administration (Burrows et al., 1986). A significant increase in elimination rate may have some implications in therapy particularly if serum kinetics are reflective of tissue kinetics. At present the applicability of the results of these studies to clinical practise are not clear. However, the kinetics of those antibiotics characterized by high volume of distribution (V_d) and lipid solubility appear to be more adversely affected by pneumonia than those with lower V_d.

CHAPTER THREE

3.0: MATERIALS AND METHODS

3.1: Animals

Seven small East African goats, 5 males and 2 females, varying in age from 14 to 26 months were used in the experiments. The animals were purchased from a local farm within the outskirts of Nairobi. The goats were healthy and remained clinically normal without any signs of respiratory disease untill the experiments commenced. The average weight of the goats was 21.0 $\pm$ 2.5 kg (range; 18 - 26 kg). Upon arrival at the Faculty of Veterinary Medicine, Kabete, the goats were acclimatized in their new environment for two weeks. During this time, they were treated with oxfendazole (Panacur^R, Hoechst), a broad-spectrum anthelmintic, and were housed indoors in pens on concrete floors. The goats were offered pelleted concentrates once daily and had free access to hay and water throughout the experiments. The same goats were used in both normal and infection experiments.

3.2: Infectious agent

The *P.haemolytica* strain was previously isolated from the respiratory tract of a goat, that had died of severe fibrinous pneumonia, at the Department of Pathology and Microbiology of the Faculty of Veterinary Medicine, University of Nairobi. *P. haemolytica* was initially passaged by growing it on blood agar plates which were checked for purity after overnight incubation at 37°C. A pure colony was transferred from blood agar plate and inoculated in 10 ml Brain Heart Infusion (BHI) broth (Difco Labs., Detroit, Michigan, U.S.A). After overnight incubation the 10 ml was added to 40 ml BHI broth. After another 6-hour incubation at 36°C the culture was used for

experimental infection. Serial dilutions were made from the 12-h culture, inoculated on blood agar plates and after 24 hours the infection dose was enumerated. The optical density of the same was measured spectrophotometrically (Spectronic- 20, Bausch & Loumb, USA.) at 537 nm wavelength.

3.3: Drug administration

Four weeks before Pasteurella challenge all the goats were injected intramuscularly on the deep gluteal muscle with DOTC (Doxycen retard, Cenevisa, S.A. Laboratories, Spain) containing 200 mg/ml active material at the dosage rate of 20 mg/kg bodyweight. Following development of pneumonia, a second intramuscular injection of DOTC was administered to the six infected goats on the twelveth day post infection.

3.4: Blood sampling

Blood samples of 5 ml each were collected by jugular venipuncture with vacutainer tubes without anticoagulant. Samples were collected at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 60, 72 and 96 hours interval following drug administration. The blood samples were allowed to clot, and were centrifuged at 3000 g for 15 minutes, and serum was harvested and stored at -20°C pending assay for DOTC.

3.5: Experimental infection

A 12-hour culture of *P. haemolytica* containing approximately 10^7 to 10^9 cfu /ml suspended in 5 ml of BHI broth was inoculated intratracheally by tracheopuncture to six goats. The control animal was inoculated with the same volume of sterile broth.

3.6: Clinical observation

Goats were examined daily for clinical signs following inoculation with the organism. The following clinical parameters were observed daily: rectal temperature, respiration rate, appetite, general behaviour, nasal discharge, cough and lung auscultation.

3.7: Haematological analysis

Blood samples of 5 ml each were collected by jugular venipuncture with heparinised vacutainer tubes. Samples were collected on day 0, 4, 8 and 12 (twice weekly at four days interval). The white blood cell (WBC) counts were performed with a coulter counter (Coulter electronics, Inc. Luton, Beds. England). Total protein concentration were measured by means of a refractometer (Atago, Japan).

3.8: Antibiotic Susceptibility test

The *in vitro* MIC of DOTC for the *P. haemolytica* isolate used was determined by the broth dilution technique (BHIB, Difco Labs. Detroit, Michigan, USA.) For the sensitivity testing an inoculum concentration of 10^6 cfu /ml from an overnight culture was used. Serial two-fold dilutions of DOTC concentrations ranging from 0.03 to 16 µg/ml were incorporated into BHI broth. All tubes were prepared simultaneously. The antibiotic containing tubes were inoculated using a micropipette which delivered an inoculum of about 10 µl, giving an inoculum size of 10^4 cfu per tube. Each concentration was tested in triplicate. The tubes were then incubated aerobically at 37°C. The MIC was read after 24 hours. The MIC was defined as the lowest concentration of antimicrobial agent at which there was no bacterial growth.

3.9: Microbiological assay of DOTC in serum

3.9.1 Preparation of media and glass plates

All media used in this study were commercially available (Difco Inc. Detroit, Michigan, USA.). Muellar Hinton Agar and broth were used for the growth of the test organism. The media were prepared by dissolving a measured amount of powder into an appropriate volume of distilled water in sterile beakers. The mixture of media and water was slightly warmed and stirred for complete dissolving, after which the beakers containing the media were autoclaved. The autoclave was set at 121°C for 15 minutes. After autoclaving, the media was ready for use. However, MHA was transferred to a hot waterbath kept at 45°C untill inoculation.

3.9.2: Preparation of inoculum

The test organism used was Bacillus Cereus Var. mycoides ATCC 11778, (Dept. Of Microbiology & Immunology, Norwegian College of Vet. Medicine, Oslo, Norway.) adapted in our laboratory. This organism was initially reactivated according to the manufacturers instructions. Single pure colonies obtained after several incubations at 37°C for 24 hours were inoculated onto cooked meat media (CMM) and also onto Tryptose soy agar (TSA) slants for storage as stock culture. The stock cultures were stored in the refrigerator and were used throughout this assay.

3.9.1.3: Sample preparation

A procedure similar to that described by Kramer et al. (1968) was used. Serum samples were diluted in the ratio of 1:2 using 0.1 M phosphate buffer (pH 4.5) which was initially prepared by dissolving 13.6 gm monobasic potassium phosphate in a litre of distilled water.

3.9.1.4: Quantitation of Inoculum

A single colony was inoculated into 10 ml of Muellar Hinton broth and incubated at 37°C for 18 hours. In order to quantify inoculum required to obtain uniform growth and clear inhibition zones, 9 ten-fold dilutions of the bacterial suspension were made using distilled water. The number of spores per millilitre in the suspension was determined by direct plate count on blood agar plates, and the corresponding optical density was determined by spectrophotometry. The original suspension (0.1 ml) and the same volume for each dilution were used to inoculate Mueller Hinton agar which was then poured onto framed glass plates and allowed to solidify. Wells of 10 mm in diameter, were dug with a cork bore. DOTC (Cenevisa, S.A. Laboratories, Spain) standard (100 mg) were weighed out and dissolved in 0.01 M HCl to give 1000 µg/ml solution. A portion of the stock solution was diluted to yield 0.1 µg/ml using 0.1 M phosphate buffer, (pH 4.5). This concentration point was used to fill the wells. The plates were then incubated at 37°C for 18 hours , after which the uniformity of growth, clarity and smoothness of the inhibition zones were inspected. From this it was concluded that 0.1 ml of an 18 hour culture (containing approximately 10^6 spores/ml) inoculated in 100 ml of the seed layer gave good growth. This concentration was used in the whole study.

3.9..5: Preparation of standard curves for doxycycline

A standard procedure described by Dornbush and Abbey (1972) was followed in preparing standard curves for serum. Blood from healthy antibiotic -free goats was collected in vacutainer tubes and centrifuged to obtain serum. A portion of the doxycycline stock solution (1000 µg/ml) was diluted in 0.1 M phosphate buffer, (pH 4.5) to yield a

concentration of 20 µg/ml. This concentration was further diluted in antibiotic free serum to obtain concentrations of 0.0375, 0.075, 0.15, 0.3, 0.6, 1.2 and 2.4 µg/ml. These concentrations were finally diluted with 0.1 M phosphate buffer to give a final concentration of 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4 and 0.8 µg/ml. Each sample concentration was assayed in triplicate with 0.1 µg/ml as the reference concentration. The plates were then incubated and later inhibition zones were read using calipers to the nearest 0.5 mm. The diameter readings for each point sample were averaged.

All reference points for any one assay and any one date were averaged and this was then the potency value for that assay. This potency diameter was used to correct the averaged sample value whose corresponding reference diameter value differed from the average potency value for that day. The correction was done such that, if the potency for that day was higher than the reference value for the point then the difference was added to the sample value; the opposite ocurred in the reverse situation. Corresponding values of the inhibition zones were then plotted against the corresponding concentration values on a semilogarithmic paper to give a linear relationship. These standard curves were used to interpret the zones of inhibition arising from the serum samples under investigation. Reference standards were prepared every time an assay was performed. The lowest concentration of doxycycline detectable in the assay was 0.05 µg/ml. The procedure used for assay of serum samples was similar to that used for standards.

The standard curves for DOTC in serum were linear within the range of 0.05 - 0.8 µg/ml (Fig 1). The day to day variations between the standard curves were insignificant. The inhibition zones were not

measurable below 0.05 µg/ml, and this concentration therefore represented the lowest sensitivity threshold of the method (Table 2).

The precision of microbiological agar diffusion method in this study was very good, with the coefficients of variation of less than 2.5% at all concentrations (Table 3). Besides being simple and inexpensive, this method was found to be accurate and sensitive.

Table 2: Diameter of the zones of inhibition (mm) obtained at various concentrations of DOTC (µg/ml) on four different occasions.

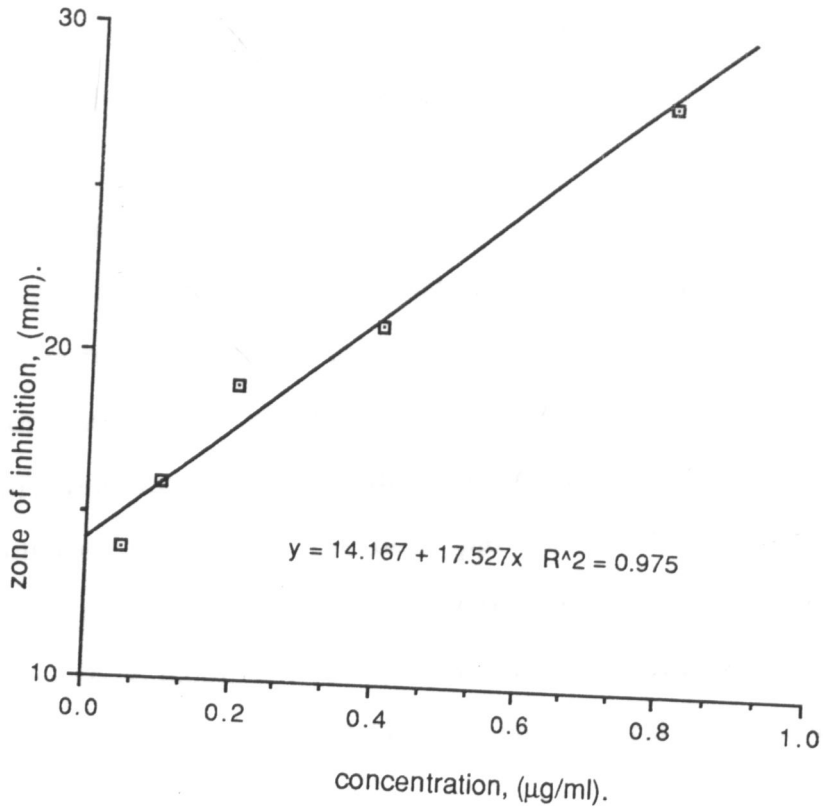
DOTC concentration (µg/ml)	Zones of inhibition (mm)			
0.05	14.0	14.0	14.0	14.5
0.10	16.0	16.0	16.0	16.0
0.20	19.0	19.0	19.0	19.0
0.40	21.0	21.0	21.0	20.0
0.80	28.0	28.0	28.0	27.5

Table 3: Coefficients for the percent(%) variation of the DOTC inhibition zones

DOTC concentration (µg/ml)	CV (%)
0.05	1.78
0.1	0
0.2	0
0.4	2.4
0.8	1.0

NB. Each concentration in serum was run four times.

Fig. 2: Standard curve for doxycycline (DOTC) microbiological agar diffusion method.



R^2 denotes r^2

NB. Diameter of the well = 10 mm

3.10.1: Pharmacokinetic analysis

Initial estimates of slopes and y-intercepts of the natural logarithm concentration-versus-time plot, using 1-, 2- or 3-compartment open pharmacokinetic model, were obtained by means of a linear- regression curve stripping program. Nonlinear- regression analysis, NONLIN (Metzler, 1969) of these estimates then determined the number of exponential terms best describing the log concentration- time curve.

The best line of fit was evaluated by regression analysis (r). The serum concentration-time curve of DOTC was best described by a two-compartment open model. The pharmacokinetic parameters were calculated from the computerized curves according to Baggot (1977). The mean pharmacokinetic parameters were calculated from values obtained for individual animals.

3.12.2: Statistical analysis

Before statistical evaluation, the distribution of all parameters was checked for normality with the method described by Shapiro and Wilk (1965). For those that were normally distributed, the effect of experimental pneumonia was calculated with the paired t- test, and for distribution free parameters, the non- parametric Wilcoxon Signed - rank test was used (Steel and Torrie, 1980). Significance was tested at the 5% level ($p < 0.05$). All calculations were performed with the microcomputer packages STATISTIX^R (NH Analytical Software, Utah,USA 1992)

CHAPTER FOUR

4.0: RESULTS

4.1: Experimental Infection

4.1.1: Virulence of *P. haemolytica*

A comparison of the virulence of the *P . haemolytica* isolate used in this study is shown in Table 4. Intratracheal inoculation of infectious doses ranging from 10^7 to 10^9 cfu/ ml produced a consistent marked febrile response, increased respiratory and accentuated bronchovesicular sounds in all infected goats. Based on the observed clinical findings, the response to challenge with *P. haemolytica* was similar in all the goats despite the fact that varying doses of the infecting organism were used. Since the severity of the infection was uniform all the goats were placed together in one group , rather than split into different groups.

Table 4: Comparison of virulence of the *P. haemolytica* isolate in goats

Infectious dose (CFU)	Number of Inoculated goats	Number of goats showing clinical signs
10^9	2	2
10^8	2	2
10^7	2	2
0	1	0

4.2.2: Clinical signs

The response to the intratracheally inoculated *P. haemolytica* was characterized by depression, coupled with an increase in rectal temperature (Fig. 3), and respiration rate (Fig. 4).

4.2.2.1: Temperature

Before the goats were infected their mean daily rectal temperature was 38.7 $\pm$ 0.1 ^{o}C. However, following infection there was a sudden rise in temperature on the sixth day which persisted over a period of six days before the animals were treated with doxycycline. This was followed by a rapid fall to normal body temperature by day fifteen post infection. Any animal with a temperature reading > 39.5 ^{o}C was considered febrile. The ratio of number of temperature readings > 39.5 ^{o}C to total number of temperature readings taken (Pyrexic Ratio) allows direct comparison and reflects both the morbidity and duration of fever and indicates the mean proportion of febrile animals on any one day following inoculation. On this basis, all the infected animals (n=6) developed pyrexia. The highest individual rectal temperature recorded during the course of infection was 40.9^{o}C on days 11 and 12 (see appendix 2).

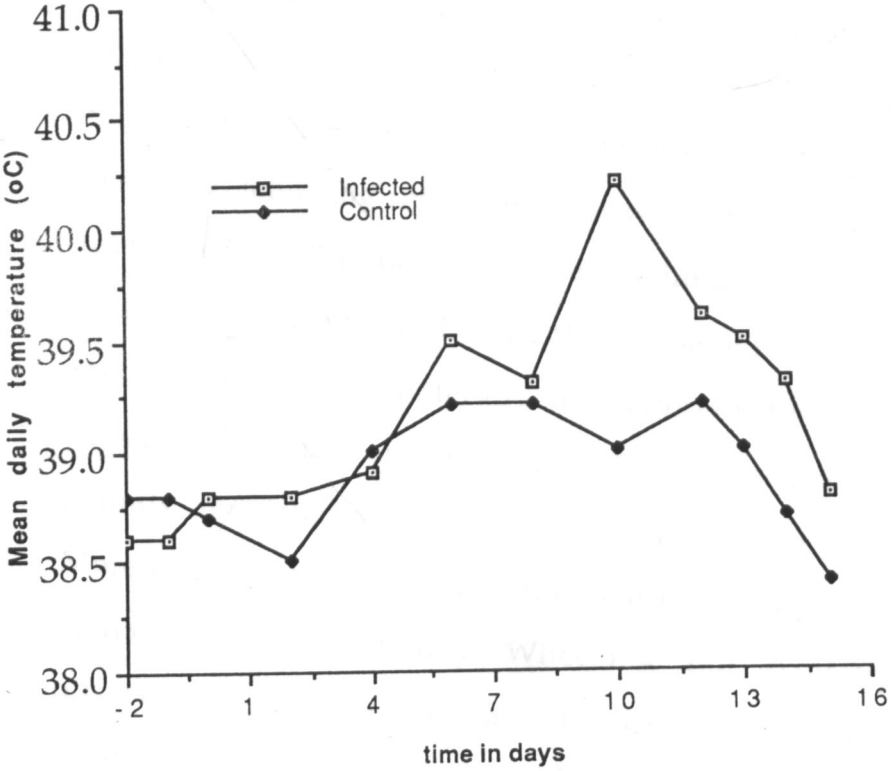


Fig 3 Mean diurnal rectal temperature following intratracheal inoculation of *P.haemolytica* in goats (n=6).

4.2.2.2: Respiratory signs

In addition to the febrile response, respiratory changes were al clinically manifested in challenged goats (Table 5). Prior to infection th mean daily respiration rates in the infected group was 22 per minut However, following infection there was a progressive rise in respirator rates from day 2 to day 6 when a maximum mean rate of 32 per minut was recorded (Figure 4). During the course of infection, 5 goats developed moist rales either unilaterally or bilaterally by the twelveth day post infection. Thoracic auscultation initially revealed increased vesicular sounds and later coarse crackles in the lower portion of the chest. Moreover, coughing coupled with a nasal discharge was observed in four goats from the tenth day. Wheezing and expiratory grunting was observed in one goat on day 11 post infection. In contrast to these findings, the control was clinically unaffected and showed only minor changes in rectal temperature and respiration rate.

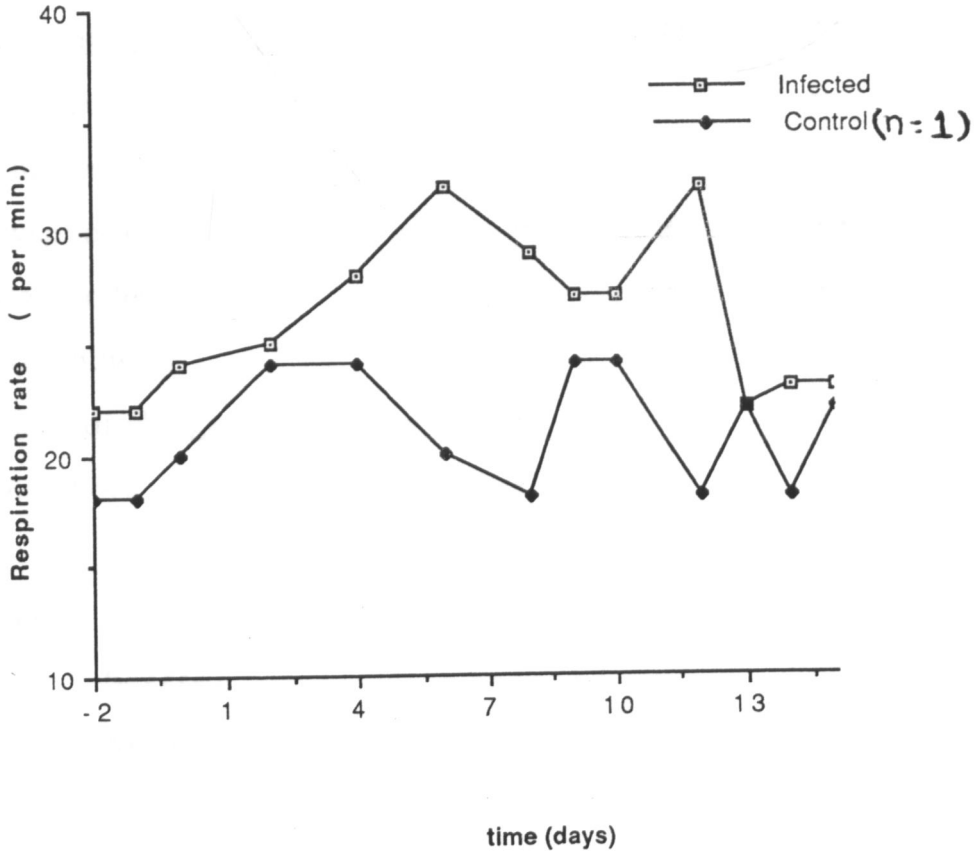


Fig. 4 Mean daily respiration rates following intratracheal inoculation of *P. haemolytica* in goats (n=6).

Table 5: Clinical findings in six goats infected with *P. haemolytica*

	Goat number					
Clinical signs	36	37	38	41	42	46
Pyrexia	+	+	+	+	+	+
Anorexia	-	-	-	+	-	-
Dyspnoea	++	++	+	++	++	++
Moist rales	++	++	++	+	++	++
Coughing	-	+	+	-	+	+
Nasal discharge	-	+	+	-	+	+
Diarrhoea	-	-	+	-	+	-

Key:

++ Severe

\+ Mild

\- Absent

4.2.3: Haematology

On the 4th day post inoculation all goats showed a progressive fall in both WBC counts and total protein concentration (88.2 ± 11.3% and 92.67 ± 6.68% of pre-infection values, respectively). There after WBC counts increased progressively through the eighth to the twelveth day p.i. (99.0 ± 9.1% to 135.0 ± 22.9% of p.i. values, respectively), while total protein (TP) decreased further to 6.43 ± 0.32 g/dl on day 12 (83.88 ± 5.88% of post infection value (See Figure 5 and Table 6).

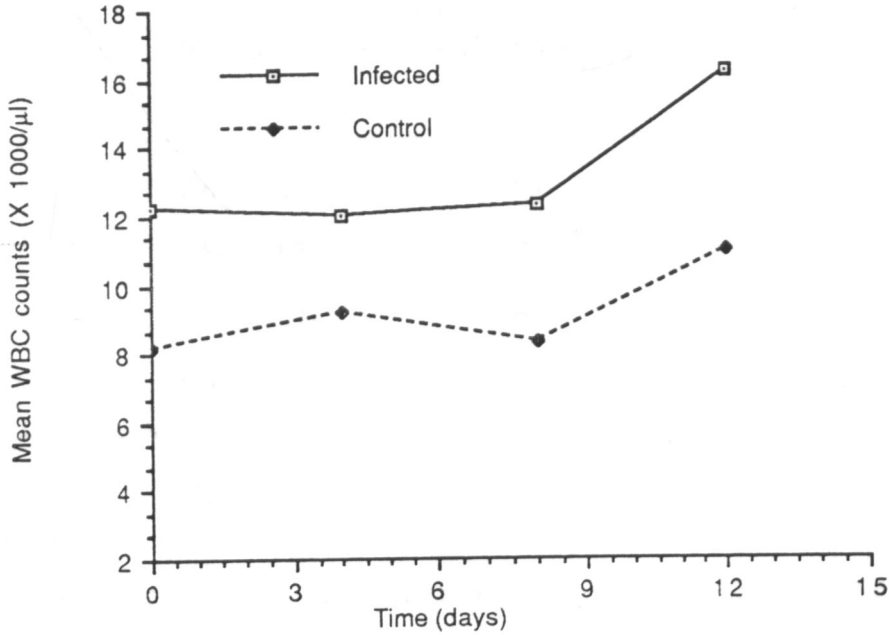


Fig 5: Mean white blood cell counts following intratracheal inoculation of *Pasteurella haemolytica* in goats (n = 6).

Table 6: Mean (± SD) total protein concentration (g/dl) of six goats after experimental infection with *P, haemolytica*..

No. of days post inoculation	Goats	
	Infected (n=6)	Control (n=1)
0	7.6 ± 0.4	8.6
4	6.86 ± 0.38	7.0
8	6.73 ± 0.37	6.4
12	6.43 ± 0.32	6.6

4.2: MIC Assay

The minimum inhibitory concentration for the *P. haemolytica* isolate used in the study is shown in Table 7. The bacteria was sensitive to concentration in the range of 0.25 to 0.5 µg/ml. Thus, the mean was found to be approximately 0.42 µg/ml.

Table 7: The MIC for *P. haemolytica* isolate

	DOTC Concentration (µg/ml)							
Replicates	<0.03	0.06	0.12	0.25	0.5	1.0	2.0	4.0
1	++	++	+	+	-	-	-	-
2	++	++	+	+	-	-	-	-
3	++	++	+	-	-	-	-	-

Key:

++ Distinct bacterial growth

+ Slight bacterial growth

- No bacterial growth

4.3: DOTC serum concentrations

The effects of respiratory tract disease on the disposition kinetics of DOTC was investigated in a group of six adult goats receiving single doses of DOTC (20mg/kg). After IM administration the absorption and distribution patterns of DOTC varied considerably between individual animals (Figs. 6a, b). DOTC was rapidly absorbed in goats before and after infection. The mean absorption rates for goats before and after infection were 1.13 ± 0.02 and 8.23 ± 3.81 hr^{-1}, respectively, showing that absorption from the site of administration was taking place rapidly, especially in infected animals. However, in one goat (before infection) a concentration of 2.18 μg/ml was obtained in serum fifteen minutes after dosing.

The mean absorption and disposition curves for DOTC following I.M. administration show different patterns (Fig. 6c). The maximum serum concentrations of DOTC (C_{max}) were apparently lower in pneumonic goats than in healthy goats. The variation in serum concentration of DOTC was greatest initially, and after peak concentrations had been attained, differences became less apparent. The serum concentrations were similar after 8 hours (0.71 ± 0.1 μg/ml) thereafter, they remained higher in the pneumonic group and were maintained for longer periods (Table 8). Thus, the mean DOTC concentration in the final serum sample taken at 48 hrs were lower in healthy group than in the pneumonic group. The mean serum level at 48 hrs for six goats before infection was 0.08 μg/ml while the corresponding value after infection was 0.22 μg/ml. The time taken to achieve peak serum concentrations (t_{max}) varied considerably between individual goats. However, the mean t_{max} before and after infection were 1.167 ± 0.167 h and 1.15 ± 0.37 h respectively, while the

corresponding C_{max} was 5.56 ± 0.58 µg/ml and 3.87 ± 0.01 µg/ml. DOTC serum concentrations in this study were on average 17% greater in pneumonic group of goat compared to the normal group.

Table 8: DOTC concentration (mean ± SD) in serum after intramuscular administration of DOTC at the dosage rate of 20 mg/kg bwt to six goats prior to and after infection with *P. haemolytica*.

Time (hrs)	Concentration in µg/ml	
	Before infection	After infection
0.25	1.34 ± 0.41	1.96 ± 0.33
0.5	4.65 ± 0.23	3.87 ±0.52
1	5.56 ± 0.58	3.3 ± 0.40
2	4.8 ± 0.55	2.87 ± 0.50
4	1.52 ± 0.02	2.33 ± 0.34
8	0.77 ± 0.02	0.71 ± 0.10
12	0.59 ± 0.02	0.68 ± 0.11
24	0.27 ± 0.05	0.50 ± 0.06
36	0.12 ± 0.02	0.39 ± 0.09
48	0.09 ± 0.01	0.20 ± 0.03

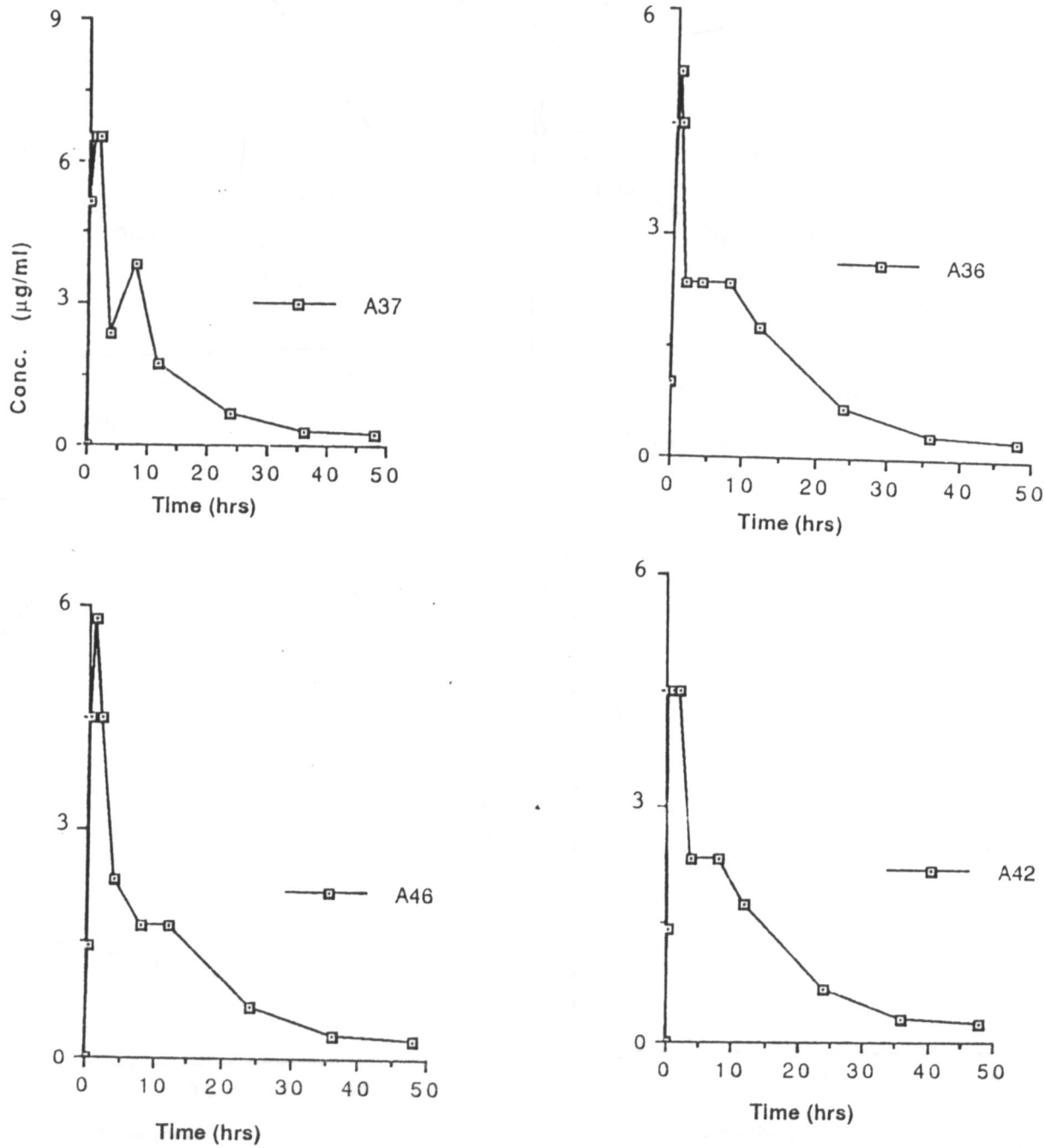
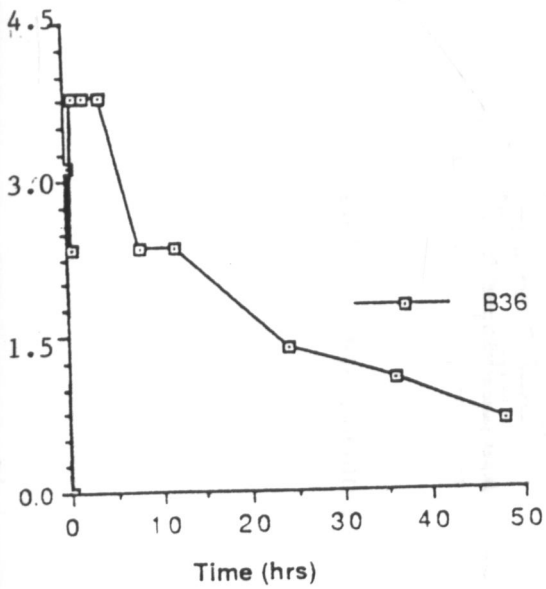
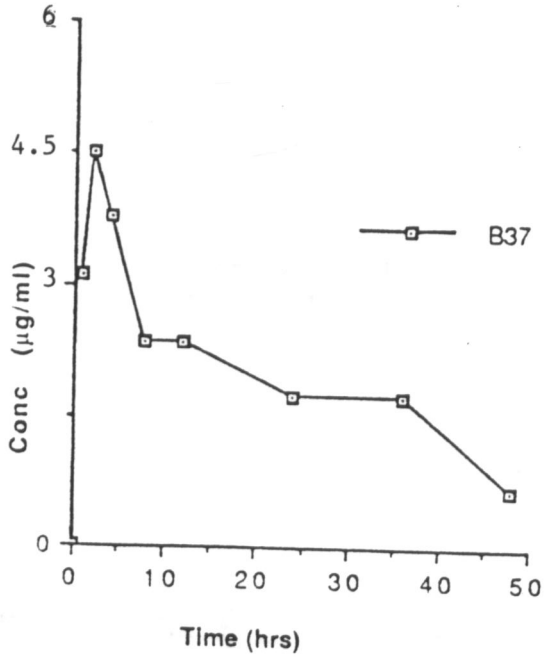
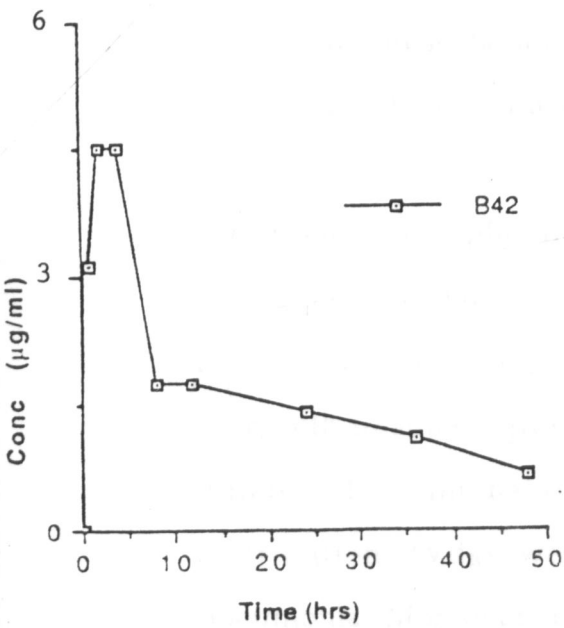


Figure 6a: Concentration of DOT in serum after intramuscular administration of 20mg/kg to four goats before *P. haemolytica* challenge. Letters denote goat number







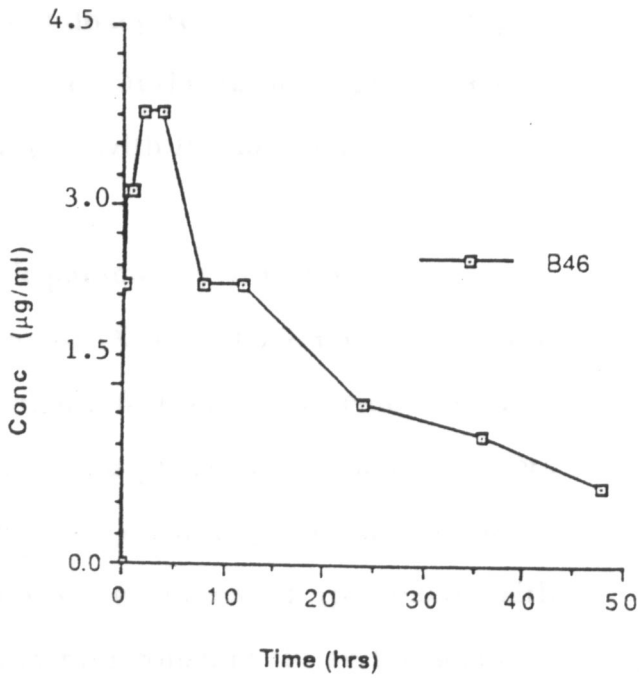


Figure 6b: Concentration of DOTC in serum after intramuscular administration of 20mg/kg to four pneumonic goats.

Letters denote goat number

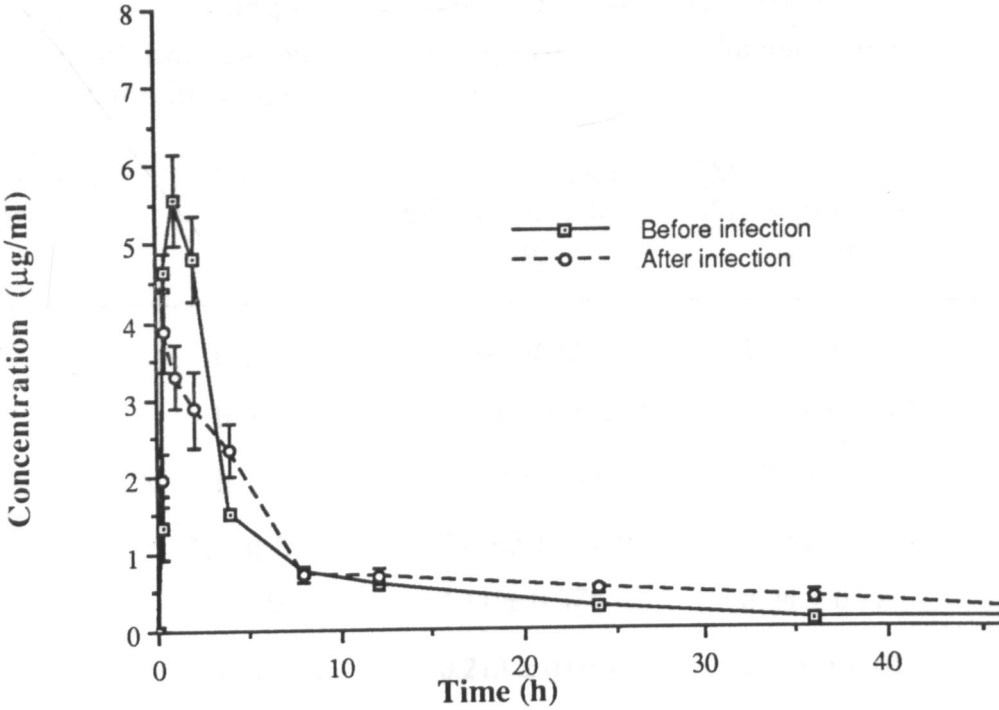


Fig 6c: Mean ± SD concentrations of doxcycline in serum following intramuscular administration of DOTC (20 mg\kg) to goats (n=6) before and after infection with *P. haemolytica*

4.5 Comparative pharmacokinetic parameters of DOTC

The pharmacokinetic parameters of DOTC after IM administration before and 13 days after experimental infection are presented in Table 9. In both treatment groups some major pharmacokinetic parameters differed. The elimination half-life ($t_{1/2ß}$) and apparent volume of distribution ($Vd_ß$) were significantly increased ($p< 0.05$) with pneumonia. Moreover, elimination rate constant (ß) and absorption half-life ($t_{1/2abs}$) were significantly decreased in pneumonic goats ($p < 0.01$).

Table 9: Pharmacokinetic parameters of DOTC after intramuscular injection of 20mg/kg bodyweight of a long acting (retard) formulation, before and after experimental *P. haemolytica* infection in six goats

Pharmacokinetic parameter	Unit	Mean ± SEM	
		Before infection (n=6)	After infection (n=5)
B	µg/ml	1.14 ± 0.02	1.52 ± 0.14
ß	h^{-1}	0.0518 ± 0.001**	0.018 ± 0.002
$t_{1/2ß}$	h	13.42 ± 0.35*	37.43 ± 0.29
AUC	mg.h/ml	70.865 ± 3.329	93.520 ±8.536
$V_{d(ß)}$	L/kg	4.11 ± 0.37*	8.24 ± 0.72
$CL_ß$	L/kg/h	0.213 ± 0.019	0.169 ± 0.01
Cmax	µg/ml	5.56 ± 0.58	3.87 ± 0.52
tmax	h	1.167 ± 0.167	1.15 ± 0.37
K_{ab}	h^{-1}	1.13 ± 0.02	8.23 ± 3.81
$t_{1/2abs}$	h	0.015 ± 0.012**	0.137 ± 0.03

Key:

** P<0.01

* P<0.05

CHAPTER FIVE

5.0: DISCUSSION

5.1: Experimental *P. haemolytica* model

Data on the pharmacokinetics of tetracyclines in diseased animals is scarce. In calves, Ames et al. (1983) and Burrows et al. (1986) used a pasteurella pneumonia model to evaluate OTC, while in goats Anika et al. (1986b) used an *Ehrlichia phagocytophila* model. Pijpers et al. (1990) used a pleuropneumonia model previously described by Van Leengoed (1988) to determine effects of pneumonia on the pharmacokinetics of OTC in pigs. Several models have been used to study the effect of infection on haemotological and biochemical changes in the target animal. In those infection models, pyrogenic cytokines like inter leuken-I (IL-1) and tumor necrosis factor-∂ (TNF∂), substances produced by activated macrophages, induced a sharp increase in the synthesis of brain prostaglandins E_2 which inhibits fore-stomach motility and raises the 'thermostat' of the heat-regulating mechanism resulting in fever (Van Miert et al., 1984; Van Miert, 1985, 1990) and an increase in WBC counts (Van Miert et al., 1982; Pijpers et al., 1990; Mevius et al., 1991). In the present study, a pneumonia model employing *P. haemolytica* was developed to determine the effects of pneumonia on the disposition kinetics of DOTC in goats. The increase in body temperature, haematological changes and respiratory changes were used to determine whether disease was present after experimental infection. The clinical signs observed after challenge were consistent with caprine pneumonic pasteurellosis reported by Mugera and Kramer (1967) and Ojo (1976). In this study, the maximum body temperature recorded was 40.9°C which is within the range reported by Ojo (1976). The incubation period following experimental

infection in this study was 6-10 days, while other workers have reported 3-7 days (Solana & Rivera, 1967; Ojo, 1976).

The pathogenesis of *P. haemolytica* infection in goats is unknown, however, in the plight of the present findings it is reasonable to conclude that caprine pasteurellosis can be experimentally reproduced by using pure cultures alone without any concurrent, extrenous stress factors. Perhaps, stress- a collective term used to describe a host of ill-defined factors may play a role in converting a latent carrier to an active state. The experimental infection study by what is likely to be the natural route gave some indication of the number of viable organisms required to set up clinical infection. Whether this is the actual dose, and as to how susceptible in-contact animals could pick such a large infective dose from an infective carrier remains to be determined. Moreover, the apparent absence of a dose response relationship suggests that the infective dose itself is a highly variable entity, presumably determined by the interaction of a variety of factors. Seemingly, this experimental model produced the desired systemic disease.

5.2 : Pharmacokinetics of DOTC in healthy goats

Following IM administration DOTC disposition was best described by a tri-exponential equation, except in one goat in which a 4 - exponential model best fitted the data and in two cases where only 2 - exponential phases were identified.

Following maximum absorption DOTC disposition was characterized by rapid distribution and slow elimination. This is in accordance with previous reports in man (Raghuram & Krishnaswamy, 1982), dogs (Wilson et al., 1988), calves (Riond et al.,

1989) pigs (Riond & Riviere, 1990) and cats (Riond et al., 1990). Pharmacokinetic Parameters of DOTC in goats have not been reported in literature. For OTC in healthy dwarf goats, Anika et al. (1986) used a two-compartment open model following intravenous administration. For DOTC in other species, Riond et al (1989) used a 2 - compartment model in calves, while Garcia-Ovando et al (1991) used a 3 - compartment model in calves. The estimate of $t^1/2ß$ (13.42 ± 0.35 h), obtained in healthy goats in this study is slightly lower than the 14.9 ± 0.9 h previously reported in calves with mature rumen function (Riond et al., 1989), 15.6 ± 0.5 h reported in man (Fourtillan et al., 1980) and 24.75 ± 10.6 h reported for ewes after IV administration (Ziv & Sulman, 1974). Following a single IV administration, a $t_{1/2ß}$ of 10.3 h was obtained by Wilson et al. (1988) in dogs while Michel et al. (1979) reported a $t^1/2ß$ value in dogs of 11.8 h after a single oral administration. In the healthy goats of this study, doxycycline $t^1/2ß$ (13.42 + 0.35 h) was somewhat longer than the previously reported value of 4.04 ± 0.58 h in pigs (Riond & Riviere, 1990), 6.99 ± 1.09 h and 4.56 ± 0.68 (SEM)h in dogs and cats, respectively (Riond et al., 1990). Such interspecies variations in the estimation of elimination half-life is probably attributable to differences in rates of metabolism and routes of administration, drug formulation, pharmacokinetic curve-fitting routines and analytical techniques used. However, close consideration of the calculated range of $t_{1/2ß}$ between two standard deviations (SD) in the study of ewes (Ziv & Sulman, 1974), of 24.75 ± 10.6 h reveals considerable overlap with the range observed in this study.

The apparent volume of distribution in the steady state $Vd_{(ß)}$ of healthy goats obtained in this study of 4.11 ± 0.37 L/kg is some what larger than the 0.93 ± 0.10 L/kg and 0.34 ± 0.03 L/kg reported in dogs

and cats, respectively (Riond et al., 1990) and 1.81 ± 0.11 L/kg in Holstein calves (Riond et al., 1989) and 0.53 ± 0.04 L/kg for young pigs (Riond & Riviere, 1990). Thus, bigger disparities in the estimation of the Vd among and within species is commonly encountered in literature. The large volume of distribution and subsequent longer elimination half-life for DOTC observed in goats of this study may be related to extensive tissue binding and/or intracellular penetration in this species. Previous studies suggest that pharmacokinetic parameters differ among species due to differences in the extent of binding to plasma proteins (Welling, 1986). The long $t^1/2ß$ of DOTC in goats and sheep compared to other species reported may be related to differences in bodyweight (Riond et al., 1990), a lower serum binding (Anika et al., 1986b) and probably due to a lower rate of metabolism as well as a high degree of ion trapping in the rumen, because total body clearance, CL_B of calves with mature rumen function was smaller than that of veal calves (Riond et al., 1989).

5.3 Effects of pneumonia on pharmacokinetics of DOTC

In evaluating the influence of disease on the disposition of tetracyclines, Burrows et al. (1986) found a significant increase in elimination rate constant (ß) between 2 h and 8 h and a decreased distribution rate constant (α) in pasteurella-challenged calves. Clark et al. (1976) reported higher serum concentrations of OTC in cattle with respiratory tract disease. In a more extensive study (Ames et al., 1983), initial serum concentrations were reported to be lower, but this was offset by a decreased ß, so that after a few hours, serum concentrations were similar for diseased and non-diseased calves. In the present study, initially serum concentrations appeared to be higher in prepneumonic goats but after 4 to 8 hours post administration serum concentration

remained the same and thereafter remained lower for prepneumonic goats (Fig. 5). In agreement with the results of the present study, Anika et al. (1986b) found a significantly increased elimination half-life of OTC in infected goats. Moreover, in this study Vd(ß) was significantly increased with pneumonia while ß was significantly decreased. An increased $t^1/_2ß$ and Vd(ß) values for Aditoprim have been reported in tickborne fever (TBF) infected goats (Knoppert et al., 1988) whereas an increased volume of drug distribution for penicillin G has been reported in dogs suffering from a generalized streptococcal or *Pseudomonas aeruginosa* infection and for quinine during febrile episodes in patients with malaria (Van Miert, 1985). Similar results were found in rabbits, pigs and in man during endotoxin induced fever (Van Miert, 1985). Although pharmacokinetic parameters are not necessarily related to specific tissues, the fact that differences in $Vd_{(ß)}$ and $Vd_{(ss)}$ were significant is indicative of an increase in distribution space during disease state. This finding is in agreement with the statement made by Baggot (1980) that disease increases the distribution volume of a drug, possibly by enhanced capacity to penetrate cellular membranes. Interestingly the increased distribution space observed in the present study did not affect serum DOTC concentrations significantly. This was because of continous absorption from injection site during the distribution phase following intramuscular injection of the repository formulation. As a result AUC_∞ was not significantly decreased. In addition, DOTC absorption from the injection site was increased significantly with infection. This is at variance with previous findings (Van Miert, 1990) that drug absorption is delayed from the injection site during febrile episodes.

The exact mechanisms involved in causing changes in distribution of DOTC in tissues following disease can only be speculated. For instance, diseases that affect plasma protein concentrations may decrease the extent of binding and alter the free drug concentration, disposition kinetics and thereby therapeutic and toxic responses (Koch-Weser and Sellers, 1976). Also, increased protein binding, owing to an increase in acute-phase reactant protein concentration, may alter the free drug fraction that is available to equilibrate with the receptor sites in the tissues (Van Miert, 1990). In the present study, after *P. haemolytica* infection total protein concentration was decreased. Thus, it could have had an effect on the extent of serum binding resulting in a high free drug concentration in serum. Shifts in pH in certain compartments, secreta or excreta during disease, as a result of local acidosis due to poor perfusion in disease states can induce substantial changes in the degree of ionization. The unchanged distribution of amoxycillin and chloramphenical during endotoxin induced fever in veal calves (Groothius & Van Miert, 1987) is consistent with this explanation since amoxycillin is an acid with a low pk_a value (2.4) while chloramphenical is a neutral drug, and therefore will hardly respond to small pH shifts. In similar studies, tick borne fever (TBF) infected goats had significantly prolonged elimination half-life values for chloramphenical (Anika et al., 1986b) and sulphadimidine (Anika et al., 1986b). The greatest changes observed with sulphadimidine and chloramphenical was attributable to a decrease in the metabolism of both drugs in febrile episodes (Anika et al., 1986a; Nouws et al., 1986). Unfortunately the metabolic profile of doxycycline in goats is yet to be determined.

Although experimental infection increased the elimination half-life of DOTC during the elimination phase, the clearance (CL_{β}), which is the sum of renal and non-renal clearance values and considered to be a better index of the efficiency of drug elimination (Baggot, 1980), was not significantly decreased. This is in agreement with the findings of Ames et al. (1983). Thus, it might be inferred that the overall elimination of the drug was not affected by the disease state, and that the change in $t_{1/2(\beta)}$ reflected a change in the volume of distribution.

5.4 Pharmacotherapeutic Implications

Despite the ease of determining serum concentrations and kinetics, these results indicate that it is not reasonable to determine dosage regimen of drugs from serum concentrations unless consideration is given to the disparity between healthy and diseased states. In this study differences were found between healthy and pneumonic goats. The MIC of DOTC for the *Pasteurella haemolytica* isolate in the study was determined to be approximately 0.4 μg/ml. A single IM administration of the drug at the recommended dosage rate of 20 mg/kg would be expected to maintain tissue concentrations of approximately 0.4 μg/ml or higher for 36 hours. An equivalent lung concentration would be expected to be marginally effective for the *Pasteurella* isolate used in the study. If the MIC were ≥ 0.5 μg/ml, a higher dose or a second dose would be necessary. However, a proportionate increase in serum or tissue concentrations with increased dosage may not be expected (Burrows, 1985; Pijpers et al., 1990). Instead, a second IM injection after 36 hours may be more effective than increasing the dose.

6.0: GENERAL CONCLUSIONS

1. The results of the present study indicate both decreased initial serum concentrations and a longer sojourn of the drug in goats suffering from respiratory disease. It is concluded that although experimental pneumonia had an effect on the pharmacokinetic profile of doxycycline, this effect is not so pronounced to necessitate alteration of dosage regimen during disease. However, further studies are required to determine actual changes in drug concentration at the site of infection. For drugs such as DOTC, with a high volume of distribution (Vd), the intravascular compartment contains a small proportion of the drug in the body. Thus determination of drug pharmacokinetics on the basis of serum concentration alone may not trully reflect tissue kinetics.

2. Based on serum concentrations and kinetic data obtained under diseased state coupled with determined *in vitro* activity, it can be concluded that DOTC could be a good alternative for OTC to treat respiratory tract infection in goats.

3. The study shows that caprine pasteurellosis can be produced experimentally by using pure cultures alone without any stress or concurrent synergistic viral infections as reported previously.

4. The minimum inhibitory concentration of doxycycline against the *P.haemolytica* isolate used in this study was found to be approximately 0.4 μg/ml.

5. Doxycycline disposition in healthy goats was best described by a 2-compartment open model with first-order absorption, a rapid distribution and a slow elimination phase.

6. Maximum serum concentration, C_{max}, time taken to attain such concentration, t_{max} and elimination half-life $t^1/_2$ ß of doxycycline in healthy goats were 5.56 ± 0.58 μg/ml, 1.167 ± 0.167 h and 13.42 ± 0.35 h respectively.

7. The elimination half-life and the apparent volume of distribution were significantly increased with pneumonia. While the elimination rate constant was significantly decreased in pneumonic goats.

7.0: REFERENCES

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Appendix.1: Experimental details of animals used in the study.

GOAT NO.	AGE (YRS)	SEX	BODY.WT(KG)	DOSE OF *P. HAEMOLYTICA*
32	1	F	21	-
36	1	M	20	10^9
37	1	M	20	10^7
38	2	F	26	10^7
41	1	M	21	10^8
42	1	M	18	10^9
46	1	M	21	10^8

Key:

M - Male

F - Female

Appendix 2: Mean daily temperature (°C) following infection with *Pasteurella haemolytica*.

	Goat number							
Days	32*	36	37	38	41	42	46	Pyrexic Ratio
0	38.8	38.6	38.9	38.8	38.6	39.4	38.6	0
1	39.0	39.8	39.6	39.8	41.2	39.6	40.0	0.83
2	38.5	38.6	38.8	38.7	38.8	38.8	39.1	0
3	38.8	38.6	38.7	38.7	38.5	38.6	38.6	0
4	39.0	39.0	39.2	38.6	38.7	39.0	39.0	0
5	38.8	38.5	39.0	38.8	38.0	38.6	38.8	0
6	39.2	39.2	39.6	39.8	39.6	39.3	39.7	0.67
7	39.1	39.3	39.6	39.4	39.4	39.5	39.6	0.33
8	39.1	39.0	39.3	39.6	39.2	39.2	39.6	0.33
9	39.1	38.7	39.9	39.2	38.6	39.8	39.1	0.33
10	39.2	40.2	40.2	39.8	40.5	40.0	40.0	1.00
11	39.2	39.1	39.6	38.7	39.4	40.9	40.2	0.50
12	38.8	38.8	40.9	39.8	38.6	39.8	39.3	0.50
13	39.0	39.3	9.3	39.5	39.3	40.0	39.2	0.33
14	38.7	39.0	40.2	39.2	38.8	39.2	39.1	0.17
15	38.4	38.8	39.1	38.9	38.6	38.9	38.5	0

*- uninfected control

Appendix 3: Mean daily respiration rates (per minute) of goats following inoculation with *Pasteurella haemolytica*.

DAYS	GOAT NUMBER.						
	32	36	37	38	41	42	46
0	18	20	30	24	15	20	24
1	20	20	28	24	24	22	24
2	24	22	32	20	20	28	26
3	22	22	30	22	20	26	24
4	20	20	30	22	20	24	24
5	20	24	32	24	24	28	28
6	20	38	34	34	24	32	32
7	18	28	38	24	32	32	34
8	18	26	36	30	22	24	34
9	24	26	36	26	22	26	32
10	24	20	34	24	20	32	22
11	24	28	34	24	22	24	32
12	18	34	34	26	22	26	32
13	18	36	34	30	28	28	34
14	20	34	42	22	24	32	28
15	22	28	32	28	20	26	28

*- uninfected control.

Appendix 4: White blood cell counts (X $10^3/\mu l$) of each goat following experimental infection with *Pasteurella haemolytica*.

	DAYS			
GOAT NUMBER	0	4	8	12
32*	8.4	9.54	8.2	10.8
36	14.9	11.92	16.0	18.06
37	10.13	15.3	11.2	18.05
38	12.2	9.56	11.9	13.74
41	12.4	10.1	12.4	17.0
42	17.8	15.94	14.4	20.01
46	10.2	10.23	8.9	13.83

* - uninfected control

Appendix 5: The trend in total protein concentration(g/dl) of each goat following inoculationwith *P. haemolytica*.

	DAYS			
GOAT NUMBER	0	2	4	8
32*	8.6	7.0	6.4	6.6
36	7.4	6.6	6.2	6.2
37	8.4	7.2	7.0	6.4
38	7.8	7.0	7.2	6.8
41	7.4	7.2	6.8	6.8
42	7.4	6.4	6.8	6.0
46	7.2	6.8	6.4	6.4

*- uninfected control

Appendix 6. Serum levels (μg/ml) of DOTC (20 mg/kg) following intramuscular administration to healthy goats (n=7)

Time (hrs)	Goat number						
	32	36	37	38	41	42	46
0	0	0	0	0	0	0	0
0.25	1.02	1.02	2.18	1.02	1.02	1.40	1.40
0.5	4.5	4.5	5.16	4.5	4.5	4.8	4.6
1	ND	5.16	6.54	5.16	5.82	4.8	5.9
2	6.0	4.5	6.0	4.5	4.5	4.5	4.8
4	1.56	1.56	1.52	1.5	1.52	1.5	1.5
8	0.58	0.78	1.26	0.58	0.78	0.78	0.58
12	0.78	0.78	0.76	0.8	0.78	0.78	0.74
24	ND	0.22	0.24	0.36	0.22	0.34	0.22
36	0.12	0.14	0.10	0.12	0.14	0.10	0.14
48	0.08	0.10	0.08	0.10	0.08	0.08	0.10

Key:

ND - Not determined

Appendix 7: Serum levels (μg/ml) of DOTC following intramuscular administration to six goats infected with *P. haemolytica* (Group B)

	Goat numbers					
Time (hrs)	36	37	38	41	42	46
0	0	0	0	0	0	0
0.25	2.08	2.0	1.5	2.2	1.4	1.56
0.50	3.12	4.16	4.2	3.16	4.2	4.4
1	3.8	3.2	2.5	3.4	3.4	3.5
2	3.15	3.65	2.6	2.3	2.3	3.2
4	2.52	2.52	1.64	2.2	2.60	2.52
8	0.78	0.78	0.78	0.58	0.58	0.78
12	0.78	0.78	0.58	0.58	0.58	0.78
24	0.46	0.58	0.58	0.46	0.46	0.46
36	0.36	0.58	0.36	0.36	0.34	0.36
48	0.22	0.18	0.16	0.18	0.22	0.24

Appendix 8: Pharmacokinetic parameters of each goat before *Pasteurella haemolytica* infection.

Kinetic parameter	GOAT NUMBER					
	32	36	38	41	42	46
B	0.93	0.97	0.79	0.94	1.02	0.95
ß	0.055	0.052	0.047	0.051	0.055	0.051
Kab	1.10	1.12	1.06	1.12	1.21	1.16
t1/2abs	0.63	0.62	0.66	0.61	0.57	0.60
t1/2ß	12.53	13.24	14.87	13.69	12.60	13.56
t1/2el	4.32	3.95	3.65	3.83	4.23	3.76
AUC	25.28	39.95	39.95	36.84	32.93	37.62
CLß	0.79	0.50	0.50	0.54	0.61	0.53
Vdß	14.4	9.6	10.6	10.6	11.1	10.4

Appendix 9: Pharmacokinetic parameters of each goat after *Pasteurella haemolytica* infection.

Kinetic parameter	GOAT NUMBER 36	37	38	41	46
B	0.30	0.93	0.89	0.67	0.29
ß	0.01	0.02	0.02	0.02	0.01
Kab	3.73	4.92	5.77	23.37	3.34
t1/2abs	0.186	0.141	0.120	0.03	0.208
t1/2ß	67.83	31.78	27.59	33.60	68.30
t1/2el	24.14	24.10	25.00	25.07	24.22
AUC	45.98	45.92	35.67	35.34	45.34
CLß	0.43	0.44	0.56	0.57	0.44
Vdß	43.50	21.78	28.03	28.30	44.10

ERATTA

Pg 2. line 19 delete "healthy and".

line 21 Insert " However, Jha et al (1989) reported pharmacokinetic data in healthy female goats following intravenous injection. Hence ...

Pg 60 Insert as line 18 reference as follows;

Jha, V.K., Jayachandran, C. Singh, M.K. and Singh S.D.(1989).Pharmacokinetic data on doxycycline and its distribution in different biological fluids in female goats. *Vet. Res. Comm.* 13, 11-16.